

Microbial ecology of the South Atlantic Subtropical Gyre: a state-of-the-art review of an understudied ocean region

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ABSTRACT

Understanding the dynamics of microbial plankton communities, their metabolic processes and taxonomic composition in oligotrophic ocean basins has been one of the central tasks of contemporary marine microbial ecology and one of the main challenges in a changing global ocean. However, despite its ecological importance, the South Atlantic Subtropical Gyre (SASG) remains poorly understood in relation to marine microbes, which comprise the major drivers of biogeochemical cycles and the largest carbon sink to the deep ocean. This review presents the state of the art of microbial ecology in the SASG, including the adjacent oligotrophic region, the Southwest Atlantic Ocean (SWAO). We have also addressed the theoretical and methodological trends in this field since 1970s, focusing on the main milestones that led to the more-detailed current knowledge of the role of oligotrophic gyres in the global carbon cycle. Finally, we discussed the general patterns of microbial community composition in the SASG, focusing on their potential responses to environmental factors. In spite of increasing efforts to investigate this region, SASG is among the least known oceanic provinces, which has hampered the debate over whether the oligotrophic ocean acts as a sink or source of CO₂ into the atmosphere.

Descriptors: Marine Microbes, Bacterial Production, Biogeochemical Cycles, Oligotrophic Oceans, Pelagic System.

INTRODUCTION

Microbial plankton is an essential component of marine ecosystems because of its key role in pelagic food-web dynamics and oceanic biogeochemical cycles, including the transport of carbon to the deep ocean (Azam et al., 1983; Cho and Azam, 1990; Gasol et al., 2008) and fixation of an important fraction of the total atmospheric carbon and nitrogen (Worden et al., 2015; Logares et al., 2020).

Marine microbes include a diverse class of heterotrophic and autotrophic prokaryotes as well as

unicellular eukaryotes (picoeukaryotes), with sizes ranging from 0.2 μm to 2.0 μm (Sieburth et al., 1978; Fuhrman, 1999). All these microbes, called picoplankton, have large population sizes, short generation times, and nearly unfathomable levels of aquatic biodiversity (Whitman et al., 1998; Thompson et al., 2017).

Bacteria and Archaea are the two domains of heterotrophic prokaryotes that, due to their ubiquity, abundance (on the order of 10⁶ cells ml⁻¹), high functional diversity and metabolic plasticity, comprise the majority of the biomass in the oceans (Fuhrman et al., 1989; DeLong, 1992). Although these two domains differ in some fundamental aspects of their metabolic features, they have been conventionally referred to as “bacteria” in prior studies. So, here we

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use the terms “bacteria” and “bacterial production” as a collective term referring to bacteria and archaea. The autotrophic picoplankton includes two main groups, photosynthetic cyanobacteria and the single picoeukaryotes, which may account for up to 64% of the total carbon biomass in oligotrophic open waters (Fogg, 1995).

The ecological and biogeochemical processes in the oceans are dependent on these microbial interactions, which comprise the major drivers of global biogeochemical cycles (Falkowski et al., 2008; 2012). The role of bacteria in marine ecosystems consists largely of consumption of dissolved organic matter, mainly as dissolved organic carbon (DOC) derived from primary production; the bacteria convert the dissolved carbon fraction into particulate organic matter by incorporating it (Fuhrman and Azam, 1980; Azam et al., 1983). Through this process, carbon that was theoretically inaccessible to heterotrophic organisms is reintroduced into food webs by microbes, making it available to higher trophic levels and the deep ocean, illustrating the fundamental role that bacteria play in the microbial loop (Azam and Malfatti, 2007; Pomeroy et al., 2007).

