UNIVERSITÀ POLITECNICA DELLE MARCHE

PhD course in Life and Environmental Sciences

Curriculum in Marine Biology and Ecology

Analysis of virus – prokaryote interactions under climate change scenarios using statistical and mathematical modelling approaches

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General introduction

Prokaryotes are an important component present in every ecosystem, from deserts to ice and hadal trenches. Besides their importance in term of abundance, they play major roles in organic matter degradation, carbon cycling and nutrient regeneration processes, sustaining primary productivity. At the same time, prokaryotes inhabiting ecosystems such as hot springs, cold seeps, and hydrothermal vents can be involved in important chemosynthetic processes, producing organic carbon through the use of inorganic compounds. Moreover, they are capable of being metabolically active even in the most extreme ecosystems on Earth, including the biosphere thousands of kilometres below terrestrial soil or the seafloor. Besides prokaryotes, also viruses have been reported in every habitat and ecosystem analyzed so far. The viral component has been especially explored in marine systems, where it exerts major control on prokaryotic dynamics through the lytic cycle. By killing prokaryotic hosts, viruses may play a key role in the carbon cycle and nutrients regeneration, indeed, the organic matter from killed cells can be used by non-infected prokaryotes. However, our knowledge on prokaryotic and viral abundance and production remains quite elusive in most habitats and need to be better evaluated also in the framework of global change scenarios. Analyzing prokaryotes and viruses is challenging and requires different approaches based on both statistics and mathematical modelling to understand virus-host interactions that can show patterns and dynamics nonlinear, oscillatory and dependent on multiple factors, as in the case of prokaryotic production that depends on both thermal and chemical energies, as elucidated in the Metabolic Theory of Ecology.

There is evidence that global climate change can induce important consequences both in marine and terrestrial ecosystem but our knowledge of its impacts on the largest ecosystem, the deep sea, is still limited. Thus, understanding the climate change effects on virus-prokaryote dynamics in the deep-sea is crucial for comprehending the biogeochemical processes that sustain the functioning of global biosphere. Moreover, the deep-sea can be vulnerable to different global change effects, one including the long term rise of bottom water temperature and the expected decrease, on most of Earth’s marine ecosystems, of the export of organic particles produced by photosynthesis from which the majority of deep sea ecosystems rely on. The other related to climate-driven episodic events that can cause sudden and significant disruptions in affected ecosystems. Therefore, it is crucial to identify specific deep sea ecosystems that can be used as model to assess the potential effects of present and future climate change scenarios.

General aims

The main aims of this PhD study were:

1. Investigating the abundance and distribution of viruses and prokaryotes in different environments (chapter 1).
2. Assessing environmental drivers that may influence viral and prokaryotic abundances and productions in different ecosystems (chapter 2).
3. Forecasting the spatial distribution of viral and prokaryotic abundances and productions in the global ocean and potential changes in relation with the future climate change scenario (chapter 2).
4. Developing mechanistic models to analyse virus-prokaryote interactions in deep-sea ecosystems under global climate change scenarios (chapter 3-4).
5. Evaluating how climate-driven (episodic) events can affect the virus-prokaryote dynamics in the deep sea (chapter 4).

Chapter 1

Viral and prokaryotic abundances and distributions on global scale

1.1 Introduction

The fundamental roles of microbes and of their viruses in all ecosystems, and especially in marine ecosystems, has been the focus of many studies in the last decades (Fuhrman, 1999; Suttle, 2005), aimed at elucidating the factors driving prokaryotic dynamics on Earth (Maranger et al., 1995). Prokaryotes (including bacteria and archaea) represent one of the largest biomass pools on earth (Rex et al., 2006; Wei et al., 2010; Bar-On et al., 2018; Bar-On et al., 2019), they serve as a substantial food source for higher trophic levels (Dell'Anno et al., 2005; Danovaro et al., 2015) and play key roles in biogeochemical cycles, largely contributing to overall ecosystem functioning at the global scale (Proctor et al., 1990; Nealson, 1997; Fuhrman, 1999; Mason et al., 2009; Arndt et al., 2013; Carreira et al., 2013; Danovaro et al., 2015; Coutinho et al., 2019; Ji et al., 2023). Bacteria and archaea are fundamental for organic matter cycling and nutrients regeneration processes in the entire biosphere, profoundly influencing global biogeochemical cycles and ecosystem functioning (Maranger et al., 1995; Madsen, 2011; Fenchel et al., 2012; Rousk et al., 2014; Maier, 2015).

Viruses represent the most abundant biological entities in the world and are presumed to infect virtually all organisms (Bergh et al., 1989; Proctor et al., 1990; Suttle et al., 1990; Whitman et al., 1998; Karner et al., 2001; Mei et al., 2004; Weinbauer, 2004; Suttle, 2007; Williamson et al., 2017; Breitbart et al., 2018; Bar-On et al., 2018; Chevallereau et al., 2022; Shiah et al., 2022; Mushegian, 2020). Determining prokaryotic mortality, viruses exert crucial influences on prokaryotic assemblages and related ecosystem dynamics (Wommack et al., 2000; Weinbauer, 2004; Suttle, 2005; Williamson et al., 2005; Danovaro et al., 2008; Rowe et al., 2008; Rohwer et al., 2009; Danovaro et al., 2015; Liang et al., 2019; Liang et al., 2021). By infecting and lysing microbial host cells and driving genetic transfers, viruses impact numerous biogeochemical and ecological processes, including nutrient cycling (particularly for nitrogen and phosphorus), energy transfer to higher trophic levels, sedimentation rates, organic matter cycling, microbial diversity, genetic transfer and overall microbial evolution (Dell'Anno et al., 2015; Fuhrman, 1999; Wommack et al., 2000; Weinbauer, 2004; Suttle, 2005; Suttle, 2007; Danovaro et al., 2008a,b; Jover et al., 2014; Danovaro et al., 2015; Wommack et al., 2015; Liang et al., 2019; Liang et al., 2021). Despite the majority of viruses can infect prokaryotes, other studies have shown that viruses do not exclusively infect prokaryotes; they can also target other organisms, including animals and plants (Gran-Stadniczeñko et al., 2019; Middelboe et al., 2017).

There is significant interest in understanding the interconnections between ongoing climate change and its impacts on ecosystems, particularly concerning viruses and prokaryotes. A comprehensive understanding of their responses to current climate change could substantially improve our ability to predict the resulting changes and related implications (Danovaro et al., 2011; Parray et al., 2022). Consequently, forecasting alterations in biological communities has emerged as a central objective in ecology, although the complexity of these communities presents several challenges. Environmental changes affect the physiology of individual organisms and their interactions, leading to shifts in the composition and functioning of biological assemblages (Suding et al., 2004; Dell et al., 2014; Burnside e al., 2014; Amarasekare, 2015; D. J. Smith et al., 2018; Tseng et al., 2019; García et al., 2023; Sun et al., 2023; Crocker et al., 2023). However, further studies are essential to provide deeper insights into the response of the microbial assemblages and of their viruses to impacts due to climate changes. Anticipating how the microbial assemblages will react and interact with global warming, ocean acidification, eutrophication, and other climate changes in the years ahead is of paramount importance (Danovaro et al., 2011; Coelho et al., 2013; Coutinho et al., 2019). Before forecasting future climate change impacts through modelling approaches, it is necessary to understand the present situation more comprehensively. Of particular significance is the quantification of relative abundances within the biosphere (Bar-On et al., 2018), where viruses and prokaryotes are the most abundant biological entities, inhabiting virtually the entire planet (Shiah et al., 2022). Prokaryotes, constitute a substantial biomass pool and are present in high abundance in nearly all ecosystems (Bar-On et al., 2019). Despite viruses represent only 1% of the total biomass in the ocean (Bar-On et al., 2019), they outnumber bacteria and archaea by about one order of magnitude across diverse habitats (Bergh et al., 1989; Proctor et al., 1990; Hara et al., 1996; Wommack et al., 2000; Hara et al., 2012; Carreira et al., 2013; Wigington et al., 2016; Lara et al., 2017). Hence, gaining a comprehensive, quantitative view of the current state of microbial standing stocks is becoming increasingly important to improve global biogeochemical models, as well as for understanding the environmental impacts and virus-prokaryote interactions with the human impacts (Bar-On et al., 2018).

In this Chapter, by integrating data from existing literature and public databases, we provide for the first time a comprehensive view not only of the global abundance of prokaryotes and viruses, but also of their viral and heterotrophic production, to implement global estimates and provide useful information for environmental modelling approaches.

1.2 Aims

The specific aims of this chapter were:

1. Collection of data from literature and public databases on the abundance and production of prokaryotes and viruses across the main ecosystems on Earth.

2. Estimating viral and prokaryotic abundance and production at global scale based on different computational approaches.

1.3 Materials and methods

1.3.1 Data collection

A literature review was conducted to find information on virus-prokaryote dynamics across different habitats at a global spatial scale (i.e., worldwide). Our search was conducted on Google Scholar in the period from March 2020 to June 2020 selecting keywords as: “virus and prokaryotes”, “viral and prokaryotic abundance”, “viral and prokaryotic production”, “virus and prokaryotes dynamics”, “virus and prokaryotes interactions”. The presence of eventual dataset duplicates was checked, and eventual replicates were removed to exclude data overlap/redundancy. Only manuscripts reporting both viral and prokaryotic components were considered.

1.3.2 Sample area

The distribution of our sampling data points covers many habitat types around the globe, including sea water, marine sediments, freshwater, freshwater sediments, soil, ice, aquifers, aerosol and basaltic fluid (Fig. 1.1).

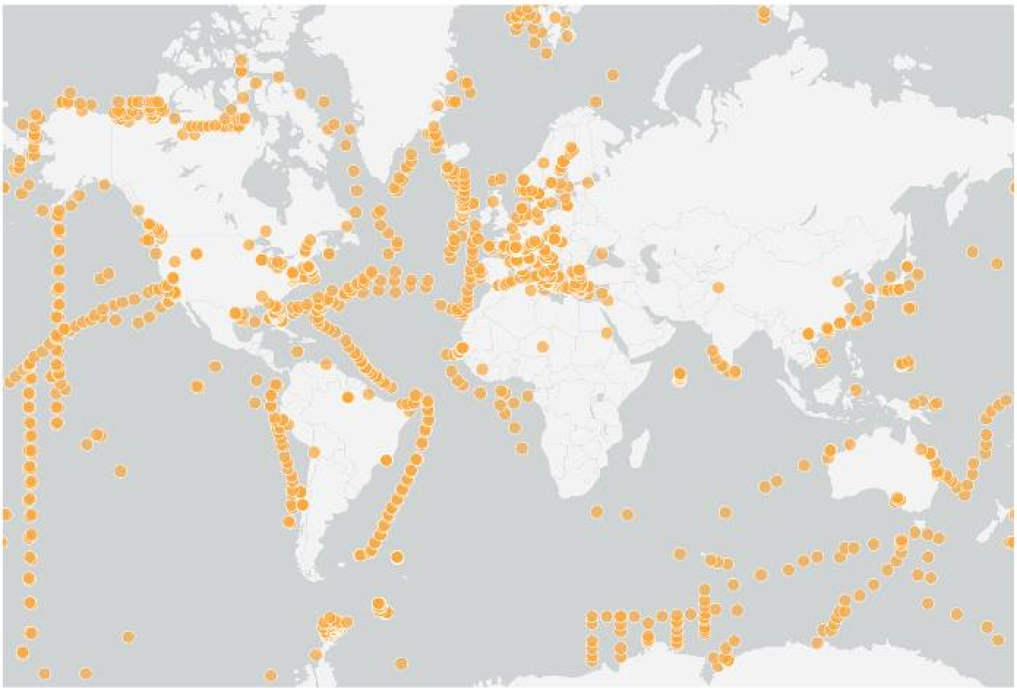

Figure 1.1 Sampling sites. Map showing the distribution of sampling sites in our global-scale study.

The number of records for each ecosystem is shown in Table 1.1.

System	number of records
Sea water	9131
Marine Sediments	417
Freshwater	411
Soil	157
Aerosol	77
Ice	71
Freshwater Sediments	38
Aquifers	18
Basaltic fluid	8

Table 1.1 Ecosystem types and relative number of sampling points. In this table are listed the types of environments considered and the number of samples per habitat type recovered and considered for the computations within this study.

1.3.3 Covariate and assumptions

The covariates analysed in this chapter have been abundances and productions. All these values collected from different studies and different ecosystems have been normalized to allow comparisons (i.e., prokaryotic abundances as number of cells per cm^3, viral abundances as virus-like particles (VLP) per cm^3, prokaryotic production as ng C per cm^3 per hour and viral production as VLP per cm^3 per hour).

To perform such normalization of the data we made the assumptions summarized in Table 1.2.

	value	**Udm**	**range**	**Reference**
Dry bulk factor for desert	1.5	$g * cm^{-3}$	1.39 - 1.69	Lan et al., 2019
Dry bulk factor for agricultural soil	1.6	$g * cm^{-3}$	1.3 - 1.7	Katuwal et al., 2020
Dry bulk factor for rhizosphere	1.25	$g * cm^{-3}$	0.9 - 1.6	Carminati et al., 2010
Dry bulk factor for forest	0.81	$g * cm^{-3}$		Prietzel et al., 2020
Average biomass for viruses	0.06	fg C	0.04 - 0.08	Dell'Anno et al., 2015
Average biomass for prokaryotes	20	fg C	5 - 75	Bar-On et al., 2018

Table 1.2 Assumptions. In this table are listed all the assumptions considered for the conversions.

1.3.4 Depth-Trends

We identified trends in cell count variability depth by plotting viral or prokaryotic abundances and productions as a function of water, sediment and soil depth. All calculations were performed on non-transformed data. Then we implemented two methods to approximate these distributions along depth.

a) In the first method we intercepted the prokaryotic and viral trends with depth by using an exponential function (Sun et al., 2021):

$$Y = a * 10^{b*x}$$

where x represents soil depth and Y are the viral and prokaryotic abundances or productions. The a, b parameters were estimated from a linear regression between depth (x, in cm) and the abundances and productions (Y, in $\log_{10}$).

b) The second method followed the approach of Kallmeyer (et al., 2012), using a power-law to describe the relationships between depth (X, in cm) and abundances or productions:

$$Y = a * X^{b}$$

Also in this method a, b parameters were estimated from a linear regression between depth (X) and the abundances or productions, both in $\log_{10}$ (Y).

1.3.5 Relationship between viral and prokaryotic abundances

To analyze the relationship between viral and prokaryotic abundances, we employed a univariate linear statistical model on a logarithmic scale for both viral and prokaryotic abundances (Wigington et al., 2016; Lara et al., 2017):

$$log_{10}V = log_{10}c + q\, log_{10}B$$

where the slope q denotes the power-law exponent that best describes the relationship between the variables, whereas c represents the multiplicative factor:

$$V = c * B^{q}$$

1.3.6 Global estimates

To obtain global estimates of the prokaryotic and viral abundances and productions, we employed three approaches. Initially, we calculated the mean concentrations and productions and multiplied them by the entire volume of the corresponding ecosystem, utilizing available data on ecosystem volumes from literature. Where feasible, we attempted to enhance detail by segmenting systems, such as in the case of sea water, where calculations were performed on three different bathymetric zones (0-200, 200-1000, >1000) where we had available volume data provided by NOAA, and in the case of marine sediments where we distinguished four sediment layers (0-20cm, 20-100cm, 1-15m, 15-60m) due to the distribution of our data. For soil, we considered a whole layer from the soil surface down to 5 m (0-5m). These estimates should be analysed with caution, as they are very much affected by the magnitude of volumes, especially in environments such as sea water and marine sediments where very large volumes lead to very large errors.

Therefore, we also utilized a dual integral approach, calculating integrals of abundance and production profiles derived from the exponential decline (Sun et al., 2021) and power-law (Kallmeyer et al., 2012) functions previously determined.

Finally, employing unit biomass values (Table 1.2), we calculated global values of viral and prokaryotic biomass based on global data of cells and VLPs number.

1.4 Results

1.4.1 Prokaryotic and viral abundance distribution

The violin plots in Fig 1.2, 1.3, 1.4 and 1.5 report the distribution of global prokaryotic and viral abundances. In the case of prokaryotes, the highest median values are observed in both sediment categories: freshwater sediments (1.3×10^9 cells cm^{-3}) and marine sediments (2.5×10^8) respectively. Soil (4.0×10^7) and freshwater (3.1×10^6) follow with a difference of almost an order of magnitude. Ice (7.9×10^5) and sea water (3.2×10^5) show intermediate values, followed by aquifers (1.3×10^5), basaltic fluid (1.0×10^4), and aerosol (5.0). Freshwater sediments and soil exhibit the widest values ranges with the first showing abundances from 2.4×10^7 to 4.6×10^{10}, and soil from 4.1×10^3 to 7.2×10^9.

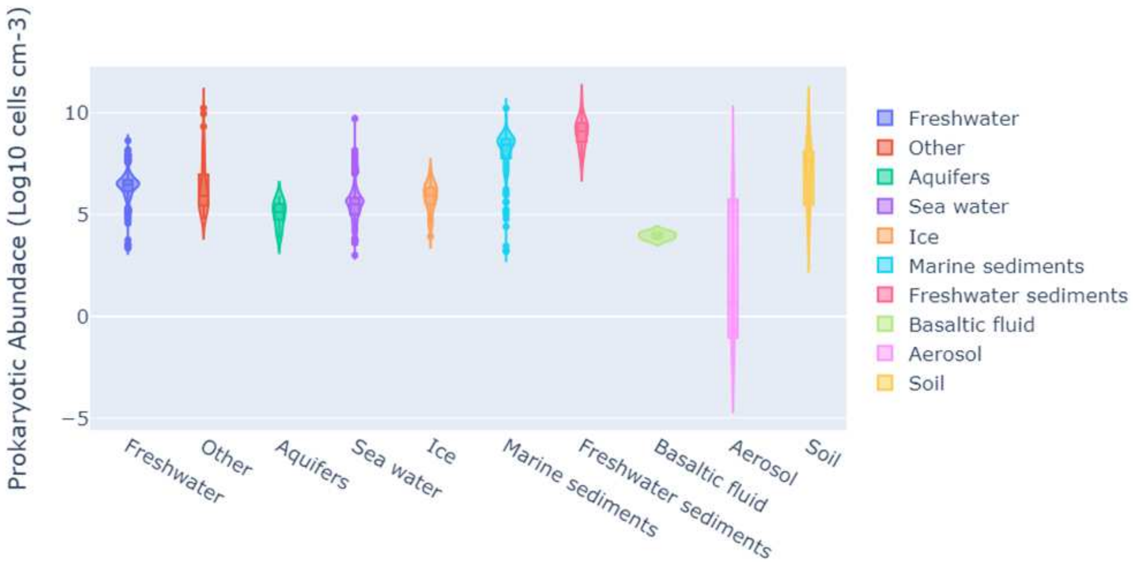

Figure 1.2 Distributions of prokaryotic abundance. Violin plots showing the distributions of prokaryotic abundances in each of the ecosystems considered in this study.

For pelagic marine ecosystems, the highest median values are found in the North Baltic Sea (7.9×10^5), followed by the Arctic (5.0×10^5) and Pacific Ocean (4.0×10^5). The Mediterranean Sea (3.2×10^5), Antarctica (2.5×10^5), and Indian Ocean (2.5×10^5) assume intermediate values, with the Atlantic Ocean displaying the lowest values (2.0×10^5). However, looking at the ranges of variability, the latter has the widest range (1.0×10^3 - 1.0×10^8), followed by the Pacific (6.6×10^3 - 5.4×10^7) and Indian (2.0×10^4 - 6.9×10^7) oceans. The Mediterranean (2.4×10^4 - 1.9×10^6) and Antarctica (2.2×10^4 - 2.6×10^6) have the lowest ranges.

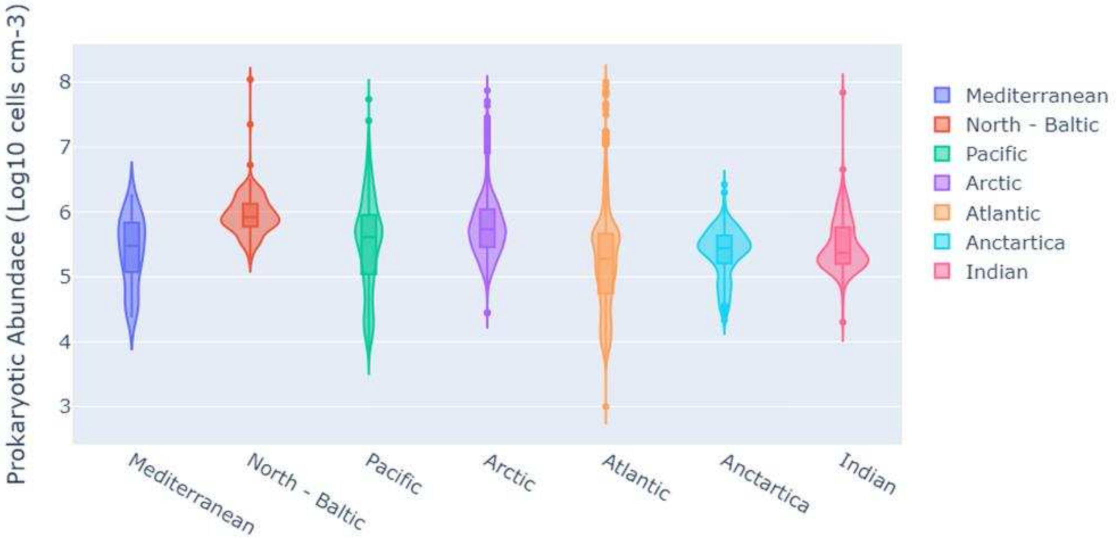

Figure 1.3 Distributions of prokaryotic abundance in sea water column. Violin plots showing the distribution of prokaryotic abundances among the seas and oceans considered.

For viruses, we observe a similar trend across different environments, in particular, the highest median values are found in both categories of sediment: freshwater sediments (4.0 × 10^9 VLPs cm^{-3}) and marine sediments (5.0 × 10^8) respectively, followed by soil (4.0 × 10^8). At more than one order of magnitude, we find freshwater (2.0 × 10^7), while ice (7.9 × 10^6) and seawater (4.0 × 10^6) fall again in the intermediate range, followed by aquifers (5.0 × 10^5), basaltic fluid (6.3 × 10^4), and aerosol (13). Considering the ranges of values, freshwater (9.5 × 10^3 - 7.3 × 10^{11}), marine sediments (1.0 × 10^4 - 8.2 × 10^{10}) and freshwater sediments (8.0 × 10^7 – 2.7 × 10^{10}) appear to have the widest ranges, followed by soil (from 3.3 × 10^3 to 1.8 × 10^{10}).

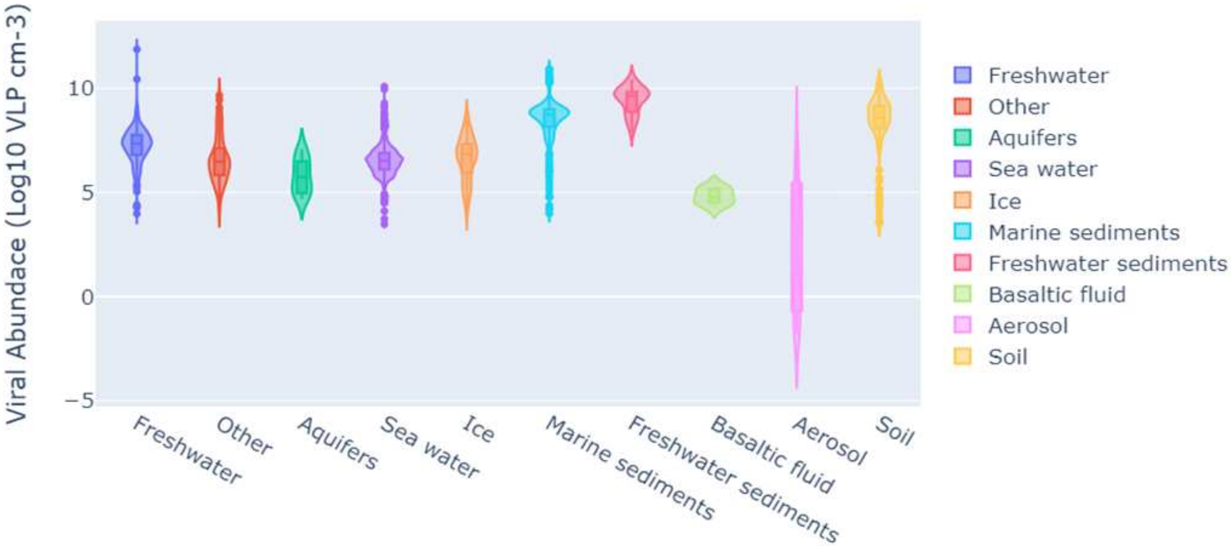

Figure 1.4 Distributions of viral abundance. Violin plots showing the distribution of viral abundances among the ecosystems considered.

Focusing on the water column, we observe again a distribution similar to that of prokaryotes, indeed, the highest median values are found in the North Baltic Sea (1.0×10^7), followed by the Arctic (6.3×10^6) and Pacific Ocean (6.0×10^6). The Mediterranean Sea (3.2×10^6) and Antarctica (3.2×10^6) show intermediate values, with the Indian Ocean (2.0×10^6) and the Atlantic Ocean (2.0×10^6) closing the list. However, when considering the value ranges, once again, the Atlantic Ocean shows the widest range ($3.0 \times 10^3 – 6.0 \times 10^8$), followed by the Indian Oceans ($3.0 \times 10^5 – 5.5 \times 10^8$), the North Baltic Sea ($1.1 \times 10^6 – 5.5 \times 10^8$) and the Pacific ($6.2 \times 10^5 – 3.8 \times 10^8$).

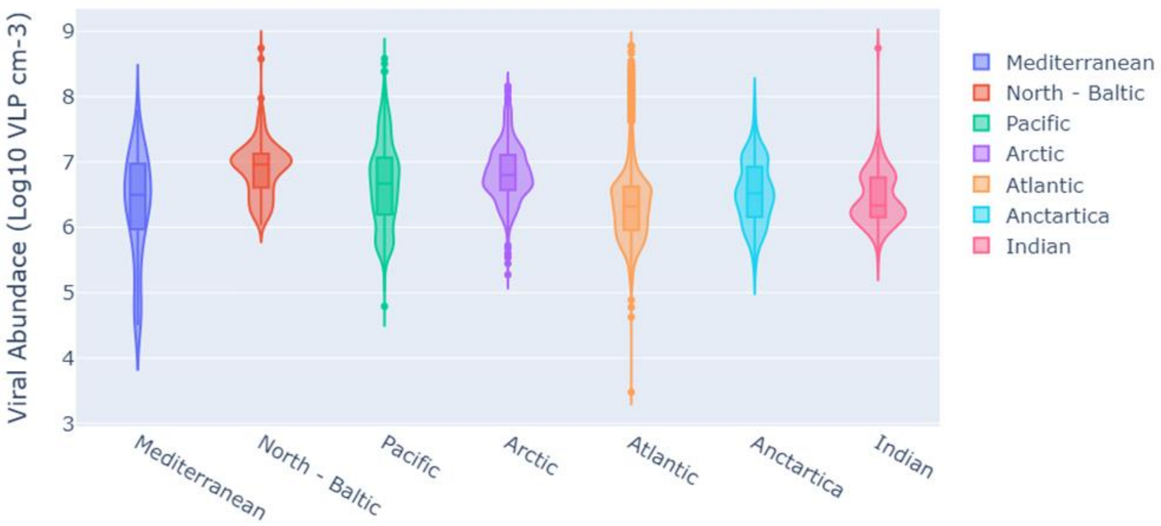

Figure 1.5 Distributions of viral abundance in sea water column. Violin plots showing the distribution of viral abundances among the seas and oceans considered.

Finally, we verified that the linear relationship between viral and prokaryotic abundances in logarithmic scale holds true across all systems. This relationship, is statistically significant ($p < 0.01$), has a coefficient of 0.72 and explains 71% of the variance in viral abundances (i.e., R-squared is equal to 0.71).

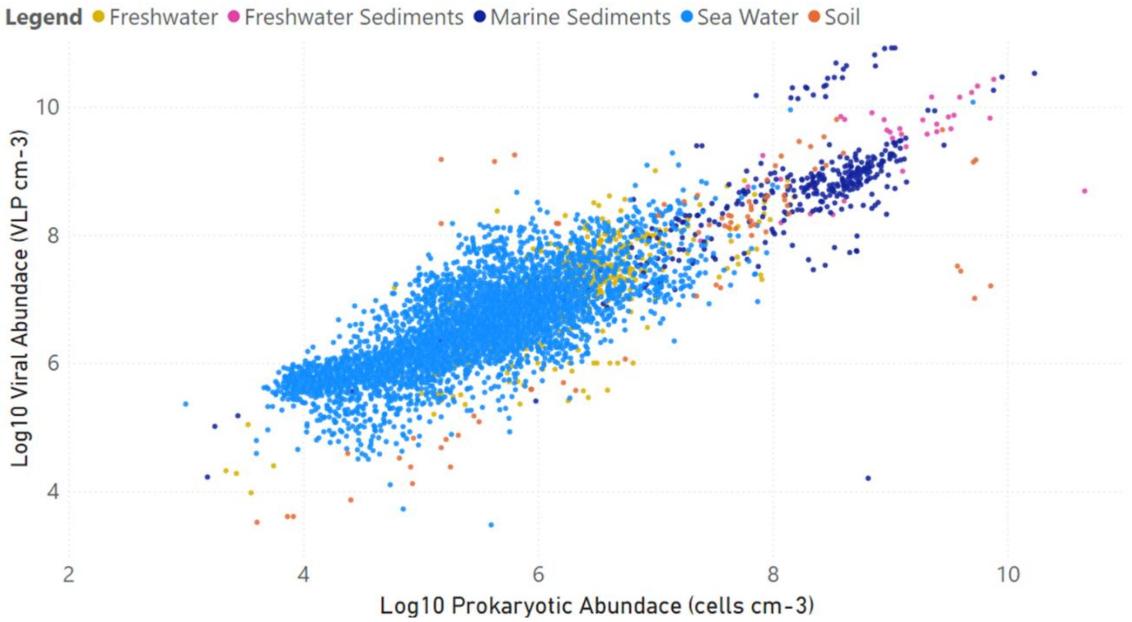

Figure 1.6 Relationship between viral and prokaryotic abundances. Scatterplot showing the relationship between viruses and prokaryotes in the ecosystems considered.

1.4.2 Prokaryotic and viral production distribution

Even in the distribution of viral and prokaryotic heterotrophic productions, we observed a certain variety, as depicted in the following violin plots (Fig 1.7), even though, due to the lack of data, we have not always obtained information for all systems. Looking at the prokaryotes, the highest median values are found in both sediment macro categories: freshwater sediments (630.96 ng C cm^{-3} h^{-1}) and marine sediments (31.62) respectively, although in the former case we have only one value in the dataset. These are followed by freshwater (0.63) and sea water (0.01). Looking at the range of variability, we observe that marine sediments have the widest values range (0.02 - 1220), followed by sea water (0.01 – 6.24), and freshwater (0.01 – 3.67).

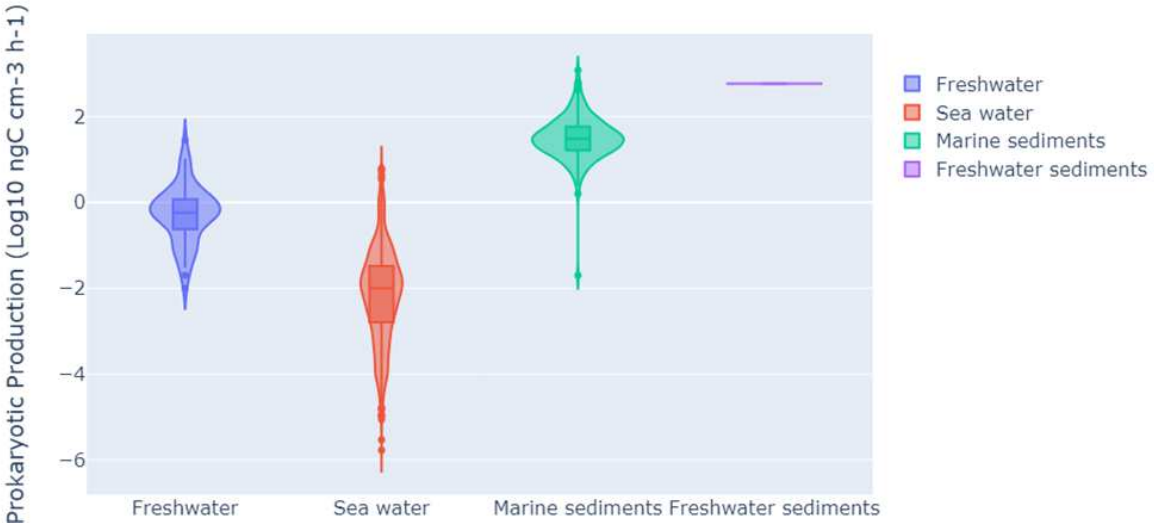

Figure 1.7 Distributions of prokaryotic heterotrophic production. Violin plots showing the distribution of prokaryotic productions among the ecosystems considered.

If we focus instead on the water column and examine the differences among various seas and oceans, the highest productions, in terms of median values, are the Mediterranean (0.79), the Atlantic (0.79), and the North Baltic Sea (0.50), followed by the Pacific Ocean (0.25), which assumes an intermediate value. The least productive are the Indian Ocean (0.10), the Arctic (0.04), and finally the Antarctic (0.02). However, when we consider the range of variability, the systems with the highest productions also exhibit greater variability, such as the Atlantic (0.01 – 5.60), followed by the Indian Ocean (0.04 – 6.24) and the Mediterranean (0.01 – 1.67). The productions of the Antarctic (0.01 – 0.96) and Nort Baltic Sea (0.15 – 0.98) set in the middle, while the Arctic (0.01 – 0.12) and Pacific (0.18 – 0.32) show the lowest values.

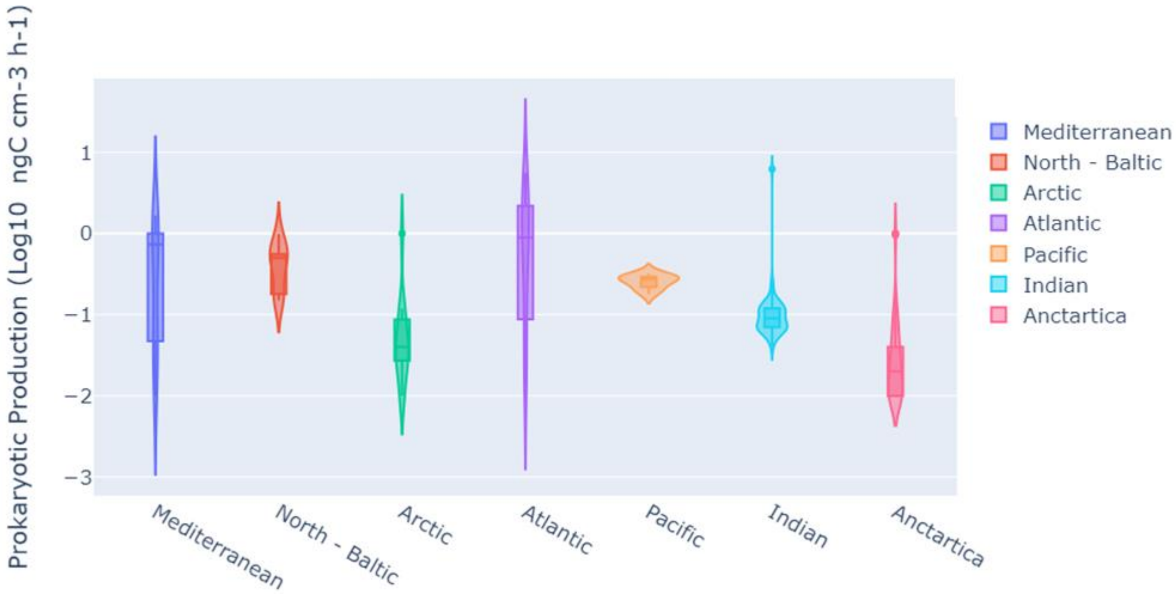

Figure 1.8 Distributions of prokaryotic production in sea water column. Violin plots showing the distribution of prokaryotic productions among the seas and oceans considered.

In the case of viruses, the highest median values are found in both sediment macro categories: freshwater sediments (7.9 × 10^7 VLP cm^{-3} h^{-1}) and marine sediments (3.2 × 10^7), respectively, although in the former case we have only one value in the dataset. Freshwater (2.5 × 10^5) and sea water (1.6 × 10^5) follow with values two orders of magnitude lower approximately, and finally ice (7.9 × 10^4). If we look at the range of values, we see that sea water (3.5 × 10 - 5.7 ×

10^8) and marine sediments (9.9×10^4 - 7.7×10^8) have the widest interval of values, followed by freshwater ($2.0 \times 10^4 – 2.0 \times 10^6$).

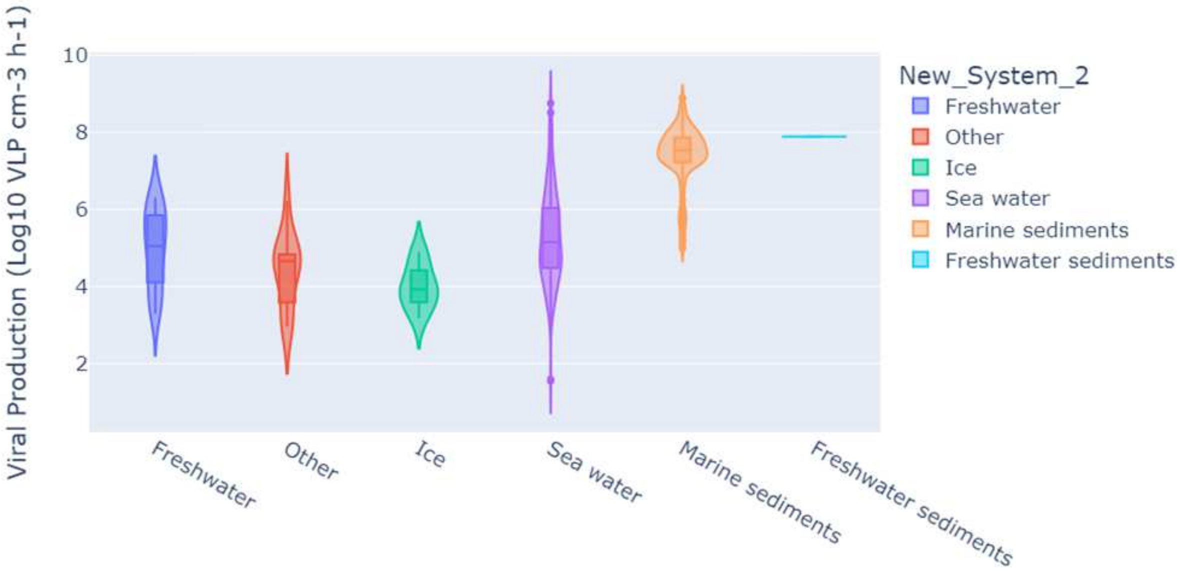

Figure 1.9 Distributions of viral production. Violin plots showing the distribution of viral productions among the ecosystems considered.

Focusing on the water column and examining the differences among various seas and oceans, the systems with the highest productions in terms of median values are Antarctica (1.0×10^6) and the Pacific (3.1×10^5). The North Baltic Sea (7.9×10^4) is followed by the Indian Ocean (5.0×10^4) and the Mediterranean Sea (5.0×10^4), which assume intermediate values. The systems with the lowest viral productions are the Atlantic Ocean (4.0×10^4) and the Arctic (3.1×10^4). Moreover, the Indian ($8.3 \times 10^3 – 1.5 \times 10^7$), Atlantic ($1.1 \times 10^3 – 1.1 \times 10^7$), and Mediterranean ($3.5 \times 10 – 4.9 \times 10^6$), for both prokaryotic and viral productions, remain the systems with the widest values ranges, meanwhile, the Arctic ($2.5 \times 10^3 – 2.8 \times 10^5$) has the narrowest values range.

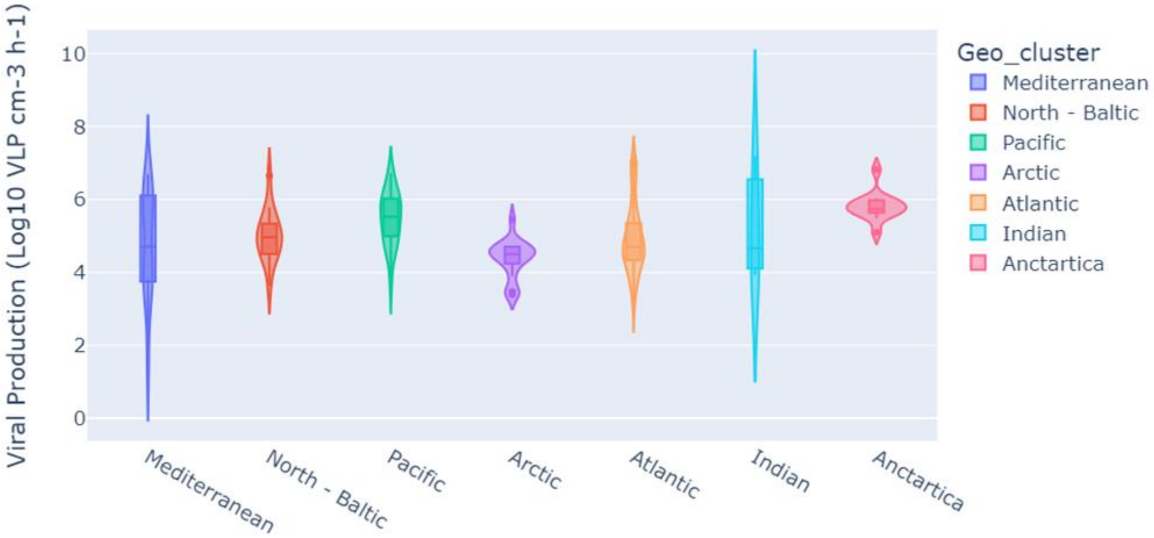

Figure 1.10 Distributions of viral production in sea water column. Violin plots showing the distribution of viral productions among the seas and oceans considered.

1.4.3 Estimates of prokaryotic and viral abundance at global scale

Global estimates of prokaryotic abundances, calculated by multiplying the average concentration of a system by its respective volume, indicate that marine sediments have the highest number of cells (1.7×10^{30}), followed by soil (3.9×10^{29}), with sea water having nearly an order of magnitude lower (1.9×10^{29}). All other systems fall between 10^{27} and 10^{26}.

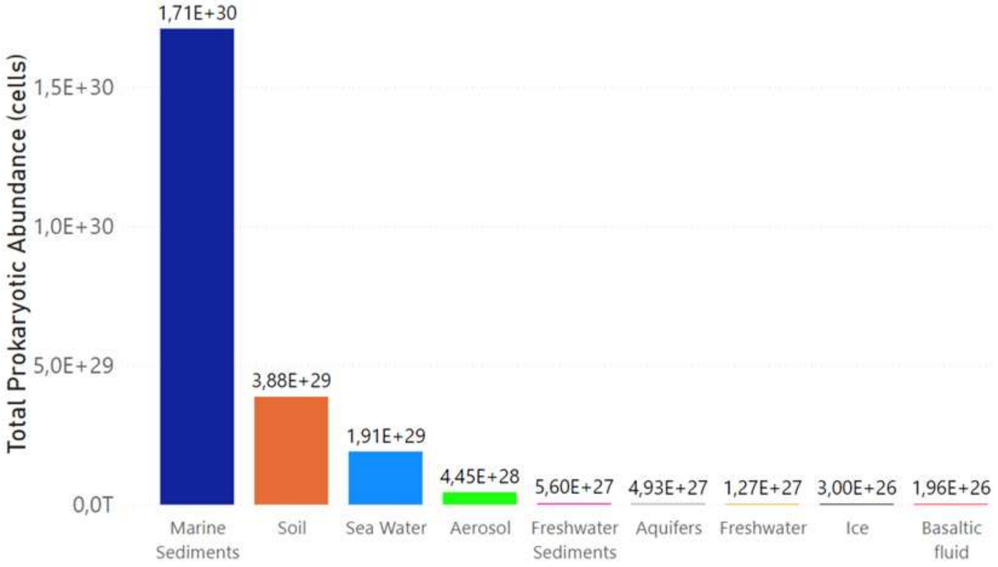

Figure 1.11 Global prokaryotic abundance distribution. Bar plot showing the distribution of total number of prokaryotes among the ecosystems considered, multiplying the mean concentrations by the entire volume of the corresponding ecosystem.

If instead we estimate using the integral of the exponential decline, the system with the highest number of cells becomes sea water (3.7 × 10^{30}), followed by marine sediments (4.7 × 10^{29}), and soil water nearly two orders of magnitude lower (5.2 × 10^{28}), along with aerosol (4.5 × 10^{28}). All other systems reported a range between 5.6 × 10^{27} and 1.9 × 10^{26}.

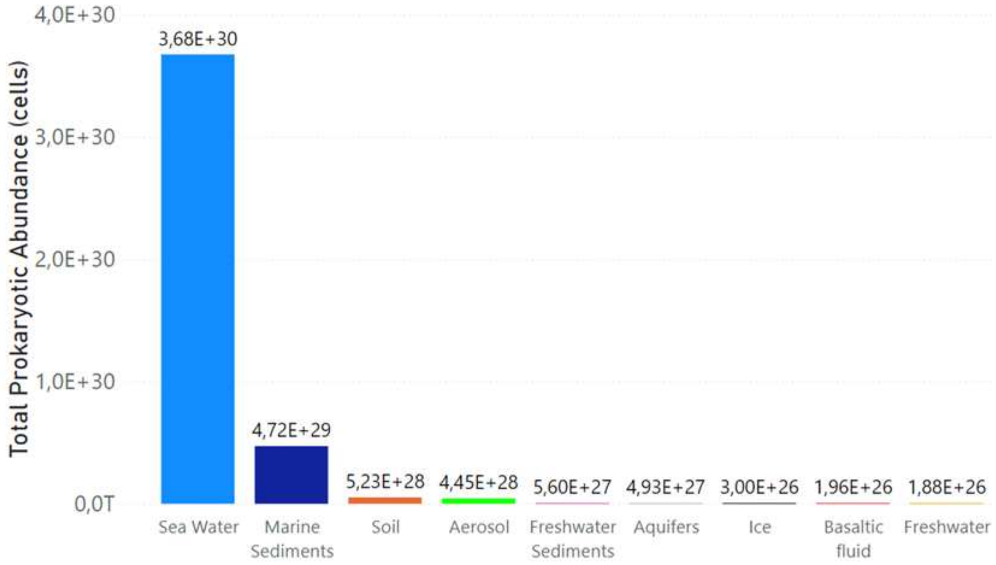

Figure 1.12 Global prokaryotic abundance distribution. Bar plot showing the distribution of total number of prokaryotes among the ecosystems considered, using the integral of the exponential decline along the depth.

Finally, if we use the power-law function for the integral estimation, the system with the highest number of viral particles becomes soil (3.6 × 10^{29}), followed by sea water (6.6 × 10^{28}), marine sediments (2.7 × 10^{28}), freshwater sediments (5.6 × 10^{27}) and freshwater (2.8 × 10^{26}).

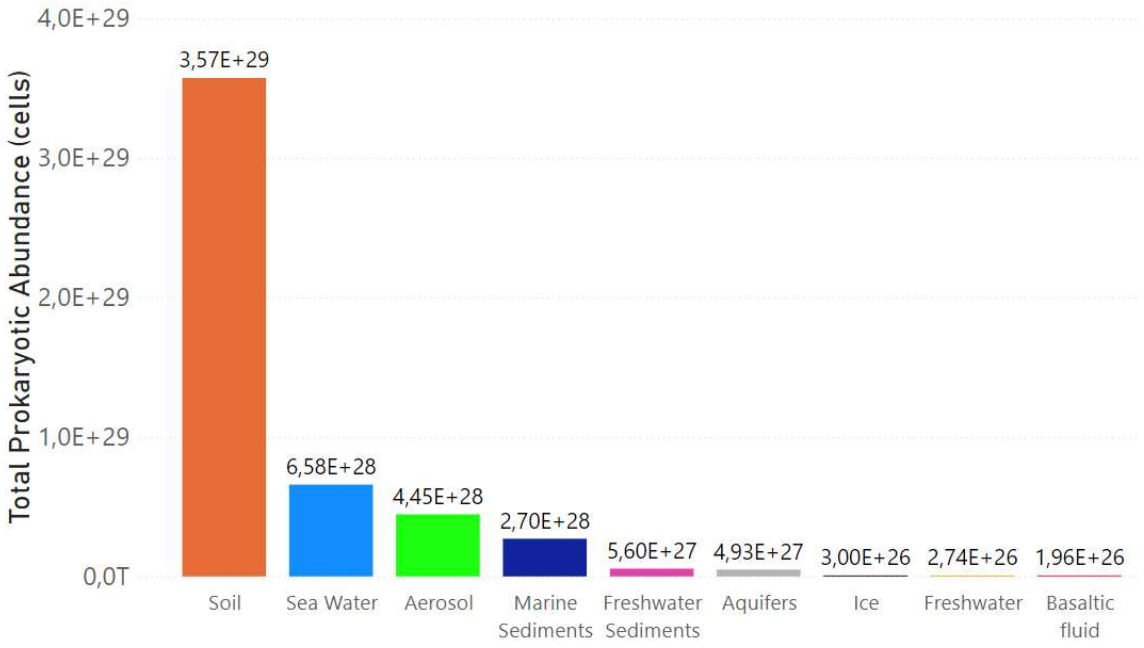

Figure 1.13 Global prokaryotic abundance distribution. Bar plot showing the distribution of total number of prokaryotes among the ecosystems considered, using the integral of the power-law.

The global estimates of viral abundances, calculated by multiplying the average concentration of a system by its respective volume, indicate that the system with the highest number of viral particles is marine sediments (2.7×10^{30}), followed by sea water (2.4×10^{30}), and soil (7.0×10^{29}). All other systems fall between 5.6×10^{28} and 1.8×10^{27}.

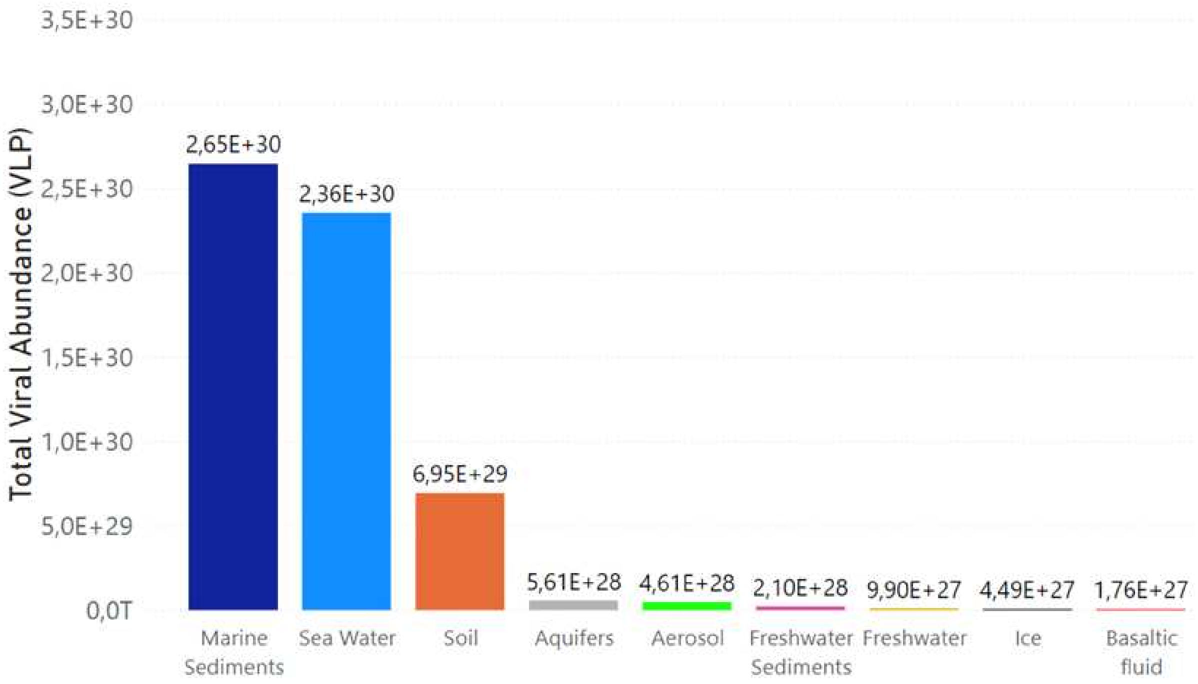

Figure 1.14 Global viral abundance distribution. Bar plot showing the distribution of total number of viruses among the ecosystems considered, obtained by multiplying the mean concentrations by the entire volume of the corresponding ecosystem.

Using the exponential integral for the estimates, the system with the highest number of viral particles again becomes sea water (2.6×10^{31}), followed by marine sediments (2.0×10^{30}), and soil water, which is nearly two orders of magnitude lower (2.3×10^{29}). All other systems fall between 5.6×10^{28} and 1.8×10^{27}.

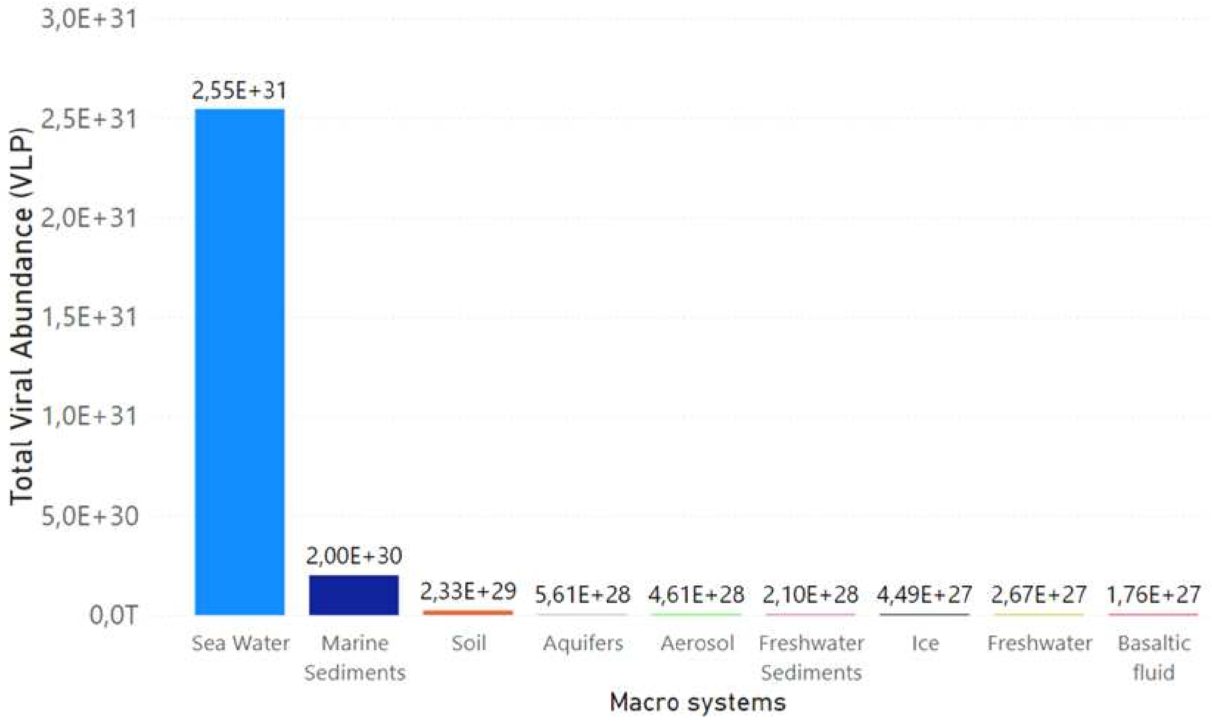

Figure 1.15 Global viral abundance distribution. Bar plot showing the distribution of total number of viruses among the ecosystems considered, using the integral of the exponential decline along the depth.

In the end, if we use the power-law function for the integral estimation, the system with the highest number of viral particles remains sea water (8.1×10^{29}), followed by soil (5.0×10^{29}), aquifers (5.6×10^{28}) and aerosol (4.6×10^{28}). All other systems fall between 3.7×10^{28} and 8.0×10^{26}.

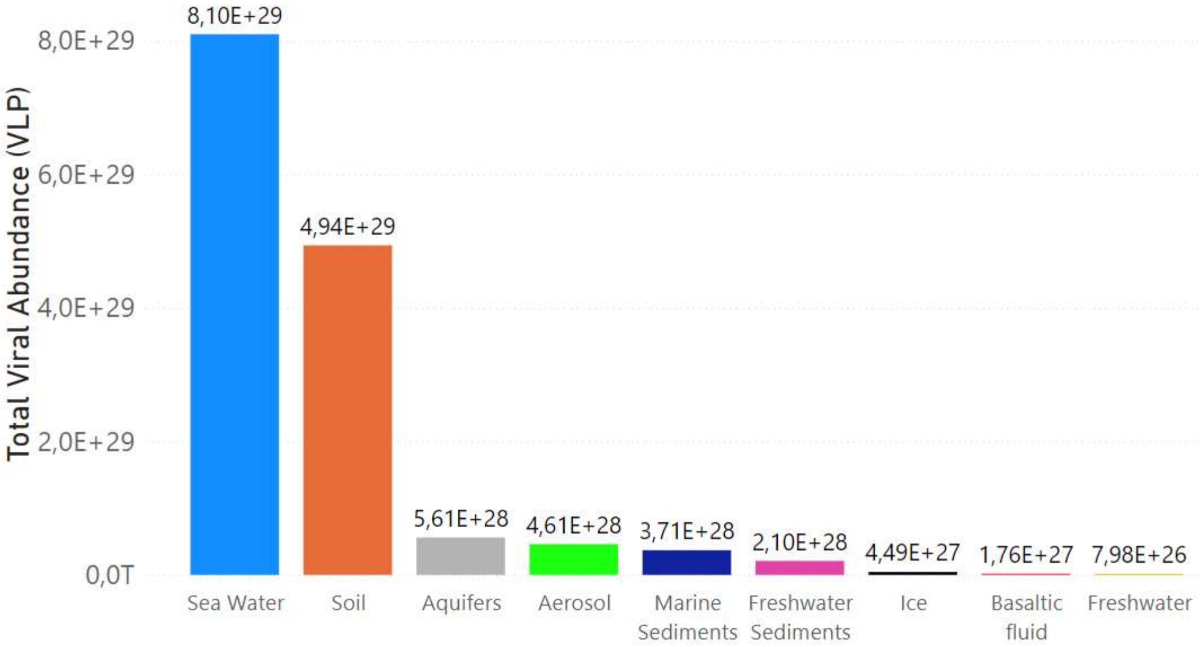

Figure 1.16 Global viral abundance distribution. Bar plot showing the distribution of total number of viruses among the ecosystems considered, using the integral of the power-law.

1.4.4 Estimates of prokaryotic and viral production at global scale

Analyzing the productions, we adopted only the approach with the integral of the exponential function, excluding the other two estimations due to the particular distribution of data points, which did not provide reliable estimates compared to the literature data. The global estimates of prokaryotic productions show that the most productive system is sea water (111.28 Mt C h^{-1}), followed by marine sediments (25.03), and freshwater (0.03).

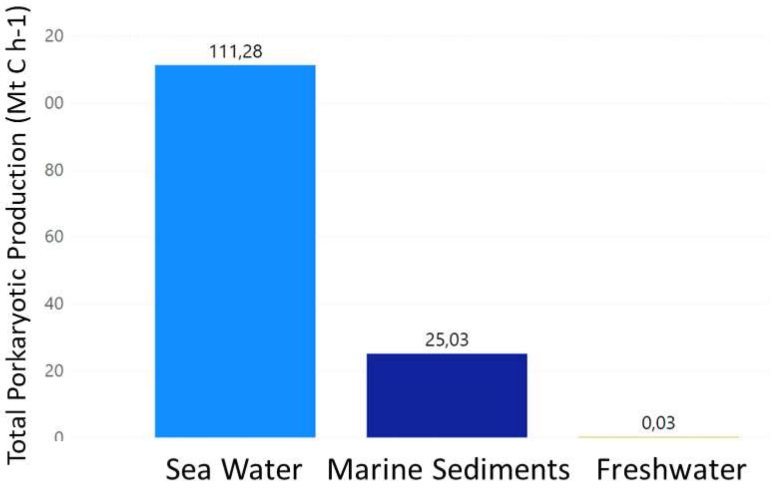

Figure 1.17 Global prokaryotic production distribution. Bar plot showing the distribution of total prokaryotic production among the ecosystems considered, using the integral of the exponential decline along the depth.

The global estimates for viral production followed a trend similar to that for prokaryotic production, highlighting that the system with the highest prokaryotic productions is sea water (48.83 Mt C h^{-1}), followed by marine sediments (4.59), and freshwater (0.01).

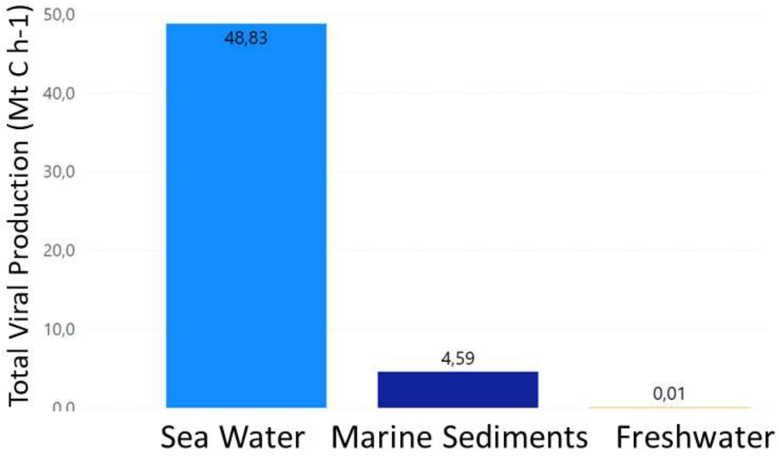

Figure 1.17 Global viral production distribution. Bar plot showing the distribution of total viral production among the ecosystems considered, using the integral of the exponential decline along the depth.

1.5 Discussion

We know that viruses are the most abundant biological entities of the oceans, outnumbering prokaryotes by at least 1 order of magnitude (Suttle, 2005; Suttle, 2007; Danovaro et al., 2015; Bar-On et al., 2018), with current estimates in the order of 10^{30} - 10^{31} viruses in the water column (Suttle, 2005; Suttle, 2007; Danovaro et al., 2015; Bar-On et al., 2018; Middelboe et al., 2006; Middelboe et al., 2011; Danovaro et al., 2008b). Together with prokaryotes they contribute to the biogeochemical cycles in several fundamental ways, playing a key role in the most relevant processes driving ecosystem functioning on the global scale (Proctor et al., 1990; Nealson, 1997; Fuhrman, 1999; Mason et al., 2009; Arndt et al., 2013; Danovaro et al., 2015; Coutinho et al., 2018). Estimating microbial and viral standing stocks and their dynamics is of paramount importance to better understand their role in global ecosystem processes and their ability to actively interact with the ongoing climate changes. The results presented here provide, for the first time, a global assessment of the microbial abundances and the associated biomass using a dataset resulting from systematic data collection that allowed to gather a dataset with over 10,000 samples distributed across different ecosystems and biomes covering a wide range of scenarios. We have to acknowledge that the most represented samples in our dataset were from marine systems (sea water, marine sediments) (Whitman et al., 1998; Bar-On et al., 2018; Mushegian, 2020), due to the relatively scarce information available at present for the other environments (Wommack et al., 2015; Williamson et al., 2017; Kuzyakov et al., 2018; Emerson et al., 2018). Nevertheless, this represents the first effort so far to comprise also viruses, besides prokaryotes, within global standing stock estimates, as well as biomass production processes. Despite information on productions is very limited and data in terrestrial environments (freshwater, soil) are still very scant, our novel estimates highlight the potential ecological importance of prokaryotes and viruses, suggesting that they could play a key role in prokaryotic dynamics and in biogeochemical cycles at global scale.

1.5.1 Comparative analysis of distributions

Due to the high number of points available, for the first time a **comparative analysis** has been possible with the aim of investigating differences among patterns of distribution for prokaryotic and viral abundances and productions in the main ecosystems. The relevance and roles of viruses infecting microbes in the environment has been investigated mainly in the oceans so far (Suttle, 2007; Brum et al., 2015; Guidi et al., 2016; Emerson et al., 2018), and remains less unexplored in other ecosystems, especially in soil (Wommack et al., 2015; Williamson et al., 2017; Williamson et al., 2017; Emerson et al., 2018; Kuzyakov et al., 2018). However, our results show that viruses reach high abundances in all ecosystems we considered, including soil.

1.5.2 Analysis of viral and prokaryotic abundances

We show that viral abundances exceed prokaryotic abundances up to more than 10 times (Cochlan et al., 1993; Maranger et al., 1995; Parkes et al., 2000; Wommack et al. 2000; Middelboe et al., 2006; Danovaro et al., 2008; Middelboe et al., 2011; Danovaro et al., 2015; Liang et al., 2021). The present study confirmed that the highest viral and prokaryotic abundances per unit volume are found in freshwater sediments, followed by marine sediments, consistent with previous literature findings (Danovaro et al., 2008b; Parikka et al., 2017). These are followed by freshwater and soil (Wilhelm et al., 2008; Parikka et al., 2017), and only then we find sea water, followed by ice. Focusing on the sea water column, high prokaryotic and viral abundances have been detected in highly productive oceanic regions as for example the North Baltic and Polar regions in line with the results from the literature (Middelburg et al., 2007; Pina et al., 2011; Danovaro et al., 2016), followed by the Arctic and the Pacific. Along with the Atlantic, the Pacific exhibits the greatest variability for both viral and prokaryotic abundances. However, the differences between the medians in seawater settle at no more than one order of magnitude. Finally, if we compare prokaryotic and viral abundances in sediments (Fig. S1), we see that freshwater is characterized by higher values (Fuhrman, 1999; Maranger et al., 1995; Clasen et al., 2008; Parikka et al., 2017), followed by coastal waters and finally by the oligotrophic open ocean, probably due to the fact that coastal and estuary waters are more productive than offshore waters (Fuhrman, 1999; Wommack et al., 2000; Corinaldesi et al., 2003; Payet et al., 2008; Evans et al., 2009; Danovaro et al., 2011; Parikka et al., 2017).

1.5.3 Analysis of viral and prokaryotic productions

The results obtained for productions partly confirm those of abundances, indeed, prokaryotic productions appear higher in sediments and freshwater, a result reflected in viral productions as well, with seawater exhibiting the greatest variability among all systems. Considering the water column, the Atlantic, the Mediterranean, and the North Baltic Sea are the systems with the highest heterotrophic prokaryotic production, although the first two show the widest ranges overall. However, the most extreme systems appear to show the lowest prokaryotic productions on average. Patterns for viral productions are entirely different, reaching the highest average values in the Indian Ocean and Antarctica, although the former, along with the Mediterranean, displays the highest variability. When comparing viral and prokaryotic production values in sediments (Fig. S1), freshwater shows the highest productions, although the available dataset, consisting of in only few observations, does not allow to obtain robust conclusions for this ecosystem. Furthermore, despite the minor discrepancy in median viral production between open and coastal ecosystems (Weinbauer et al., 2004; Parikka et al., 2017), the latter exhibits a broader range of variability. In contrast, prokaryotic productivity appears to be higher in nutrient-rich coastal waters, which confirms previous evidence (Fuhrman, 1999).

1.5.4 Relationship between viral and prokaryotic abundances

Viral abundance is usually positively correlated to the abundance of potential host cells in marine ecosystems (Wommack et al., 2000; Weinbauer, 2004; Danovaro et al., 2008a), as frequently reported by previous studies (Maranger et al., 1995; Weinbauer et al., 1995; Anesio et al., 2007; Danovaro et al., 2008b; Danovaro et al., 2011; Helton et al., 2012; Parikka et al., 2017; Weinbauer et al., 2003). Previous evidence based on multiple linear regressions showed that the variation in prokaryotic abundance can primarily explain the variability in viral abundance and can be used as the sole predictor to explain changes in virus abundance (Cochlan et al., 1993; Paul et al., 1993; Boehme et al., 1993; Fuhrman et al., 1998). More recently, also Wigington (et al. 2016) and Lara (et al. 2017) found a significant and positive relationship between viral and prokaryotic abundance. Similarly, also in other environments such as soil and different aquatic environments, it was found that viral abundance was positively correlated with bacterial abundance (Williamson et al., 2005; Rowe et al., 2008; Wommack et al., 2015; Wigington et al., 2016; Liang et al., 2019). However, our understanding of the virus-prokaryote relationship at global scale is still poor (Wilhelm et al. 2003; Danovaro et al., 2011); therefore, another important result of this chapter is that it revealed that this linear prokaryote-virus relationship in logarithmic scale (base 10), already identified by Wigington (et al., 2016) and Lara (et al., 2017) in sea water, is still valid across all the main ecosystems. Additionally, the value of scaling exponent for the viral abundances (0.72) as power of prokaryotic abundances is consistent with a previous study on seawater (Wigington et al., 2016; Knowles et al., 2016; Lara et al., 2017), and, in our analysis, is still valid across major ecosystems on Earth. The difference with the value of 0.91 in Lara (et al., 2017), suggest a that viral abundance is lower than that predicted by Lara (et al., 2017).

1.5.5 Patterns with depth

The graphs in Fig. S2 show that microbial abundances decrease with depth in most of systems considered. Our results align with the literature (Lloyd et al., 2013; Danovaro et al., 2015; Rex et al., 2006), indicating higher abundance of viruses and bacteria in sea surface waters ranging from 0 to 200 m, where they appear to be favored by higher energy flows and biological productivity in the upper ocean (Bratbak et al., 1990; Proctor et al., 1990; Cochlan et al., 1993). In deeper zones, microbial assemblages could be disadvantaged by more extreme conditions due to high pressures and low temperatures, as well as the limited availability of nutrients whose reduced quantity may decrease the growth of prokaryotes (Hara et al., 1991; Cochlan et al., 1993; Weinbauer et al., 1993; Maranger et al., 1995; Wommack et al., 2000; Danovaro et al., 2000; Middelboe et al., 2006; Wilhelm et al., 2008; Jacquet et al., 2010; Danovaro et al., 2008a; Parikka et al., 2017). Additionally, the decline seems to follow a power-law curve or an exponential decline (Rex et al., 2006; Parada et al., 2007; Winter et al., 2009; Danovaro et al. 2008; Middelboe et al., 2011; Kallmeyer et al., 2012; De Corte et al., 2012; Lloyd et al., 2013; Liang et al., 2014; Yang et al., 2014; Li et al., 2014; Danovaro et al., 2015; Lara et al., 2017; Suttle, 2007; Sun et al., 2021) although we were able to observe this pattern clearly only for some systems (sea water and freshwater along water depth, agricultural soil). We noticed a decline pattern with depth in marine sediment as well, which could also be linked to the presence of increasingly refractory resources with increasing depth (Danovaro et al., 2000; Middelboe et al., 2006; Borrel et al., 2012; Parikka et al., 2017; Hewson et al., 2001; Glud et al., 2004; Middelboe et al., 2011; Lloyd et al. 2013). However, we observe, from the violins in Fig. S3, that viral and prokaryotic abundances in sediment do not change with the water column depth, as already seen in Danovaro (et al., 2016). Finally, if we look at the productions, we notice a difference between the 0-200m range and the other two (200-1000m and >1000m) characterized by similar production distributions.

1.5.6 Global stocks

Our estimates of total viral and prokaryotic abundances and productions are consistent with literature data, although exhibiting some differences since no study until now had evaluated the abundances of viruses on such a large dataset and with estimation techniques based on interpolations and integrals. Specifically, in the case of global viral abundances, our data ranges from 1.3 × 10^{30} - 2.8 × 10^{31}, with the maximum value positioned almost midway between the estimates of Cobián (et al., 2016) (4.8 × 10^{31}) and Mushegian (2021) (1.1 × 10^{31}). On the lower end of our interval, it approaches the estimate of Bar-On (et al., 2018) (3.0 × 10^{30}). Utilizing the conversion factor described in Table 1.2, we obtain a total biomass value ranging from 0.35 to 1.67 Gt C, exceeding the estimate of Bar-On (et al., 2018) by 0.2 Gt C. The main difference between the three global estimates lies in the estimation of viral abundance in the global ocean. Here, I obtain three different estimates. The lowest is 8.1 × 10^{29}, obtained using the integral of the power-law (Kallmeyer et al., 2012). This function adheres well to the data but tends to underestimate in shallower bathymetric zones where several points exceed the curve with abundances higher than those estimated. Conversely, using the exponential decline it behaves in the opposite manner, overestimating the abundances, as seen from the distances between the measured points and the estimated curve in the 0-2000m depth range. Therefore, the estimate obtained through this second methodology is 2.6 × 10^{31}, is 32 times higher than the first integral. In between lies the estimate calculated using average abundances in the three abovementioned depth ranges. This value (2.4 × 10^{30}), in terms of magnitude, aligns with the literature data (Suttle, 2007; Cobián et al., 2016; Mushegian, 2020). In marine sediments, it was not possible to calculate the integral using the power-law approach, but the results obtained with the other two methods are almost identical and fall within the literature values, lower than the value in Cobián (et al., 2016) (4.2 × 10^{31}), where the number is based on Whitman's estimates that extend to 4 km in the subsurface, compared to our 60 m, but higher than

Danovaro (et al., 2015) estimate (9.8×10^{28}) that considers only the top 50 cm. On soil, we obtain results with the same order of magnitude for the three approaches, also due to the limited number of data points and shallow depths (5m). In this case, the estimate with the mean concentrations assumes the highest value, whereas the integral with the exponential function is almost three times lower. These results are consistent with the value reported by Mushegian (2020) (6.0×10^{29}), but lower by 7.4 to 20 times compared to the estimate by Cobián (et al., 2016) (4.9×10^{30}).

If we consider prokaryotic abundances, our global data range from 4.3×10^{29} to 4.2×10^{30}, consistent with literature values falling within the range 3×10^{30} - 5×10^{30} (Whitman 1998, Cobián et al., 2016; Bar-On et al., 2018). Using the conversion factors described in Table 1.2, we obtain total biomass values ranging from 12 to 176 Gt C, an interval that encompasses Bar-On (et al., 2018) estimate of 77 Gt C. In this case as well, the main difference between the three estimates lies in the estimation of prokaryotic abundance in the global ocean. The lowest estimate is 6.6×10^{28} obtained using the integral of the power-law which, as mentioned earlier, tends to underestimate the data in the shallower bathymetric zones but it is consistent with the data of Karner (et al., 2001). Conversely, the use of the exponential decline overestimates the data, therefore, the estimate obtained through this second methodology is $3,7 \times 10^{30}$, which in this case is 55 times higher than the first integral. The estimate calculated using the average abundances in the three bathymetric zones (1.9×10^{29}) falls between these values and is consistent with literature data (1.2×10^{29} in Whitman et al., 1998; 1.0×10^{29} in Guemes et al., 2016). As with viruses, it was not possible to calculate the integral using the power-law in Marine sediments. However, the results obtained with the other two approaches do not show the same large differences as in sea water. Both estimates (4.7×10^{29} - 1.7×10^{30}) are lower than those of Whitman (et al., 1998) ($3.6 * 10^{30}$) and higher than those of Danovaro (et al., 2015) (4.9×10^{28}) due to the different layer depth considered as with viruses, but higher than the estimate of Kallmeyer (et al., 2012) (2.9×10^{29}), which considers the same depth of 4km like Whitman (et al., 1998). On land, we obtained very similar results between the integral approach with the power-law (3.6×10^{29}) and the average concentration (3.9×10^{29}), consistent with Whitman's data (2.6×10^{29}), even if he considered soil depth down to 4km. However, the integral with the exponential has values six times lower (5.2×10^{28}).

Finally, we implemented the first global evaluation for prokaryotic and viral global production that highlights how viruses and prokaryotes are crucial components in the carbon cycle. In particular, the sea water ecosystem is characterized by the highest productions, especially prokaryotic global production that reaches values of 111 Mt C h^{-1} and it is about twice the viral production, whereas the prokaryotic production in marine sediments is about five times the viral production (25.03 Mt C h^{-1}).

These results confirm that a global estimate for viruses and prokaryotes is still difficult to achieve, because, in this case, despite having a broader and more distributed database compared to previous estimates (Whitman et al., 1998; Karner et al., 2001; Kallmeyer et al., 2012; Danovaro et al., 2015; Bar-On et al., 2018; Mushegian, 2020), the outcome largely depends on the mathematical approach used, which, in ecosystems characterized by larger volumes, leads to variations in total estimates even up to an order of magnitude. Moreover, despite the large number of data points, our dataset is still limited, especially when considering non-marine environments, and the distribution of points is not uniform in time and space. However, despite these limitations, our study confirms that viruses and prokaryotes play a key role in the food web and biogeochemical fluxes, given the significant carbon reservoir they represent and make available to higher trophic levels.

Viral abundance	**Danovaro et al. 2015**	**Suttle 2007**	**Bar-On et al. 2018**	**Mushegian 2020**	**Danovaro et al. 2008**	**Cobian et al. 2016**	**Mushegian 2021**	**Mean values**	**Integral of the exponential**	**Integral of the power law**
Marine Sediments	9,80E+28				2.87E+29	4.18E+31	4,00E+29	2,65E+30	2,00E+30	

Global Ocean		1,00E+30				1.29E+30	2,00E+30	2,36E+30	2,55E+31	8,10E+29
Freshwater						1.76E+27		9,90E+27	2,67E+27	7,98E+26
Freshwater Sediments					5.0E+27			2,10E+28	2,10E+28	2,10E+28
Soil						4.88E+30	6,00E+29	6,59E+29	2,33E+29	4,94E+29
All systems			3,00E+30	3,00E+30		4.80E+31	1,10E+31	5,70E+30	2,77E+31	1,33E+30

Table 1.3 Viral abundances among different systems. In this table are listed values of viral abundance collected from different papers and different ecosystems.

Prokaryotic abundance	**Danovaro et al. 2015**	**Whitman et al. 1998**	**Karner et al. 2001**	**Bar-On et al. 2018**	**Mushegian 2020**	**Lipp et al. 2008**	**Kallmeyer et al. 2012**	**Parkes et al. 2000**	**Cobian et al. 2016**	**Mushegian 2021**	**Mean values**	**Integral of the exponential**	**Integral of the power law**
Marine Sediments	4,90E+28	3,55E+30				5,00E+30	2,90E+29	1,00E+29	3.80E+30	4,00E+29	1,71E+30	4,72E+29	
Global Ocean		1,20E+29	4,40E+28				1,20E+29		1.01E+29	1,20E+29	1,91E+29	3,68E+30	6,58E+28
Freshwater		2,30E+26							1.26E+26		1,27E+27	1,88E+26	2,74E+26
Freshwater Sediments											5,60E+27	5,60E+27	5,60E+27
Soil		2,60E+29					2,60E+29		2.50E+29	3,00E+29	3,88E+29	5,23E+28	3,57E+29
All systems		5,00E+30		3,00E+30	3,00E+30				4.15E+30	3,00E+30	2,30E+30	4,21E+30	

Table 1.4 Prokaryotic abundances among different systems. In this table are listed values of prokaryotic abundance collected from different papers and different ecosystems.

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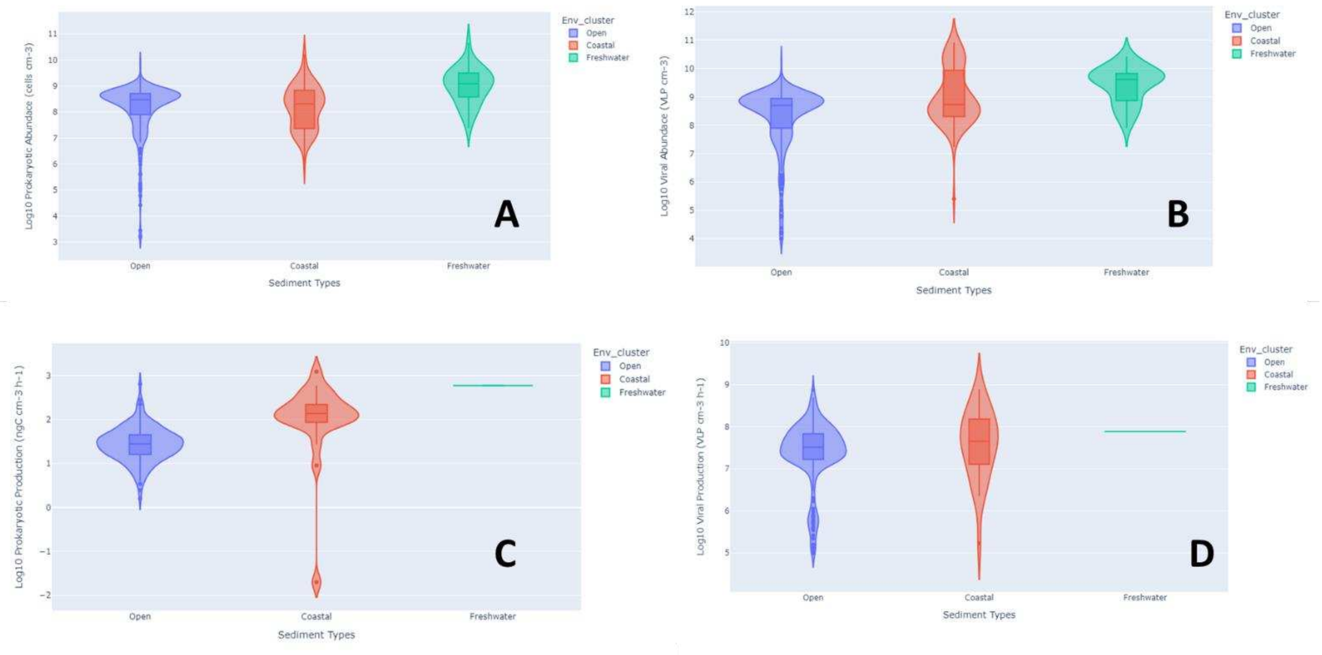
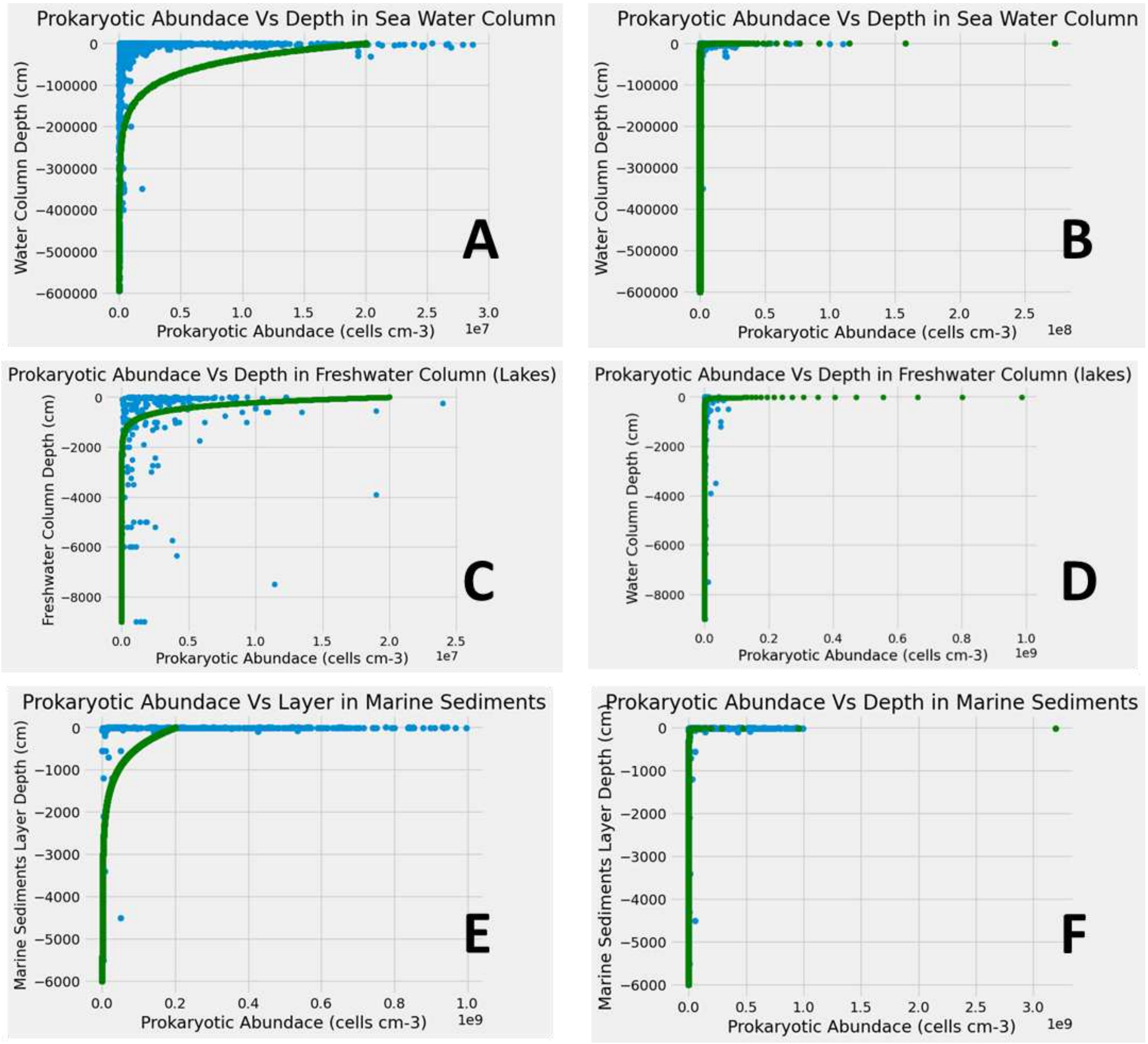
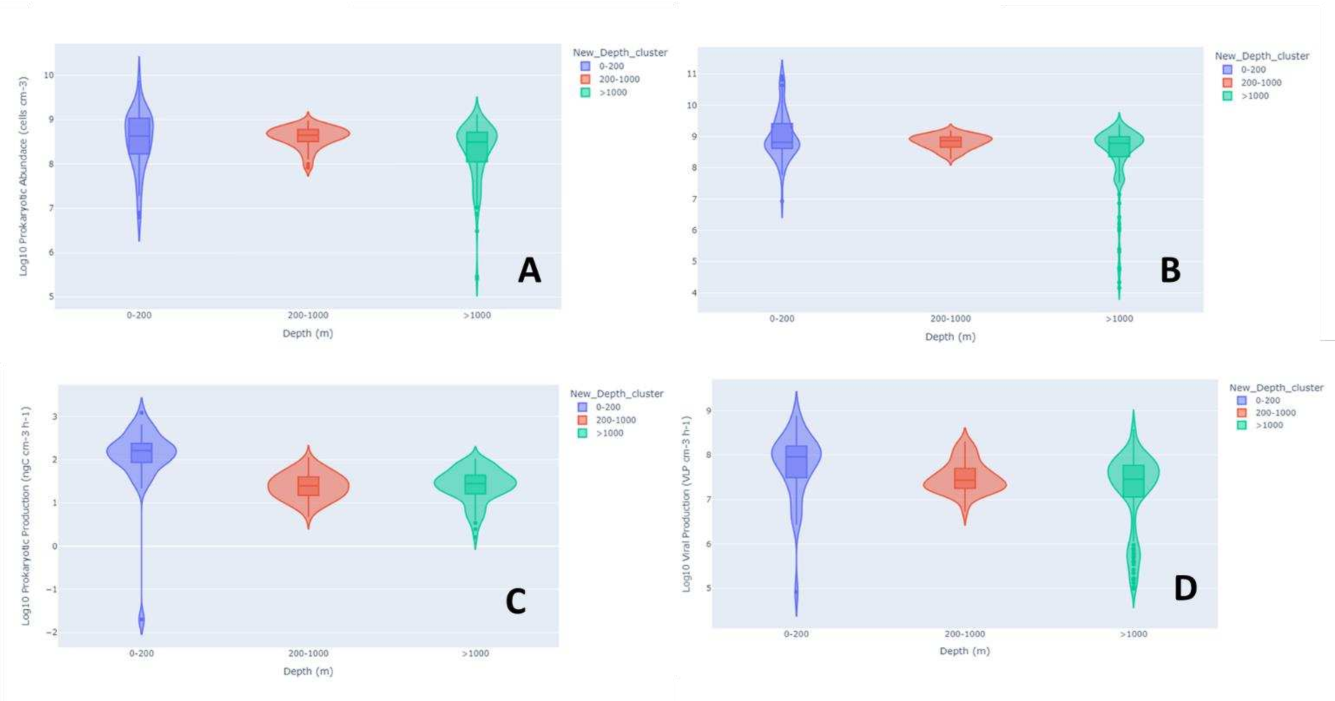
Supplementary Materials



Supplementary Figure 1.1 Distributions of prokaryotic and viral abundances and productions in sediments. Violin plots showing the distribution of prokaryotic (A) and viral (B) abundances, and of prokaryotic (C) and viral (D) productions in different kinds of sediments.