Understanding the dynamics of microbial plankton communities, their taxonomic composition and their metabolic processes has been one of the central tasks of contemporary marine microbial ecology and a main challenge in better understanding the role of microbes in a changing global ocean. During the last five decades, the growing efforts in this area have been impelled mainly by technological advancements and improvements that resulted in the findings that have led to the well-established concepts regarding the marine food web and carbon flow. Most recent approaches have yielded new insights into the role of heterotrophic bacterial production in oligotrophic oceans and in the biological carbon pump, and have also driven the development of new theories, such as the recently proposed microbial carbon pump (Jiao et al., 2014; Legendre, 2015; Zhang et al., 2016).

The implications of global changes for the bacterial community and carbon sinks have been discussed (Morán et al., 2015; Huete-Stauffer et al., 2016; Allen et al., 2020) and studies concerning marine microbial ecology in open ocean basins and subtropical oligotrophic gyres have assumed new importance.

The subtropical gyres, because of their immense size (~ 40% of the surface of the earth) play a significant role in the global carbon cycle and in sequestering atmospheric CO₂ through physical and biological processes (McClain et al., 2004; Brix et al., 2006). Therefore, understanding the interactions between these two processes within the subtropical gyres is a central task for determining the magnitude and variability of the carbon exported from the surface to the deep ocean, as well as the CO₂ exchange between the atmosphere and the ocean (Signorini et al., 2015).

The South Atlantic Subtropical Gyre (SASG), centered at approximately 50°W and 30/40°S, is a distinct biogeochemical province, with unique physical and biological characteristics relative to adjacent regions (Longhurst, 2010; Signorini et al., 2015). This anticyclonic gyre of approximately 4,500 km diameter is bordered by a number of major surface ocean currents and by one of the most oligotrophic areas in the entire ocean on the Brazilian continental shelf (Alves Junior et al., 2015), the Southwest Atlantic Ocean (SWAO) (Figure 1). Despite its ecological importance, the SASG remains little studied compared to other ocean basins.

In this review, we address the state of the art of microbial ecology in the SASG, including the oligotrophic adjacent region (SWAO). We also briefly address methodological trends and conceptual advances in this field, which have led to current well-established models of carbon flows in oligotrophic pelagic ecosystems, and the need for understanding whether the oligotrophic ocean is predominantly net autotrophic or heterotrophic. Finally, we discuss the general patterns found in the SASG based on the literature review, focusing on the mechanisms that shape microbial metabolism in this region.

MARINE MICROBIAL ECOLOGY - METHODOLOGICAL TRENDS AND CONCEPTUAL ADVANCES

Initially, in an attempt to quantify research contributions to marine microbial ecology over the last five decades, we searched for articles published in peer-reviewed and indexed journals from 1974 through 2020 by the Web of Science portal (www.webofscience.com). The first step of our search was conducted by crossing the terms “bacterioplankton”, “marine bacteria”, “microbial plankton”, “heterotrophic bacteria” or “marine bacterial production”, with the

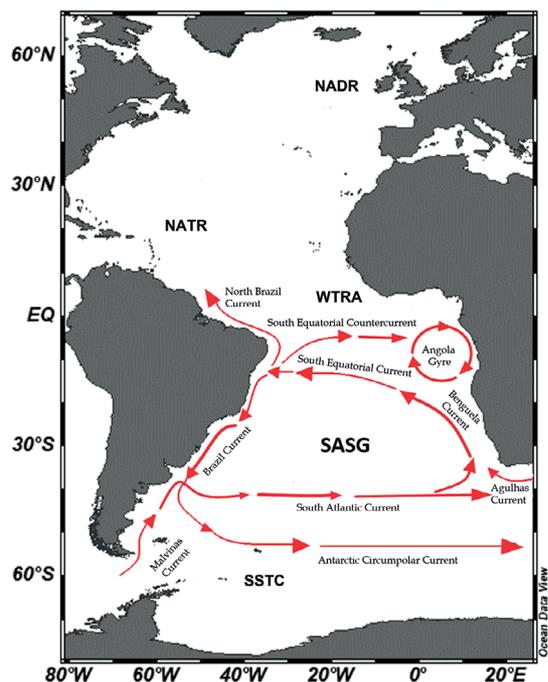


Figure 1. Schematic representation of major current systems that affect the South Atlantic Subtropical Gyre, and the five major biogeochemical provinces of the Atlantic Ocean: North Atlantic Drift Province (NADR), North Atlantic Tropical Gyre (NATL), Western Tropical Atlantic (WTRA), South Atlantic Subtropical Gyre (SASG) and the South Subtropical Convergence (SSTC). Data were plotted in Ocean Data View (Schlitzer, 2020).