Supplementary Figure 1.2 Distributions of prokaryotic and viral abundances along the depth. Violin plots showing the distribution of prokaryotic and viral abundances along the depth for different ecosystems and in green the exponential decline curve used as approximation: prokaryotic abundance in sea water column (A), viral abundance in sea water column (B), prokaryotic abundance in freshwater column (C), viral abundance in freshwater column (D), prokaryotic abundance in marine sediments layer (E), viral abundance in marine sediments layer (F),



Supplementary Figure 1.3 Distributions of prokaryotic and viral abundances and productions in sediments. Violin plots showing the distribution of prokaryotic (A) and viral (B) abundances, and of prokaryotic (C) and viral (D) productions considering three different water column depths (0-200m, 200-1000m, >1000m).

Chapter 2

Macroecological drivers of viral and prokaryotic distribution and dynamics on a global scale

2.1 Introduction

Large part of the biomass in the global biosphere is accounted for by microorganisms, among which prokaryotes (including bacteria and archaea) represent the most abundant components (Bar-On et al., 2019; Rex et al. 2006, Wei et al. 2010). Prokaryotes, as well as the viruses able to infect them, play key roles in global biogeochemical cycles and can respond and actively interact with the ongoing climate change (Danovaro et al., 2011; Jover et al., 2014). For a better understanding of the effects and implications of current climate change on the functioning of the entire biosphere (Hutchins et al., 2017; Jansson et al., 2019), we have to deepen our knowledge on the patterns and drivers of the distribution of prokaryotes and viruses, in terms of abundance, biomass and functions (Cochlan 1993; Clasen et al., 2008; Danovaro et al., 2016; Wigington et al., 2016), which remain currently largely undescribed. The distribution and dynamics of prokaryotic assemblages are usually driven by multiple environmental variables, such as temperature and nutrients availability (Pecl et al., 2017; Ripple et al., 2017; Timmis et al., 2019), as well as by biotic interactions (e.g., predation, parasitism, mutualism, or competition), mainly with other prokaryotes, or with phytoplankton, protists, and viruses (Karner et al., 2001; Jørgensen et al., 2007; Azam et al., 2007; Kallmeyer et al., 2012; Sunagawa et al., 2015; Danovaro et al., 2015). To date, studies that comprehensively include all these biotic components into the assessment of prokaryotic ecological drivers are virtually absent, highlighting the need for further research (Yeh, 2022). This Chapter focuses on the macroecological drivers of viral and prokaryotic distribution and dynamics on a global scale, particularly concerning the factors influencing virus–host interactions (Rohwer et al., 2009; Jover et al., 2014; Dell'Anno et al., 2015; Danovaro, 2015). This driver analysis is crucial for: i) understanding the role of viruses in the regulation of prokaryotic abundances (Cochlan et al., 1993; Danovaro et al., 2008; Dell'Anno et al., 2015; Lara et al., 2017), ii) evaluating the importance of system that are "hotspots" of prokaryotes and viruses, and iii) predicting prokaryotic and viral responses to environmental changes due to climate change pressures (Danovaro et al., 2011). By integrating data from hundreds of studies, including recent studies, and from the Copernicus web-platform (Copernicus, Oregon), we provide a comprehensive overview of the current global prokaryotic and viral distributions and we forecast changes in microbiological stocks under different climate change scenarios (CMIP6). The analysis of the drivers of viral and prokaryotic distribution and dynamics developed in this chapter is useful to implement food web and global biogeochemical models, that currently do not take these factors into account (Wei et al., 2010).

2.2 Aims

The main aims of this chapter were to investigate:

1. the main drivers influencing prokaryotic and viral distribution in marine (water column and sediments) and terrestrial (freshwater and soil) ecosystems.
2. the possibility to develop a statistical model to estimate the prokaryotic and viral spatial distribution at a global scale.
3. the prokaryotic and viral responses under climate change scenarios using statistical models.

2.3 Materials and methods

2.3.1 Sample area and dataset

The dataset used in this chapter was the same validated in chapter 1.

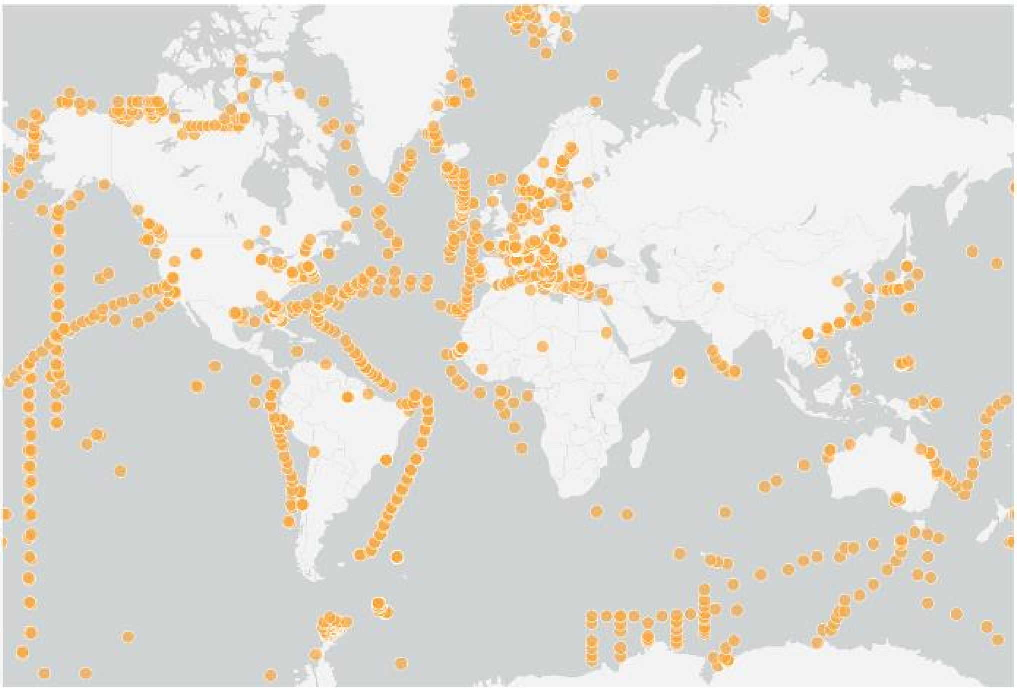


Figure 2.1 Sampling sites. Map showing the global distribution of sampling sites.

2.3.2 Drivers import from external sources

Since the bibliographic research, conducted across various studies, did not provide sufficient information about the drivers we intended to analyze, we utilized two platforms to address these gaps: Copernicus and Oregon. The following table summarizes all the source databases used, along with the respective imported drivers. To reduce the complexity of the data-integration process, the temporal component was not considered in the analysis, so external data were imported as average values considering the whole of 2019 as reference period.

Database	**Platform**	**Drivers**	**Reference**
Global Ocean Physics Analysis and Forecast [Reanalysis]	Copernicus Marine Service	Temperature (°C), Salinity (psu)	46. E.U. Copernicus Marine Service Information. Global Ocean 1/12° Physics Analysis and Forecast updated Daily. *Copernicus Marine* *Service Web site* https://doi.org/10.48670/moi-00016 (2021).
Global Ocean Biogeochemistry Analysis and Forecast	Copernicus Marine Service	Chl a (mg m^{-3}), NO3 (mmol m^{-3}), PO4 (mmol m-^{3}), O2 (mmol m^{-3}), Primary Production (PP, mg m^{-3} d^{-1}), pH	47. E.U. Copernicus Marine Service Information. Global Ocean Biogeochemistry Analysis and Forecast. *Copernicus Marine Service* *Web site* https://doi.org/10.48670/moi-00015 (2021).
Global Ocean 3D Chlorophyll-a concentration, Particulate Backscattering coefficient and Particulate Organic Carbon	Copernicus Marine Service	POC (mg m^{-3})	https:// data.marine.copernicus.eu/ product/ MULTIOBS_GLO_BIO_BGC_3D_REP_015_010/ description
VGPM Standard - Modis	Oregon - Ocean Productivity	Primary Production (mg m^{-2} d^{-1}) *we named this variable "Carbon flux" to distinguish from Copernicus data on PP	https:// sites.science.oregonstate.edu/ ocean.productivity/ index.php Behrenfeld, Michael J., and Paul G. Falkowski. "Photosynthetic rates derived from satellite-based chlorophyll concentration." *Limnology and oceanography* 42.1 (1997): 1-20.
Lake Surface Water Temperature	Copernicus Land Service	Temperature (°C)	https://land.copernicus.eu/global/products/lswt
Lake water quality	Copernicus Land Service	Turbidity (ntu), Trophic state (%), Chl a (ng m^{-3})	https://land.copernicus.eu/global/products/lwq
Land Surface Temperature	Copernicus Land Service	Temperature (°K)	https://land.copernicus.eu/global/products/lst
Soil Water Index	Copernicus Land Service	Soil water index (%)	https://land.copernicus.eu/global/products/swi
Surface Soil Moisture	Copernicus Land Service	Surface Soil Moisture (%)	https://land.copernicus.eu/global/products/ssm
Dry Matter Productivity	Copernicus Land Service	Dry Matter Productivity (kg ha^{-1} d^{-1})	https://land.copernicus.eu/global/products/dmp
Fraction of green Vegetation Cover	Copernicus Land Service	Fraction of green Vegetation Cover (%)	https://land.copernicus.eu/global/products/fcover

Table 2.1 External databases. In this table are listed the external sources used to integrate drivers when they were not present in the articles.

2.3.3 Architecture and pipeline of data-integration

In order to standardize all the data and consolidate them into a single database, we developed an automated pipeline that, in addition, associated, to the values extracted from the articles (regarding prokaryotic and viral abundances, productions and some environmental parameters), the drivers extracted from previously described web platforms, based on geographic coordinates. In cases where a value for a driver was unavailable, the value from the nearest available geographic area was used. Given the complexity of this operation, particularly when dealing with multi-array netCDF-4 data sources, a specific Python algorithm was developed for marine data. For terrestrial data (freshwater and soil), the QGIS tool was employed using the zonal statistics mode.

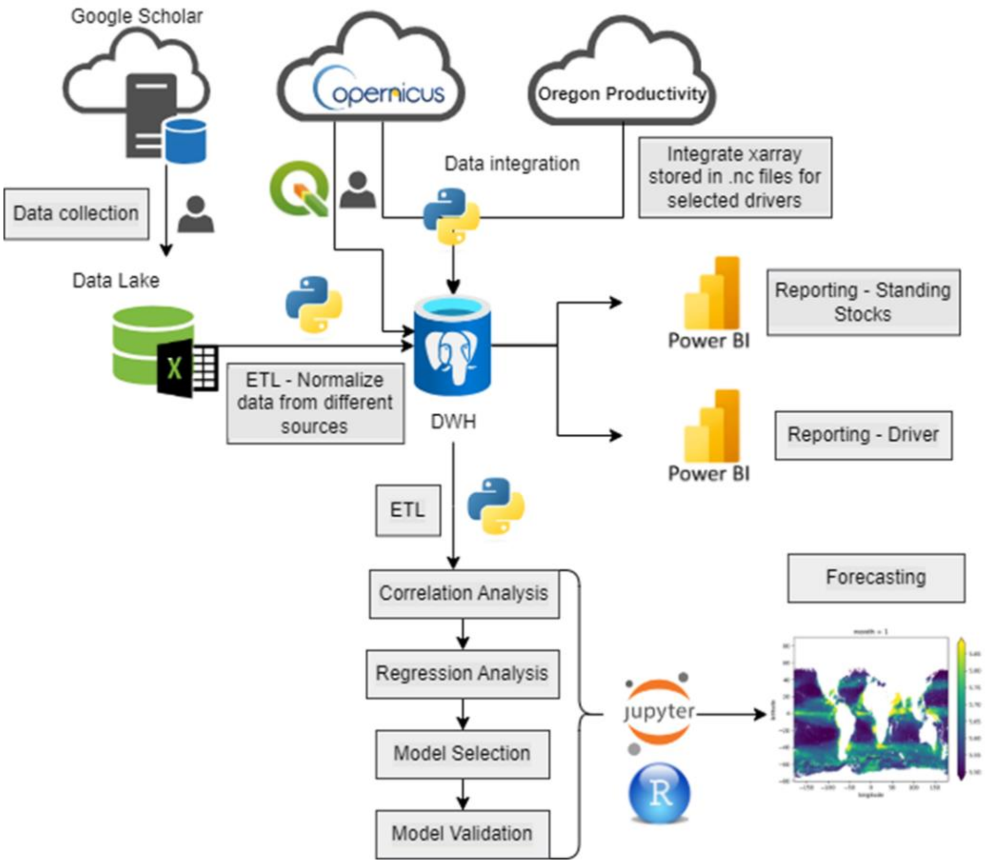


Figure 2.2 Design of the workflow. This figure summarizes the architecture implemented with all the frameworks used and the workflow followed to develop all the analysis.

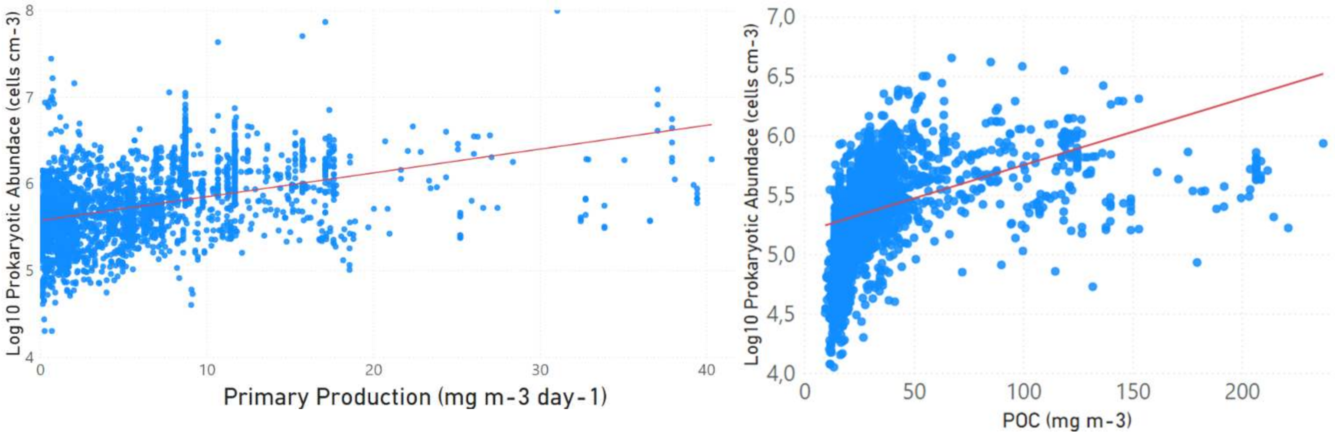
2.3.4 Statistical Analysis and Forecasting

To analyse relationships between viral and prokaryotic abundances and productions, treated as response variables, and macroecological drivers as predictor variables, we conducted a multivariate statistical analysis. We considered sea water, marine sediments, freshwater, freshwater sediments, and soil separately due to the different set of drivers used for each of these systems. Moreover, we log-transformed the dependent variables to homogenize variance and reduce the effect of the outliers (Tittensor et al., 2010). Our approach began with an initial exploratory phase (Exploratory Data Analysis – EDA) where we examined univariate patterns with the covariates. We then addressed collinearity and multicollinearity issues using the Pearson Correlation Coefficient (Cohen etal., 2009; Zuur et al., 2010) with a threshold of 0.75 and Variance Inflation Factors (VIF; Rawlings et al., 1998; Faraw, 2015) with a threshold of 7, as multicollinearity could undermine the reliability of multivariate regressions. Subsequently, we assessed the multivariate effects of drivers to explain the variability of abundances and productions, employing linear models (LM, both in linear and logarithmic scale with base 10) and generalized linear models (GLM; McCullagh, 2019) with various distributions (Poisson and Negative Binomial) to compare patterns and results among different regressions. For each distribution, a model selection was then performed to identify the best model (Zuur et al., 2010), minimizing the Akaike information criterion value (AIC; Johnson et al., 2004; Burnham et al., 2011), excluding models with no significant coefficients and those violating the assumption of independence of the response observations (Burnham et al., 2011). Models with differences in AIC less than 2 were considered equivalent and summarized in the best models recap tables (Johnson et al., 2004; Burnham et al., 2011). Finally, some of these models were used to project abundance values and distributions across the global ocean in a 3D spatial model using Copernicus data of 2019, averaged over 12 months, as input for the predictor variables (spatial forecasting, Bar-On 2018). We attempted projections for the year 2100 using one of the CMIP6 scenarios (scenario SSP2 for sea surface temperature: +1.5 °C, scenario SSP5 for primary production: -3%), exploring both spatial and temporal forecasting, and creating maps where the results for the response variables were averaged over three bathymetric zones (0-200, 200-1000, >1000). All visualizations were generated in Power BI, while statistical analyses were conducted in Python 3.9 using the pandas and statsmodels packages (McKinney, 2011, 2012).

2.4 Results

2.4.1 Univariate regressions between prokaryotic and viral abundances and productions and environmental drivers

The distribution of prokaryotic abundances in seawater, when considered in relation to trophic resources, represented in this figure by primary production and POC, and temperature, shows significant positive relationships. Moreover, in the case of POC, this trend appears to be asymptotic.



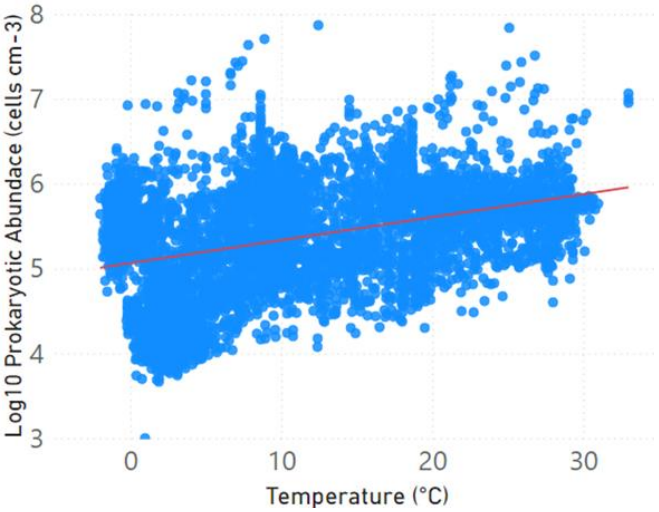
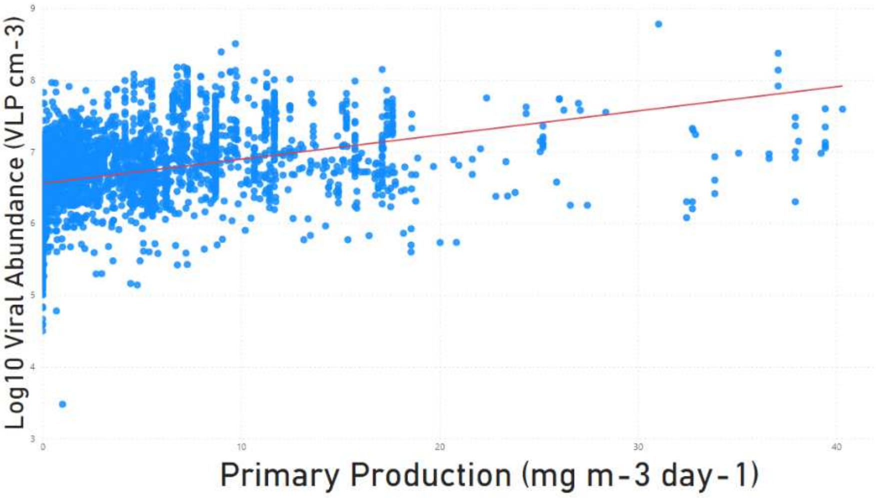


Figure 2.3 Prokaryotic abundance and drivers in sea water column. These scatterplots show the relationships between prokaryotic abundance in logarithmic scale and some drivers: primary production, POC and temperature, with the univariate regression line in red.

If we analyze viral abundances in seawater, we notice similar relationships to those observed for prokaryotes.



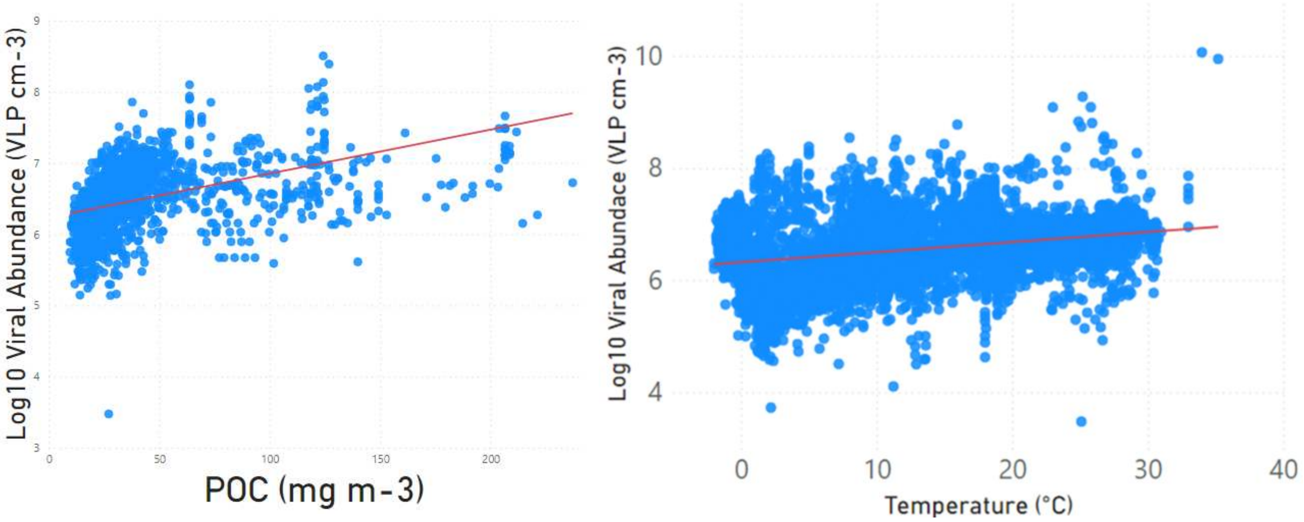


Figure 2.4 Viral abundance and drivers in sea water column. These scatterplots show the relationships between viral abundance in logarithmic scale and some drivers: primary production, POC and temperature, with the univariate regression line in red.

Our data for prokaryotic abundances in marine sediments confirm the presence of a positive correlation with carbon flux, in addition, zooming on flux values below 80 reveals an asymptotic pattern of abundances.

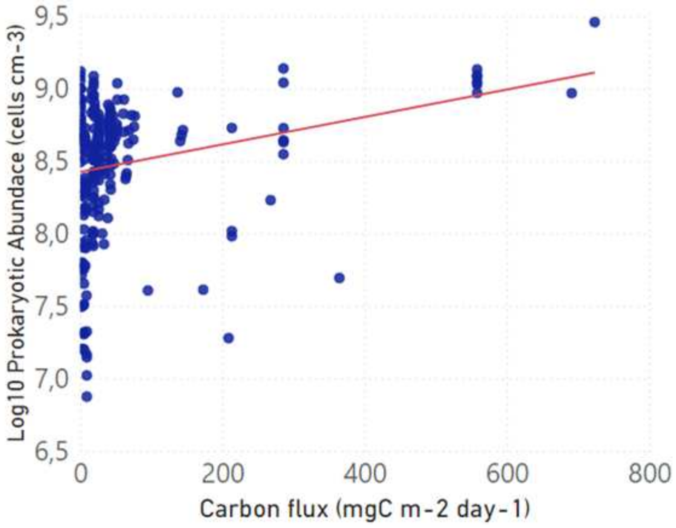


Figure 2.5 Prokaryotic abundance and carbon flux in marine sediments. These scatterplots show the relationships between prokaryotic abundance in logarithmic scale and the carbon flux (i.e., primary production from Oregon), with the univariate regression line in red.

Analyzing prokaryotic productions in the sea water column, we observe positive trends both with resources, represented by chlorophyll, and temperature.

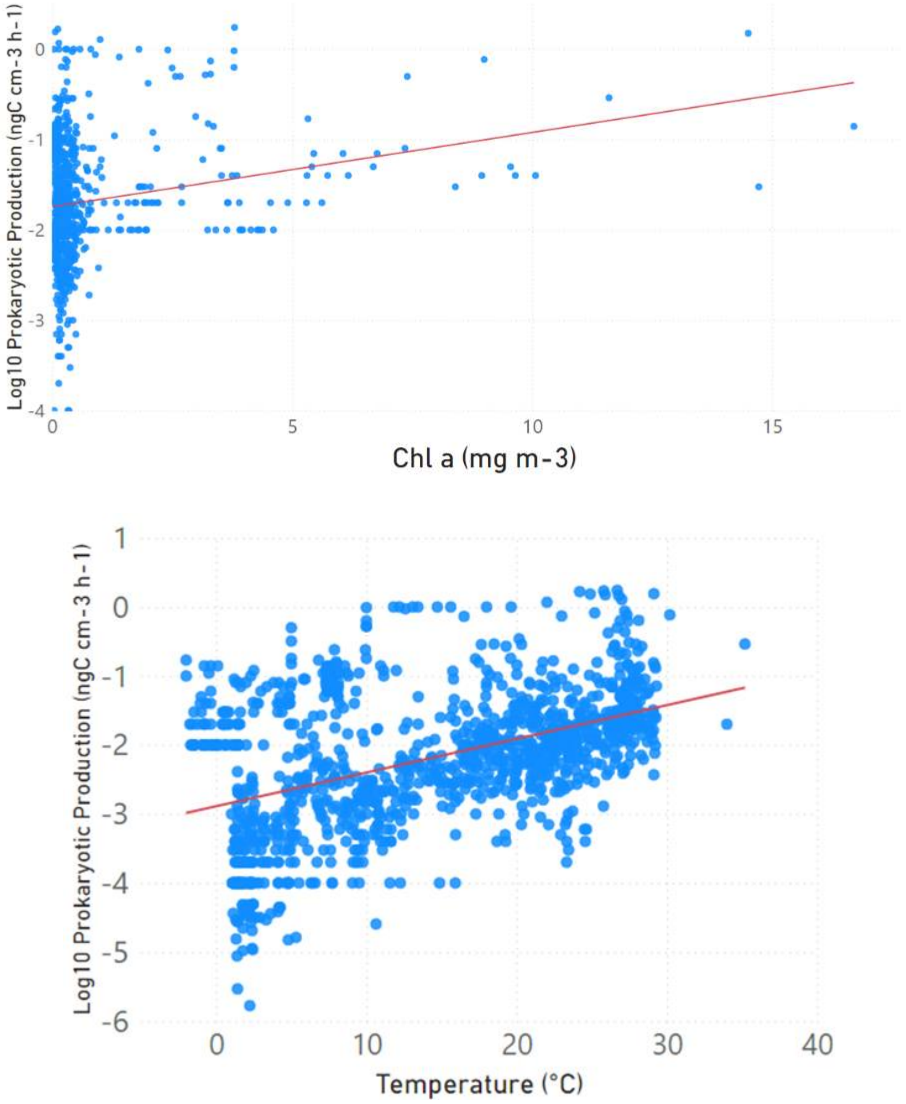


Figure 2.6 Prokaryotic production and drivers in sea water column. These scatterplots show the relationships between prokaryotic production in logarithmic scale and some drivers: chlorophyll and temperature, with the univariate regression line in red.

Similarly, viral productions in sea water column also exhibit positive correlations with trophic resources, represented by both chlorophyll and POC. Additionally, the relationship with temperature appears to be directly proportional.

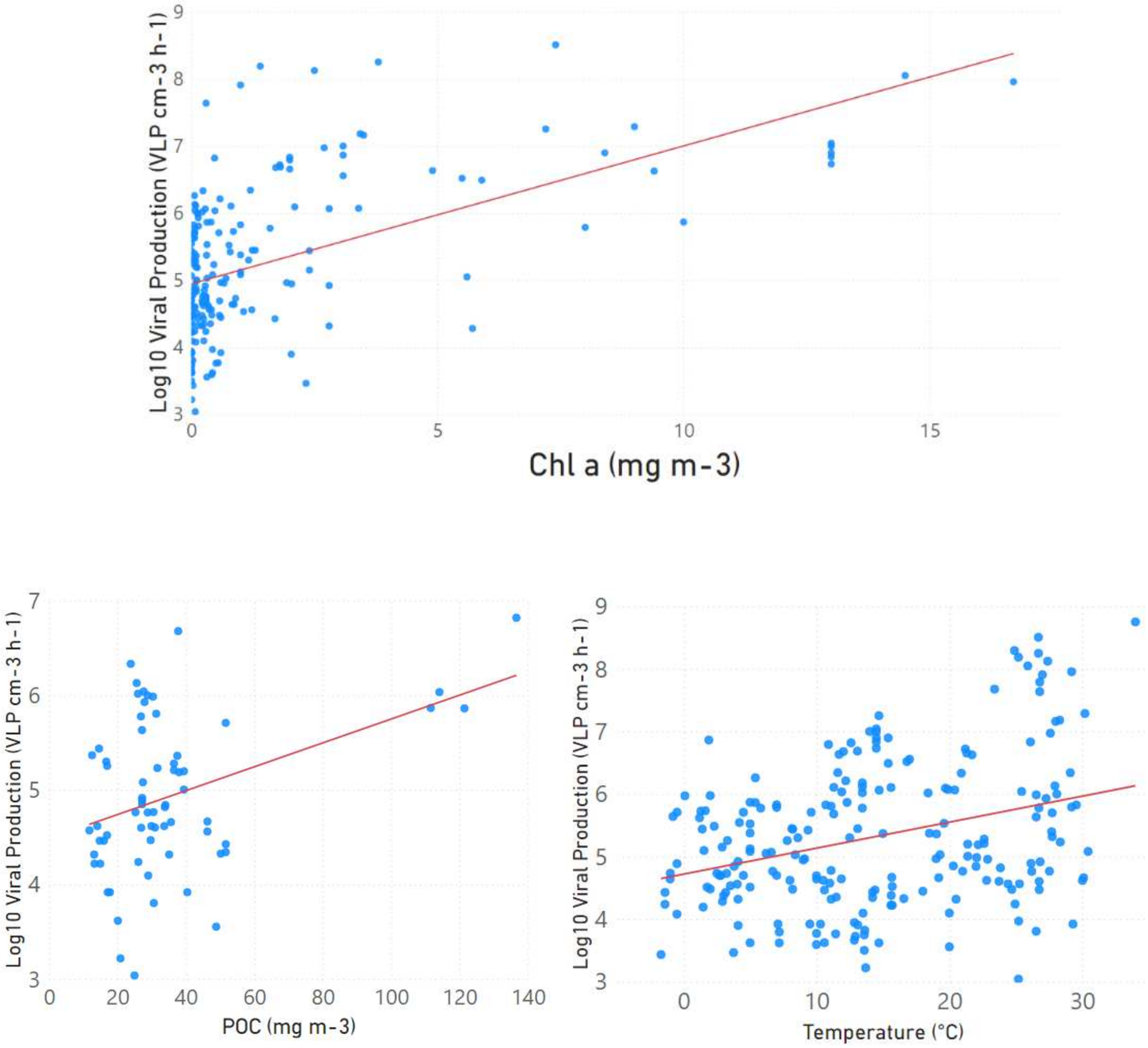


Figure 2.7 Viral production and drivers in sea water column. These scatterplots show the relationships between viral production in logarithmic scale and some drivers: chlorophyll, POC and temperature, with the univariate regression line in red.

In marine sediments, the positive relationships between prokaryotic production and food availability, represented by carbon flux in this case, persist, as well as the positive correlation with temperature.

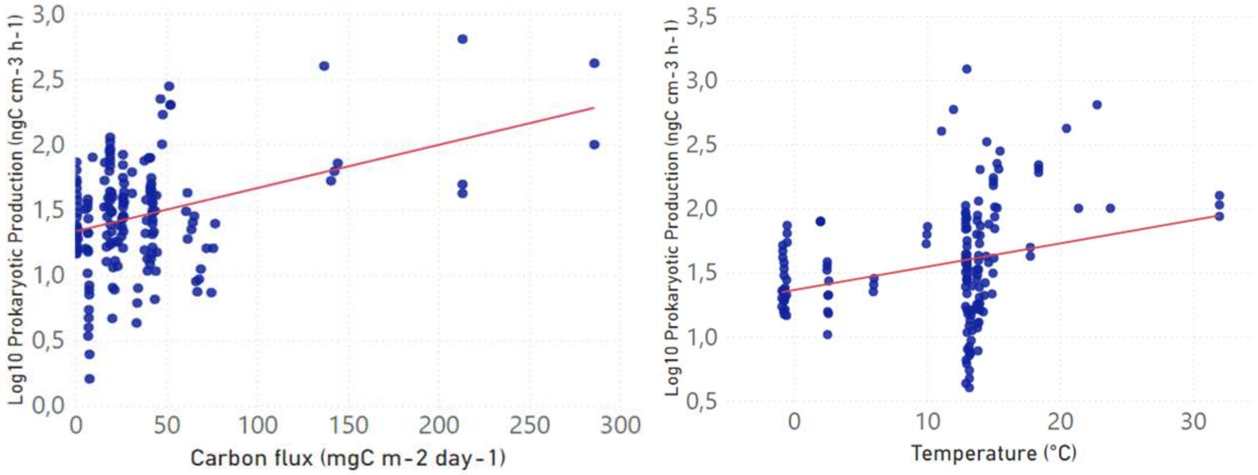


Figure 2.8 Prokaryotic production and drivers in marine sediments. These scatterplots show the relationships between prokaryotic production in logarithmic scale and some drivers: carbon flux and temperature, with the univariate regression line in red.

Finally, regarding the marine environment, the same patterns observed in the case of prokaryotic production are still valid for viral productions.

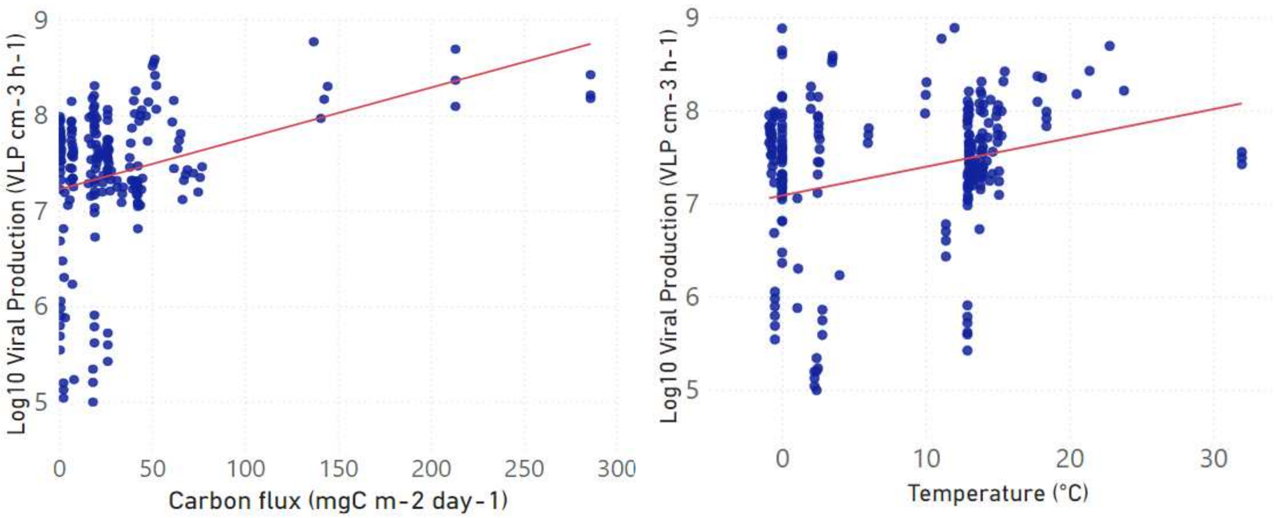


Figure 2.9 Viral production and drivers in marine sediments. These scatterplots show the relationships between viral production in logarithmic scale and some drivers: carbon flux and temperature, with the univariate regression line in red.

In the terrestrial environment, specifically in freshwater, prokaryotic abundances show positive correlations with temperature and chlorophyll, the latter exhibiting an asymptotic pattern.

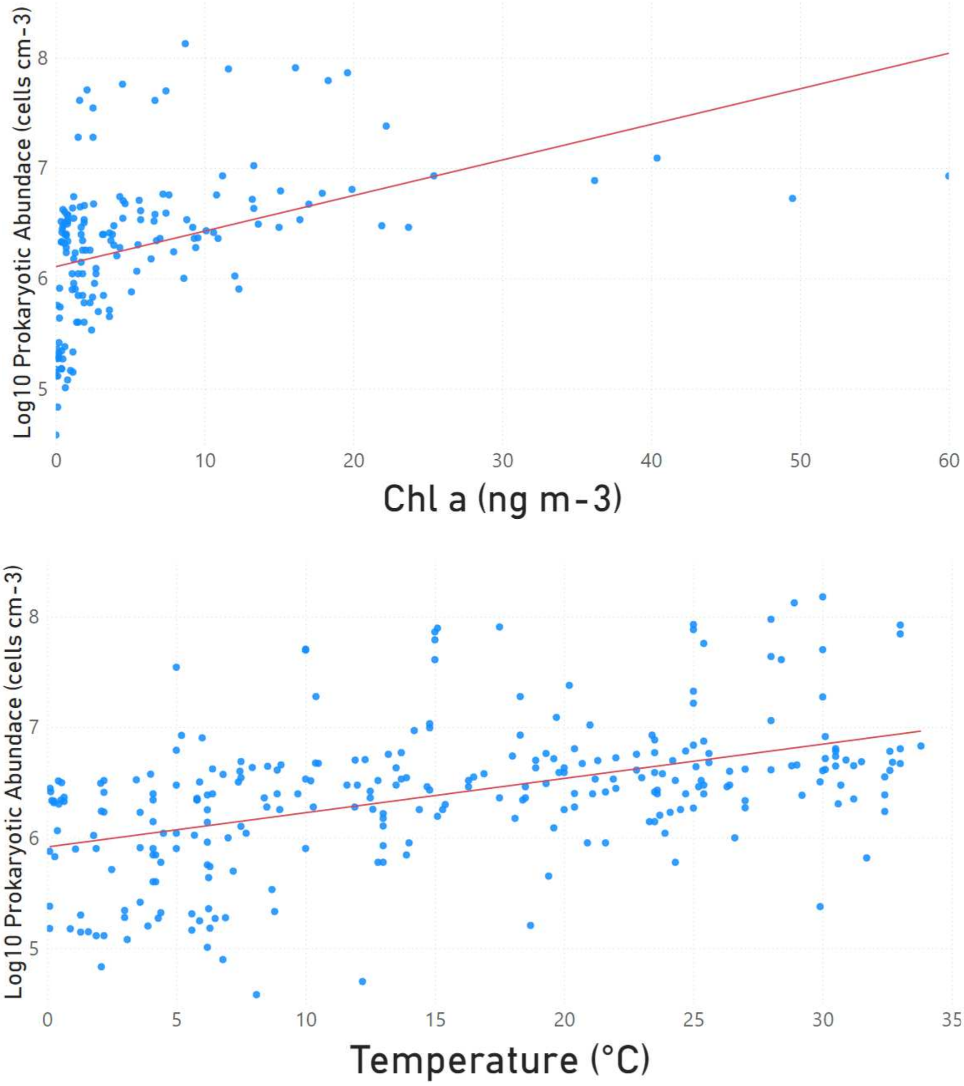
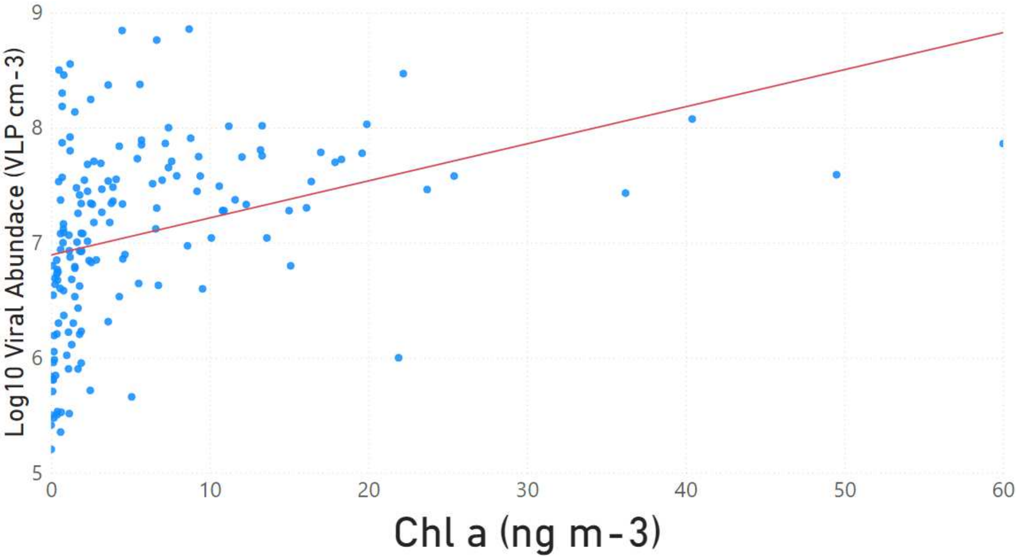


Figure 2.10 Prokaryotic abundance and drivers in freshwater. These scatterplots show the relationships between prokaryotic abundance in logarithmic scale and some drivers: chlorophyll and temperature, with the univariate regression line in red.

Similarly, in the case of viral abundances in freshwater, we observe the same trends with chlorophyll and temperature. The results obtained in this phase have provided us with valuable insights to initiate a multivariate analysis that is necessary, since from the univariate patterns analyzed so far, we are unable to disentangle the effect of individual covariates on the response variables, especially when the predictors are correlated with each other.



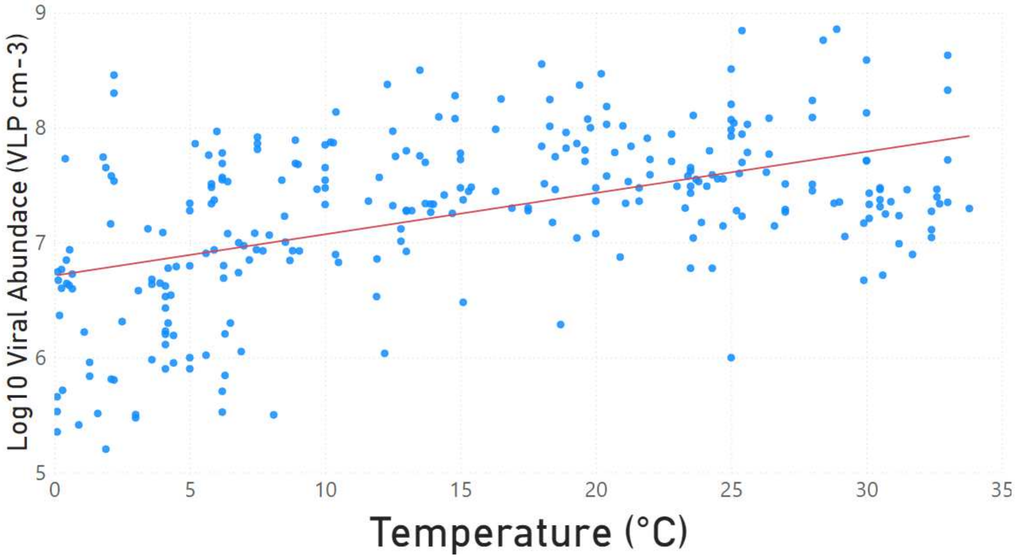


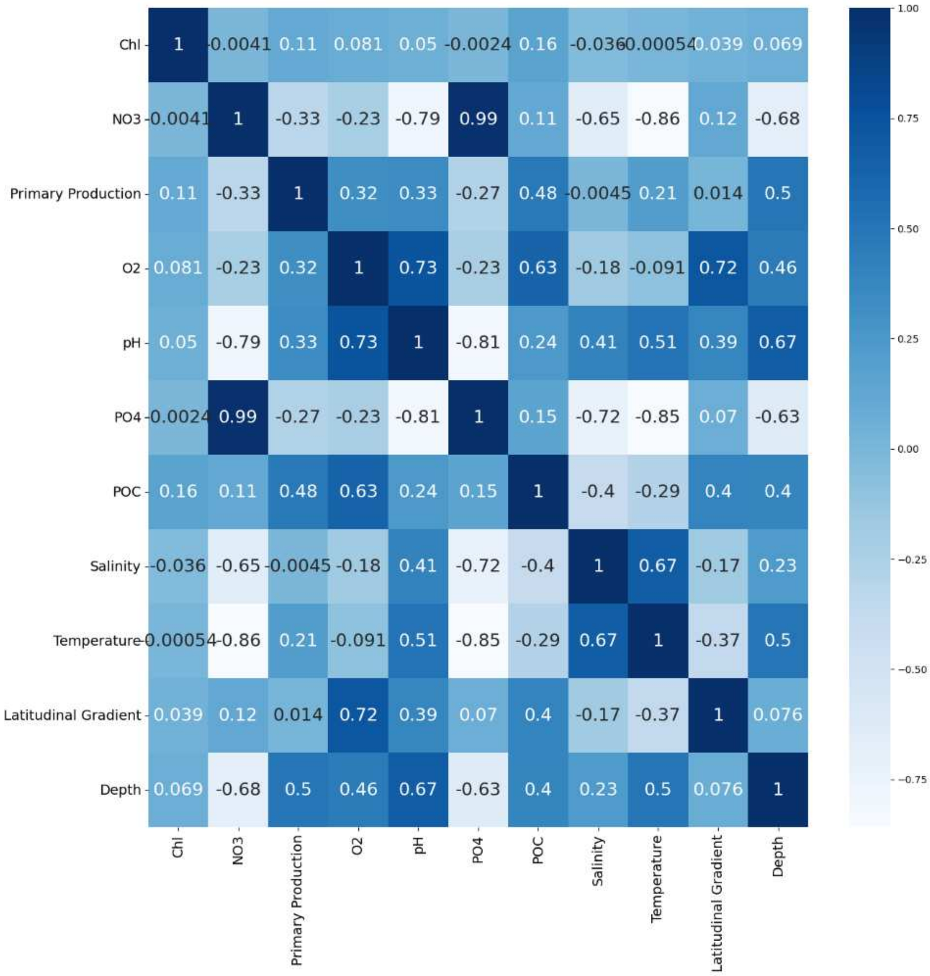
Figure 2.11 Viral abundance and drivers in freshwater. These scatterplots show the relationships between viral abundance in logarithmic scale and some drivers: chlorophyll and temperature, with the univariate regression line in red.

System	Response	udm	Predictor	udm	coeff	p-value	R2
Sea water	Prokaryotic abundance	cells cm-3	Primary production	mg m-3 d-1	0.0692	<0.001	0.279
			POC	mg m-3	0.0057	<0.001	0.159
			Temperature	°C	0.0254	<0.001	0.123
	Viral abundance	VLP cm-3	Primary production	mg m-3 d-1	0.0568	<0.001	0.246
			POC	mg m-3	0.0062	<0.001	0.187
			Temperature	°C	0.0124	<0.001	0.039
Marine sediments	Prokaryotic abundance	cells cm-3	Carbon flux	mg m-2 d-1	0.0006	0.013	0.025
Sea water	Prokaryotic production	ng cm-3 h-1	Chl a	mg m-3	0.0443	0.010	0.031
			Temperature	°C	0.0471	<0.001	0.285
	Viral production	VLP cm-3 h-1	Chl a	mg m-3	0.1942	<0.001	0.239
			POC	mg m-3	0.0140	0.002	0.144
			Temperature	°C	0.0182	0.033	0.024
Marine sediments	Prokaryotic production	ng cm-3 h-1	Carbon flux	mg m-2 d-1	0.0015	<0.001	0.146
			Temperature	°C	0.0180	0.004	0.054
	Viral production	VLP cm-3 h-1	Carbon flux	mg m-2 d-1	0.0013	0.001	0.046
			Temperature	°C	0.0309	<0.001	0.071
Freshwater	Prokaryotic abundance	cells cm-3	Chl a	ng m-3	0.0313	<0.001	0.166
			Temperature	°C	0.0279	<0.001	0.143
	Viral abundance	VLP cm-3	Chl a	ng m-3	0.0303	<0.001	0.103
			Temperature	°C	0.0325	<0.001	0.157

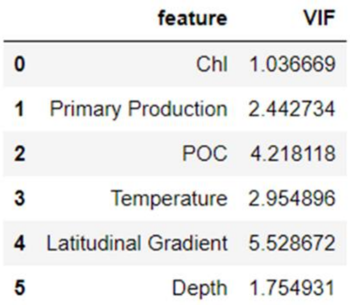
Table 2.2 Univariate regressions for prokaryotic and viral abundances and productions in different systems. This table summarizes the results of the univariate regressions analyzed in this paragraph, showing for each regression the associated information on coefficient, p-value and R2.

2.4.2 Collinearity and multi-collinearity detection

In sea water column, due to the strong correlation with temperature (Pearson Correlation Coefficient >0.75), NO_3 and PO_4 were removed. Moreover, to reduce multicollinearity, we discarded salinity, O_2, and pH, showing a VIF (Variance Inflation Factors) value greater than 7.



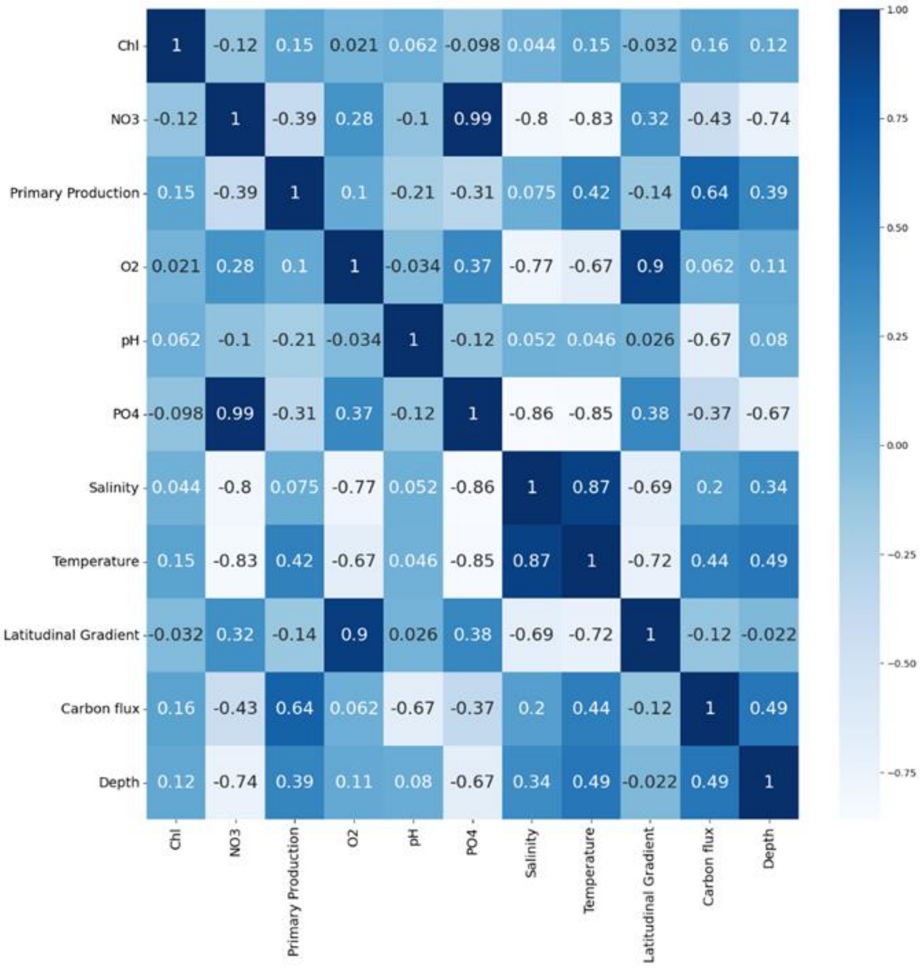
	Chl	NO3	Primary Production	O2	pH	PO4	POC	Salinity	Temperature	Latitudinal Gradient	Depth
Chl	1	-0.0041	0.11	0.081	0.05	-0.0024	0.16	-0.036	-0.00054	0.039	0.069
NO3	-0.0041	1	-0.33	-0.23	-0.79	0.99	0.11	-0.65	-0.86	0.12	-0.68
Primary Production	0.11	-0.33	1	0.32	0.33	-0.27	0.48	-0.0045	0.21	0.014	0.5
O2	0.081	-0.23	0.32	1	0.73	-0.23	0.63	-0.18	-0.091	0.72	0.46
pH	0.05	-0.79	0.33	0.73	1	-0.81	0.24	0.41	0.51	0.39	0.67
PO4	-0.0024	0.99	-0.27	-0.23	-0.81	1	0.15	-0.72	-0.85	0.07	-0.63
POC	0.16	0.11	0.48	0.63	0.24	0.15	1	-0.4	-0.29	0.4	0.4
Salinity	-0.036	-0.65	-0.0045	-0.18	0.41	-0.72	-0.4	1	0.67	-0.17	0.23
Temperature	-0.00054	-0.86	0.21	-0.091	0.51	-0.85	-0.29	0.67	1	-0.37	0.5
Latitudinal Gradient	0.039	0.12	0.014	0.72	0.39	0.07	0.4	-0.17	-0.37	1	0.076
Depth	0.069	-0.68	0.5	0.46	0.67	-0.63	0.4	0.23	0.5	0.076	1



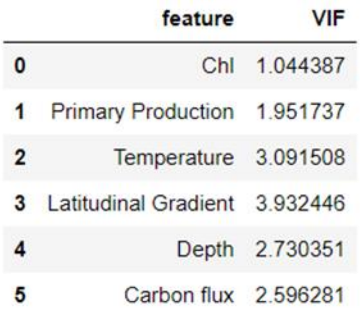
	feature	VIF
0	Chl	1.036669
1	Primary Production	2.442734
2	POC	4.218118
3	Temperature	2.954896
4	Latitudinal Gradient	5.528672
5	Depth	1.754931

Figure 2.12 Multi-collinearity detection in sea water. The heatmap shows the Pearson Correlation Coefficient for each couple of drivers considered. The Table shows the VIF values for the final set of covariates for the regression analysis.

In marine sediments, due to the strong correlation with temperature, NO3, PO4 and salinity were removed, while O2 was eliminated due to its correlation with the latitudinal gradient. In addition, to reduce multicollinearity, we discarded pH, showing a VIF value greater than 7.



	Chl	NO3	Primary Production	O2	pH	PO4	Salinity	Temperature	Latitudinal Gradient	Carbon flux	Depth
Chl	1	-0.12	0.15	0.021	0.062	-0.098	0.044	0.15	-0.032	0.16	0.12
NO3	-0.12	1	-0.39	0.28	-0.1	0.99	-0.8	-0.83	0.32	-0.43	-0.74
Primary Production	0.15	-0.39	1	0.1	-0.21	-0.31	0.075	0.42	-0.14	0.64	0.39
O2	0.021	0.28	0.1	1	-0.034	0.37	-0.77	-0.67	0.9	0.062	0.11
pH	0.062	-0.1	-0.21	-0.034	1	-0.12	0.052	0.046	0.026	-0.67	0.08
PO4	-0.098	0.99	-0.31	0.37	-0.12	1	-0.86	-0.85	0.38	-0.37	-0.67
Salinity	0.044	-0.8	0.075	-0.77	0.052	-0.86	1	0.87	-0.69	0.2	0.34
Temperature	0.15	-0.83	0.42	-0.67	0.046	-0.85	0.87	1	-0.72	0.44	0.49
Latitudinal Gradient	-0.032	0.32	-0.14	0.9	0.026	0.38	-0.69	-0.72	1	-0.12	-0.022
Carbon flux	0.16	-0.43	0.64	0.062	-0.67	-0.37	0.2	0.44	-0.12	1	0.49
Depth	0.12	-0.74	0.39	0.11	0.08	-0.67	0.34	0.49	-0.022	0.49	1



	feature	VIF
0	Chl	1.044387
1	Primary Production	1.951737
2	Temperature	3.091508
3	Latitudinal Gradient	3.932446
4	Depth	2.730351
5	Carbon flux	2.596281

Figure 2.13 Multi-collinearity detection in marine sediments. The heatmap shows the Pearson Correlation Coefficient for each couple of drivers considered. The Table shows the VIF values for the final set of covariates for the regression analysis.

2.4.3 Multivariate regressions between microbial abundances and productions and environmental drivers

The best models identified through the model selection procedure, as described in the Methods and Materials section, are summarized in the Table 2.2 below for sea water. Some of these models consider logarithmic prokaryotic abundance and employ multivariate linear regressions, while others maintain the response variable in linear scale and implement generalized linear models with Poisson distribution. It is noteworthy that in all models, trophic resource is consistently included, with primary production mainly, followed by POC, both exhibiting statistically significant positive correlations, consistent with univariate analysis findings. Additionally, temperature emerges as a predictor in almost all models, while the relationships between viruses and prokaryotes demonstrate significant positive associations in 4 out of 6 models, such as depth. Finally, the latitudinal gradient shows directly proportional relationships with abundances in only three over six models, none of which include viral abundance. It is crucial to note that the relationship with depth is directly proportional since depth is considered with a negative sign.

response	**distribution**	**temp**	**pp**	**poc**	**grad_lat**	**depth**	**Log_Viral_Abund**	**Viral_Abund**	**Log_Prok_Abund**	**Prok_Abund**
Log_Prok_Abund	linear	0.0104	0.0304				0.4796		▨	▨
Log_Prok_Abund	linear	0.0056	0.0224	0.0012	0.0020	0.0010			▨	▨
Prok_Abund	poisson	0.0118	0.0319	0.0023	0.0027	0.0032		4.363E-06	▨	▨
Prok_Abund	poisson	0.0124	0.0331	0.0029	0.0028	0.0032			▨	▨
Log_Vir_Abund	linear		0.0047	0.0032			▨	▨	0.5579	
Vir_Abund	poisson	0.0026	0.0272	0.0086		0.0024	▨	▨		3.98E-04

Table 2.3 Best LM and GLM for prokaryotic and viral abundances in sea water column. This table summarizes the best models found through the regression analysis of the abundances for prokaryotes and viruses using different distributions. Each number corresponds to the coefficient of the covariate in the regression model, all covariates represented are statistically significant, grey cells indicate that the covariate has not been included in the regression analysis for that particular response variable.

The summary table for the abundance's best models in marine sediments, show that no covariate compares in all models selected. However, trophic resource is included in most regressions, although, through different metrics. Specifically, carbon flux calculated by Oregon is included in 6 out of 11 models, both for prokaryotic and viral abundances, as observed in univariate analysis. Chlorophyll and primary production, provided by Copernicus, are both included in 2 models each, with the former showing statistically significant positive trends only with prokaryotes and the latter appearing solely in viral models. Temperature is a predictor in 5 out of 11 models, both for prokaryotic and viral variables, while the relationships between viruses and prokaryotes is significant and positive in 7 out of 11 models, with a frequency near that of trophic resources. Depth, on the other hand, no longer appears among the predictors since, as observed from the violins in Chapter 1, there seem to be no sediment variations of the abundances associated with water column depth.

Finally, the latitudinal gradient shows directly proportional patterns in 4 models, with only 1 involving viral abundance.

response	**distribution**	**temp**	**pp**	**chl**	**grad_lat**	**Carbon flux**	**Log_Viral_Abund**	**Viral_Abund**	**Log_Prok_Abund**	**Prok_Abund**
Log_Prok_Abund	linear	0.0316			0.0207	0.0005				
Log_Prok_Abund	linear						0.6895			
Prok_Abund	poisson	0.0466		0.0003	0.0305			4.77E-10		
Prok_Abund	poisson	0.0371		0.0002	0.0299	0.0014				
Log_Vir_Abund	linear				0.0041	0.0010				
Log_Vir_Abund	linear					0.0009				
Log_Vir_Abund	linear					0.0004			0.5993	
Log_Vir_Abund	linear		0.0232						0.6544	
Vir_Abund	poisson					0.0006				1.20E-09
Vir_Abund	poisson	4.75E-05	0.0156							1.37E-09
Vir_Abund	poisson	0.0022								1.36e-09

Table 2.4 Best LM and GLM for prokaryotic and viral abundances in marine sediments. This table summarizes the best models found through the regression analysis of the abundances for prokaryotes and viruses using different distributions. Each number corresponds to the

coefficient of the covariate in the regression model, all covariates represented are statistically significant, grey cells indicate that the covariate has not been included in the regression analysis for that particular response variable.

Looking at the productions, for the best models in sea water, we observe that both temperature and resources are included in 8 out of 9 models, moreover, 7 models consider primary production and one model of viral abundance considers chlorophyll as proxies for trophic resources. These findings are consistent with those from univariate exploratory analyses. The latitudinal gradient and depth appear only in one model of viral productions, indicating a nearly insignificant role compared to that for the abundances. Lastly, the relationships between viruses and prokaryotes in 4 models, both for viral and prokaryotic production, demonstrate how productions also depend on the interconnections between viruses and hosts. In this case, abundances were used instead of productions as covariates due to the lack of data points containing both prokaryotic and viral production data.

response	**distribution**	**temp**	**pp**	**chl**	**grad_lat**	**depth**	**Viral_Abund**	**Log_Prok_Abund**	**Prok_Abund**
Prok_Prod	poisson	0.0979							1.28e-06
Prok_Prod	poisson	0.1102	0.0527						
Prok_Prod	poisson	0.1085	0.0479				3.563e-08		
Prok_Prod	linear	0.0251	0.0108						3.614e-07
Prok_Prod	linear	0.0317	0.0139						
Prok_Prod	linear	0.0305	0.0128				5.577e-09		
Log_Vir_Prod	linear		0.0253		-0.0093	0.0006			
Log_Vir_Prod	linear	0.0164	0.0259					0.3047	
Viral_Prod	poisson	0.0646		0.7140					4.244e-08

Table 2.5 Best LM and GLM for prokaryotic and viral productions in sea water. This table summarizes the best models found through the regression analysis of the abundances for prokaryotes and viruses using different distributions. Each number corresponds to the coefficient of the covariate in the regression model, all covariates considered are statistically significant.

For the productions in marine sediments, the temperature appears in only 2 out of 11 best models, while depth gains greater relevance, appearing in 7 out of 11 models, the majority of which are for prokaryotic production. However, the importance of trophic resources remains fundamental, although with different proxies. Specifically, 7 models include carbon flux as a predictor, all with prokaryotic productions, while 6 models show Copernicus primary production as covariate for both viral and prokaryotic productions. Chlorophyll appears in 5 models again, all for prokaryotic production. Finally, the relevance of depth increases, since it is present in 6 out of 7 models for prokaryotic production and in one for viral production, while the virus-prokaryote relationship is significant in 5 models, equally distributed between the two production responses.

response	**distribution**	**temp**	**pp**	**chl**	**depth**	**Carbon flux**	**Log_Vir_Prod**	**Viral_Prod**	**Log_Prok_Prod**	**Prok_Prod**
Log_Prok_Prod	linear			0.0002	0.0001	0.0011				
Log_Prok_Prod	linear				0.0001	0.0012				
Log_Prok_Prod	linear				7.526e-05	0.0008	0.6405			
Prok_Prod	poisson	0.0352	0.0791	0.0003	0.0002	0.0010				
Prok_Prod	poisson		0.0973	0.0003	0.0003	0.0011				
Prok_Prod	poisson	0.0500	0.0868	0.0003		0.0014				
Prok_Prod	poisson			0.0003	0.0001	0.0020		5.149e-09		
Log_Vir_Prod	linear		0.0735							
Log_Vir_Prod	linear		0.0300						0.5290	
Log_Vir_Prod	linear								0.5869	
Viral_Prod	poisson		0.0474		0.0001					0.0027

Table 2.6 Best LM and GLM for prokaryotic and viral productions in marine sediments. This table summarizes the best models found through the regression analysis of the abundances for prokaryotes and viruses using different distributions. Each number corresponds to the coefficient of the covariate in the regression model, all covariates represented are statistically significant, grey cells indicate that the covariate has not been included in the regression analysis for that particular response variable.

If we describe the results for the abundances in terrestrial environments as freshwater, temperature is the most prevalent covariate in our regressions, appearing in 5 out of 6 models. Chlorophyll is the sole proxy for trophic resources and appears in 4 models, all related to prokaryotic abundance. Both patterns are consistent with the findings in the univariate analysis mentioned earlier. To account for virus-prokaryote relationships, abundances were used as predictors in 4 regressions.

response	distribution	temp	chl	Log_Viral_Abund	Viral_Abund	Log_Prok_Abund	Prok_Abund
Log_Prok_Abund	linear		0.0157	0.4555			
Log_Prok_Abund	linear	0.0200	0.0188				
Prok_Abund	poisson	0.0290	0.0261		3.563e-09		
Prok_Abund	poisson	0.0725	0.0182				
Log_Vir_Abund	linear	0.0389				0.4561	
Viral_Abund	poisson	0.0678					1.245e-08

Table 2.7 Best LM and GLM for prokaryotic and viral abundances in freshwater column. This table summarizes the best models found through the regression analysis of the abundances for prokaryotes and viruses using different distributions. Each number corresponds to the coefficient of the covariate in the regression model, all covariates represented are statistically significant, grey cells indicate that the covariate has not been included in the regression analysis for that particular response variable.

For freshwater productions, patterns with trophic resources are represented in two out of five models by chlorophyll, both related to viral production, and in one model of prokaryotic production by the trophic state. Finally, both best models for viral productions include prokaryotic abundances among their covariates.

response	distribution	chl	trophic_state	Log_Prok_Abund	Prok_Abund
Log_Prok_Prod	linear			1.0071	(6.356e-08, if we used the covariate in linear scale)
Prok_Prod	poisson				1.374e-07
Prok_Prod	poisson		0.0188		
Log_Vir_Prod	linear	0.0015		0.3715	

Viral_Prod	poisson	0.0061			5.43e-09

Table 2.8 Best LM and GLM for prokaryotic and viral productions in freshwater column. This table summarizes the best models found through the regression analysis of the abundances for prokaryotes and viruses using different distributions. Each number corresponds to the coefficient of the covariate in the regression model, all covariates represented are statistically significant.

In freshwater sediments, due to the low number of points, we are able to find only few significant patterns as those with the latitudinal gradient. Finally, the relationships between viruses and prokaryotes are included in each of the best models, for both productions considered.

response	distribution	grad_lat	Log_Viral_Abund	Viral_Abund	Log_Prok_Abund	Prok_Abund
Log_Prok_Abund	linear		0.6727			
Prok_Abund	poisson	0.0078		1.56e-11		
Log_Vir_Abund	linear				0.6089	
Viral_Abund	poisson	0.0598				1.311e-11

Table 2.9 Best LM and GLM for prokaryotic and viral abundances in freshwater sediments. This table summarizes the best models found through the regression analysis of the abundances for prokaryotes and viruses using different distributions. Each number corresponds to the coefficient of the covariate in the regression model, all covariates represented are statistically significant, grey cells indicate that the covariate has not been included in the regression analysis for that particular response variable.

In the end, considering the abundances in soil, due to the reduced amount of data and the high multicollinearity among the covariates, the most frequent driver was the soil water index, which shows statistically significant and positive relationships with both abundances. Also, the virus-prokaryote relationships are present and significant as driver, but only in the models for prokaryotic abundance.

Response	**distribution**	**swi**	**Log_Viral_Abund**	**Viral_Abund**
Log_Prok_Abund	linear		0.6999	
Prok_Abund	poisson	0.0107		8.147e-12
Log_Vir_Abund	linear	0.0808		
Viral_Abund	poisson	0.0479		

Table 2.10 Best LM and GLM for prokaryotic and viral abundances in soil. This table summarizes the best models found through the regression analysis of the abundances for prokaryotes and viruses using different distributions. Each number corresponds to the coefficient of the covariate in the regression model, all covariates represented are statistically significant, grey cells indicate that the covariate has not been included in the regression analysis for that particular response variable.

In conclusion, the results of the multivariate regression analysis conducted on different environments, in particular seawater and marine sediments, using various statistical approaches, indicate that the variance of viral and prokaryotic abundances and productions is significantly explained by temperature, trophic resources (using different proxies), and virus-prokaryote interactions. However, significant differences exist in each system considered due to variations in the number of data points and predictors considered.

2.4.4 Maps of prokaryotic and viral abundances and productions spatial-temporal forecasting

In this paragraph, I show the outcomes of the modelling approach used to forecast the prokaryotic and viral abundances and productions based on the changes in environmental parameters predicted by the climate change scenario we selected. Results are illustrated in maps that report the projected values at global scale. For prokaryotic abundance we selected one of the best models (Table 2.2), in particular we chose the model incorporating predictors such as primary production (pp), particulate organic carbon (POC), temperature, latitudinal gradient, and depth. This model, computed in logarithmic scale for sea water using a 3D input provided by Copernicus values averaged on 12 months, resulted in a global estimate of the mean prokaryotic abundance of approximately 2.2×10^4 (cells cm^{-3}). Comparing this data with the results of the same model computed with a temperature increase and a primary production decrease both based on CMIP6 scenarios, we observed a mean abundance value increasing of over 0.134%. We have also computed maps where each grid point corresponds to a spatial average across three bathymetric zones (as explained in the Materials and Methods above). The epipelagic zone displayed the highest number of observations, consequently making the predictions more robust. In the Figure 2.14, we observe that areas characterized by higher productions are those near the coasts, especially those of the Indian Ocean, Africa, Latin America, and Indonesia, followed by areas crossed by the Gulf Stream and those near Antarctica.

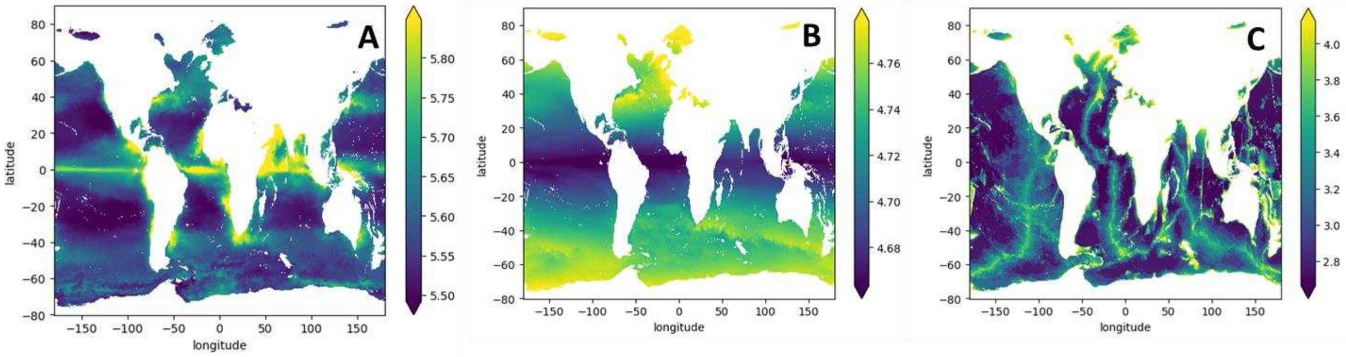


Figure 2.14 Maps of prokaryotic abundance spatial distribution. These maps show the distribution of prokaryotic abundances calculated using the best model with predictors: primary production (pp), particulate organic carbon (POC), temperature, latitudinal gradient, and depth, averaging the values in three bathymetric zones (0-200m [A], 200-1000m [B], >1000m [C])

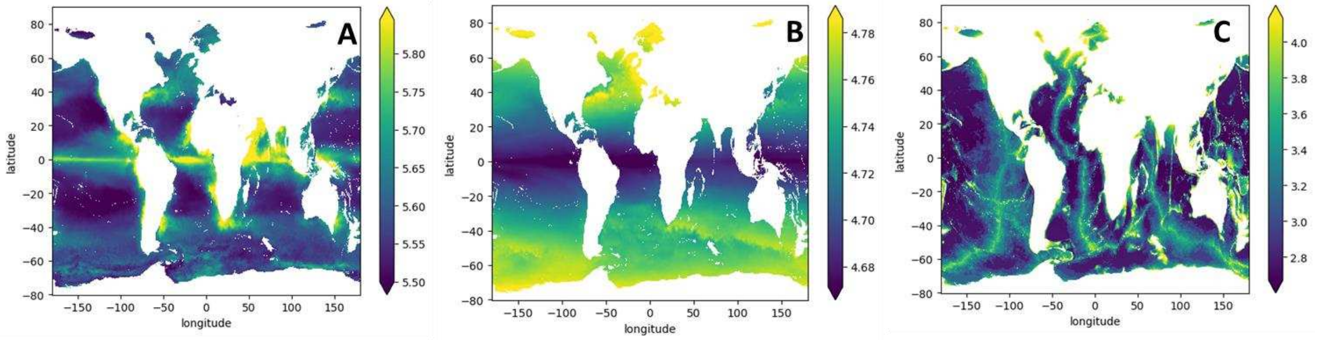


Figure 2.15 Maps of predicted prokaryotic abundance for 2100. These maps show the predicted distribution of prokaryotic abundances calculated using the best model with covariates: primary production (pp), particulate organic carbon (POC), temperature, latitudinal gradient, and depth, averaging the values in three bathymetric zones (0-200m [A], 200-1000m [B], >1000m [C]), for the year 2100, considering the CMPI6 scenario indicated in Material and Methods.

For the maps with viral abundance, we used a different set of predictors again suggested by one of the best models calculated before including: primary production, POC and prokaryotic abundance in logarithmic scale. To calculate the values of prokaryotes we used the estimates derived from the previous model, and we obtained a value of global average estimate for the viral abundance around 7.2×10^5 (VLP cm^{-3}). If we compare this average value with that of the same model calculated under CMIP6 scenario, we obtain a lower average abundance value of 0.003%. In addition, from the figures we notice that the Figure 2.16 A shows important differences if compared to the Figure 2.14 A, in fact, despite we found a high number of viruses where we also had many prokaryotes as we noticed comparing Figure 2.14 with Figure 2.16, the areas of highest intensity are in the viral abundance case those around Antarctica and some spots along the coasts, but much smaller in extent than those we saw for prokaryotes. Finally, in the Figure 2.16 B the highest viral abundances are those near the higher latitudes, as for the prokaryotic case.

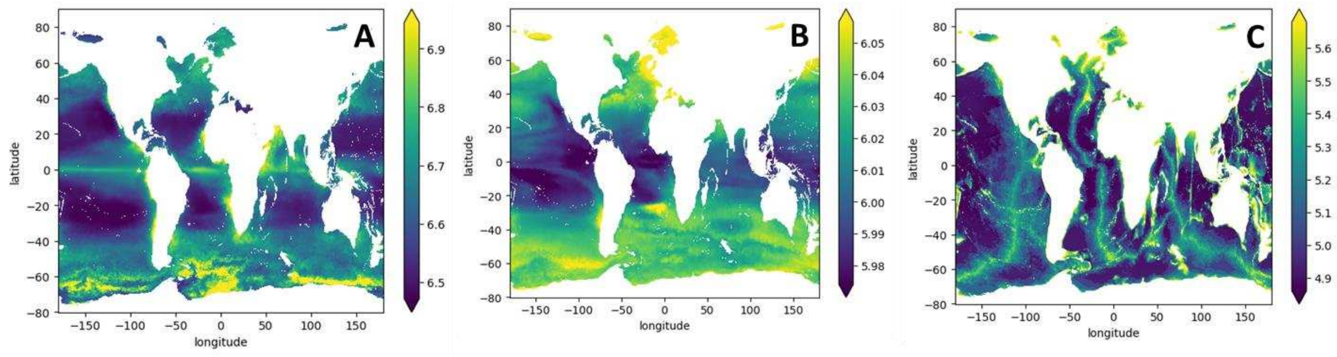


Figure 2.16 Maps of viral abundance spatial distribution. These maps show the distribution of viral abundances calculated using the best model with predictors: primary production, POC and prokaryotic abundance, averaging the values in three bathymetric zones (0-200m [A], 200-1000m [B], >1000m [C])

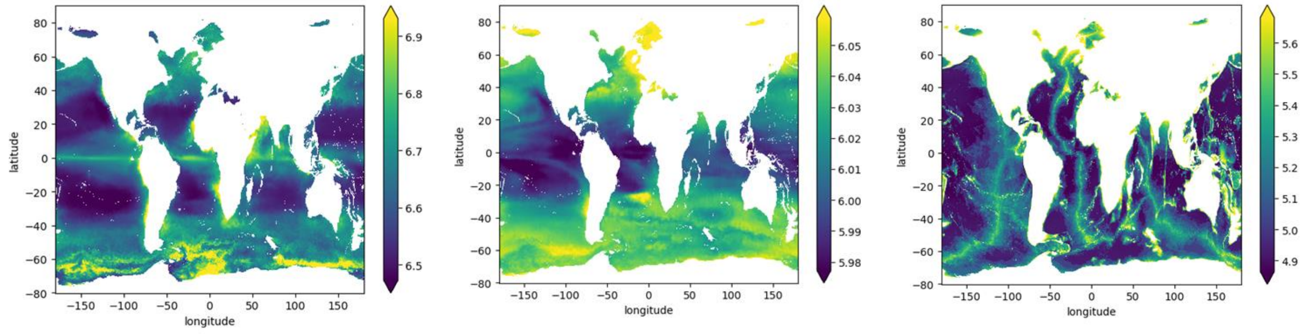


Figure 2.17 Maps of predicted viral abundance for 2100. These maps show the predicted distribution of viral abundances calculated using the best model with covariates: primary production, POC and prokaryotic abundance, averaging the values in three bathymetric zones (0-200m [A], 200-1000m [B], >1000m [C]), for the year 2100, considering the CMPI6 scenario indicated in Material and Methods.

For the distributions of prokaryotic productions, we used the Poisson's best model having as covariates: temperature and prokaryotic abundance in linear scale. Therefore, to calculate the values of prokaryotic abundances, we used a best model with the same covariates as the previous one (primary production (pp), particulate organic carbon (POC), temperature, latitudinal gradient, and depth), but with a Poisson distribution. From these figures we note that the range 0-200 shows similarities with respect to the prokaryotic abundance map, especially in the lower latitudinal ranges, while the areas with the higher latitude show important differences, since we find low production where they have many prokaryotes. Looking at the 200-1000 range, the highest productions are detected in the Gulf Stream area and in the Mediterranean basin, with a few yellow areas in the Indian Ocean off southern Africa. If we calculate again the difference between the average global prokaryotic production at the present time and the average global production in the CMIP6 scenario we get an increase of more than 16%.

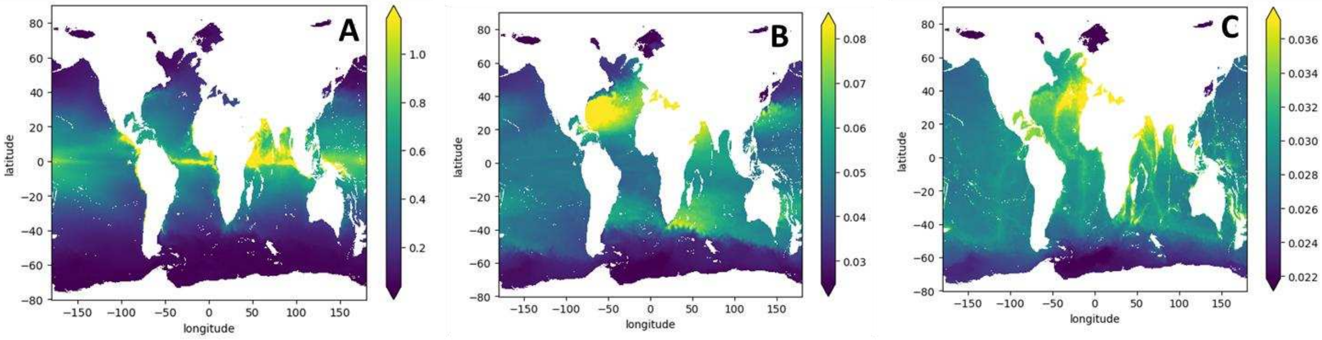


Figure 2.18 Maps of prokaryotic production spatial distribution. These maps show the distribution of prokaryotic production calculated using the best model with predictors: temperature and prokaryotic abundance, averaging the values in three bathymetric zones (0-200m [A], 200-1000m [B], >1000m [C])

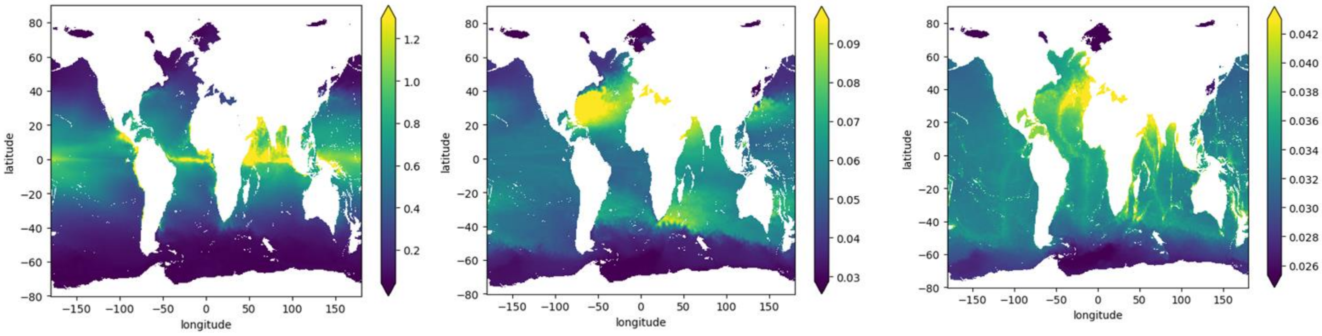


Figure 2.19 Maps of predicted prokaryotic production for 2100. These maps show the predicted distribution of prokaryotic productions calculated using the best model with covariates: temperature and prokaryotic abundance, averaging the values in three bathymetric

zones (0-200m [A], 200-1000m [B], >1000m [C]), for the year 2100, considering the CMPI6 scenario indicated in Material and Methods.

Finally, for the distributions of viral productions, we used the Poisson's best model having as covariates: primary production, depth and latitudinal gradient. The 0-200 range shows similarities with the prokaryotic production map, although the areas in yellow with the highest productions appear geographically wider in the viral case. In addition, the patterns that we see in the epipelagic layer (Figure 2.20 A) also carry over to the other two figures (Figure 2.20 B, C) although each has peculiarities. If I calculate again the difference between the average global viral production at the present time and the average global production in the CMIP6 scenario I get a 0.775% reduction.

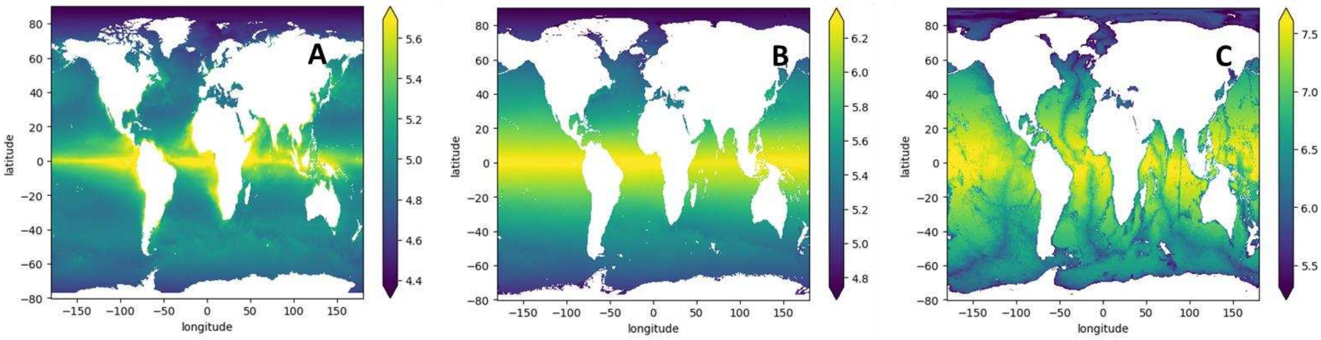


Figure 2.20 Maps of viral production spatial distribution. These maps show the distribution of viral production calculated using the best model with predictors: primary production, depth and latitudinal gradient, averaging the values in three bathymetric zones (0-200m [A], 200-1000m [B], >1000m [C])

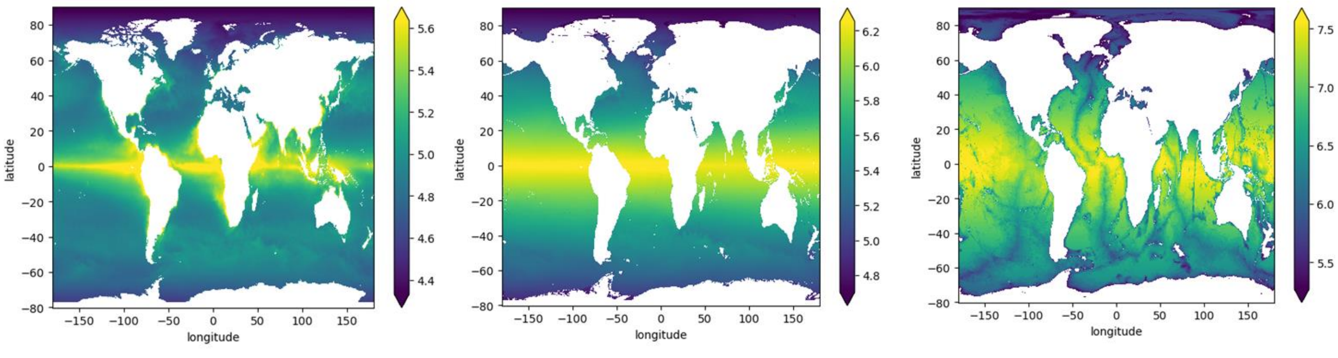


Figure 2.21 Maps of predicted viral production for 2100. These maps show the predicted distribution of viral productions calculated using the best model with covariates: primary production, depth and latitudinal gradient, averaging the values in three bathymetric zones (0-200m [A], 200-1000m [B], >1000m [C]), for the year 2100, considering the CMPI6 scenario indicated in Material and Methods.

2.5 Discussion

We know that prokaryotes and viruses play a pivotal role in the functioning of both pelagic and benthic ecosystems, influencing microbial food webs, controlling prokaryotic diversity, and impacting biogeochemical cycles. Specifically, prokaryotes represent one of the largest biomass pools on earth (Bar-On et al., 2018), serving as an enormous food source for higher trophic levels (Dell'Anno et al., 2005; Danovaro et al., 2015). Viruses represent an essential component of the ecological community, influencing microbial dynamics either through viral lysis or regulating microbial physiology via temperate infections and gene transfer between microbes, profoundly influencing biogeochemical cycles in the ocean (Bergh et al., 1989; Proctor et al., 1990; Suttle et al., 1990; Howard-Varona et al., 2017; Kavagutti et al., 2019; Zimmerman et al., 2020; Ji et al., 2023). Since our understanding of the controlling factors of prokaryotic and viral abundances and productions over larger spatial scale in the oceans (thousands of kilometers) is still poor (Wilhelm et al. 2003; Danovaro et al., 2011), it is important to explore the interconnection between the shifts in different environmental variables due to climate change and the effects on marine microbial assemblages and overall ecosystem functioning (Hughes, 2000; Hays et al., 2005; Doney et al., 2009). In fact, a better understanding of their response to present climate change would enhance our ability to predict and adapt to the consequences of such changes, especially regarding impacts on viruses whose knowledge is still largely absent (Danovaro et al., 2011). Numerous studies have explored the intricate relationships between viruses and prokaryotes and their interactions with both biotic and abiotic environmental factors (Li et al., 2004; Mojica et al., 2014; Wigington et al., 2016; Sweetman et al., 2017; Lara et al., 2017; López-Sandoval et al., 2019; Morán et al., 2020; Lønborg et al., 2020; LaBrie et al., 2021; Liang et al., 2021; Cao et al., 2023; Antunes et al., 2023). Additionally, mechanistic models like biogeochemical models have investigated the specific and temporal dynamics of viruses and prokaryotes in relation to other components within food webs. However, a comprehensive analysis of prokaryotic and viral abundances and productions across all ecosystems has been lacking so far. Previous investigations (Wiggington et al., 2016; Lara et al., 2017), have been confined to marine environments and have primarily focused on limited number of drivers such as depth, latitude, and prey-predator dynamics.