keywords regarding to the main methods that contributed to the evolution of marine microbial ecology science: “epifluorescence microscopy”, or “thymidine”, or “leucine”, or “flow cytometry”, or “omics” (“metagenomics”, “metatranscriptomics”, “metaproteomics”, “metabolomics”, or “metabarcoding”). Since the pioneering work of Pomeroy (1974) was the starting point of this review, the sequential search included publications from 1974 through 2020. In a second step, to select only those studies concerning microbial ecology in marine environments, we conducted a new screening through a search of keywords in the title, and when this was not sufficient, by reading the publication abstract. Our compilation resulted in a total of 758 studies published in marine microbial ecology. However, the first published record on the Web of Science portal dates only from 1978.

In an attempt to provide an overview of conceptual advances that have led to current well-established models of carbon flows in oligotrophic

pelagic ecosystems, as well as the current debate over whether the oligotrophic ocean is predominantly net autotrophic or net heterotrophic, we provided a timeline highlighting the theoretical and methodological milestones over the last five decades (Figure 2). For this, we also considered the relevant studies in this field that were not registered in our search. In addition, for an overview of methodological trends, we compiled the results from our search by the annual number of publications involving each of the main methods (Figure 3 and Table S1). The main advances in microbial ecology from a historical perspective are addressed below.

MICROBIAL ECOLOGY IN OLIGOTROPHIC OPEN OCEAN BASINS - A HISTORICAL PERSPECTIVE

From the first perception of the high abundance of bacteria in marine environments to the current post-genomic age, great leaps in marine microbial ecology since the late 1970s have dramatically increased our knowledge in this area, which has become an extremely dynamic field of research in the last five decades. This exponential rate of progress, closely related to the development of technologies and advancement of precision methods, has led to the current high level of understanding of key ocean processes, including bacterial production (BP), respiration (BR), growth efficiency (BGE) and carbon flow through trophic interactions.

After the initial perception of the high abundance of bacteria in marine environments using the epifluorescence microscope and fluorescent dyes (Hobbie et al., 1977; Porter and Feig, 1980), the introduction of radioactive-isotope methods for measuring heterotrophic bacterial production (Fuhrman and Azam, 1980; 1982; Kirchman et al., 1985) inaugurated a new era in studies of microbial dynamics in aquatic environments.

Some early studies using the ^3H -thymidine and ^3H -leucine incorporation methods also investigated the coupling between BP and phytoplankton primary production (Hobbie and Cole, 1984; Kirchman et al., 1989). These first investigations were conducted in coastal waters (Pomeroy and Deibel, 1986), inner shelf regions (Fuhrman et al., 1985; Cho and Azam, 1988), estuarine plumes (Ducklow and Kirchman, 1983; Kirchman and Hoch, 1988; Malone and