2.5.1 The role of our study

In this chapter, for the first time, we conducted a comprehensive analysis of the drivers influencing prokaryotic and viral abundance and production on a global scale. The study employs a robust data integration approach across various platforms, including Copernicus and Oregon, to reveal the primary potential drivers that can elucidate the distributions of prokaryotic and viral abundances and productions in major ecosystems. Here we analysed physical and chemical characteristics of different ecosystems (e.g., temperature, salinity, turbulence and mixing regimes, nutrient availability) selecting those that are considered important covariates influencing prokaryotic and viral dynamics, because they have a major effect on the abundance, distribution and metabolism of assemblages (Wommack et al., 2000; Weinbauer, 2004). To identify these drivers, the regression analysis was chosen as technique to discover recurrent patterns among environmental covariates and prokaryotic and viral response variables in a multivariate context. The relationships identified do not represent mechanistic cause-and-effect dynamics but reveal trends that can provide insights for further analyses and a reevaluation of biogeochemical models in light of significant drivers that may have been previously overlooked or understudied. Lastly, different types of models were employed, considering that the nature of information in response variables or the distribution of data, in some cases, posed challenges. The driver analyses reveal in some cases non-linear patterns (as for chlorophyll and carbon flux, Fig. 2.5-2.6) and slope variations between the ecosystems considered (as for POC, Fig. 2.3-2.4); however, the need for three-dimensional predictive maps coupled with the difficulty of integrating multidimensional data from Copernicus led us at this early stage to opt for multivariate generalized linear regression models without introducing random effects or more complex models.

2.5.2 The importance of temperature as a driver for viruses and prokaryotes

The results of the model selection presented in this study, confirm that temperature is one of the primary drivers of ecosystem functioning in seawater, marine sediments, and freshwater, capable of explaining part of the variability in both abundances and productions, for both viral and prokaryotic components. The positive relationships observed with prokaryotic productions are consistent with findings in the literature (Kirchman e al., 2009; Lønborg et al., 2016; Sweetman et al., 2017; Morán et al., 2017, 2020) and with the Metabolic Theory of Ecology (Brown et al., 2004), which posits an increase in metabolism with rising temperatures according to the Arrhenius equation (López-Urrutia et al., 2007). The increase in prokaryotic productions leads to a growth in viral productions, as viral dynamics are linked to the metabolism of their hosts (Danovaro et al. 2008, 2011). Moreover, even though the results regarding the relationships between abundances and temperature in the literature are not always the same (Lara et al., 2017), at a global level, we have found a positive correlation with prokaryotic abundances already highlighted in previous studies (Li et al., 2004). The positive correlation with the viral abundance may be a consequence of the positive effect of temperature on prokaryotic production (White et al., 1991; Jiang et al., 1994; Maranger et al., 1995).

2.5.3 The role of trophic resources as a driver of the spatial distribution of viruses and prokaryotes

Another driver significant in many of the best models in the systems analyzed (sea water, marine sediments, freshwater, and freshwater sediments) is trophic resources. Various proxies were used for this driver, such as primary production (Tittensor et al., 2010) and chlorophyll concentration (Cochlan et al., 1993; Paul et al., 1993; Boehme et al., 1993; Fuhrman, 1999) along the water column, while carbon flux (Sweetman et al., 2017) was employed for marine sediments. Several studies conducted at a regional scale and in different ecosystems have pointed out that the distributions and dynamics of microbial assemblages depend on pelagic primary production, vertical particle flux, and chlorophyll, indicating a possible causal relationship between both prokaryotes and viruses with trophic state (Cochlan et al., 1993; Hewson et al., 2001; Weinbauer et al., 2004; Clasen et al., 2008; Danovaro et al., 2011; Lønborg et al., 2019). Our results confirm this hypothesis, as we identified a consistent positive and significant correlation between food availability and prokaryotic abundance across various environments. Indeed, prokaryotes are considered significant consumers of labile organic compounds in the ecosystems. (Hewson et al., 2001; Li et al., 2004; Middelboe et al., 2006; Carreira et al., 2013; Danovaro et al., 2016; Holman et al., 2021). Moreover, recent studies based on the metabolic theory provided evidence of the importance of chemical energy coupled with thermal energy (Morán et al., 2019, 2020), according to the re-adapted Arrhenius law temperature and resource dependent (López-Urrutia et al., 2007). Therefore, the increase in resources enhances prokaryotic productions by providing organic sources for heterotrophic prokaryotic metabolism (López-Urrutia et al., 2007; Danovaro et al., 2011; Lonborget al., 2020; LaBrie et al., 2021), a pattern confirmed also in the best models. Our finding of increasing viral abundances in areas of higher trophic status may be related to an increase in burst size per lytic event in more eutrophic zones as observed in many previous studies

(Weinbauer et al., 1993, 2004; Maranger et al., 1995). Finally, in the case of viral productions, this relationship with resources could be a consequence of the increase in prokaryotic productions, since viral replication depends on the activity of the host organisms (Glud et al., 2004; Danovaro et al., 2008; Middelboe et al.,2011; Danovaro et al., 2011; Lara et al., 2017;). These findings support the importance of surface primary production processes and the associated downward fluxes of particulate organic carbon in influencing the distribution of prokaryotic and viral standing stocks in the global ocean. Specifically, the export of organic carbon from surface waters to the deep-sea floor via sinking particles is expected to play a significant role in regulating the metabolism and standing stocks of benthic assemblages in the deep sea (Fuhrman et al., 1999).

2.5.4 Virus-prokaryote interaction as a driver of viral and prokaryotic distribution

As seen in chapter 1, microbial assemblages are closely related to biotic factors such as viral abundance, suggesting that virus-host interactions as top-down regulatory factors are important in driving prokaryotic dynamics in all ecosystems and need to be included for accurate models of ecosystem functioning (Williamson et al., 2017; Kuzyakov et al., 2018; Liang et al., 2021). Moreover, previous analyses indicate that the abundance of viruses is closely linked with the abundance of their hosts, so that any change in the abundance, metabolic state, and doubling time of the prokaryotic hosts will affect viral abundance (Fuhrman, 1999; Wommack et al., 2000; Weinbauer, 2004; Middelboe et al., 2006; Danovaro et al., 2008; Clasen 2008; Carreira et al., 2013; Wigington et al., 2016; Knowles et al., 2016; Lara et al., 2017). Our statistical results show how this relationship is an important driver in many of the best models and across all systems, soil and aquatic environments included (Williamson et al., 2005; Rowe et al., 2008; Wommack et al., 2015; Wigington et al., 2016; Williamson et al., 2017; Liang et al., 2019, 2021;). Moreover, in marine sediments, where we have a sufficient number of observations with both viral and prokaryotic production values, we found a relationship between these two productions, possibly due to the dependence of the viral replication on the host metabolism (Middelboe et al., 2003; Glud et al., 2004; Suttle, 2007; Clasen et al., 2008; Danovaro et al., 2008, 2011; Middelboe et al., 2011; Carriera et al., 2013), confirming viruses as a dynamic component of marine sediments communities with a large impact on prokaryote mortality and biogeochemical cycling (Hewson et al. 2001; Fischer et al. 2003; Glud et al., 2004; Carreira et al., 2013).

2.5.5 The role of depth as a driver of the spatial distribution of viruses and prokaryotes

Our best models for abundances in sea water and for both viral and prokaryotic productions in marine sediments highlight a decreasing pattern with depth, then, we can conclude that depth is also an important environmental driver. This pattern is not only a consequence of food supply, given the lack of collinearity of depth with trophic resources, indeed, in deeper zones, more extreme conditions due to high pressures and low temperatures, as well as the limited availability of nutrients, could disadvantage microbial assemblages (Hara et al., 1991; Weinbauer et al., 1993; Maranger et al., 1995; Danovaro et al., 2000; Wommack et al., 2000; Middelboe et al., 2006; Rex et al., 2006; Danovaro et al., 2008b; Wilhelm et al., 2008; Jacquet et al., 2010; Lloyd et al., 2013; Danovaro et al., 2015; Parikka et al., 2017).

2.5.6 Latitude as a driver to explain viral and prokaryotic distribution

Our data point out that prokaryotic abundances in both sea water column and marine sediments increase with latitudes that is a driver included in many best models. Indeed, the highest abundances of prokaryotes were reported in North Baltic and Arctic, which were characterized by a higher availability of trophic resources (Danovaro et al., 2016). Other drivers such as pH, O2 (Tittensor et al., 2010; Carreira et al., 2013; Sweetman et al., 2017), NO3, PO4 (Taylor et al., 2010; Zeng et al., 2016; Zhang et al., 2017a, 2017b; Liang et al., 2021), and salinity

(Husson-Kao et al., 2000; Lunde et al., 2005; Gnezda-Meijer et al., 2006; Williamson et al., 2006), although important, were excluded due to their multicollinearity with other covariates. Microbial community dynamics in soil remain understudied (Williamson et al., 2005; Rowe et al., 2008; Wommack et al., 2015; Wigington et al., 2016; Liang et al., 2019, 2021) and the role of viruses largely unexplored (Emerson et al. 2018), moreover, the associations between viral abundances and dynamics with soil properties is still poorly understood (Liang et al., 2021). However, even though the number of points is low, our best models in soil highlight that soils water index is an important factor contributing to viral abundances (Williamson et al., 2005), since viruses persist longer in wetter soils (Hurst et al., 1980; Williamson et al., 2005).

2.5.7 Copernicus data-integration to generate 3D maps of viral and prokaryotic distribution

Lastly, for the first time, a 3D model for the global ocean prokaryotes and viruses has been created based on a global analysis of drivers, capable of providing a forecasted distribution of both abundances and productions. This model estimates prokaryotic and viral values even in areas and depths where, until now, measurements were lacking, thus providing crucial insights. These estimations are crucial as they offer a global-scale understanding of stock distribution. However, these maps are obtained from a multivariate statistical analysis affected by both the uncertainties in the modelled inputs taken from web platforms (as Copernicus) and the high level of simplification provided by the regressions in describing the relationships between drivers and response variables. Therefore, these maps should be regarded only as indicative of the potential real distributions, considering that increasing the number of studies/sampling points will enhance the accuracy of this results over time.

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Chapter 3.

Assessing the impacts of climate change on viral-prokaryotic interactions in deep-sea ecosystems using theoretical models

3.1 Introduction

Deep-sea ecosystem is the largest biome on Earth constituting over 65% of the Earth's surface and around 95% of the global biosphere (Gage et al., 1991; Danovaro et al., 2015; Sweetman et al., 2017). With the exception of cold seeps and hydrothermal vents, life in the deep sea depends on organic matter fluxes produced in the ocean surface and settling to the sea bottom through vertical fluxes and advection processes (Danovaro et al., 2014). Only a limited fraction of the surface primary production estimated at 48.5 to 54 Pg C/year reaches the deep seafloor (approximately 1-3 %; Meysman et al., 2006; Dunne et al., 2007), thus deep-sea ecosystems, especially those far the continental margins, are considered food-limited environment (Gage et al., 1991; Druffel et al., 1999; Bartlett, 1992; Jørgensen et al., 2007).

In benthic deep-sea ecosystems, life is dominated by prokaryotes which play a key role in C cycling and nutrient regeneration processes, thus influencing the functioning of the entire Biosphere (Falkowski et al., 2004; Dell'Anno e al., 2005; Arrigo, 2005; Karl, 2007; Falkowski et al., 2008; Levin et al., 2009; Karsenti et al., 2011;Armstrong et al., 2012; Thurber et al., 2014; Mengerink et al., 2014; Danovaro et al., 2014, 2015; Levin et al., 2015; Danovaro et al., 2017; Sweetman et al., 2017). Increasing evidence suggests that viral infection plays a significant role in controlling prokaryotic dynamics both in shallow and deep-sea ecosystems (Fuhrman et al., 1999; Wommack et al., 2000; Suttle et al., 1990; Suttle, 2007; Danovaro et al., 2008), viruses, by infecting and killing their prokaryotic hosts, transform living biomass into organic detritus which can be used again by non-infected prokaryotes (Fuhrman et al., 1999; Wommack et al., 2000; Suttle, 2007). This process, called viral shunt, reduce the energy transfer to the higher trophic level, but can accelerate C cycling and nutrient regeneration rates (Fuhrman et al., 1999; Suttle 2005). The importance of viruses in controlling prokaryotic dynamics is particularly evident in deep-sea ecosystems where viruses can shunt on average, up to 80% of prokaryotic C production (Danovaro et al., 2008). Therefore, integrating the viral component into trophodynamic and biogeochemical models is essential for enhancing our understanding of the functioning of the world oceans (Suttle, 2007). However, the incorporation of virus impacts on ecosystem processes into biogeochemical models is still in its infancy (Weitz et al., 2015; Record et al., 2016; Mateus et al., 2017; Talmy et al., 2019; Zimmerman et al., 2020).

3.1.1 Modelling prokaryote-virus dynamics

The microbial ecosystem of the deep sea is characterized by high complexity and dynamic nature, making it challenging to gain deeper insights into its composite functioning without the support of advanced mathematical models, such as the Generalized Lotka-Volterra models (Freedman et al., 1977; Beddington et al., 1975; Thingstad et al., 1985; Skalski et al., 2001; Xiao et al., 2001; Kar et al., 2004; Zhang et al., 2012; Sayekti et al., 2017, Kuntal et al., 2019). The complexity of the system lies not only in the dense network of interconnections but also in the cause-and-effect relationships which are prevalent not linear (Thingstad, 1998). These interactions play a crucial role in maintaining ecosystem balance, particularly in the deep sea, and has encouraged the development of methods and tools for their analysis (Fisher et al., 2014; Bucci et al., 2016; Shaw et al., 2016; Baksi et al., 2018). In recent years, there has been significant interest in prokaryote-virus modelling due to its potential to forecast future dynamics and enhance our understanding of prokaryote-virus interactions, including viral infections (Beretta et al., 1998; Beretta et al., 2001; Beretta et al., 2002; Hilker et al., 2006;

Hilker et al., 2006b; Siekmann et al., 2008; Berry et al., 2014; Fisher et al., 2014). However, only a limited number of food-web models that incorporate the virus component have been proposed (Thingstad et al., 1997; Thingstad, 2000; Miki et al., 2008), and the processes through which viruses influence carbon and nutrient cycling have yet to be fully integrated into large-scale climate and Earth system models (Follows et al., 2007; Stock et al., 2014; Weitz et al., 2015), particularly within deep-sea sediment ecosystems. Most of the dynamics of both virulent phages and their hosts have primarily been described using extensions of Lotka–Volterra models (Levin et al. 1977; Lenski et al., 1988; Beretta et al., 2001; Weitz et al., 2008), which have become a conceptual foundation for modern theoretical ecology. However, the quantitative exploration of host-virus dynamics is still in its early stages (Wichels et al., 1998; Comeau et al., 2005; ; Holmfeldt et al., 2007; Flores et al., 2011; Poisot et al., 2011), and despite decades of discussion on the potential role of viruses in marine biogeochemical cycles (Fuhrman, 1999) and the viral shunt (Wilhelm et al., 1999), the precise quantitative impact of viruses on regional and global-scale processes remains largely unresolved (Poorvin et al., 2004; Winget et al., 2005; Rowe et al., 2008; Winget et al., 2009; Weinbauer et al., 2009; Holmfeldt et al., 2010; Matteson et al., 2012; Rowe et al., 2012; Weitz et al., 2012).

3.1.2 Modelling virus-prokaryote dynamics under climate change

Climate change is impacting water temperature and nutrient availability, which in turn affect the interactions between viruses and prokaryotes (Bauer et al., 2013; Urban et al., 2016; Finke et al., 2017; Zhang et al., 2021; Krishna et al., 2023). Temperature, in particular, influences nearly all metabolic rates and alters the dynamics of the assemblages by modifying their interactions (Petchey et al., 1999; Wilmers et al., 2007; Tylianakis et al., 2008; Rall et al., 2010; Öhlund et al., 2015; Zimova et al., 2016; Sekerci, 2020). Higher temperatures increase the rates at which viruses encounter their hosts (Murray et al., 1992; Krishna et al., 2023), thereby enhancing viral lysis and consequently elevating predation rates (Post et al., 1999). Thus, understanding the role of viruses-prokaryotes relationships in the ecosystem functioning (Pomeroy et al., 2001; Weitz et al., 2015; Serra-Pompei et al., 2019) is not sufficient since the acceleration of global climate change makes these relationships not static. Therefore, we need to develop models quantifying and predicting the biological responses to climate change also in the prokaryotic and viral components (Cherabier et al., 2022; Parmesan et al., 2022; IPCC). However, the literature still lacks a comprehensive mathematical understanding of how changing climate and virus-prokaryote dynamics interplay (Sekerci et al., 2020). To comprehend the evolving nature of these interactions, an increasing array of numerical approaches have been implemented (Mouquet et al., 2015; Petchey et al., 2015). Despite the development of numerous models aimed at understanding the impacts of climate change (Urban, 2015), forecasting biological responses still remains challenging (Davis et al., 1998; Angert et al., 2011; Urban et al., 2016), since the complexity of these processes (Suding et al., 2004; Dell et al., 2014; Burnside et al., 2014; Amarasekare, 2015; D. J. Smith et al., 2018; Tseng et al., 2019; García et al., 2023; Crocker et al., 2023; Sun et al., 2023). Nevertheless, a mechanistic, quantitative framework for including viruses in large-scale biogeochemical models is needed and requires substantial knowledge from both laboratory and field studies, which to date exists for few virus–host systems (Zimmerman et al., 2020).

3.1.3 The role of the Metabolic Theory of Ecology

Marine microbes play pivotal roles in biogeochemical cycles, and given the context of global warming, their response to temperature holds critical importance for our understanding of the ocean's structure and functioning in the future (Sarmiento et al., 2004; O'Connor et al., 2009; Huete-Stauffer et al., 2015). It is imperative to reliably document the direct response of Earth's biota to temperature changes to comprehend the impacts of global warming and the biosphere's feedback to climate change (López-Urrutia et al., 2007). The Metabolic Theory of Ecology (MTE; Brown et al., 2004) provides a framework for studying the effects of temperature and trophic resources on marine viruses and prokaryotes, offering a simple theoretical approach to link energetics across multiple biological scales (del Río, 2008; Price et al., 2012; Glazier, 2015; Huete-Stauffer et al., 2015). Specifically, in the deep sea, we employ MTE to investigate the relative roles of carbon flux and temperature in influencing parameters such as growth rate. We aim to assess the effects of thermal kinetic energy availability (i.e., temperature) and chemical potential energy (i.e., POC), especially in an extremely energy-limited system, to predict how deep-sea virus-prokaryote dynamics may be impacted by climate change (Houghton, 2001; Gillooly et al., 2001; Brown et al., 2004; López-Urrutia et al., 2006; Harris et al., 2006; López-Urrutia et al., 2007; Regaudie-de-Gioux et al., 2012; McClain et al., 2012).

3.1.4 The models developed

The work presented in this study aims to develop a comprehensive mathematical model of virus-prokaryote dynamics in the deep sea. All the models employed are designed revisiting the well-known prey-predator model proposed by Lotka and Volterra (Lotka, 1925; Volterra, 1927; Rosenzweig et al., 1963; Thingstad et al., 1974; Roughgarden, 1979; Thingstad et al., 1985; Bratbak et al., 1985; Thingstad et al., 1990; Iannelli and Pugliese, 2015; Sayekti et al., 2017; Constable et al. 2017; Sekerci, 2020; Cherniha, 2022) and are kept sufficiently simple to explore the consequences on virus-prokaryote dynamics within deep-sea sediments as temperature and resource availability undergo shifts due to climate change. To achieve this goal, we utilized a permanent temperature gradient between two regions in the Pacific Ocean as an approximation for ocean warming, and carry out this analysis on the virus-prokaryote dynamics under climate change using two numerical approaches:

- one based on the Generalized Lotka-Volterra (GLV), considering viruses, prokaryotes and organic carbon as solutions of the system of equations;

- the other integrating the Metabolic Theory of Ecology on a Lotka-Volterra (LV) model, describing virus-prokaryote dynamics (Laws et al., 2000; Yoshino et al., 2007; May et al., 2007; Solé et al., 2012; Tikhonov et al., 2017; Bunin, 2017).

3.2 Aims

The main aims of this chapter were:

- ✓ evaluation of the role of temperature and carbon availability on virus-host interactions in benthic deep-sea ecosystem.
- ✓ development of a theoretical model to investigate the virus-prokaryote interactions and forecast their potential responses under future global change scenarios.
- ✓ enhancement of our knowledge of the microbial food-web dynamics in the deep sea and its potential responses to climate change.

3.3 Materials and methods

3.3.1 Study areas and sample collection

For this study we used unpublished data of deep-sea sediments that were collected in the Pacific Ocean at ca. 33°S. Sampling stations were located at ca. 3000 m depth in an area (from 76 to 79°W) characterized by the presence of a ridge, which divides the deep-sea floor into a warmer (northern) sector and a colder (southern) sector, differing on average in bottom temperature of ca. 0.2°C. Four sampling stations were located in the northern sector and four in the southern one. All analyses were carried out on the top 1 cm of sediment.

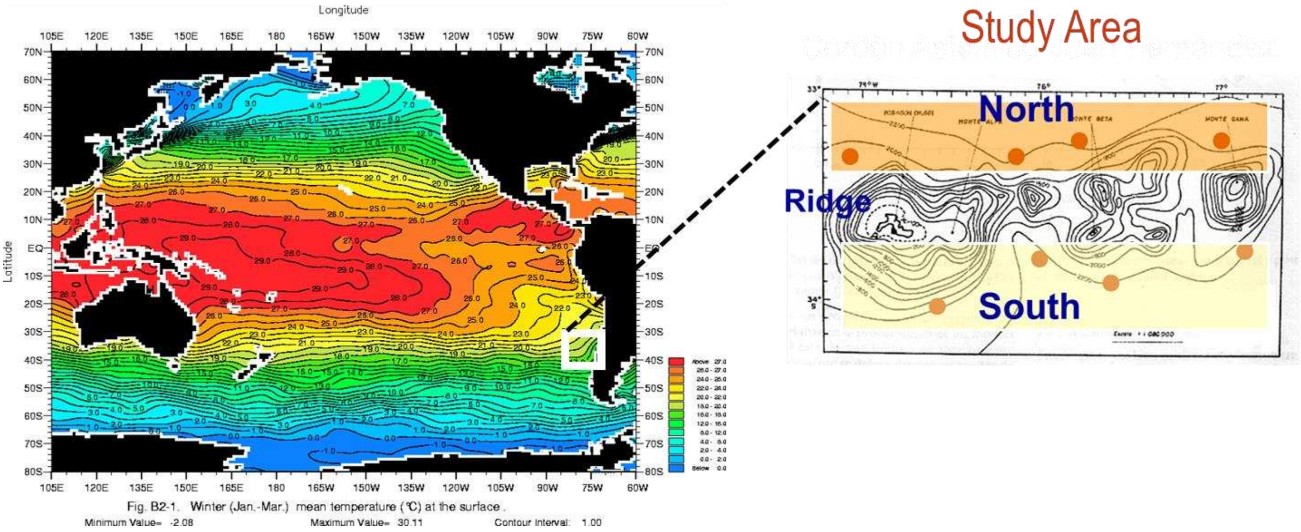

Figure 3.1 Map of the study area in the Pacific Ocean. In the enlarged map on the right, the eight stations.

3.3.2 Environmental, trophic, microbial and viral variables

The temperature of the bottom waters at each sampling site were determined by CTD. The trophic characteristics of the benthic deep-sea ecosystems were investigated through the analysis of the biopolymeric C (BPC) content (used as a proxy of the more bioavailable fraction of organic C for heterotrophic consumers) in the sediment (Dell'Anno et al., 2015; Danovaro, 2010). Values of photosynthetic primary production of the surface waters were extracted from the ocean productivity database (www.science.oregonstate.edu) and referred to the same year and the same area of the Pacific Ocean where deep-sea sediments were collected. The variables used to describe the virus-prokaryote dynamics were prokaryotic abundances as number of cells g^{-1}, viral abundances as VLP (virus-like particles) g^{-1}, prokaryotic heterotrophic C production as ng C g^{-1} h^{-1} and viral production as VLP g^{-1} h^{-1}, prokaryotes killed by viruses as cells g^{-1} h^{-1} and Carbon released by killed prokaryotes as ng C g^{-1} h^{-1}.

3.3.3 Metabolic Theory, mechanistic models and regression analysis

For our analysis, we developed two numerical approaches trying to integrate the effect of sea water warming and carbon flux reduction, into models of virus-prokaryote dynamics, to predict the consequences of climate change in the deep sea microbial food web.

In the first approach we started from a Lotka-Volterra model where the prey function $B(t)$ was represented by prokaryotes, while the predators $V(t)$ were viruses, with the following form:

$$\frac{d\boldsymbol{B}}{dt} = \alpha * \boldsymbol{B} - \beta * \boldsymbol{B} * \boldsymbol{V}$$

$$\frac{d\boldsymbol{V}}{dt} = \delta * (\beta * \boldsymbol{V} * \boldsymbol{B}) - \Omega * \boldsymbol{V}$$

where δ (the burst size), Ω (the viral decay rate) and the percentage of chemosynthetic production (used to adjust the value of prokaryotic production) were estimated for each area. To estimate these parameters, we created an algorithm that, started from a range of values for each parameter, solved the system of equations for different combinations of parameter values and calculated the difference between each solution and the measured data. Then, we selected the parameters combination of values that minimized the differences between the simulated solutions and the real data for both systems (north and south). Finally, to include the effect of climate change, we tried to express the system coefficients as functions of temperature and food availability (Laws et al., 2000; Abiodun et al., 2016, Wieczynski et al., 2023). Specifically, both β (attack rate) and Ω (Parikka et al., 2017) were approximated by the formula $e^{(coef1* T\, +\, coef2*C\, +\, intecept)}$, where T is the temperature, C is the labile organic carbon in logarithmic scale in the sediment, and the tuple $(coef1,\ coef2, intecept)$ is the result of the log-liner regression. The growth rate (α) was calculated using the Arrhenius formula from the Metabolic Theory of Ecology (MTE, López-Urrutia et al., 2007). Therefore, in this first approach, the trophic resource is not considered as a solution of the system, but as an input to be provided to obtain the growth rate, the attack rate and the decay rate. In addition, we assumed that all prokaryotic mortality depends solely on viruses (Lara et al., 2017) and that trophic resources consist of the sum of the flux and carbon released by killed prokaryotes, along with a fraction of organic carbon in sediment. We also assumed that burst size (δ) is constant. The choice of a Lotka-Volterra model, even in the absence of fluctuations in the source data, was driven by two main reasons, the lack of a reliable estimation method for the carrying capacity, and the need to have a sufficiently simple model without explicit references to trophic resources, so that these could also vary discontinuously, as in the case of alterations due to extreme episodic events.

In the second approach, we started from a Generalized Lotka-Volterra (GLV) model in which the trophic resource is included as a system solution:

$$\frac{d\boldsymbol{B}}{dt} = \alpha * \boldsymbol{C} * \boldsymbol{B} - \beta * \boldsymbol{B} * \boldsymbol{V}$$

$$\frac{d\boldsymbol{V}}{dt} = \delta * (\beta * \boldsymbol{V} * \boldsymbol{B}) - \Omega * \boldsymbol{V}$$

$$\frac{d\boldsymbol{C}}{dt} = \theta * \beta * \boldsymbol{V} * \boldsymbol{B} + \lambda * \boldsymbol{V} - \sigma * \boldsymbol{B} * \boldsymbol{C} + C\_flux$$

Assuming $\theta = 20$ fg C, we estimated δ (burst size) and the percentage of chemosynthetic production through optimization using the same algorithm described before. Additionally, we assumed $\alpha,\ \beta,\ \Omega, \sigma,\ \lambda$ to be equal to $\boldsymbol{e}^{(\boldsymbol{coef} * \boldsymbol{T} + \boldsymbol{intecept})}$, where T represents temperature and each pair $(coef, intecept)$ corresponds to the outcome of the log-linear regression between each dynamic parameter (i.e. $\alpha,\ \beta,\ \sigma,\ \Omega,\ \lambda$) and T. Similarly, we assumed that all prokaryotic mortality depends solely on viruses and that trophic resources consist of the sum of flux and carbon released by killed prokaryotes, along with a fraction of organic carbon in sediment.

3.3.4 Climate change scenarios and forecasting

For the first model, we had two input parameters to define (temperature and trophic resources) in order to configure each system of equations. Thus, we created a grid of parameters combination based on a CMIP6 scenario: the SSP5 scenario with a 20% reduction in carbon flux that we projected to the bottom resulting in an equal reduction in trophic resources in the sediment (strong assumption) for both sites. As for the temperature increase, we tested with a +0.3°C both for North and South, a value that mimics the difference between the two sites. Subsequently, we developed an algorithm that, for each combination of parameters, set the model, computed the solutions, and plotted the averages of the solutions. Since the solutions are periodic, we assumed that the measured data, being an average of replicates, could be compared with the averages of each periodic solution. Then we generated a 3D graph for both viruses and prokaryotes depicting the variation of their average abundances with changes in temperature and trophic resources.

For the second model, the changing parameter was only temperature and we considered a potential increase of 0.6°C to generate 2D scatterplots depicting the variation of viral and prokaryotic abundances steady states with changes in temperature.

3.4 Results

3.4.1 Differences in environmental settings and in prokaryotic and viral variables between the North and South areas

Analyzing the data collected between the two areas, we observe that the bottom water temperature between the northern and southern sites exhibits significant differences (ANOVA, $p>0.05$), whereas the BPC does not vary significantly, as we can seen from the Figure 3.2.

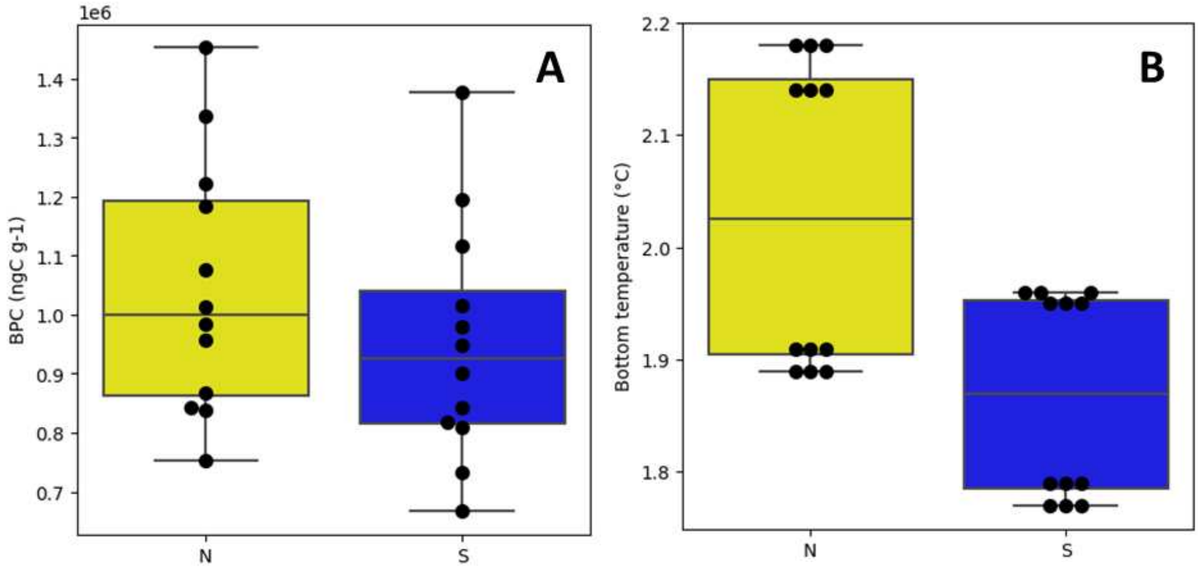

Figure 3.2 Differences of trophic resource availability and bottom water temperature at the investigated sites. The plot A) shows the availability of trophic resources (expressed as biopolymeric C concentrations) in surface sediments collected in the northern (warmer, in yellow) and southern (colder, in blue) stations of the Pacific Ocean. The plot B) shows the differences in bottom water temperature between the two sites.

If we analyze the prokaryotic and viral abundances, we notice that there are no significant differences in prokaryotic abundances, while in the northern site, viral abundances are statistically higher (ANOVA $p<0.05$).

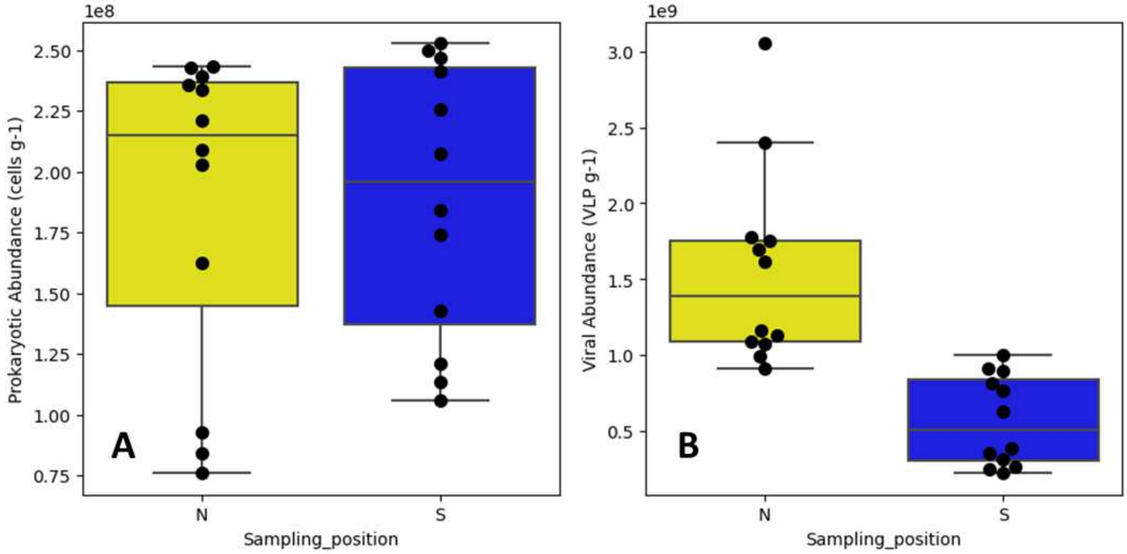

Figure 3.3 Differences in prokaryotic and viral abundances at the investigated sites. The plot A) and B) show the prokaryotic and viral abundances, respectively, in surface sediments collected in the northern (warmer, in yellow) and southern (colder, in blue) stations of the Pacific Ocean.

If we analyze the productions, the differences are significant both for prokaryotes and viruses, with the northern site showing higher values than the southern site.

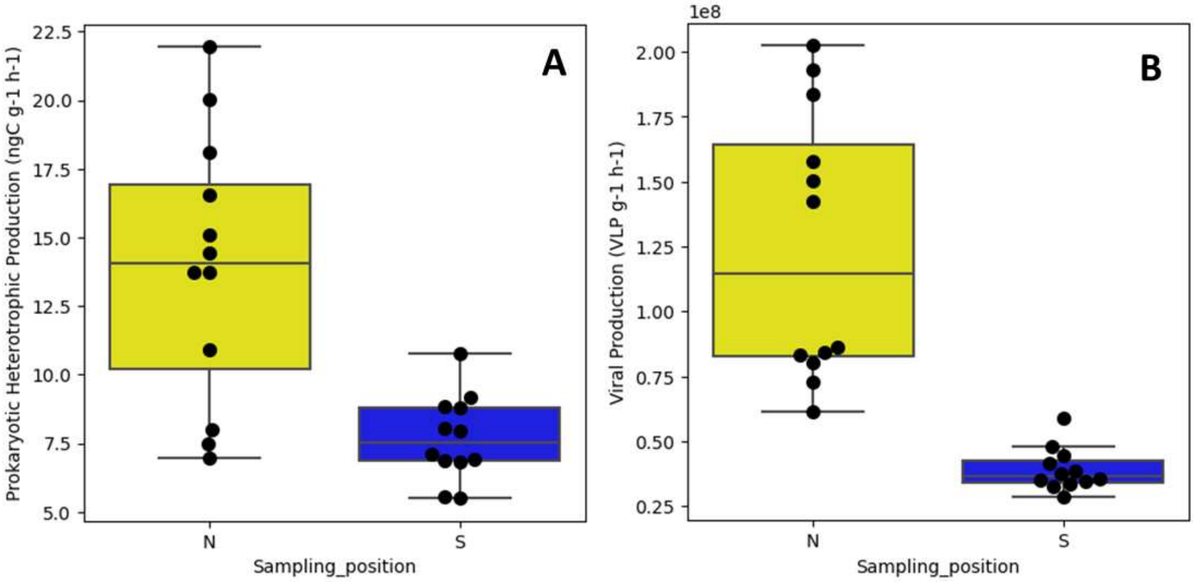

Figure 3.4 Differences in prokaryotic heterotrophic C production and viral production at the investigated sites. The plot A) and B) show the prokaryotic heterotrophic C production and viral abundances, respectively, in surface sediments collected in the northern (warmer, in yellow) and southern (colder, in blue) stations of the Pacific Ocean.

Finally, if we analyze the number of cells killed by viruses and the released carbon, we notice that the northern site exhibits statistically higher values (ANOVA, p<0.05).

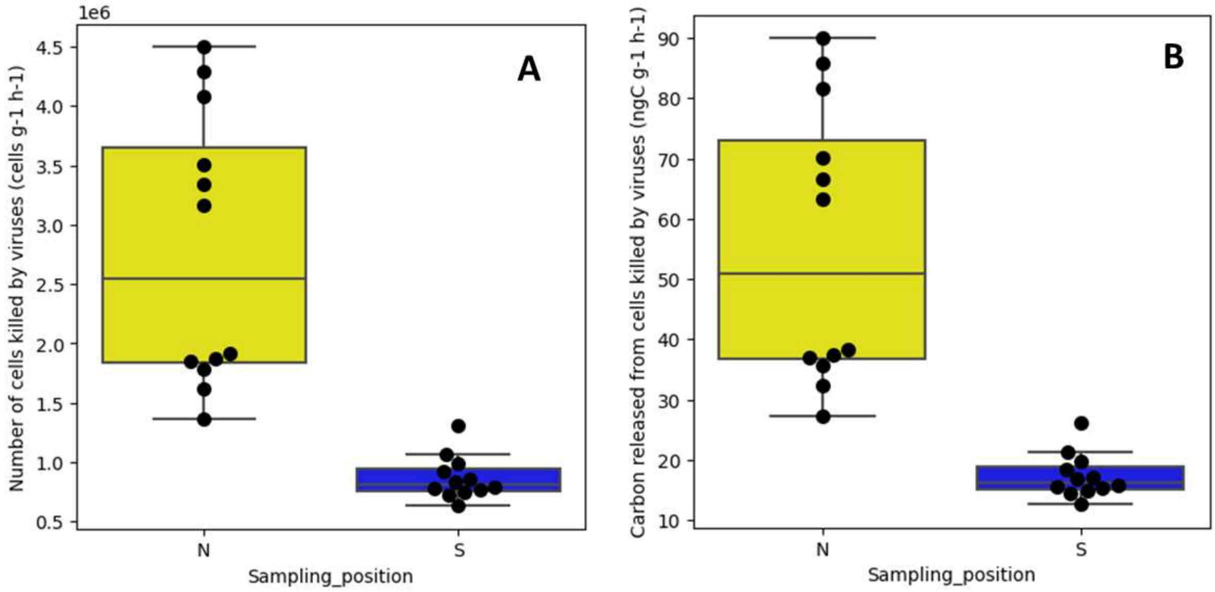

Figure 3.5 Differences in the number of cells killed by viruses and C released from killed cells at the investigated sites. The plot A) and B) show both the number of cells killed by viruses and the carbon released from these killed cells, in surface sediments collected in the northern (warmer, in yellow) and southern (colder, in blue) stations of the Pacific Ocean.

3.4.2 Lotka-Volterra models for North and South

Starting from the measured data we constructed two LV models to describe the behavior of viruses and prokaryotes in the two sites, integrating data of prokaryotic and viral abundances (static) and data of production (dynamic) into a single system that numerically modeled interactions and cause-effect mechanisms. In the first model obtained for the southern site, we observe how our measured data aligns with the periodic oscillations of the theoretical solutions. Specifically, for prokaryotes, our measured data (an average of all replicates from the 4 stations) lies close to the maximum value of the periodic solution, and compared to the average of the theoretical solutions, it shows a delta of approximately +34%. Similarly, there is a delta of approximately +31% for viruses, even though our measured data is far from the extremes of the oscillatory solution.

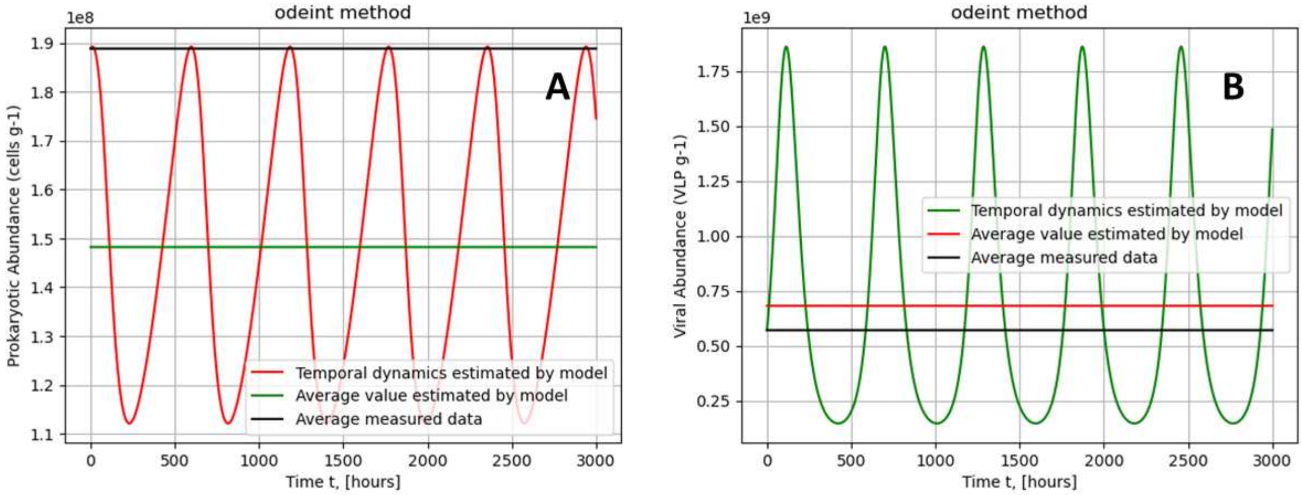

Figure 3.6 Analysis of solutions of the Lotka-Volterra model for South. The plot A) shows the theoretical solution for prokaryotes in red, its mean value in green and the average of measured data in black. The plot B) shows the theoretical solution for viruses in green, its mean value in red and the average of measured data in black.

In the model configured for the northern site, we observe again how our measured data for prokaryotes lies near the highest values of the periodic oscillations. In particular, the difference of this measured data when it is compared to the average of the theoretical solutions is approximately +21%, a similar delta (+19%) observed also for viruses, which however exhibit a measured data point more centered in the range of values of the oscillatory solutions. In addition, the system dynamic appears to be more accelerated, compared to that of the southern site, with both solutions showing a higher frequency of oscillation than in South. Finally, the value range of the prokaryotic solution increases by +45%, differently from the range of viral abundance that shows a +99% increase if compared to the South.

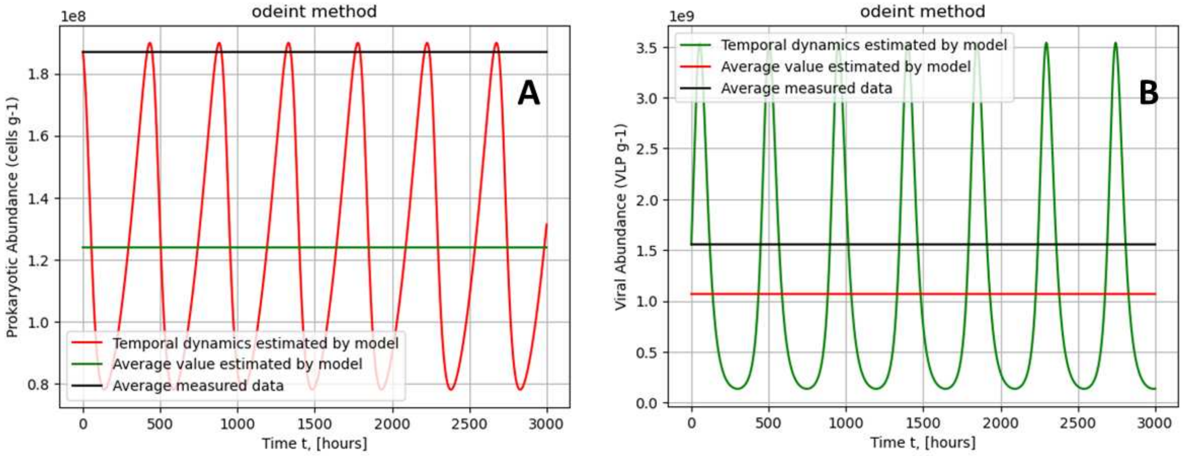

Figure 3.7 Analysis of solutions of the Lotka-Volterra model for North. The plot A) shows the theoretical solution for prokaryotes in red, its mean value in green and the average of measured data in black. The plot B) shows the theoretical solution for viruses in green, its mean value in red and the average of measured data in black.

3.4.3 Lotka-Volterra model with MTE integration and analysis of solutions

The second step was to create a model both temperature and resource dependent, through the Metabolic Theory of Ecology (MTE), expressing the rates in the system of equations as functions of both temperature and resources.

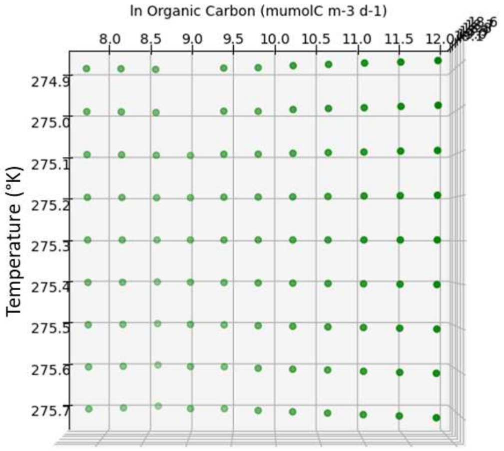

Figure 3.8 Grid of temperature and carbon values used to configure the Lotka-Volterra model with MTE integration. The plot shows all the couples of temperature and food

availability parameters, used to set the LV models with the MTE integration. Each point corresponds to a system of equations for which the prokaryotic solution has been computed.

In the previous grid, each point represents the average of the periodic solution of prokaryotic abundance corresponding to a specific set of parameters. Slicing this 3D scatterplot to focus on individual trends with respect to a single parameter, we notice that with a reduction in carbon, prokaryotes initially increase, but for x-values between 8.5 and 9, they reverse their trend and begin to decline (Fig. 3.9A). Differently, as the temperature increases we observe irregular growth with a reversal in trend in the range between 275.2°C and 275.4°C, where they seem to decrease before resuming an upward trajectory (Fig. 3.9B).

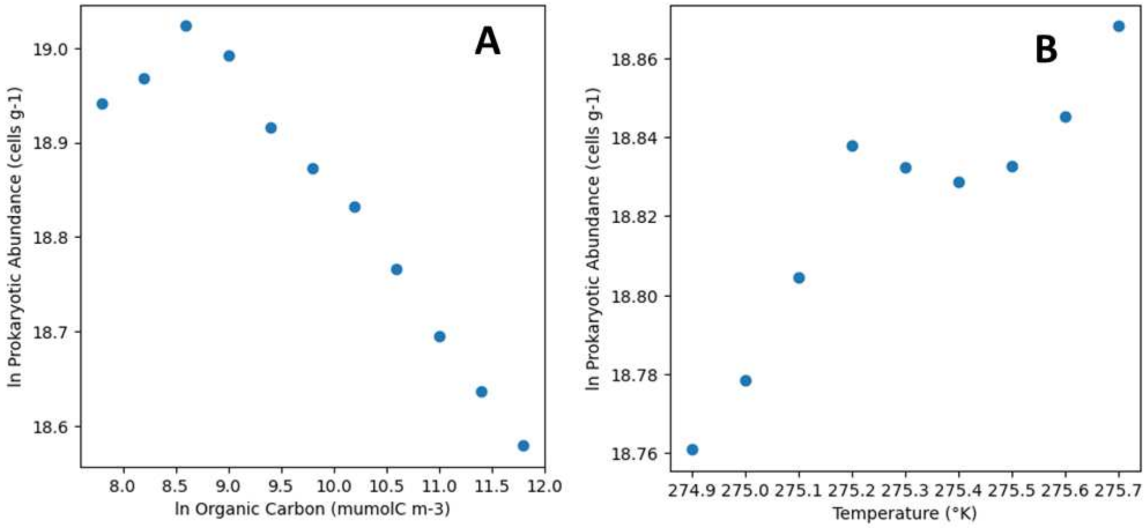

Figure 3.9 Analysis of average prokaryotic solutions changing temperature or trophic resources. The plot A) shows the behavior of the average of the prokaryotic solutions as food availability changes. The plot B) shows the prokaryotic solutions trends with respect to temperature change.

The two graphs in Fig. 3.10 highlight how the combined effect of temperature and organic carbon is more complex than considering only one single environmental variable, with abundance values ranging from 18.6 to 19.1.

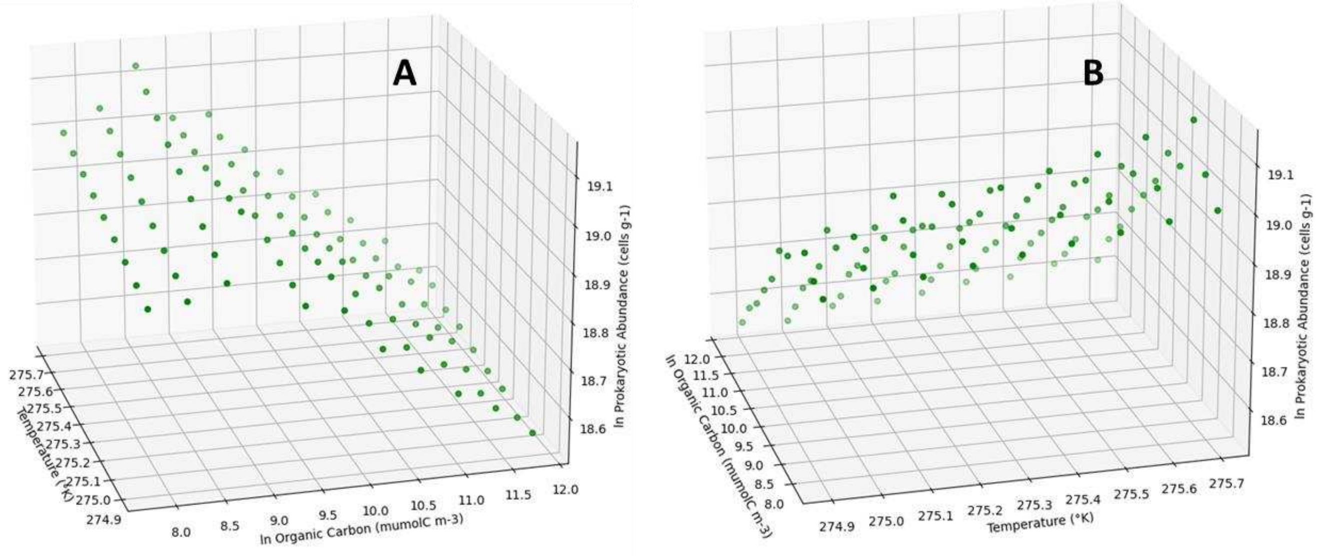

Figure 3.10 Analysis of average prokaryotic solutions changing both temperature and trophic resources. Both plots show the behavior of the average of the prokaryotic solutions as temperature and food availability changes according to different scenarios.

The same analysis of the solutions patterns considering variations in temperature and resources has also been conducted for viruses, which constitute the second component of our model. The grid dimensions are the same as those used for prokaryotes, as we can see from the Figure 3.11.

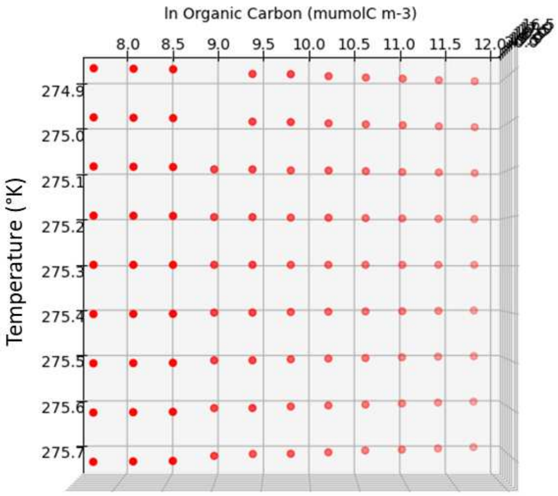

Figure 3.11 Grid of temperature and carbon values used to configure the Lotka-Volterra model with MTE integration. The plot shows all the couple of temperature and food availability parameter to set the LV model with the MTE integration. Each point corresponds to a system of equations for which the viral solution has been computed.

Each point on this grid represents the average of the periodic solution of viral abundance corresponding to a specific set of parameters (temperature and trophic resources). Slicing this 3D scatterplot to focus on individual trends considering a single environmental parameter, we notice that viruses tend to decrease with a reduction in carbon, although with a non-constant velocity, as evidenced by the lower slope of points at lower carbon values. The dynamics with temperature also show an irregular pattern, with a very sudden growth between 275.1°C and 275.4°C, before and after which the trend appears to be constant.

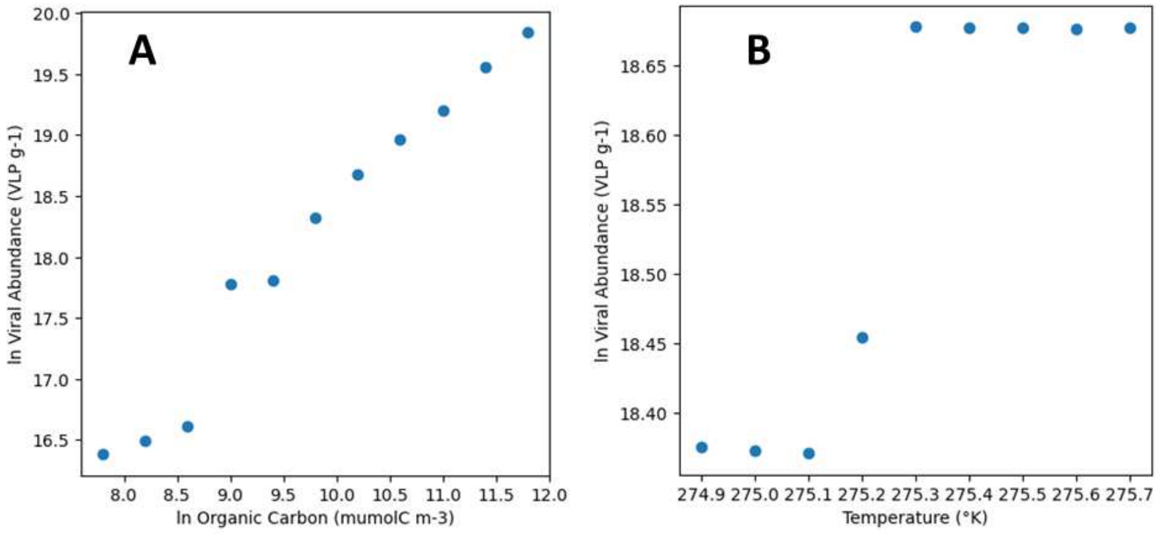

Figure 3.12 Analysis of average viral solutions changing temperature or trophic resources. The plot A) shows the behavior of the average of the viral solutions as food availability changes. The plot B) shows the viral solutions trends with respect to temperature change.

In the two graphs in the Fig. 3.13, we observe how the combined effect of temperature and organic carbon appears more complex than considering the change of a single environmental variable, with values of viral abundances ranging from 16.5 to 20.0. However, we notice a greater predominance of trophic resources in driving the behavior of viral solutions, with temperature-induced changes appearing to be less visible.

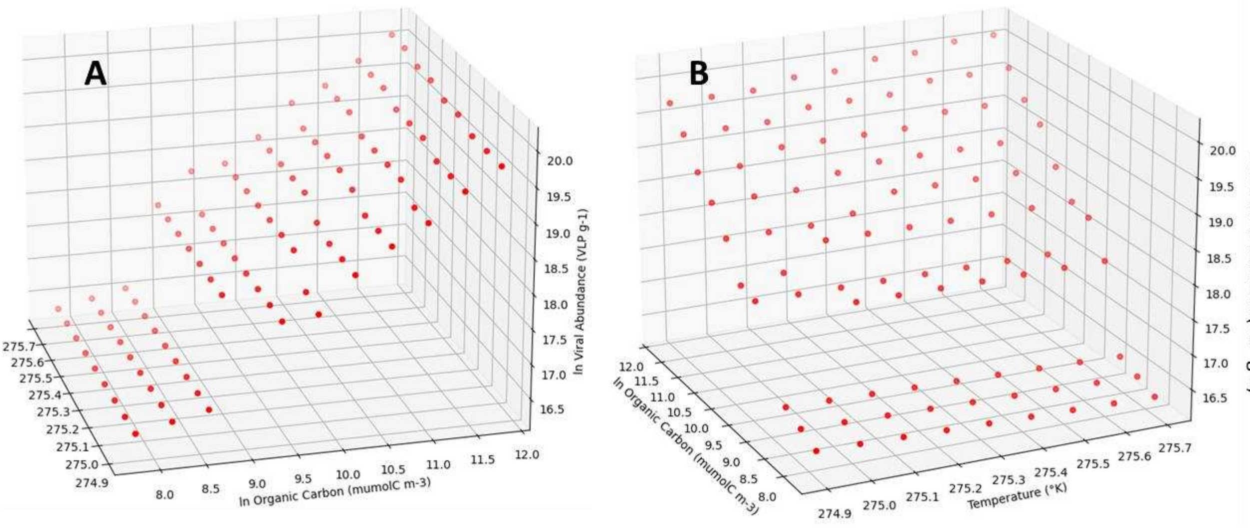

Figure 3.13 Analysis of average prokaryotic solutions changing both temperature and trophic resources. Both plots show the behavior of the average of the viral solutions as temperature and food availability changes according to different scenarios.

3.4.4 Generalized Lotka-Volterra models for North and South

Since the system is not affected by changes in external carbon input as observed from the values of Oregon ocean productivity, we attempted to model the virus-prokaryote dynamics of the two sites using a GLV, including trophic resources among the system's solutions. In this case as well, we initially developed a system for each site, calculating the rates using both measured data and computational estimation. In this second modeling approach, we notice how the theoretical solutions tend toward a steady state, with the initial oscillations becoming less pronounced until they approach the stationary theoretical solution. Analyzing the differences between our measured data and the steady states in the southern site, we observe that for prokaryotes, this difference is +22%, while for viruses it is -47%, and for trophic resources, it is -78%. Furthermore, we also notice how the measured data for prokaryotes lies near the highest values of our theoretical oscillations, while the resource exhibits an opposite behavior, lying among the lowest values of the numerical solution.

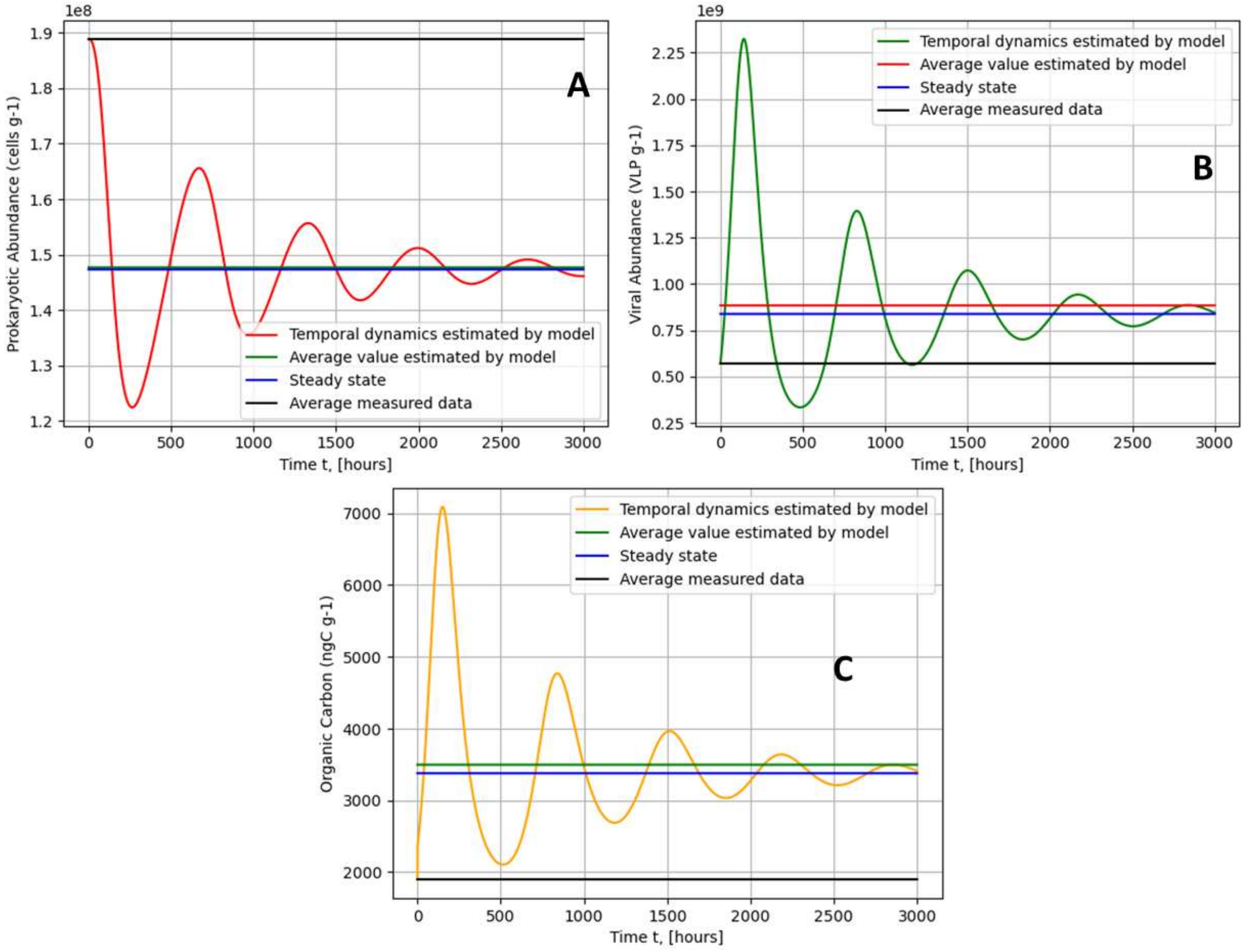

Figure 3.14 Analysis of solutions of the Generalized Lotka-Volterra model for South. The plot A) shows the theoretical solution for prokaryotes in red, its mean value in black, the average of measured data in green and the steady state in blue. The plot B) shows the theoretical solution for viruses in green, its mean value in black, the average of measured data in red and the steady state in blue. The plot C) shows the theoretical solution for the organic carbon in yellow, its mean value in black, the average of measured data in green and the steady state in blue.

Analyzing the differences between our measured data and the steady states for the GLV configured for the northern site, we observe that for prokaryotes, the same difference increases to +32%, while for viruses, the difference reverses sign, rising to +45%. Meanwhile, for trophic resources, the delta decreases in absolute value, reaching -17%. Additionally, in this site as well, we notice how the measured data for prokaryotes lies near the highest values of our theoretical oscillations.

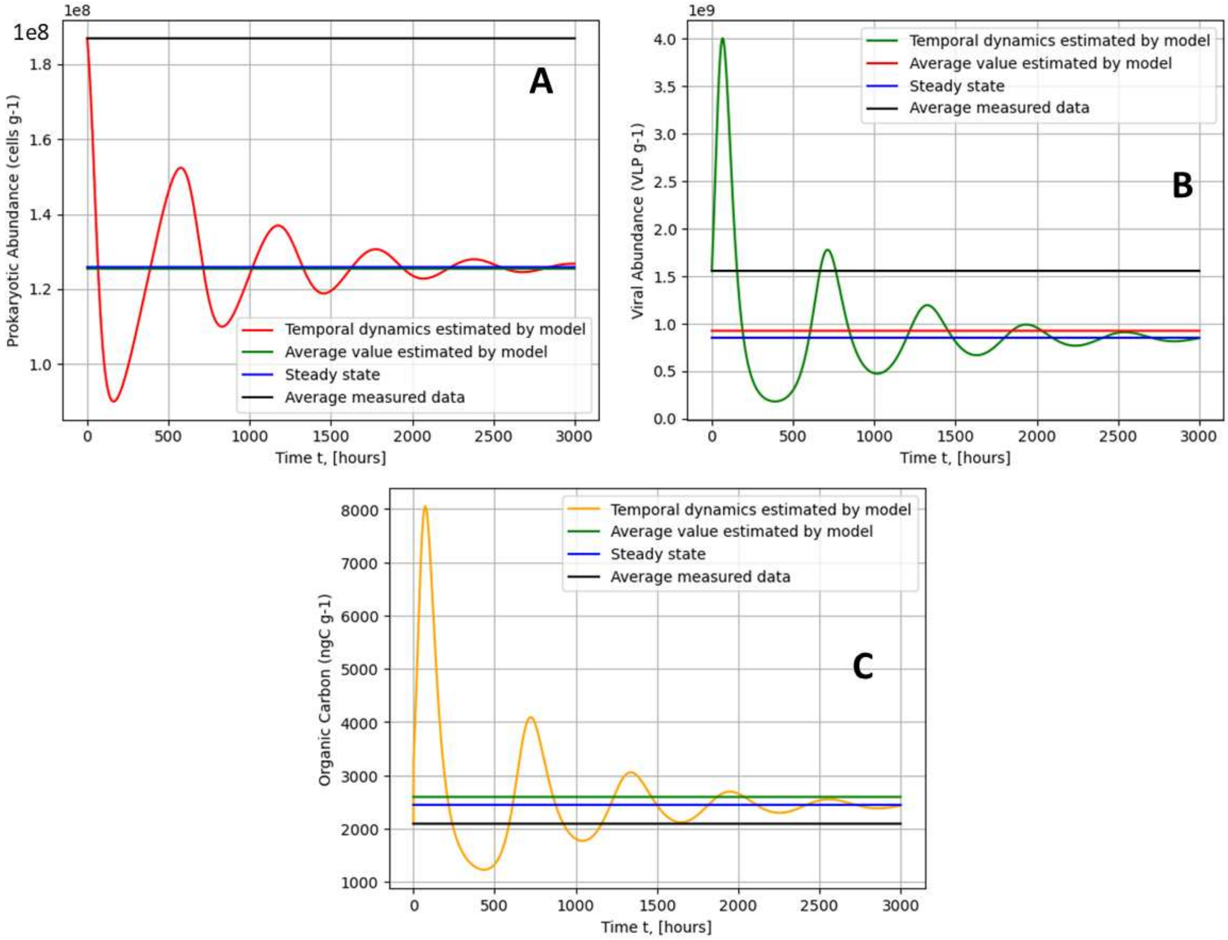

Figure 3.15 Analysis of solutions of the Generalized Lotka-Volterra model for North. The plot A) shows the theoretical solution for prokaryotes in red, its mean value in black, the average of measured data in green and the steady state in blue. The plot B) shows the theoretical solution for viruses in green, its mean value in black, the average of measured data in red and the steady state in blue. The plot C) shows the theoretical solution for the organic carbon in

yellow, its mean value in black, the average of measured data in green and the steady state in blue.

3.4.5 Generalized Lotka-Volterra model using Temperature as parameter and analysis of solutions

After verifying that the numerical solutions of the GLV exist for each site, we expressed each coefficient of the system as an exponential function of temperature (see Materials and Methods) and studied the behavior of the steady states as temperature varied within a range of 0.6°C. Each point on the blue line represents a steady state, i.e. a constant solution that does not vary with respect to time, but as the temperature varies, our system changes so that the steady state associated with it no longer remains the same. This variation of systems and therefore of the relative steady states as the temperature varies draws the curves we see in Fig. 3.15. From the results, we observe that viral abundance steady states increase as temperature increases, following a curve concave downward, as carbon steady states, even if their curve is concave upward, while prokaryotic steady states decrease.

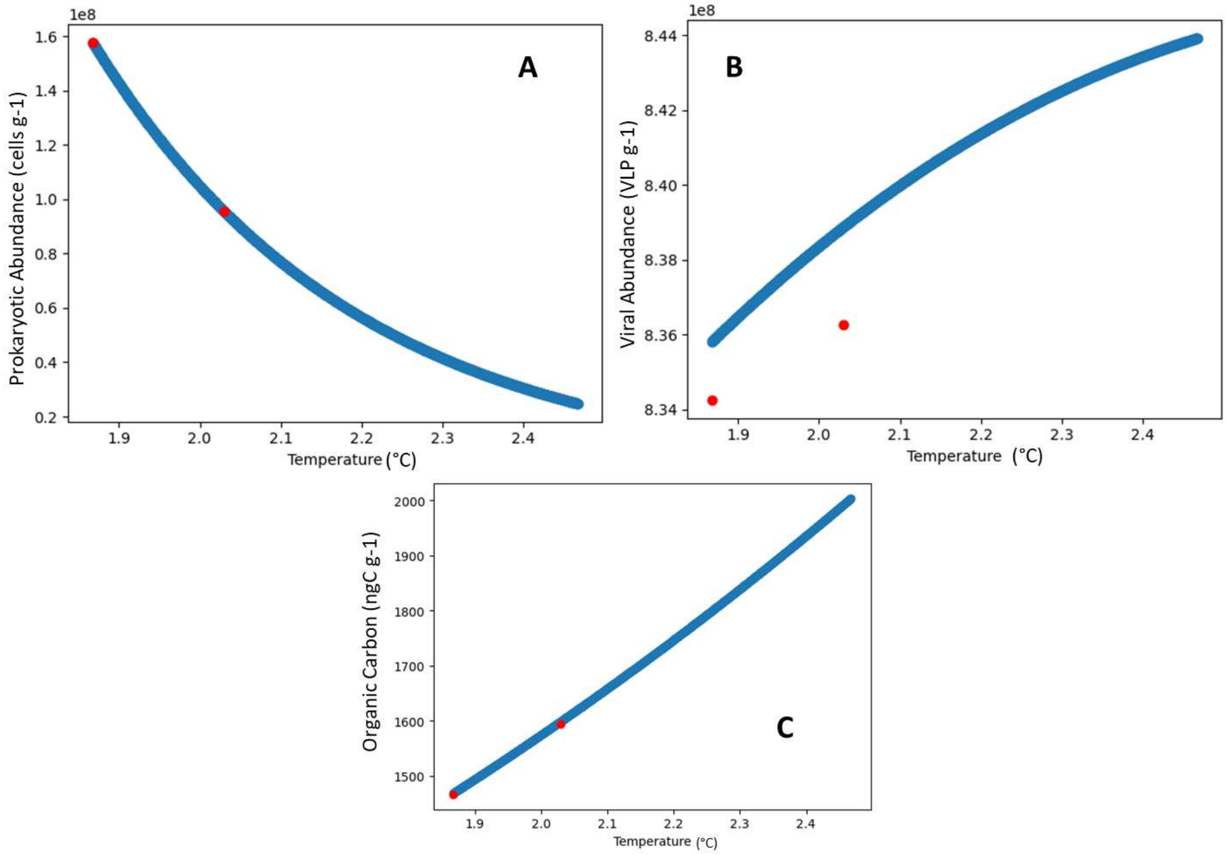

Figure 3.15 Analysis of steady states solutions of the Generalized Lotka-Volterra as temperature changes. The plot A) shows the behavior of steady states solutions for prokaryotes, each point in blue represents a solution of the system with a particular value of temperature, the red points are the steady states of the GLV models configured for each site starting from the measured data. The plot B) shows the theoretical solution for viruses in green, its mean value in black, the average of measured data in red and the steady state in blue. The plot C) shows the theoretical solution for the organic carbon in yellow, its mean value in black, the average of measured data in green and the steady state in blue.

3.5 Discussion

Total benthic biomass, in deep sea sediments, is dominated by prokaryotes that play a pivotal role in C production (either heterotrophic or chemoautotrophic), nutrient cycling, and energy transfer to the higher trophic levels (Danovaro et al., 2014; Jørgensen et al., 2007). However, the responses of the deep sea viral and prokaryotic components to global climate change are, so far, largely unknown (Danovaro et al., 2001; Sarmento et al., 2010; Danovaro et al., 2011; Mora et al., 2013; Thakur et al., 2019; Vaqué et al., 2019; Kuppardt-Kirmse et al., 2020; Wieczynski et al., 2023). The findings presented in this study are needed to gain a better picture of the functioning of deep-sea ecosystems and understand how the microbial and viral assemblages will respond to changes in environmental variables such as temperature and organic matter input. Moreover, our results aim to contribute to biogeochemical models' improvement, especially for viral-mediated processes in the deep sea (Wilhelm et al., 1999; Middelboe et al., 2003; Middelboe et al., 2006; Suttle, 2007), providing a framework able to enhance the forecasting of both benthic processes and biogeochemical cycles in the largest ecosystem on Earth under different climate change scenarios.

3.5.1 System setting and Metabolic Theory of Ecology

In this study, we analyzed an area of the South Pacific characterized by a permanent thermal gradient, due to an oceanic ridge, chosen as a proxy for climate change, to evaluate, through a mechanistic approach, the dynamics of viruses and prokaryotes, considering the effects depending on environmental variables. From the data, it is evident that the northern site is characterized by statistically higher temperatures, while trophic resources show no significant differences. These differences seem to have impacts on viral and prokaryotic productions, significantly higher in the north, as well as on the higher number of viruses in the north. The system, therefore, appears to be accelerated by this higher thermal energy. This result is consistent with the Metabolic Theory of Ecology (MTE), which highlights how productions are linked through the Arrhenius equation to temperature and resources. The application of this theory to our data has shown an activation energy of -2.71 eV, higher than the activation energy for water column but in line with literature data (Kirchman et al., 2009; Lønborg et al., 2016; Morán et al., 2017; Šolić et al., 2017; Lønborg et al., 2019; Morán et al., 2020).

3.5.2 The role of temperature

Despite temperature being the key component of global change, attention has only recently turned to the direct effects of temperature on viruses (Nagasaki et al., 1998; Wieczynski et al., 2023), hosts (Kendrick et al. 2014) and their associated ecosystem processes (Allen et al. 2005; López-Urrutia et al., 2007; Toseland et al. 2013; Edwards et al. 2016; Sommer et al. 2017; Thomas et al. 2017). In fact, it affects most of metabolic processes (Eppley, 1972; Raven et al., 1988) including growth rate, which increase with warming (Eppley, 1972; Serra-Pompei et al., 2019]. Moreover, warming could have positive or negative effects on virus-host encounter and attack rates (Maeda et al., 1976; Murray et al., 1992; Wilhelm et al. 1999; Atkinson et al. 2003; Savage et al. 2004; Daufresne et al. 2009; Dell et al. 2011, 2014; Gibert et al. 2016; Bernhardt et al. 2018; Martin et al., 2020), however, although more studies are needed, in our case system, we assessed a positive relationship between temperature and both attack and decay rate through the regression analysis. Then, we assumed that global warming increases both the rates at which viruses encounter their hosts and their metabolism (Murray et al, 1992; Laws, 2017; Krishna et al., 2023), enhancing the predation rate (Corvalan et al., 2005; Tylianakis et al., 2008; Sentis et al., 2017; Laws et al., 2017).

3.5.3 The role conjunct of temperature and trophic resources

Commonly, temperature and substrate availability are considered interrelated limiting factors influencing heterotrophic prokaryotes in various oceanic regions (Pomeroy et al., 2001; López-Urrutia et al., 2007; Huete-Stauffer et al., 2015; Lønborg et al., 2022). This implies that examining only one limiting factor may be misleading; in fact, for example, growth tends to depend not only on temperature but also on the availability of resources (Clarke, 2003; López-Urrutia et al., 2007; Marañón et al. 2018; Morán et al. 2018). However, direct studies on the influences of food availability and temperature on viral and prokaryotic communities are rare (McClain et al., 2012) and no study has undertaken a comprehensive cross-comparison of the effects of changing temperature and organic matter on deep sea viral and microbial processes. Global sea surface temperature has increased by 0.7°C for the last 100 years and is expected to increase over the next century (Bindoff et al., 2007) with consequences on the net primary production (NPP), inorganic nutrients, and organic substrates (Behrenfeld et al., 2001, 2006; Kwiatkowski et al., 2020). Studies suggest that prokaryotes respond sensitively to changes in temperature and quality or quantity of organic substrates (Sarmento et al., 2010; Ducklow et al., 2011; Engel et al., 2014; Thornton, 2014). However, it is still uncertain whether temperature is a major controlling factor (Lønborg et al., 2021) since some studies suggest that viral and microbial processes are already functioning close to their optima in waters; therefore, increasing temperatures further should not have any large impact or a negative influence on biogeochemical process rates (Wiebe et al., 1992; Johnson et al., 2007; Wei et al., 2010; Morán et al., 2017). Nevertheless, other studies suggest that tropical marine microbes increase their activity under warmer conditions (McKinnon et al., 2017; Lønborg et al., 2019; Lønborg et al., 2022). Therefore, understanding the effects of multiple drivers acting synergically in marine ecosystems is receiving more attention (Adams, 2005; Crain et al., 2008; Andersen et al., 2020; Gissi et al., 2021) and our study precisely moves in that direction.

3.5.4 Modelling virus-prokaryote dynamics under climate change

Indeed, this study aimed at elucidating virus-prokaryote dynamics in the deep sea under climate change scenarios, and, to do so, we hypothesized three types of controls in the mechanistic model formulation, including: temperature, substrate availability (also referenced as bottom-up control), and viral loss/mortality (or top-down control, Eppley et al., 1977; Harrison, 1983; López-Urrutia and Morán, 2007; Kirchman et al., 2009; Morán et al., 2017; Pham et al., 2022; Kim et al., 2023). Therefore, in our food web we considered only three components: organic carbon, prokaryotes and viruses; this simplification enabled us to make specific predictions on the range of effects that viruses have on viral and microbial ecosystems in deep sea surface sediments, given suitable parameterizations.

Then, we devised two methodologies based on these assumptions. In the first model, we constructed a system of equations delineating the dynamics of virus-prokaryotes in the deep sea, incorporating the principles of the Metabolic Theory of Ecology (MTE) to estimate coefficients, including both temperature and trophic resources in each equation (Laws, 2000; López-Urrutia et al., 2007; Abiodun et al., 2016; Wieczynski et al., 2023). For the second model, we devised a system of three equations describing the dynamics of virus-prokaryotes-resource interactions, with the trophic resources included in the solutions set. These models were developed following the methodology outlined in Motta (et al. 2012) and, considering the influence of environmental factors on virus-host dynamics, they address a significant gap in the current scientific literature (Sekerci et al., 2020). Unlike recent studies focusing solely on the impacts of climate change on consumer-resource interactions, our research also explores variations in viral predation rates due to increased predator metabolism as a consequence of rising temperatures (Tylianakis et al., 2008).

The rationale behind our selection of Lotka-Volterra models (LV) or generalized Lotka-Volterra models (GLV) lies in their suitability for elucidating nonlinear dynamics, which are characteristic of ecological systems such as the deep sea (Thingstad et al., 1974, 1990, 1997, 2000).

3.5.5 Modelling virus-prokaryote dynamics using Lotka-Volterra models with MTE integration

The results obtained in the initial phase of our analysis, where we estimated virus-prokaryote dynamics separately for each of the two areas, revealed differences between real and estimated solutions ranging from 33% to 20%. These results served as the foundation for the following integration of the two models into a unified framework, where parameter estimation was conducted using the Metabolic Theory of Ecology (MTE). It's worth noting that these differences may appear substantial, but it's essential to consider that our model did not account for the effects of grazers, moreover, some coefficients in the equations (as burst size, viral decay rate and percentage of chemosynthetic production) rely on assumptions or approximations since data were unavailable. Within the MTE framework, we specifically focused on estimating the growth rate, which became a function of temperature and trophic resources, following the formula outlined by Urrutia-Moràn. Similarly, the attack rate and decay rate of viruses were estimated using analogous assumptions (Tylianakis et al., 2008). However, due to the absence of explicit formulas, we employed a multivariate regression approach to estimate the patterns of these coefficients in relation to temperature and trophic resources. Our simulations using the final unified model, which incorporated both warming and organic resources, revealed a complex dynamic. As expected, with rising temperatures, both prokaryotic and viral abundance increased, even though not monotonically. This finding aligns with sample data since we observed both a viral increase and a slight prokaryotic increase, in terms of abundances, passing from the southern site to the northern. In addition, this model allows us to analyze climate change scenarios as trophic resources change, in particular, when reducing the carbon input (i.e., for example transitioning from North to South), we observed that viruses decreased while prokaryotes increased. This trend continued until reaching values of trophic resources ranging between 8.5 and 9, the reduction in viruses begin to slow down and prokaryotes start to decline. Since the attack rate of viruses exhibits a positive pattern with resources (i.e., fewer resources result in fewer viruses), as the growth rate, we can hypothesize from our model that, at high resource values, top-down control is more predominant than bottom-up control, leading a prokaryotic increase as the number of viruses decreases. Conversely, below a certain threshold, bottom-up control becomes more dominant, as evidenced by the reduction in prokaryotes number despite viruses diminishing.

3.5.6 Modelling virus-prokaryote dynamics using Generalized Lotka-Volterra models

In this second approach, we devised a system of three equations describing the dynamics of virus-prokaryotes-resource interactions. Trophic resources were included among the solutions, with temperature as the only changing parameter. Following the same analytical steps, we evaluated the results of numerical solutions for each system calculated separately in the northern and southern region, obtaining differences between measured data and theoretical steady states ranging from 21% to 47% (from 16% to 77% when considering carbon estimation errors as well). Consequently, we transitioned to a unified model expressing the coefficients as exponential functions of temperature. Simulations with increasing temperature revealed a growth in virus number, as the attack rate increased, leading to a decline in prokaryotes and a consequent rise in carbon. Under this temperature change scenario, the bottom-up control provided by the resources was unable to counteract the decline associated with heightened viral activity (top-down control).

These GLV steady states patterns are more similar to our sampled data making the GLV approach more realistic in estimating the dynamics with changing temperature. A possible explanation could be the stable carbon dynamics which make trophic resource suitable for the integration into the GLV as solution. However, both numerical approaches support previous empirical and modeling findings highlighting the importance of consider both the effects of viral presence and temperature warming to predict virus-prokaryote dynamics in deep-sea ecosystems (Murray et al., 1992; Weitz et al., 2015; Mojica et al., 2016; Piedade et al., 2018; Biggs et al., 2021; Demory et al.; 2021). Moreover, our results provide a further quantitative support for the hypotheses regarding the key role of viruses and climate change impacts in global nutrient cycles (Fuhrman, 1999; Wilhelm et al., 1999; Suttle, 2005; Brussaard et al., 2008; Weitz et al., 2012).

3.5.7 Limits and future perspectives

We acknowledge that there are some uncertainties inherent in our modeled processes and parameters, leading to uncertainties in our modeling results. This study represents only a preliminary step toward constructing a model capable of fully elucidating the role of temperature, organic matter and associated microbial and viral processes in global marine biogeochemical cycles under climate change. Other further steps are necessary before reaching that objective. Firstly, other factors that can influence microbial and viral dynamics have to be included in our models, such as oxygen (O2) levels, inorganic nutrients (NO3, PO4), grazers, lysis vs. lysogeny, latent period, and biodiversity. Indeed, it has been shown that the impacts of temperature raise are influenced by the composition of the community (Striebel et al., 2016). Moreover, variation in viral life strategies and latency are ecologically important (Stough et al. 2017, Correa et al. 2021) and exhibit unique trends with temperature that are currently unresolved (Shan et al. 2014), determining a crucial gap in our model and in general in the understanding of the temperature dependencies of viral infection. Additionally, certain parameters were assumed to be relatively constant in our model, including burst size, respiration rate, and chemosynthesis percentage. However, increasing temperature can potentially impact burst size, as documented by several studies (Hadas et al. 1997; Nagasaki et al.,1998; Demory et al., 2017; Maat et al., 2017; Piedade et al. 2018), therefore, these parameters should be considered dynamic in future model refinements (Wieczynski et al., 2023). Furthermore, the potential implications of temperature dependencies for microbial food web dynamics and functioning may be multifaceted, context-specific, and subject to variation across ecosystems. Predicted effects of warming on microbial and viral abundances in marine systems, as suggested in Danovaro (et al., 2011), are likely to differ among oceanic regions, and a uniform response to increasing temperatures across environments is improbable. Finally, due to the lack of direct measurements for all parameters needed in the systems of equations, some were estimated through optimization techniques, as detailed in the Materials and Methods section. This reliance on estimation introduces uncertainties into our models, impacting the alignment between numerical solutions and empirical data (Suttle 2007). Additionally, the regression-based interpolations adopted to describe the trends of the rates with respect to temperature and trophic resources are affected by errors due to the reduced number of points. These limitations underscore the need for more extensive research efforts in the deep sea, which can provide additional data points for statistical approximation and develop more precise mechanistic formulas, as that for growth rate (Arrhenius, López-Urrutia et al., 2007). Moreover, these integrations underscore the complexity of climate change effects on deep-sea virus-prokaryote dynamics; elucidating these relationships and impacts requires mechanistic models capable of detailing the functioning of microbial ecosystems. In conclusion, the significance of the microbial and viral component in the deep sea and in biogeochemical cycles, emphasizes the necessity of further analyses and developments for a better understanding and an improved theoretical representation of the impacts of virus-microbe interactions on ecosystem processes under warming conditions.

3.6 References

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Chapter 4.

Climate-driven episodic events exacerbate the viral impact on benthic deep-sea ecosystem functioning

4.1 Introduction

Climate change is expected to alter the functioning, composition and biodiversity of marine communities, especially viral and microbial ones, and associated ecosystem processes, such as primary production, trophic transfer to higher trophic levels, and carbon sequestration (Danovaro et al., 2004; Orr et al., 2005; Hoegh-Guldberg et al., 2007; Hoegh-Guldberg et al., 2010; Steinacher et al., 2010; Doney et al., 2012; Yasuhara et al., 2012; Cocco et al., 2013; Mora et al., 2013). The impacts occur through changes in the physical environment and through the direct effect of temperature on physiological processes (Serra-Pompei et al., 2019). Ocean warming, acidification, oxygen depletion, and reduction in primary production have all been highlighted as potentially having negative biological consequences (Danovaro et al., 2004; Orr et al., 2005; Hoegh-Guldberg et al., 2007; Hoegh-Guldberg et al., 2010; Steinacher et al., 2010; Doney et al., 2012; Yasuhara et al., 2012; Cocco et al., 2013; Mora et al., 2013). Changes in temperature can affect metabolism, reproduction, and survival (Peck et al., 2009; Doney et al., 2012), which is already evident in multiple shallow and deep-sea ecosystems (Danovaro et al., 2001; Hoegh-Guldberg et al., 2010), mainly for aquatic microorganisms (White et al., 1991; Pomeroy et al., 2001; Šolić et al., 2017). Even parameters regarding food supply, such as primary productivity and sinking organic-carbon flux, and dissolved oxygen can influence metabolism, body size, reproduction, and thus control, in part, the biomass in any area of the oceans (Ruhl et al. 2008). Taken together, the changes in ecosystem functioning are the results of a delicate balance between positive and negative effects due to climate-driven changes in the physical environment (Serra-Pompei et al., 2019), but the global dynamics is complex, therefore, it is possible that even minor changes as those at the base of the food web, could be amplified through the trophic chains affecting structure and functioning of marine ecosystems (Edwards et al., 2004; Wiltshire et al., 2008; Montoya et al., 2010; Sarmiento et al., 2010). We have a relatively good understanding of the potential changes in ocean biogeochemical parameters expected under different scenarios (Caldeira et al., 2003; Cao et al., 2008; Steinacher et al., 2010; Cocco et al., 2013), and conceptually we know some of the mechanisms through which ecological and social systems may be impacted by such changes. However, we lack a synthetic global quantification of biogeochemical changes on the ocean and how they may pertain to marine biota.

4.1.1 The Mediterranean Sea as a model for investigating climate-driven impacts in the deep sea

The Mediterranean Sea is one of the most vulnerable regions to temperature rise (Bethoux et al., 1999; Cacho et al., 2002; Somot et al., 2006, 2008; Giorgi et al., 2008), which are expected to increase on average by 2–4 °C over the next few decades (Timmermann et al., 1999; Meehl et al., 2007; Šolić et al., 2017). Multidecadal observations reported that temperature rise in the Mediterranean Sea is not only confined to surface waters but also occurs in deep bottom waters (Bethoux et al., 1999). Besides long term trends of changes in thermohaline conditions of the deep waters of the Mediterranean Sea likely related to global warming (Krahmann et al., 1998; Béthoux et al., 1999; Duarte et al., 1999; Giorgi, 2006; Giorgi et al., 2008; Skliris et al., 2014; Mayot et al., 2016), it has been observed changes in deep water masses also in relation to episodic events related to climate-driven forcing (Borghini et al., 2014; Schroeder et al., 2008, 2016).