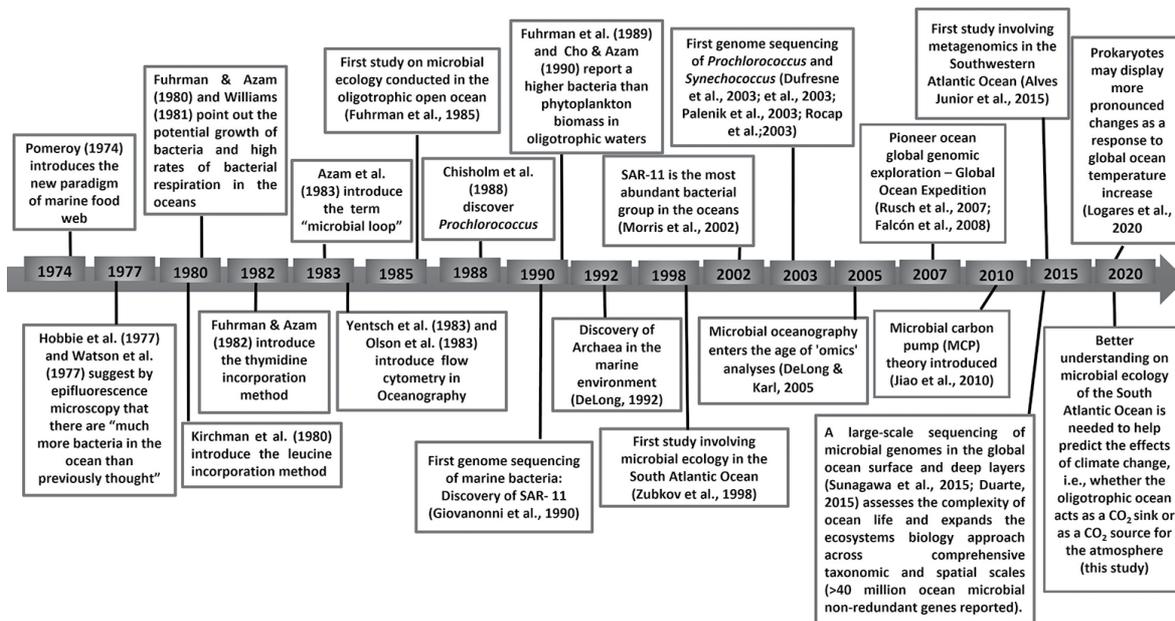


Figure 2. Timeline including the theoretical and methodological milestones over the last five decades (1974-2020) that led to current well-established models of carbon flows in oligotrophic pelagic ecosystems, and the need for understanding whether the oligotrophic ocean is predominantly net autotrophic or net heterotrophic.

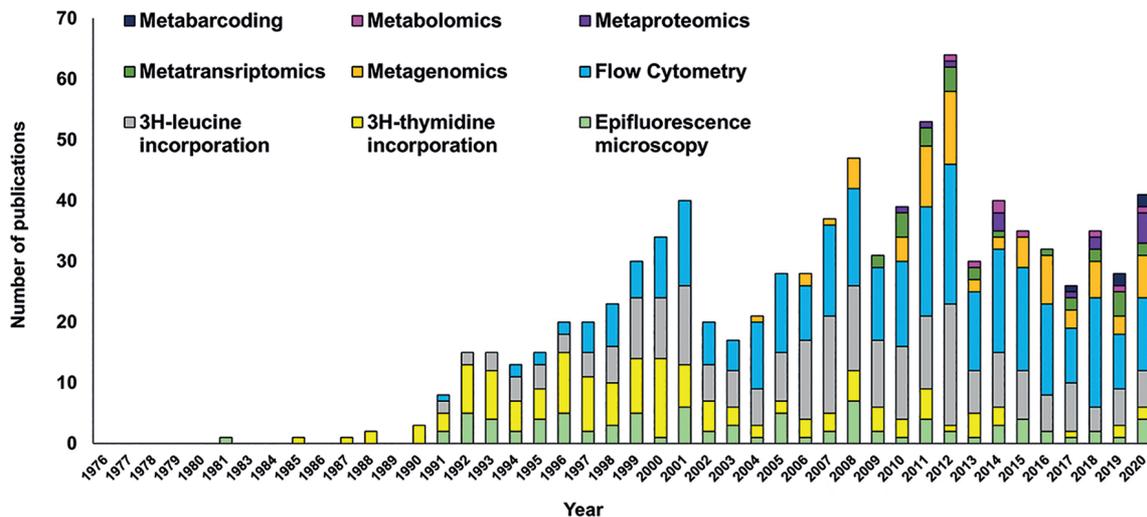


Figure 3. Annual number of citations of each methodology approach resulting from a search for publications on marine microbial ecology by the Web of Science website, from 1974-2020. The keywords "epifluorescence microscopy, 3H-thymidine, 3H-leucine, flow cytometry, metagenomics, metatranscriptomics, metaproteomics, metabolomics and/or metabarcoding" were crossing with "bacterioplankton", "marine bacteria", "microbial plankton", "heterotrophic bacteria" or "marine bacterial production".

Ducklow, 1990) and upwelling regions (McManus and Peterson, 1988) of the North Pacific and North Atlantic. Overall, the observations reported in these studies indicated changes in the microbial community along the open ocean to the coastal gradient, i.e.,

that bacterial biomass and activity were highest near the coast, tending to comprise <20% of the phytoplankton biomass and the total particulate organic carbon. However, the first measurements of BP in oligotrophic open oceans, conducted in the euphotic

zone of the Sargasso Sea and North Pacific Gyre, reported instances where bacterial biomass equaled the phytoplankton biomass (Li et al., 1992) or even exceeded the phytoplankton primary production (Fuhrman et al., 1989; Cho and Azam, 1990).

In part because of this variability, the differences between oceanic regions in microbial loop dynamics and in the fraction of primary production processed by heterotrophic bacteria remained unclear until the discovery of *Prochlorococcus* cyanobacteria (Chisholm et al., 1988), which completely changed the knowledge of the microbial plankton composition in oligotrophic waters.

Overall, these discoveries stressed the need to investigate the energy balance in different trophic systems and the major oligotrophic oceanic provinces. The energy balance can be expressed by the balance between gross primary production by phytoplankton (GPP) and community respiration (CR), in which bacterial respiration (R) can comprise a large proportion (50 to 90%) (Rivkin and Legendre, 2001). If $GPP > R$, the system is in a state of net autotrophy and can sustain the export, or sequestration, of dissolved oxygen, reduced carbon, and the potential energy that this represents. Otherwise, if $GPP < R$, the system is in a state of net heterotrophy and must import reduced carbon and oxygen to sustain life, also even suggesting an output of CO_2 from parts of the ocean to the atmosphere (Karl, 2007).

Until the end of the 1990s, however, microbial ecology in the SAO was completely unknown (Figure 4 and Table S3), and most of the studies of organic-carbon flow through bacterioplankton used empirical models (Del Giorgio et al., 1997; Del Giorgio and Cole, 1998). These studies suggested that the relationship between bacterial growth efficiency (BGE) and BP is variable and that BGE varies systematically with BP, and the trophic richness of the environment increases from marine regions to estuaries (Cole et al., 1988; Cho and Azam, 1988).

In attempt to better understand the coupling between phytoplankton and heterotrophic bacteria and the growing interest in the carbon cycle in the face of ocean global changes, in the last two decades, direct measurements of GPP and R came to be widely used to derive estimates of net community metabolism in the oligotrophic open ocean (Serret et al., 2001), including the first metabolic state assessments of the SASG (Gonzalez et al., 2002; Hoppe et al., 2002; Serret et al., 2002). These studies sometimes report a prevalence of heterotrophy ($GPP < R$) (Serret et al., 2001; Gonzalez et al., 2002; Hoppe et al., 2002; Duarte et al., 2013), and others a prevalence of autotrophy ($GPP > R$) (Williams, 1998; Serret et al., 2002; Williams et al., 2013; Tilstone et al., 2015).