Recent studies of the Mediterranean Sea and, more specifically, of its Western basin (WMb) have disclosed temperature and salinity trends in the Western Mediterranean Deep Water (WMDW) and Levantine Intermediate Water (Rixen et al., 2005; Schroeder et al., 2006, 2008). Every winter, the strong water buoyancy loss caused by cold and dry winds over the Gulf of Lion (GoL), drives the WMDW formation by open ocean convection (Medoc, 1970; Marshall et al., 1999) and by cascading of shelf water via submarine canyons (Puig et al., 2013; Houpert et al., 2016). The volume of new WMDW is anomalously high in years when both processes occur. In particular, the very harsh winters of 2004–2005 and 2005–2006, combined with anomalously salty and warm Levantine Intermediate Water (LIW) due to increased heating and evaporation on Eastern Mediterranean influenced by the Eastern Mediterranean Transient (EMT), formed large volumes of saltier, warmer, and denser WMDW in the GoL (Schroeder et al., 2008) that reached the seafloor, spread laterally, and gave rise to a new vertical pattern of the deep layers in the WMb. These major events have been identified with the starting of a Western Mediterranean Transition (WMT) (Zunino et al., 2012), in analogy with the EMT during the 90s of last century (Roether et al., 1996; Naranjo et al., 2017).

4.1.2 Deep winter convection

Winter convection allows particulate (and dissolved) matter to be vertically exported. The efficiency of this process also determines the concentration of nutrients brought to surface waters and, therefore, the intensity of the subsequent phytoplankton bloom. The sequence ‘convection-bloom’ is the main driving force of vertical export flux in this region (Heimbürger et al., 2013). Very strong winter convection events were recorded in winters 1996–1997, 2004–2005, 2005-2006 and 2007–2008, whereas shallower winter convection events occurred in winter 1990–1991, 1991–1992, 1992–1993, 1994–1995 and 2001–2002. Some winters were characterized by short, intense convection episodes (e.g., 2005–2006), whereas others were defined by long-lasting winter convection. The sharp and intense peaks of winter 2004–2005 and 2005–2006 probably yielded high vertical export fluxes. Atmospheric inputs do not generally determine the occurrence of vertical export fluxes (Migon et al., 2002; Passow, 2004; Passow et al., 2006), the work of Heimburger (et al., 2013) suggests that the high vertical export flux in the DYFAMED region is determined by winter convection and by the subsequent phytoplankton bloom.

4.1.3 The role of temperature and substrate availability in the viral and microbial dynamics

Among variables that may influence microbial and viral dynamics, the role of temperature and substrate availability aroused the interest of scientists for years (Pomeroy et al., 2001; Calvo-Díaz et al. 2014, Lønborg et al. 2016; Franzo et al., 2019). Temperature is a dominant controller of biological process rates at all levels of biological organizations (Arroyo et al., 2022; Cruz-Paredes et al., 2023), thus, changes in thermal regimes will influence how C is processed through all ecosystems. Microorganisms dominate the metabolic activity underpinning decomposition and therefore constitute a major determinant of C fluxes in the biosphere. Thus, to accurately predict how warming will alter C dynamics we need to understand how microorganisms depend on temperature. However, while some studies investigated only the effect of temperature on microbial and viral production (Lønborg et al. 2016), others tried to relate the observed microbial metabolic changes to both temperature and resources (Calvo-Díaz et al. 2014). Overall, these kinds of studies highlighted a variable susceptibility of prokaryotes to both temperature and substrate availability, making quite difficult the extrapolation of any general description about their interaction (Pomeroy et al., 2001). To date, the majority of investigations pertaining to this issue have been carried out on bacterioplankton while organic matter processing in the sediments remained almost neglected, notwithstanding some exceptions in freshwater (Zoppini et al., 2010; Pohlon et al., 2013, Hill et al., 2017) and estuarine environments (Danovaro et al., 2002). The general lack of studies focusing on marine sediments represents an important knowledge gap because the sediment-water interface not only represents one of the largest interfaces on Earth (Middelburg, 2018), but it is also the site where all physicochemical and biological processes become more intensive (Frankowski et al., 1999; Franzo et al., 2019). Microbial-mediated processes in the sediments acquire, therefore, a very important role from a biogeochemical point of view. This highlights the need to study the potential influence of temperature and substrate availability on benthic microbial activities and C uptake. The issue is particularly pressing nowadays because understanding microbial and viral dynamics dependence from temperature and substrates may help to detect any biogeochemical alteration due to large-scale, climate change-related events. (Franzo et al., 2019). In particular in the Mediterranean marine ecosystems which are supposed to assist to strong modifications and regime shifts in their dynamics (Group et al., 2011; Coll et al., 2008; Conversi et al., 2010; Mayot et al., 2016)

4.1.4 Extreme climate-driven events

Over the last three decades, there has been growing concern on Earth's climate changes, since, in addition to a general increase in temperature, it has been predicted that there will be changes in the geographical distribution, intensity and frequency of extreme climate events (ECEs) (Griggs et al., 2002; Mitchell et al., 2006; IPCC, 2007; Planton et al., 2008; Stott, 2016). The extremes events that are generally considered in the impact studies of climate change are either rare, or severe through their consequences (Beniston et al., 2007), but there are many possible definitions of extreme events (Smith, 2011; Stott et al., 2004; Babcock et al., 2019; Swain et al., 2020), leading to some difficulties in comparing different analyses. According to the most recent studies future climate change will be accompanied with a change in the statistics of extreme events becoming more frequent ant more intense (Herring et al 2018; Smale et al. 2019), but the picture is highly dependent on the type of extremes and on the regions that are considered. (Planton et al., 2008). However, to understand climate change, it is imperative to understand how (and why) these climate-related extremes are changing in a warming world. ECEs differs from a gradual climate change driven solely by changes in mean temperature assumed in most of the biogeochemical models (Burrows et al., 2014; Lenoir et al., 2015; Ling, 2008; Fischer et al., 2013; Christidis et al., 2015; Vergés et al., 2016; Brown et al., 2016; Mann et al., 2017; Harris et al., 2018; Oliver et al., 2018; Babcock et al., 2019). They have serious implications for ecosystems (Field, 2012), especially marine ones, becoming important drivers of change in the dynamics of biotic communities (Easterlin et al 2000; Parmesan et al., 2000; Gutschick et al., 2003; Jentsch et al., 2007); in fact, discrete events of novel extreme magnitude and frequency can drive ecosystems beyond stability and resilience. However, ECEs will have significant consequences not only for ecosystems but also for the human economic, social, and political systems that depend on them. Then we urgently need to advance research on extreme events and their consequences, combining our efforts to forecast the climate changes with our understanding of marine ecosystem responses to assess and minimize the impacts of ECEs (Scheffer et al., 2001; van de Pol et al., 2017; Babcock et al., 2019; Lionello et al., 2006; Basterretxea et al., 2018).

In this chapter, we aimed to elucidate the effects of climate-driven extreme events on microbial and viral dynamics by using the same approach as in Chapter 3 with Lotka-Volterra models. We integrated concepts and formulas from the Metabolic Theory and followed similar steps in the development of the models. Creating mechanistic models, unlike statistical analysis, allows us to understand the cause-and-effect mechanisms that arise within our simplified food web, considering the variations over years and accounting for the effects resulting from the episodic event of deep winter convection contributing to the Western Mediterranean Transition (WMT).

4.2 Aims

The main aims of this chapter were:

- ✓ investigating the responses of virus-prokaryote interactions in relation with climate-driven episodic events through mechanistic models;
- ✓ forecasting changes in microbial and viral dynamics under global climate change scenarios taking into account the effects of episodic events.

4.3 Materials and methods

4.3.1 Study areas and sample collection

For this study we used unpublished data of deep-sea sediment samples that were collected over a decade from 1999 to 2008 in a bathyal plain of the Western Mediterranean Sea located at ca. 3000 m depth in an area (39.523°N 5.902°W). All analyses were carried out on the top 1 cm of sediment.

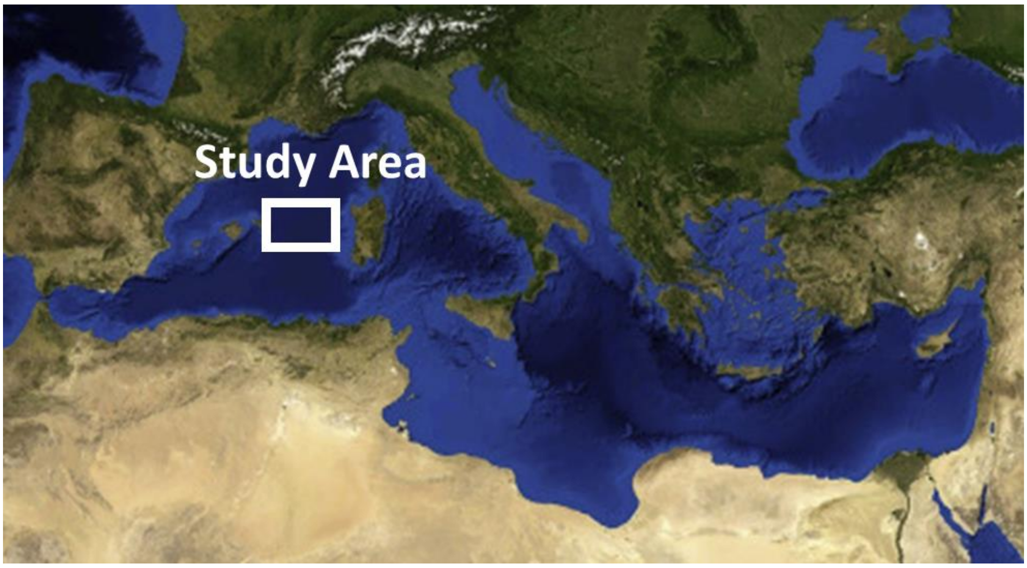


Figure 4.1 Map of the study area in Western Mediterranean Sea. The study area in the white quadrant.

4.3.1 Environmental, trophic, microbial and viral variables

The temperature of the bottom waters at each sampling site were determined by CTD. The trophic characteristics of the benthic deep-sea ecosystems were investigated through the analysis of the biopolymeric C (BPC) content (used as a proxy of the more bioavailable fraction of organic C for heterotrophic consumers) in the sediment. Values of photosynthetic primary production of the surface waters were extracted from the ocean productivity database (www.science.oregonstate.edu) and referred to the same year and the same area of the Mediterranean Sea where deep-sea sediments were collected. To estimate the increase in bottom flux generated by the episodic event of strong deep water convection that ultimately caused the Western Mediterranean Transition, we imported and analyzed data from Copernicus. Specifically, we utilized the following two databases:

Database	**Platform**	**Covariates**	**Reference**
Global Ocean Physics Analysis and Forecast [Reanalysis]	Copernicus Marine Service	Temperature (°C), Salinity (psu)	46. E.U. Copernicus Marine Service Information. Global Ocean 1/12° Physics Analysis and Forecast updated Daily. *Copernicus Marine* *Service Web site* https://doi.org/10.48670/moi-00016 (2021).
Global Ocean Biogeochemistry Analysis and Forecast	Copernicus Marine Service	Chl a (mg m^{-3}), Primary Production (PP, mg m^{-3} d^{-1})	47. E.U. Copernicus Marine Service Information. Global Ocean Biogeochemistry Analysis and Forecast. *Copernicus Marine Service* *Web site* https://doi.org/10.48670/moi-00015 (2021).

Table 2.1 External databases. In this table are listed the external sources used to integrate environmental covariates when they were not present in the articles.

The variables used to describe the microbe-virus dynamics were prokaryotic abundances as number of cells g^{-1}, viral abundances as VLP (virus-like particles) g^{-1}, prokaryotic heterotrophic C production as ng C g^{-1} h^{-1} and viral production as VLP g^{-1} h^{-1}, prokaryotes killed by viruses as cells g^{-1} h^{-1} and Carbon released by killed prokaryotes as ng C g^{-1} h^{-1}.

4.3.2 Metabolic Theory, mechanistic models and regression analysis

For our analysis, we developed two numerical approaches trying to integrate the effect of sea water warming and carbon flux reduction, into models of population dynamics, to predict the consequences of climate change in the deep sea microbial food web.

In the first approach we started from a Lotka-Volterra model where the prey function $B(t)$ is represented by prokaryotes, while the predators $V(t)$ are viruses, with the following form:

$$\frac{d\boldsymbol{B}}{dt} = \alpha * \boldsymbol{B} - \beta * \boldsymbol{B} * \boldsymbol{V}$$

$$\frac{d\boldsymbol{V}}{dt} = \delta * (\beta * \boldsymbol{V} * \boldsymbol{B}) - \Omega * \boldsymbol{V}$$

where δ (the burst size), Ω (the viral decay rate) and the percentage of chemosynthetic production (used to adjust the value of prokaryotic production) were estimated through optimization for each sample year. To estimate these parameters, we created an algorithm that, started from a range of values for each parameter, solved the system of equations for different combinations of parameter values and calculated the difference between each solution and the measured data. Then, we selected the parameters combination of values that minimized the differences between the simulated solutions and the real data for all years considered. Finally, to include the effect of climate change, we tried to express the system coefficients as functions of temperature and food availability (Laws et al., 2000; Abiodun et al., 2016; Wieczynski et al., 2023). Specifically, both β (attack rate) and Ω (Parikka et al., 2017) were approximated by the formula $e^{(coef1 * T + coef2 * C + intecept)}$, where T is the temperature, C is the labile organic carbon in logarithmic scale in the sediment and the tuple $(coef1,\ coef2, intecept)$ is the result of the log-liner regression. The growth rate (α) was calculated using the Arrhenius formula from the Metabolic Theory of Ecology (MTE, López-Urrutia et al., 2007). Therefore, in this first approach, the trophic resource is not considered as a solution of the system, but as an input to be provided to obtain the growth rate, the attack rate and the decay rate. In addition, we assumed that all prokaryotic mortality depends solely on viruses (Lara et al., 2017) and that trophic resources consist of the sum of the flux and carbon released by killed prokaryotes, along with a fraction of organic carbon in sediment. We also assumed that burst size (δ) is constant. The choice of a Lotka-Volterra model, even in the absence of fluctuations in the source data, was driven by two main reasons, the lack of a reliable estimation method for the carrying capacity, and the need to have a sufficiently simple model without explicit references to trophic resources, so that these could also vary discontinuously, as in the case of alterations due to extreme episodic events.

In the second approach, we started from a Generalized Lotka-Volterra (GLV) model in which the trophic resource is included as a system solution:

$$\frac{d\boldsymbol{B}}{dt} = \alpha * \boldsymbol{C} * \boldsymbol{B} - \beta * \boldsymbol{B} * \boldsymbol{V}$$

$$\frac{d\boldsymbol{V}}{dt} = \delta * (\beta * \boldsymbol{V} * \boldsymbol{B}) - \Omega * \boldsymbol{V}$$

$$\frac{d\boldsymbol{C}}{dt} = \theta * \beta * \boldsymbol{V} * \boldsymbol{B} + \lambda * \boldsymbol{V} - \sigma * \boldsymbol{B} * \boldsymbol{C} + C\_flux$$

Assuming $\theta = 20$ fgC, we estimated δ (burst size) and the percentage of chemosynthetic production through optimization using the same algorithm developed as above. Additionally, we assumed $\alpha,\ \beta,\ \Omega, \sigma,\ \lambda$ to be equal to $\boldsymbol{e}^{(\boldsymbol{coef} * \boldsymbol{T} + \boldsymbol{intecept})}$, where T represents temperature and each pair $(coef, intecept)$ corresponds to the outcome of the log-linear regression between each dynamic parameter (i.e. $\alpha,\ \beta,\ \sigma,\ \Omega,\ \lambda$) and T. Similarly, we assumed that all prokaryotic mortality depends solely on viruses and that trophic resources consist of the sum of flux and carbon released by killed prokaryotes, along with a fraction of organic carbon in sediment.

4.3.2 Climate change scenarios and forecasting

For the first model, we had two input parameters to define (temperature and trophic resources) in order to configure each system of equations. We thus created a grid of parameters combinations based on a CMIP6 scenario: the SSP5 scenario with a 20% reduction in carbon flux that we projected to the bottom resulting in an equal reduction in trophic resources in the sediment (strong assumption) for all sites. As for the temperature increase, we tested with the same +0.3°C as in the chapter 3. Subsequently, we developed an algorithm that, for each combination of parameters, set the model, computed the solutions, and plotted the averages. Since the solutions are periodic, we assumed that the measured data, being an average of replicates, could be compared with the averages of each periodic solution. This allowed us to generate a 3D graph for viruses and prokaryotes depicting the variation of their average abundances with changes in temperature and trophic resources.

For the second model, the changing parameter was only temperature and we considered the same potential increase of 0.3°C to generate 2D scatterplots depicting the variation of viral and prokaryotic abundances steady states with changes in temperature.

4.4 Results

4.4.1 Temporal changes over years

Our data reveals an increasing pattern for temperature over the years. In detail, this pattern is not constant but experiences a significant surge between 2005 and 2006. The organic resources in sediments, on the other hand, exhibit a decreasing trend, which stabilizes from 2002 onward, as evidenced by ANOVA tests that do not report statistically significant differences between 2002, 2006, and 2008.

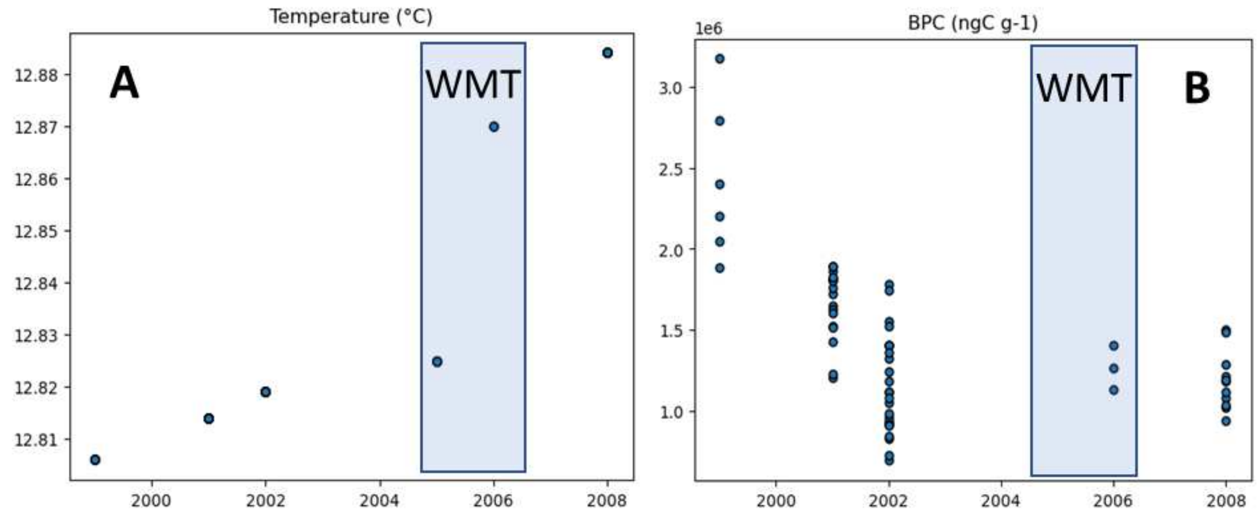


Figure 4.2 Temporal pattern of deep-sea water temperatures and organic resources' availability. The plot A shows the temporal pattern of bottom water temperature in the bathyal plain of the Western Mediterranean Sea where sediment samples have been collected, the row indicates the year when the Western Mediterranean Transition happened. The plot B shows the temporal pattern of labile organic C (as biopolymeric C) concentrations in surface sediments of the bathyal site of the Western Mediterranean Sea.

Looking at the microbial and viral abundances, we observe that prokaryotes decrease but with a changing trend. Specifically, between 2002 and 2005, there are no significant differences, as well as between 2006 and 2008, while the difference between 2005 and 2006 is statistically significant. Conversely, for viruses, the trend is increasing, and again, the trend is not monotonic. Between 2005 and 2006, there are no significant differences (ANOVA $p>0.05$), while the differences between 2006 and 2008 are significant, as also evident from the Figure 4.3.

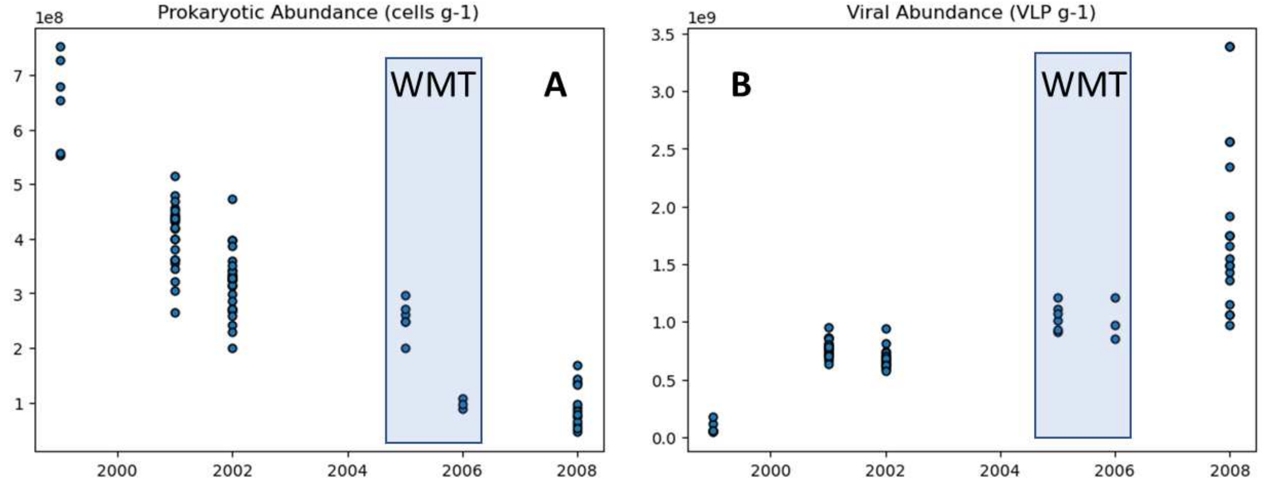


Figure 4.3 Changes of benthic deep-sea prokaryotic and viral abundances in the Mediterranean Sea. Reported are temporal changes of the abundances of prokaryotes (A) and viruses (B) in surface deep-sea sediments of the Western Mediterranean Sea. The light blue band indicates the time interval in which the episodic event of strong deep water convection occurred, causing ultimately the Western Mediterranean Transition.

If we analyse both viral and prokaryotic productions, they show an increasing pattern, initially mild, as ANOVA tests do not indicate significant differences for the first few years. However, both viral and prokaryotic production tests show a $p<0.05$ when comparing 2006 and 2008, where values increase both in terms of mean and range amplitude.

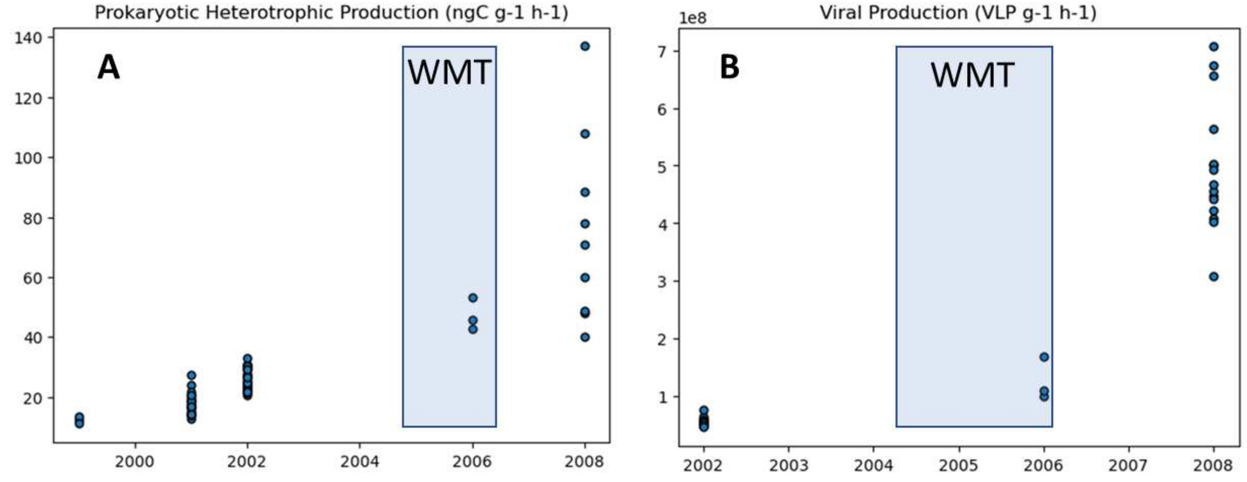
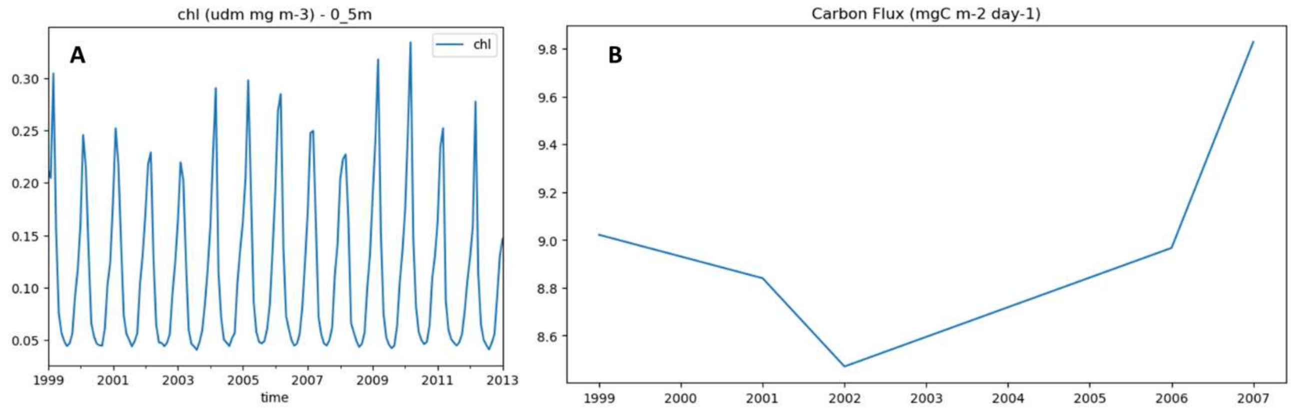


Figure 4.4 Changes of benthic deep-sea prokaryotic and viral productions in the Mediterranean Sea. Reported are temporal changes of the productions of prokaryotes (A) and viruses (B) in surface deep-sea sediments of the Western Mediterranean Sea. The light blue band indicates the time interval in which the episodic event of strong deep water convection occurred, causing ultimately the Western Mediterranean Transition.

Finally, from the data of Oregon and Copernicus, we noticed that at the surface, the flux remains unchanged, as does the quantity of chlorophyll that can be considered as a resources indicator. However, at the bottom, the ratio between pre deep winter convection chlorophyll (considering the year 2003) and post deep winter convection chlorophyll, ranged between 41 and 95 times, indicating that the deep winter convection phenomenon had a significant impact at the bottom.



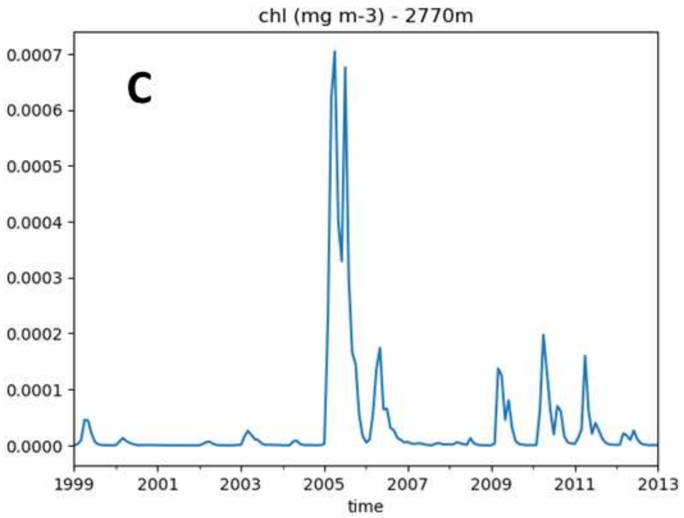


Figure 4.5 Temporal pattern of surface water chlorophyll and ocean productivity. The plot A) shows the temporal pattern of chlorophyll in the first 5 meters of the water column, as predicted by Copernicusl. Plot B) shows the temporal pattern of carbon flux in the deep obtained from the Oregon estimates on the sea surface (years 1999, 2001, 2002, 2006, 2007) and then using the formula of exponential decline. The plot C) shows the temporal pattern of chlorophyll in the deep (2770 meters) from Copernicus data.

4.4.2 Combined effect of temperature and trophic resources in the Metaboli Theory of Ecology

To analyze the combined effects associated with the increase in temperature and resources due to the deep winter convection, we used the Arrhenius equation revised by López-Urrutia (et al., 2007). However, lacking measured data on both how many time the flux increases due to the deep winter convection and on chemosynthetic percentage, we calculated different scenarios based on these two parameters, obtaining activation energy values ranging from -3.3 eV to -13 eV, values up to 4 times higher than the values found in literature for water column.

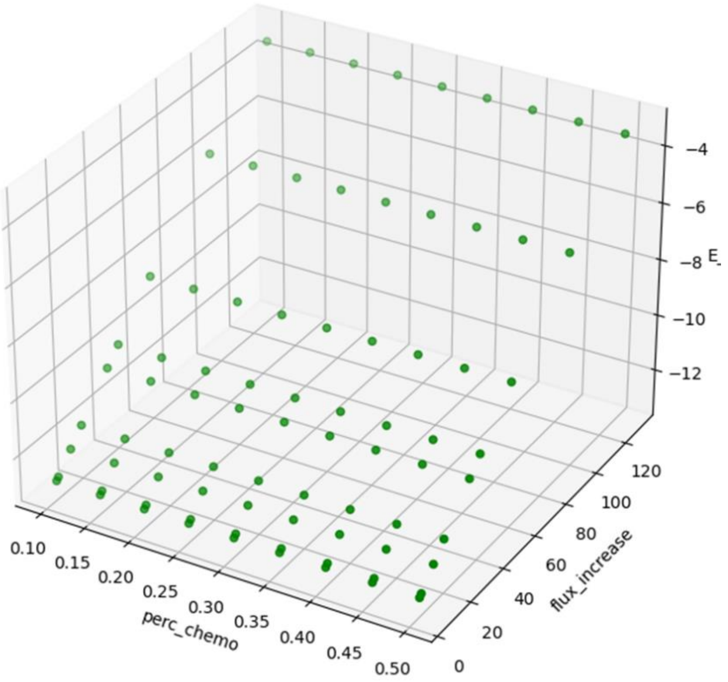


Figure 4.6 Activation energy for prokaryotic production as the percentage of chemosynthesis and the multiplication factor for flux varies. The 3D scatterplot shows the different values of activation energy in the Arrhenius equation for the prokaryotic production as the percentage of chemosynthesis and the Oregon flux multiplicative coefficient change.

4.4.3 Lotka-Volterra models by years

Starting from the measured data, we constructed different LV models to describe the behavior of viruses and prokaryotes over the years, unifying data of microbial and viral abundances (static) and production (time-dependent) into a single system that numerically modeled interactions and cause-and-effect mechanisms. In the model obtained for the year 2001, we observe how our measured data aligns with the periodic oscillations of the theoretical solutions. Specifically, for prokaryotes, our measured data positions around the maximum value of the periodic solution, showing a delta of approximately +34% compared to the average of the theoretical solutions. Conversely, there is a delta of approximately -3% for viruses. For the year 2002, the difference in prokaryotic abundance remains similar (+32%), but there is an increase in the absolute value of the viral abundance difference since the viruses are now lower than the predicted value of almost -49%. Moreover, we begin to notice an increase in the frequency of periodic solutions, and, while the amplitudes of the viral solutions remain the same, the amplitudes of the prokaryotic solutions tend to contract.

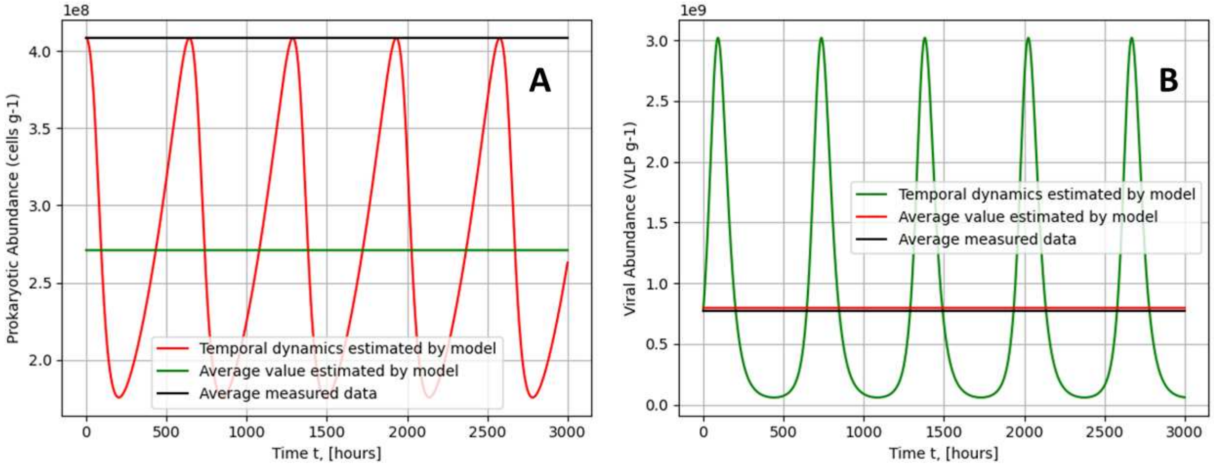


Figure 4.7 Analysis of solutions of the Lotka-Volterra model for 2001 year. The plot A shows the theoretical solution for prokaryotes in red, its mean value in green and the average of measured data in black. The plot B shows the theoretical solution for viruses in green, its mean value in red and the average of measured data in black.

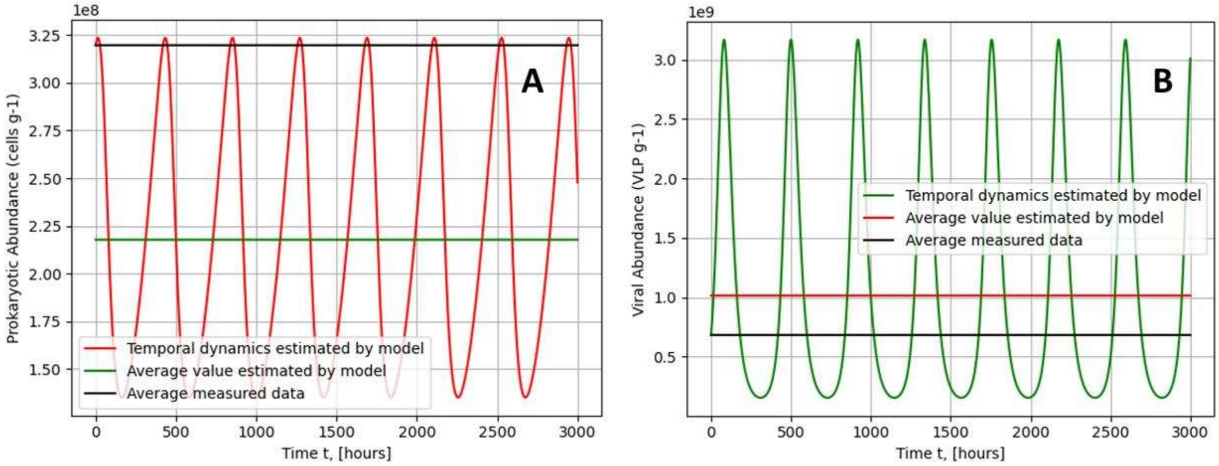


Figure 4.8 Analysis of solutions of the Lotka-Volterra model for 2002 year. The plot A shows the theoretical solution for prokaryotes in red, its mean value in green and the average

of measured data in black. The plot B shows the theoretical solution for viruses in green, its mean value in red and the average of measured data in black.

In the two models configured after the deep winter convection and at the beginning of the Western Mediterranean Transition (WMT), we observe how the dynamic appears to be significantly accelerated, with the number of oscillations increased by more than double compared to 2002. Analyzing the various solutions specifically, in 2006, the difference between the real data and the simulated data in prokaryotic abundances increased to +55%, while in viral abundances, this delta decreased in absolute value to -14%. Additionally, the range of amplitude of the prokaryotic solutions continues to contract, while that of the viral solutions remains approximately the same. If we observe what happens in 2008, the difference for prokaryotes further increases and reaches +76%, while the delta for viruses rises to +54%. Observing the oscillations ranges, we see that the amplitude of the prokaryotic solution grows slightly, while that of the viral solution increase by approximately 49% respect to 2006.

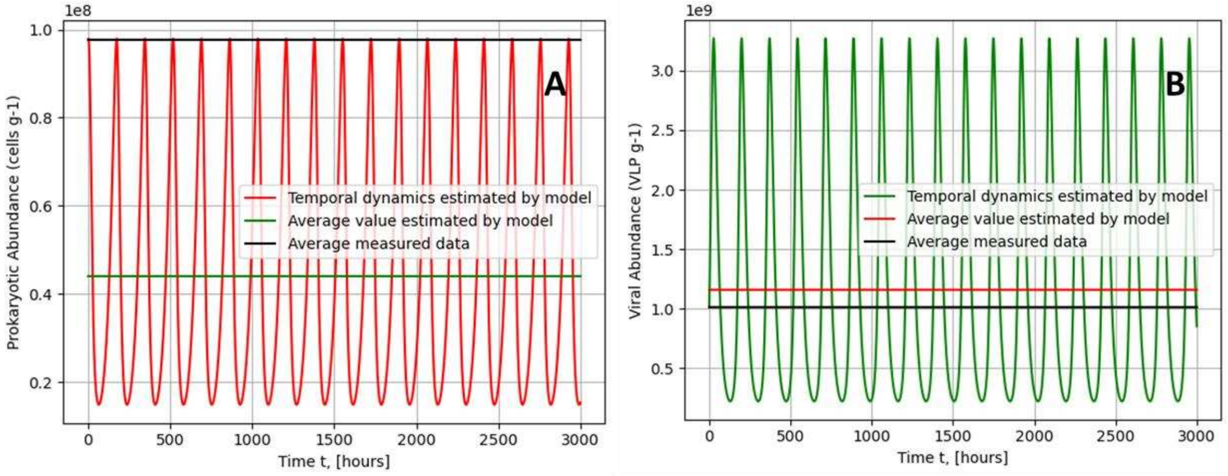


Figure 4.9 Analysis of solutions of the Lotka-Volterra model for 2006 year. The plot A shows the theoretical solution for prokaryotes in red, its mean value in green and the average of measured data in black. The plot B shows the theoretical solution for viruses in green, its mean value in red and the average of measured data in black.

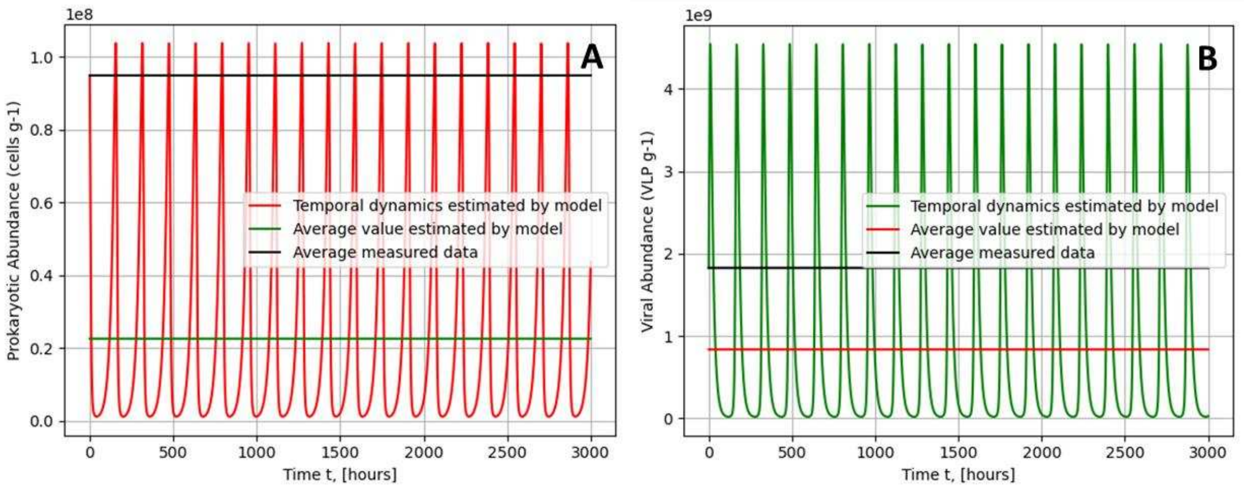


Figure 4.10 Analysis of solutions of the Lotka-Volterra model for 2008 year. The plot A shows the theoretical solution for prokaryotes in red, its mean value in green and the average of measured data in black. The plot B shows the theoretical solution for viruses in green, its mean value in red and the average of measured data in black.

4.4.4 Lotka-Volterra model with MTE integration and analysis of solutions

Taking into account the irregular dynamics of both temperature and carbon flux from the surface due to the episodic event of strong deep winter convection, to study the behavior of the solutions with unpredictable changes of environmental parameters as temperature and food availability, we created a model that is both temperature and resource-dependent through the Metabolic Theory of Ecology (MTE). Subsequently, we expressed the LV coefficients as functions of these two parameters and created a grid, as shown in the Figure 4.11, considering different scenarios, as explained in the Materials and Methods section. We note that not all nodes of the grid have a green dot, indicating that the algorithm used to compute the numerical solution did not always converge.

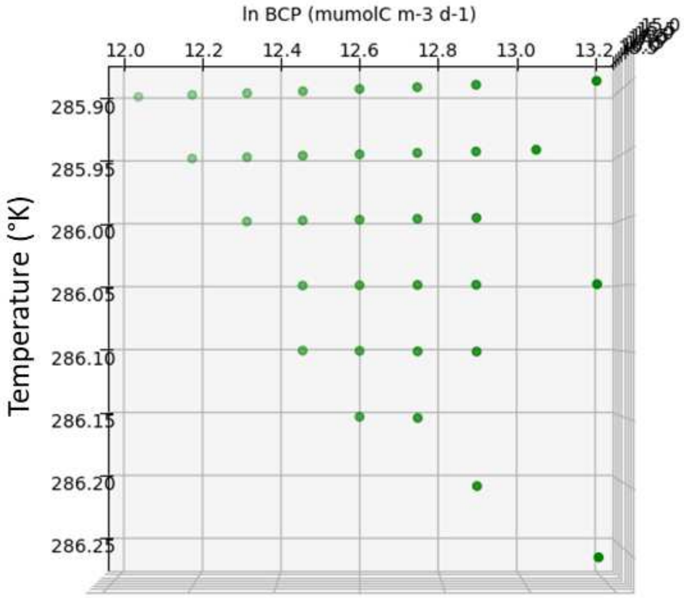


Figure 4.11 Grid of temperature and carbon values used to configure the Lotka-Volterra model with MTE integration. The plot shows all the couples of temperature and food availability parameters, used to set the LV models with the MTE integration. Each point corresponds to a system of equations for which the prokaryotic solution has been computed.

In the previous grid, each point represents the average of the periodic numerical solution of prokaryotic abundance corresponding to a specific set of parameters (temperature, resources). Slicing this 3D scatterplot to focus on trends with respect to a single parameter, we notice that prokaryotes decrease monotonically both with temperature increase and trophic resources increase. This data highlights the importance of viruses because the growth rate increases under both warming and carbon increase since it follows the Arrhenius formula (López-Urrutia et al., 2007).

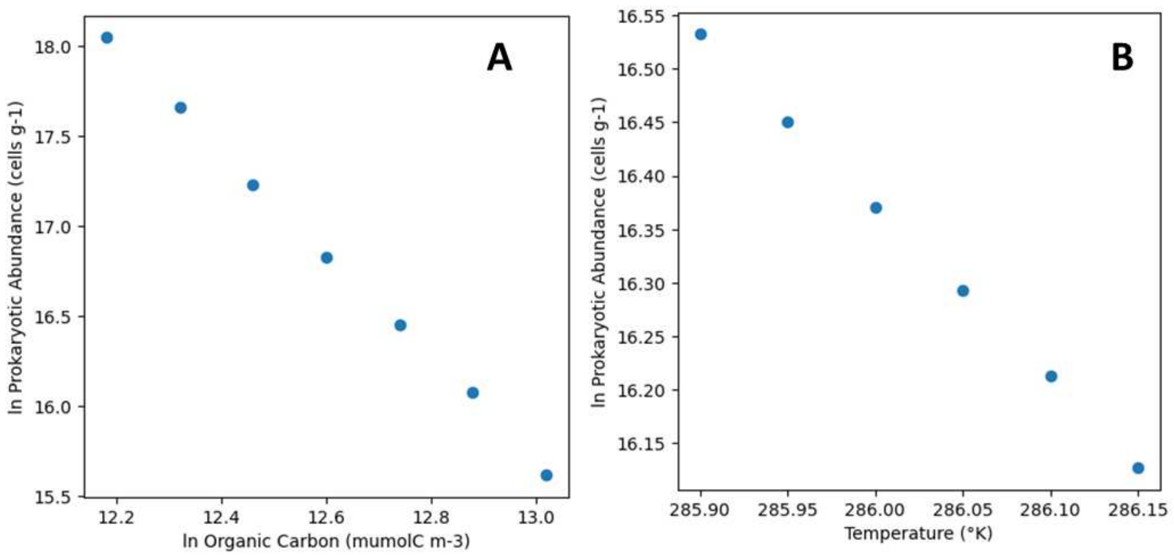


Figure 4.12 Analysis of average prokaryotic solutions changing temperature or trophic resources. The plot A shows the behavior of the average of the prokaryotic solutions as food availability changes. The plot B shows the prokaryotic solutions trends with respect to temperature change.