In turn, some studies conducted along Atlantic transoceanic transects, have reported alternating patches between heterotrophic and autotrophic net community metabolism on both temporal (Aristegui and Harrison, 2002) and latitudinal scales (González

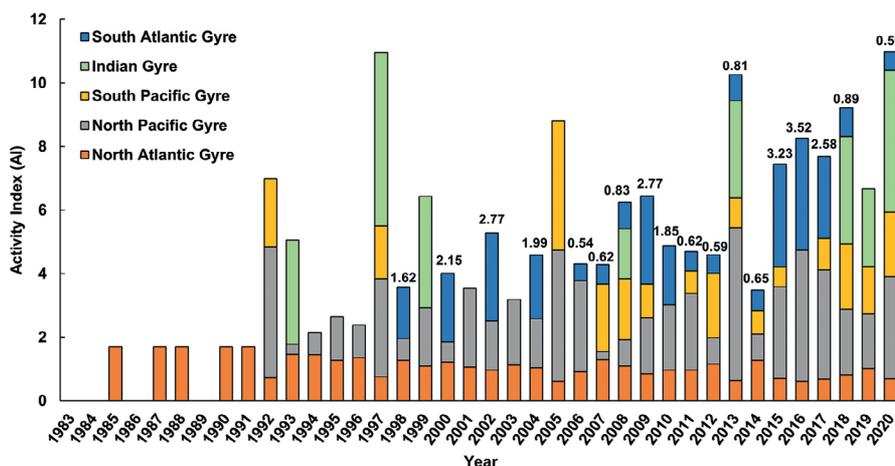


Figure 4. Cross comparison of Activity Index (AI) on marine ecology research from 1985 (year corresponding to the first study in the oligotrophic open ocean) to 2020, between the five major oligotrophic subtropical gyres: North Atlantic Gyre, North Pacific Gyre, South Pacific Gyre, Indian Gyre and the South Atlantic Gyre. The numbers above the bars correspond to AI calculated for SASG.

et al., 2002; Hoppe et al., 2002), with large net-heterotrophic regions. Overall, these studies have revealed seasonal and geographical patterns, suggesting that the plankton community structure influences the net ecosystem metabolism, thus limiting the predictive ability of GPP:R relationships. Further investigations conducted in the Pacific, Atlantic and Arctic oceans corroborated previous studies, showing that bacterioplankton communities differ among the temperate, subtropical, tropical and polar provinces (Falcón et al., 2008; Gasol et al., 2009; Westberry et al., 2012). These studies also suggest that the variability of R, and not only of primary production, needs to be considered in estimations of the ocean metabolic status (Serret et al., 2015).

Despite being intensively investigated, the metabolic balance of oligotrophic gyres remains controversial and an active debate about the carbon balance in oligotrophic pelagic marine ecosystems is still ongoing (Duarte et al., 2013; Williams et al., 2013; Tilstone et al., 2015), highlighting the need to improve knowledge about microbial metabolism in subtropical oligotrophic gyres.

Most recent approaches based on “omics” analyses (metagenomics, metatranscriptomics, metaproteomics, metabolomics and metabarcoding) have provided new insights into the metabolic potential of the oceans and biogeographic patterns (Morales et al., 2011; Stegen et al., 2013; Aylward et al., 2015; Lechtenfeld et al., 2015; Logares et al., 2020). Although these types of analyses do not provide direct information on bacterial production, they are essential to infer microbial community dynamics for the carbon cycle based on its genetic composition and metabolism (DeLong et al., 2006). Genomic and post-genomic analyses, paired with other approaches to evaluate bacterial production, have refined investigations of marine microbial ecology and biogeochemical processes in unprecedented detail. These analyses have also provided important insights to reach a predictive understanding of shifts of microbial communities in a changing global ocean, and consequently of potential changes in carbon flow in the pelagic system (Hutchins and Fu, 2017; Manrique and Jones, 2017; Allen et al., 2020; Grossart et al., 2020).

In turn, metagenomic analyses, designed to address questions related to genetic and biochemical microbial diversity, have revealed the astounding

diversity and heterogeneity contained in microbial communities, as well as biogeographic patterns based on metagenomic sequence similarity pertaining to the temperate Atlantic, tropical Atlantic and tropical Pacific oceans, including both coastal and open-ocean sites (Rusch et al., 2007; Falcón et al., 2008). In the last 15 years, important contributions in this field have been provided by ocean expeditions such as the Global Ocean Sampling (GOS) (Rusch et al., 2007), the Malaspina Circumnavigation (Duarte et al., 2015) and the Tara Oceans Project (Sunagawa et al., 2020), which also made it possible to address the question of how microbial communities adjust to global environmental variations on a planetary scale.