The two graphs in Figure 4.13 show how the combined effect of temperature and organic carbon creates a flat surface, as the trends with respect to each of the environmental covariates are monotonically decreasing. Therefore, the overall pattern for prokaryotic abundance is decreasing as temperature and carbon increase, with ranges from 18.5 to 15.0 in logarithmic scale.

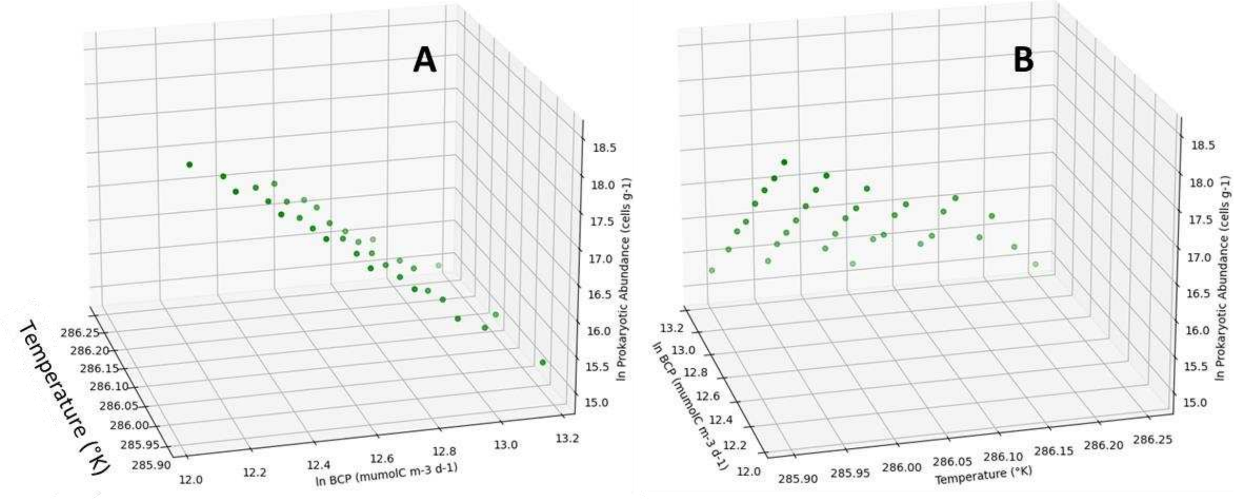


Figure 4.13 Analysis of average prokaryotic solutions changing both temperature and trophic resources. Both plots show the behavior of the average of the prokaryotic solutions as temperature and food availability changes according to different scenarios.

The same analysis of the solutions patterns, considering variations in temperature and resources, has also been conducted for viruses, which constitute the second component of our model. The grid dimensions are the same as those used for prokaryotes, as we can see from the Figure 4.14.

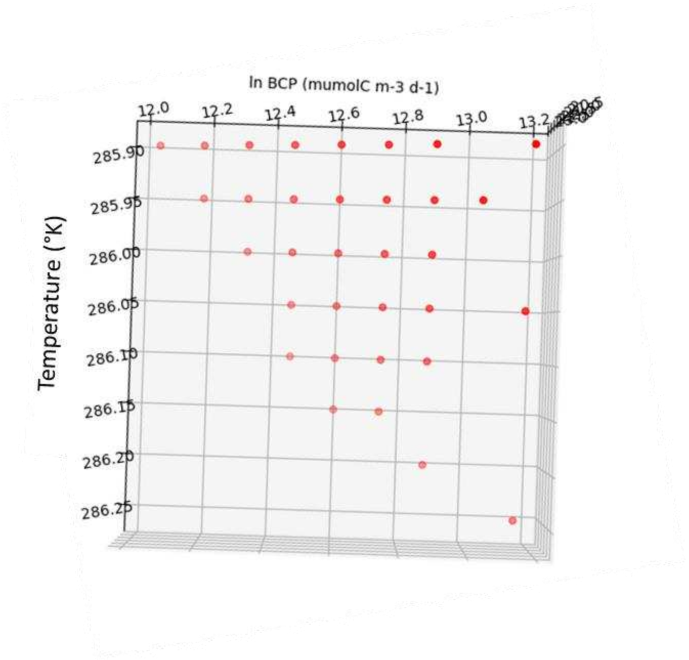


Figure 4.14 Grid of temperature and carbon values used to configure the Lotka-Volterra model with MTE integration. The plot shows all the couple of temperature and food availability parameter to set the LV model with the MTE integration. Each point corresponds to a system of equations for which the viral solution has been computed.

Each point on this grid represents the average of the periodic solution of viral abundance corresponding to a specific set of parameters (temperature and trophic resources). If we slice the 3D scatterplot to focus on individual trends considering a single environmental parameter, we notice two opposite patterns. In particular, viruses tend to decrease following an increase in carbon availability, and this aspect needs to be further elucidated since from the measured data we observe an increase in the number of viruses after the deep winter convection. Moreover, the prokaryotic decline is in contrast with a reduction in viral pressure and an increase in food availability. The dynamic with temperature is opposite and coherent with measured data since the number of viruses increases with increasing temperature. Additionally, the range of viral abundances connected to each environmental parameter variation is approximately the same, suggesting that in an episodic event, it's difficult to distinguish which environmental factor influences the viral dynamics the most.

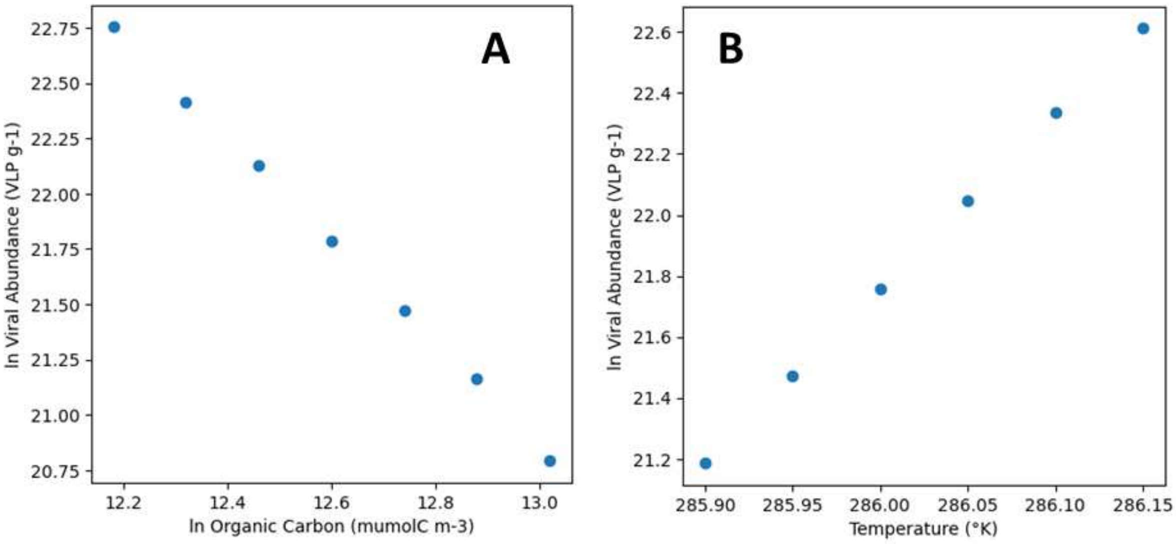


Figure 4.15 Analysis of average viral solutions changing temperature or trophic resources. The plot A shows the behavior of the average of the viral solutions as food availability changes. The plot B shows the viral solutions trends with respect to temperature change.

In the two graphs of Fig. 4.16, we observe how the combined effect of temperature and organic carbon appears more complex in 3D, since viruses present opposite trends with respect these two environmental parameters, with values of viral abundances ranging from 20.5 to 23.0 in logarithmic scale.

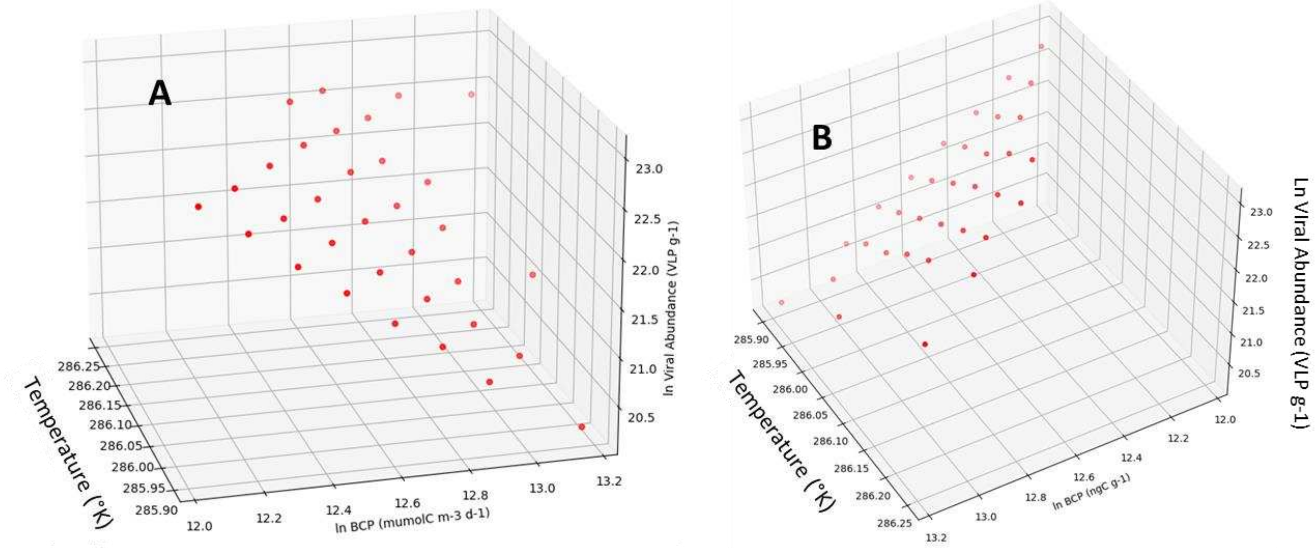


Figure 4.16 Analysis of average prokaryotic solutions changing both temperature and trophic resources. Both plots show the behavior of the average of the viral solutions as temperature and food availability changes according to different scenarios.

4.4.5 Generalized Lotka-Volterra models by years

In the last part of our analysis, we attempted to utilize GLV models, incorporating trophic resources among the system's solutions to observe the differences between the two modelling approaches, as in chapter 3. In this case as well, we initially constructed a system for each year, calculating the rates using both measured data and computational estimation. We noticed how the theoretical solutions tend toward a steady state, with their initial oscillations becoming less pronounced until they approach the stationary theoretical solution. Analyzing the differences between our measured data and the steady states in the year 2001, we observed that for prokaryotes this difference was +32%, for viruses it was +4%, while for trophic resources, it was -145%. Furthermore, we also observed how the average measured data for prokaryotes lays near the highest values of our theoretical oscillations, while the resource exhibited an opposite behavior, lying among the lowest values of the numerical solution. For the year 2002, we noticed a similar dynamic with a very similar difference in the number of prokaryotes, approximately +33%, and +9% for viruses, while the difference for organic carbon decreased to -55%. Additionally, in 2002, we observed how the oscillations tended towards the steady state more rapidly, with oscillations diminishing quickly.

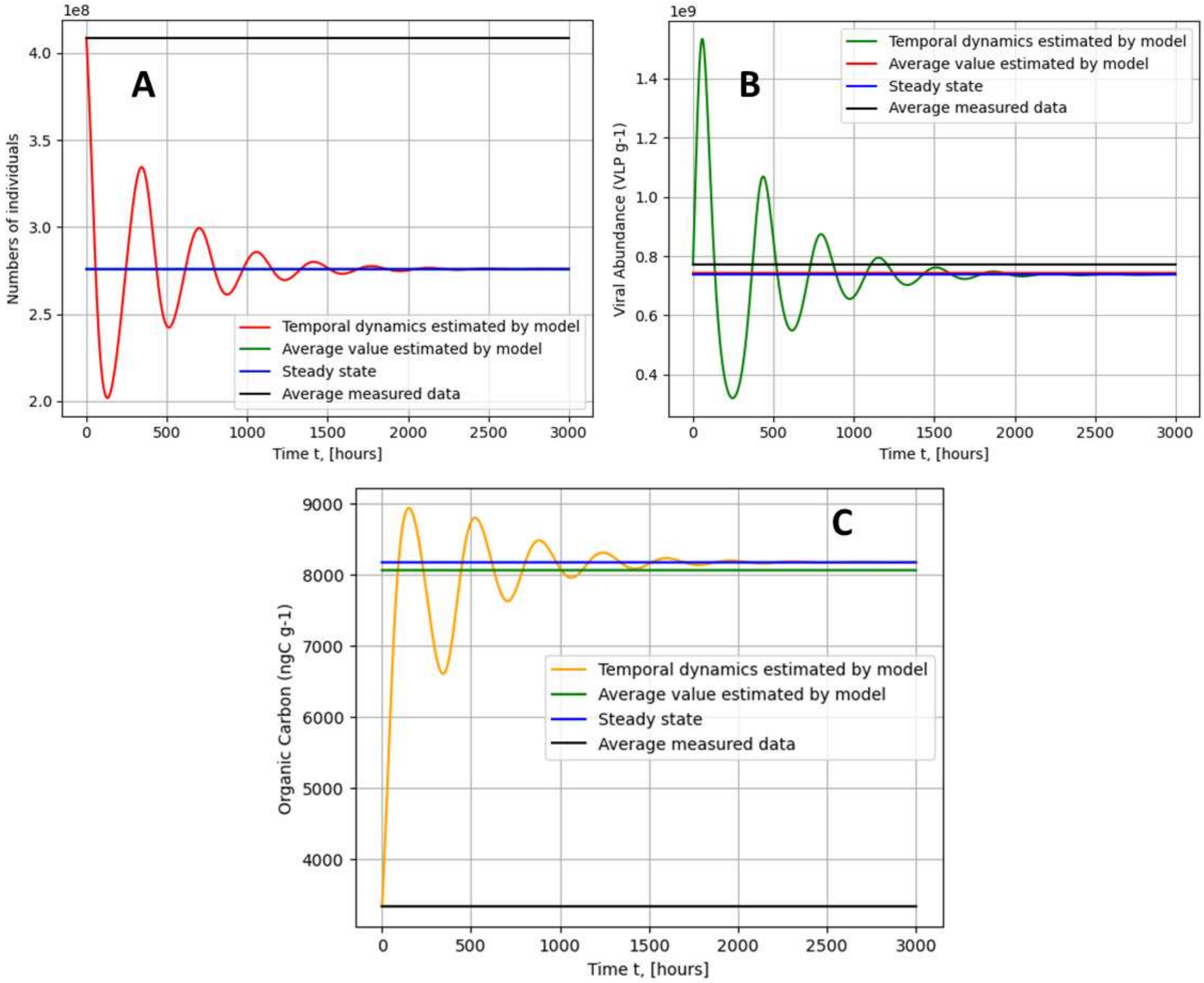


Figure 4.17 Analysis of solutions of the Generalized Lotka-Volterra model for 2001. The plot A shows the theoretical solution for prokaryotes in red, its mean value in black, the average of measured data in green and the steady state in blue. The plot B shows the theoretical solution for viruses in green, its mean value in black, the average of measured data in red and the steady state in blue. The plot C shows the theoretical solution for the organic carbon in yellow, its mean value in black, the average of measured data in green and the steady state in blue.

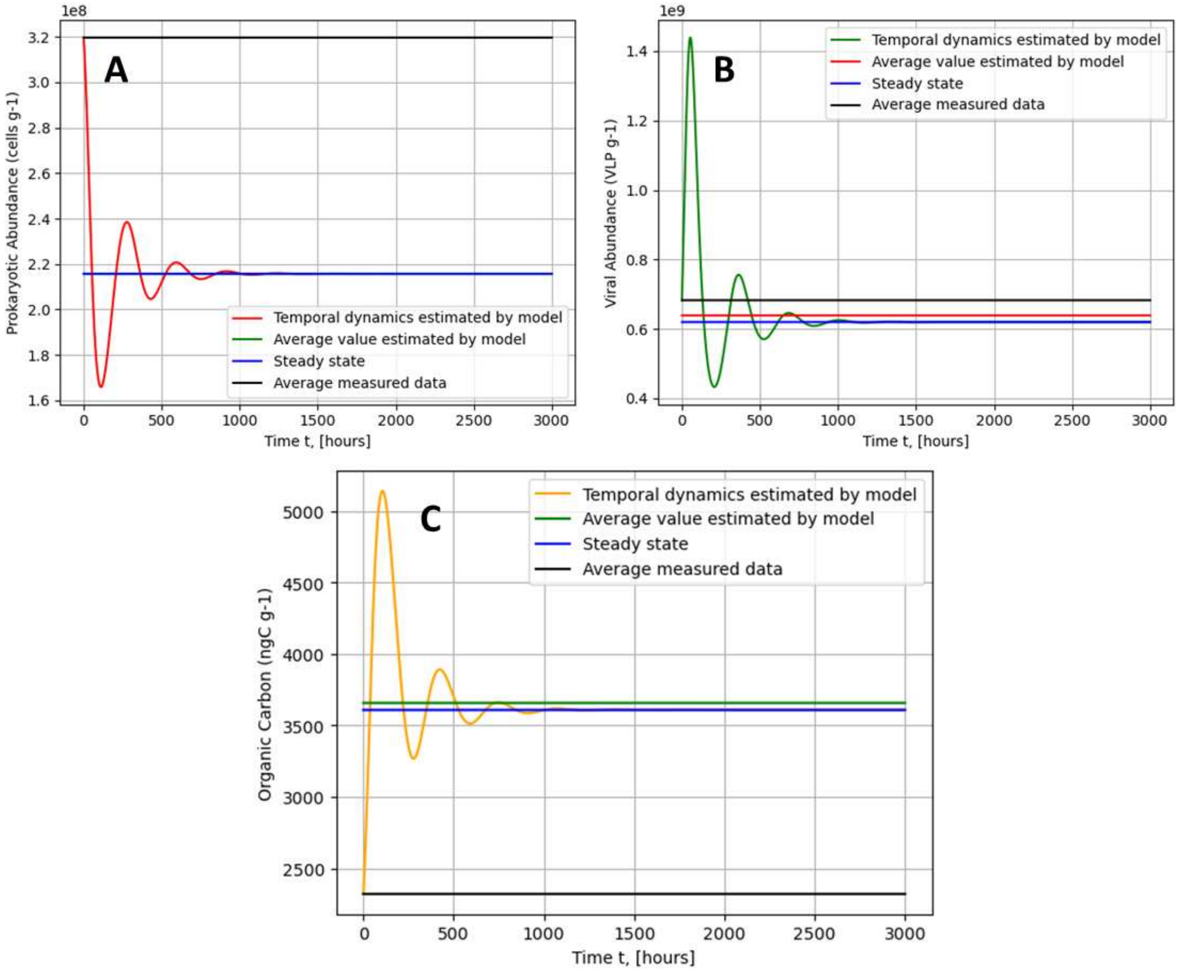


Figure 4.18 Analysis of solutions of the Generalized Lotka-Volterra model for 2002. The plot A shows the theoretical solution for prokaryotes in red, its mean value in black, the average of measured data in green and the steady state in blue. The plot B shows the theoretical solution for viruses in green, its mean value in black, the average of measured data in red and the steady state in blue. The plot C shows the theoretical solution for the organic carbon in yellow, its mean value in black, the average of measured data in green and the steady state in blue.

Analyzing the differences between our measured data and the steady states of the GLV after the strong deep winter convection and the following WMT, we observe that the dynamics show differences with the systems analyzed before, especially for viruses whose steady state solution is now far more distant from the measured data. The particular trends exhibited in the 2006 suggest that the system is affected by the rapid shift of environmental parameters (temperature and trophic resources), making the results in this year less reliable. Looking instead at the system in 2008, no longer affected by the climate-drive episodic event, the dynamics appear to be more regular, following dynamics similar to those of 2001 and 2002. In this case, the differences between our measured data and the steady states are: +76% for prokaryotes, +75% for viruses, while for trophic resources -37%.

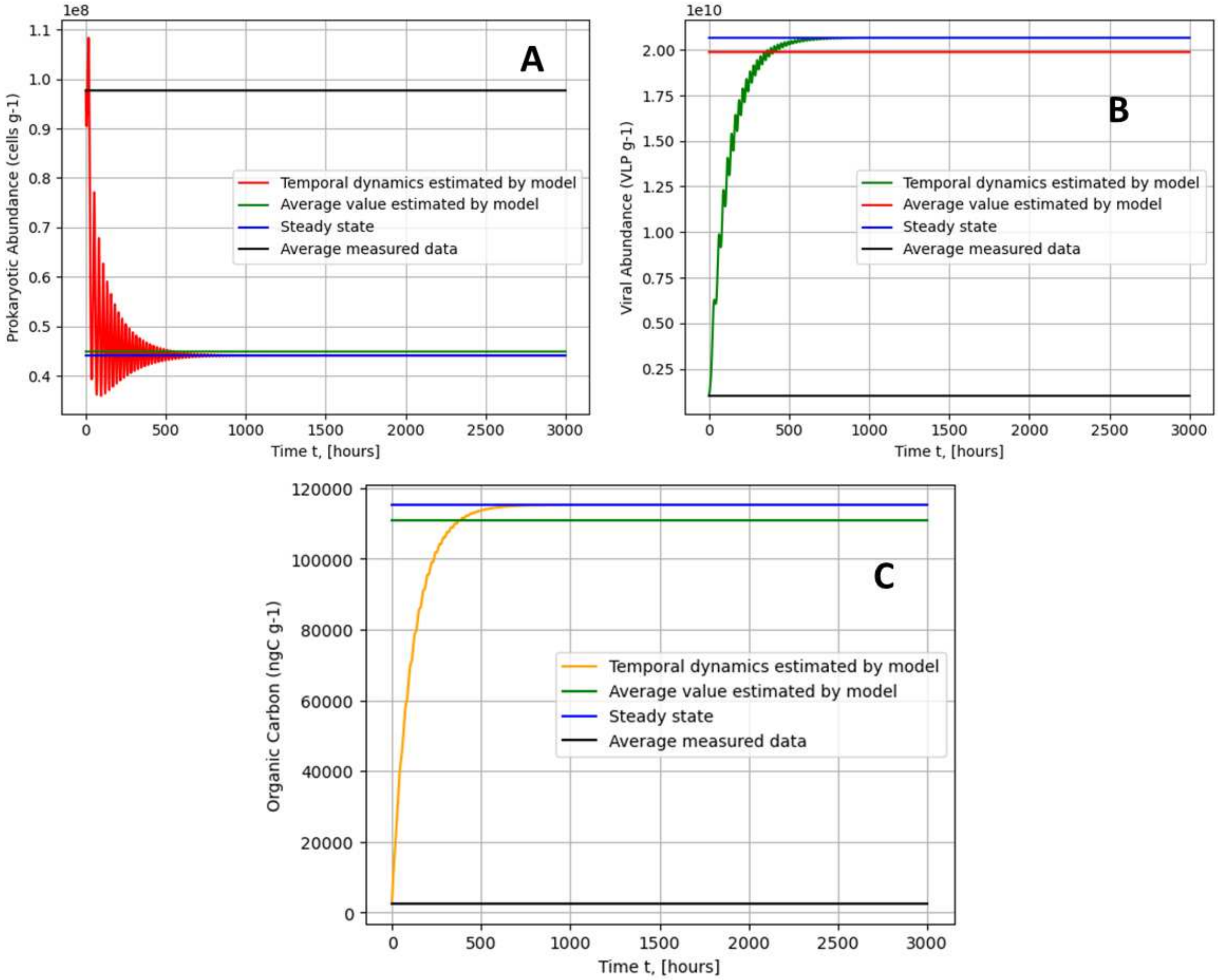


Figure 4.19 Analysis of solutions of the Generalized Lotka-Volterra model for 2006. The plot A shows the theoretical solution for prokaryotes in red, its mean value in black, the average of measured data in green and the steady state in blue. The plot B shows the theoretical solution for viruses in green, its mean value in black, the average of measured data in red and the steady

state in blue. The plot C shows the theoretical solution for the organic carbon in yellow, its mean value in black, the average of measured data in green and the steady state in blue.

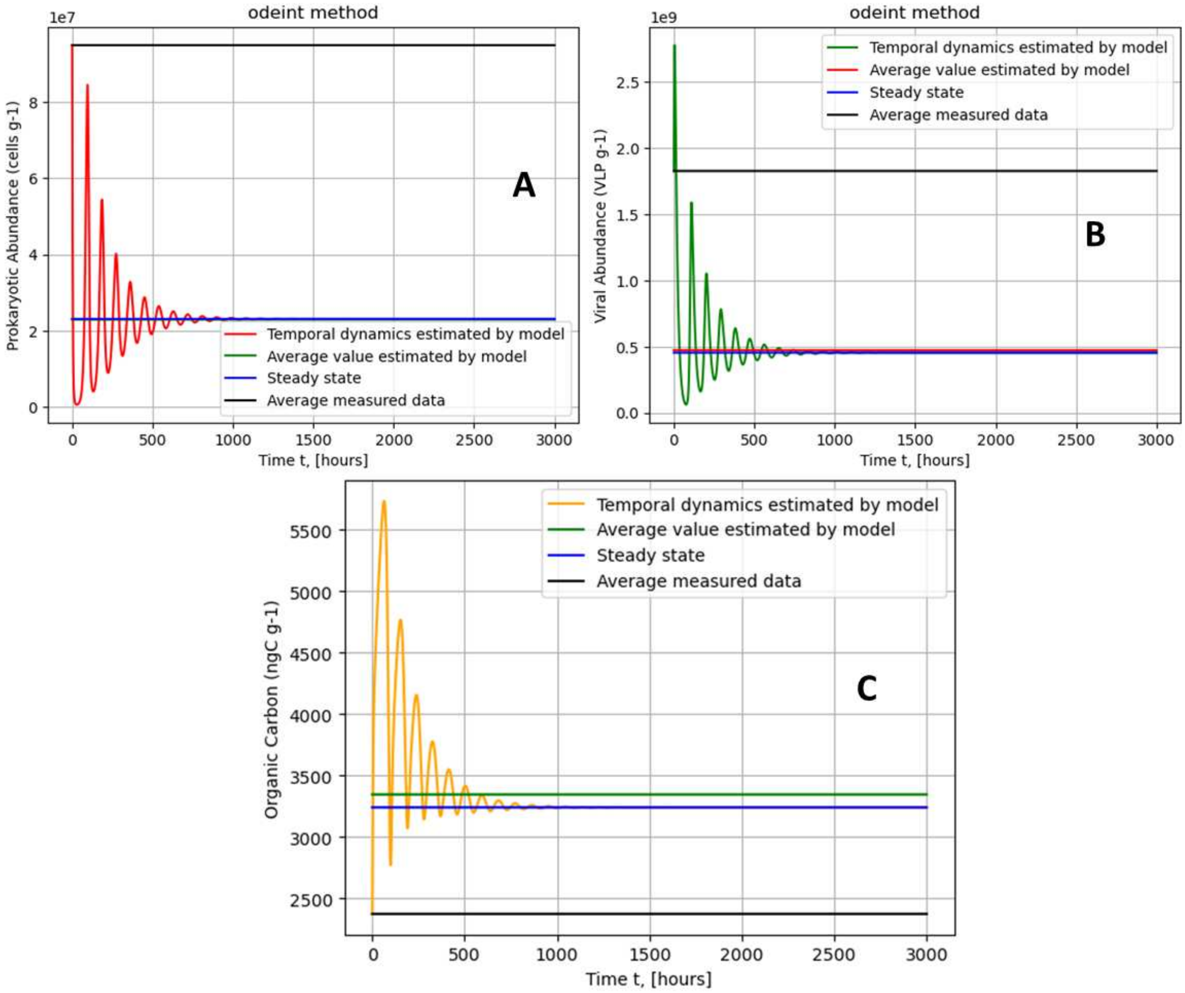


Figure 4.20 Analysis of solutions of the Generalized Lotka-Volterra model for 2008. The plot A shows the theoretical solution for prokaryotes in red, its mean value in black, the average of measured data in green and the steady state in blue. The plot B shows the theoretical solution for viruses in green, its mean value in black, the average of measured data in red and the steady state in blue. The plot C shows the theoretical solution for the organic carbon in yellow, its mean value in black, the average of measured data in green and the steady state in blue.

4.4.6 Generalized Lotka-Volterra model using Temperature as parameter and analysis of solutions

After verifying that the numerical solutions of the GLV exist for each year, we expressed each coefficient of the system as an exponential function of temperature (see Materials and Methods) and studied the behavior of the steady states as temperature varied within a range of approximately 0.3°C. From the results, we observed that viral abundance steady states decrease as temperature increases, following a curve concave downward, also prokaryotic steady states decrease but at a much higher velocity, while resources increase. However, the viral dynamic, contrary to what we would expect from the sampled data, suggests that this second approach fails to accurately explain the dynamics of our food web. Additionally, as we observed in the GLV of 2006, the episodic increase of carbon sinking from the surface creates a singularity in the dynamics of the trophic resources, which this approach is unable to intercept.

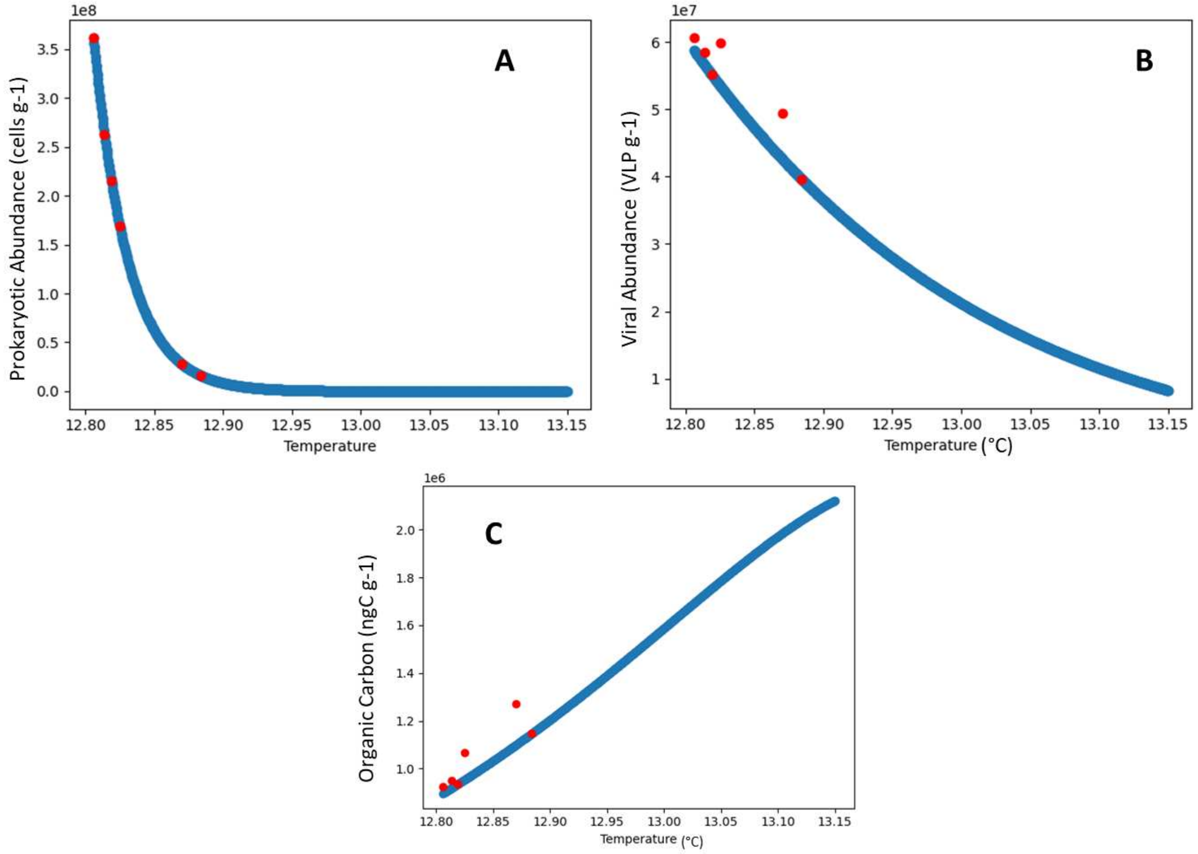


Figure 4.21 Analysis of steady states solutions of the Generalized Lotka-Volterra as temperature changes. The plot A shows the behavior of steady states solutions for prokaryotes, each point in blue represents a solution of the system with a particular value of temperature, the red points are the steady states of the GLV models configured for each site starting from the measured data. The plot B shows the theoretical solution for viruses in green, its mean value in black, the average of measured data in red and the steady state in blue. The plot C shows the theoretical solution for the organic carbon in yellow, its mean value in black, the average of measured data in green and the steady state in blue.

4.5 Discussion

It is now evident that climate change is generating various impacts globally (Levitus et al. 2005; Danovaro et al., 2011; Cherabier et al., 2022), even in the deep sea where the constancy of temperature over time is a well-known characteristic of the ecosystems (Danovaro et al., 2020). These impacts have also been observed in the water masses of the Mediterranean Sea where the deep water has been documented to become warmer since 1959 (Group et al., 2011; Kessouri et al., 2018), with a further acceleration after the mid-80s, attributed to a variety of forcing factors, including global warming. Moreover, the impacts of climate change may include the frequency and intensity of deep convections (Kessouri et al., 2018), such as the one documented in the Western Mediterranean basin in winter 2004/2005 (Schroeder et al., 2006; Borghini et al., 2014; Schroeder et al., 2016). All these phenomena have led to a change in the physical properties of the water masses known as the Western Mediterranean Transition, with increased salinity and temperature in the bottom layer (Zunino et al., 2012). Our study aimed to analyze for the first time the ecological consequences at the microbial and viral levels, where temperature and resource changes affect various ecological aspects, including metabolism and relationships (Llodra, 2002; McClain et al., 2012; Yasuhara et al., 2016).

Our data confirms that the phenomenon of deep winter convection and the consequent Western Mediterranean Transition (WMT) result in a shift of the system, with a much higher temperature increase compared to previous years (Fig 4.2; Schroeder et al., 2016). This change leads to consequences within the deep sea microbe-virus dynamics, both in terms of abundances and productions, as confirmed by the results of ANOVA, especially for the viruses (Fig. 4.3 and 4.4). These changes are linked to the increase in temperature and carbon flux to the bottom, two key factors capable of influencing the metabolism of both viruses and hosts such as the growth rate (López-Urrutia et al., 2007) and the attack rate (Morán et al., 2017; Lønborg et al., 2019; Morán et al., 2020). We have verified that the increase in carbon flux is not linked to an increase in primary productivity of the surface waters because the data of chlorophyll and Oregon primary productivity do not show anomalous variations over the decade. However, the Copernicus data of chlorophyll at the bottom shows how the effects of the deep winter convection (Zúñiga et al. 2008; Schroeder et al., 2008, 2016) lead to an increase both in bottom flux (up to 95 times) and in environmental variables as temperature and salinity. To relate the stock variables of abundances and the time-dependent measurements of productions, we adopted LV and GLV models since these mechanistic models allowed us to precisely understand the dynamics of our food web. The statistical approach through the sole identification of patterns would not have allowed us to delve into causal mechanisms, nor to explore more complex and nonlinear relationships, nor to understand which type of control weighed more on prokaryotic abundances between the bottom-up control of resources and the top-down control of viruses. Finally, based on the literature for biogeochemical models (Aumont et al., 2015; Kim et al., 2023), this approach based on differential equations allowed us to simulate scenarios in benthic deep sea ecosystems due to the effects of climate change on temperature and food availability (Wieczynski et al., 2023). Viruses play a crucial role in prokaryotic mortality in the world's oceans (Suttle, 2007), moreover, they release organic compounds from lysed cells, providing labile carbon for prokaryotes and enhancing their production (Danovaro et al., 2008; Shelford et al., 2014). This dynamic is particularly relevant in benthic deep-sea ecosystems, where viruses significantly contribute to prokaryotic mortality and exert important controls on microbe-virus dynamics and biogeochemical cycles (Danovaro et al., 2008; Danovaro et al., 2016b). Our study reveals, for the first time, that the increase in bottom water temperatures and carbon flux in deep Mediterranean Sea was accompanied by a rise in the abundance of benthic viruses. This increase, as indicated by our models, appears to be primarily linked to the raise of temperature (as shown in Fig 4.15B) that leads to higher viral productions due to the increased host metabolism (prokaryotic production), while the behavior concerning trophic resources requires further investigation. These findings broaden our understanding of the role of viruses in deep-sea sediments, highlighting that viruses not only regulate prokaryotic mortality but also respond sensitively to changes in both temperature and resource dependent prokaryotic metabolism. The impacts of viruses on deep-sea ecosystems and food webs can manifest both directly and indirectly. The reduction in prokaryotic abundance with increasing temperature (Fig 4.12B) is attributed to a stronger top-down control effect, leading to increased prokaryotic mortality at higher deep water temperature regimes. This direct effect of viruses as temperature increases opposes the increase in the growth rate, as described by the Arrhenius equation and confirmed by measured data. Prokaryotic dynamic depends on temperature and resources, nevertheless, the numerical solution requires further investigation, because the model suggests a decrease in the number of prokaryotes that cannot be explained concurrently with a decrease in viruses and an increase in trophic resources. Finally, we can observe in both approaches, but especially with the LV models (Fig 4.7,4.8,4.9,4.10), a substantial increase in the system's velocity. This creates a positive feedback mechanism, since the increase in carbon released from killed prokaryotes supports the growth of prokaryotic production, as explained by the López-Urrutia formula, and ultimately drives further viral production. Then, the higher viral production provides labile carbon that sustains and stimulates prokaryotic growth, given the reduced availability of organic resources in deep-sea sediments. Furthermore, considering the scarcity of resources, the increase in carbon linked to deep winter convection provides an additional boost to the system dynamic, that previously was mainly based on carbon released by prokaryotes compared to the contribution of carbon from external sources. When the bottom flux increases, due to the climate-driven episodic event, the system undergoes a change in the carbon dynamics with an increase in the weight of carbon flux that allows the transition to a new state of the system more accelerated. Once the flux effect subsides, the system returns to rely mainly on the carbon from killed organisms as source of food, but with a dynamic that remains accelerated, determining more carbon released from killed organisms compared to that of the years before 2006. In fact, if we exclude 1999 and 2001, the BPC (Bottom Particulate Carbon) at the bottom remains stable and unaffected, the only carbon consumed and released is the one from dead prokaryotes and sinking from the surface. And it is precisely the importance of carbon increase due to climate-driven episodic event that makes the approach with GLV inadequate, since a prerequisite of this model is that the carbon solution has to be continuous and regular. In conclusion, our results indicate that deep-sea ecosystems are sensitive to both temperature and resource changes and that climate-driven episodic events can create shift in the environmental parameters that can reflect in the microbe-virus dynamics with consequences as system dynamics acceleration and rise of viral abundance.

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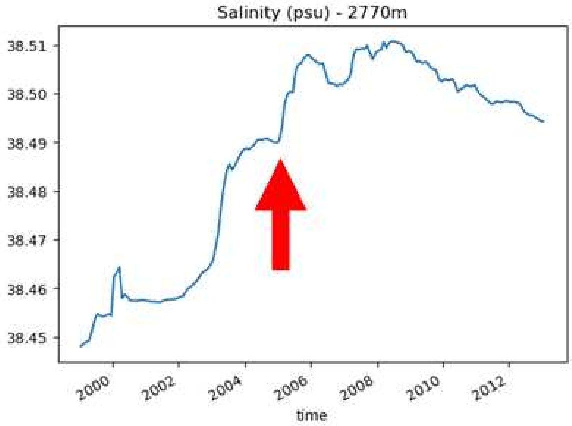
Supplementary Materials



Supplementary Figure 4.1 Temporal pattern of deep-sea water salinity. The plot A shows the temporal pattern of bottom water salinity in the bathyal plain of the Western Mediterranean Sea where sediment samples have been collected, the row indicates the year when the Western Mediterranean Transition happened.

Final conclusions

Viruses and prokaryotes are fundamental components of biogeochemical cycles and provide nourishment to higher trophic levels through their biomass. Therefore, investigating the relationships between them and the external environment is crucial, especially in light of climate change, which can lead to alterations in temperature and resource availability, causing irreversible effects. Indeed, it is important to understand these dynamics deeply to predict and prevent impacts even on these smallest components, which can then have cascading effects on the entire trophic network and consequently on ecosystem services.

However, the complexity of biological systems and climate change, which can provoke gradual as well as episodic effects, requires the use of increasingly sophisticated modeling and analytical techniques. This manuscript fits precisely within this framework, exploring with the assistance of biostatistical and biomathematical approaches the dynamics of virus-prokaryote interactions under different climate change scenarios, combining measured data with modeling information from platforms such as Copernicus and Oregon.

Our analysis of virus-prokaryote interactions under the effects of climate change began with an examination of microbial and viral, abundance and production, stocks to understand the current contribution of these two components to the total biomass of the biosphere, aiming to provide a more detailed estimate compared to previous ones by using a dataset among the largest and most detailed used so far. It was confirmed that viruses and prokaryotes are important components in the global carbon budget and its cycling. Furthermore, relationships between viruses and prokaryotes were investigated through regression analysis, revealing statistically significant and consistent relationships among ecosystems in terms of abundances. This confirms that viral abundance and distribution are closely dependent on prokaryotes in all the major ecosystems investigated on Earth.

The part of my study concerning global environmental drivers, despite our dataset being much more representative of marine ecosystems, has allowed us to understand which other relationships, beyond virus-host interactions, were significant and common across different ecosystems. This analysis indicated the minimum set of factors that a biogeochemical model must consider if it aims to offer reliable predictions for microbe-virus dynamics, especially in light of climate change. In this section, we have observed that temperature, bottom-up control of resources, and virus-prokaryote relationships are very important drivers with significant patterns in both viral and prokaryotic abundances and productions.

The information about the main drivers governing virus and prokaryote dynamics were used as the starting point to develop precise and detailed mechanistic models to explain microbe-virus interactions among themselves and with the external environment in the benthic deep sea ecosystem under different climate change scenarios. These insights were applied in a specific area of the Pacific Ocean, used as a proxy for a climate change scenario where only temperature was considered. The most effective approach in describing our data was a GLV system where the trophic resource was a solution of the system and temperature varied as a parameter. This allowed us to highlight how, in the deep sea, an increase in temperatures due to climate change could lead, for limited ranges of variation that do not alter the community, to a reduction in the prokaryotes number and an increase in viruses.

Finally, we further complicated our study by considering the effects of a climate-driven episodic event in the deep Mediterranean Sea, which led to an increase in temperature and resources at the bottom and thus caused a shift in the deep sea ecosystem (WMT). In this second scenario, climate change affected both temperature and resources, and in this case, the model that performed best was the LV with the MTE integration, which successfully explained the dynamics of viruses' growth and prokaryotes' reduction as temperature increases. However, it requires further investigation for the effects of the resources, since intercepting the effects of an episodic event using a model is not straightforward, given its velocity and magnitude. Nevertheless, it has shown that an increase in both prokaryotic and viral productions, due to the deep winter convection, leads to an increase in the oscillatory dynamics of microbial and viral abundances, persisting with the permanent shift in environmental conditions due to the WMT.

Overall, the findings reported in this PhD thesis indicate that climate change is an important driving force already affecting microbial and viral assemblages, even in the deep sea. Therefore, enhancing modeling approaches can certainly provide valuable insights for predicting the effects of climate change on microbial and viral dynamics which are fundamental for the proper functioning of biogeochemical cycles.