Most recent investigations are also focusing on advancing the understanding of how ocean changes such as warming and acidification may affect the biological carbon pump (BCP) and the microbial carbon pump (MCP), as well as the potential transition from dominance of the BCP to the MCP in oligotrophic oceans (Zhang et al., 2017). BCP depends on photosynthetic fixation of CO₂ and subsequent carbon export, driven mainly by sinking of particulate organic carbon. The MCP is a component of the BCP concept, used to describe the microbial production of refractory DOM (RDOM), which can be stored for millennia in the deep sea, rather than being respired as dissolved inorganic carbon and returned to the atmosphere (Jiao et al., 2010; Jiao and Azam, 2011). Both processes have significant effects on the air-sea CO₂ fluxes on century time scales (IPCC, 2013) and are crucial to ocean carbon flow and climate control.

CURRENT KNOWLEDGE OF MICROBIAL ECOLOGY IN THE SOUTH ATLANTIC SUBTROPICAL GYRE

DATA ASSESSMENT

To assess current knowledge of microbial ecology in the South Atlantic Subtropical Gyre, we performed a new screening from the results of the first step of our search (i.e. methodological approaches) on the Web of Science portal (www.webofscience.com). Through a search of keywords in the title, and when this was not sufficient, by reading the publication abstract, or yet, methodologies, we selected only studies conducted in oligotrophic areas of the SAO: the South Atlantic Subtropical Gyre (SASG) and the Southwestern Atlantic Ocean (SWAO) (Figure 5).

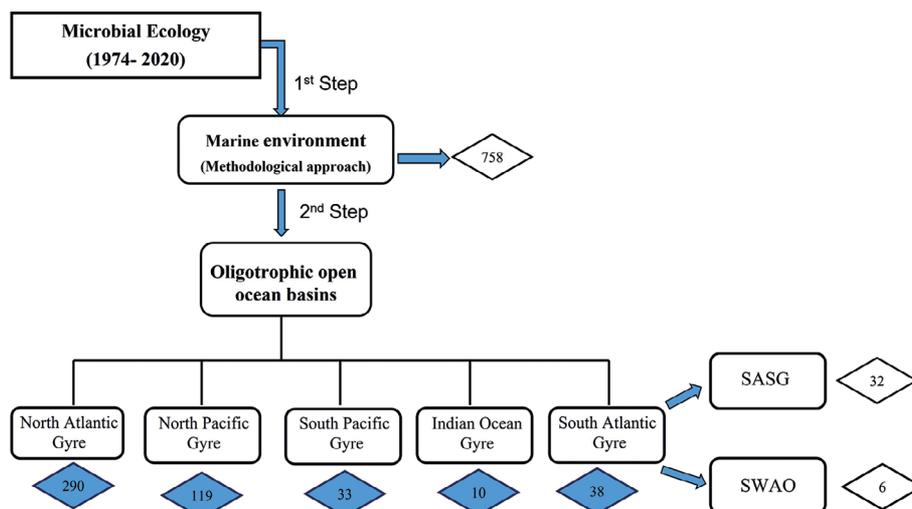


Figure 5. Schematic representation of the main steps of our searching strategy for publications on marine microbial ecology on SASG, through the Web of Science Portal. Black diamonds represent the total number of publications. Blue diamonds represent the total number of citations for the five major subtropical gyres: North Atlantic Gyre, North Pacific Gyre, South Pacific Gyre, Indian Gyre and the South Atlantic Gyre, from 1974 to 2020.

Our search involving microbial ecology in this region resulted in 38 studies, of which 32 were conducted in the open ocean area of the SASG and six in the southwestern Atlantic, comprising the South Brazilian Bight (SBB). This total number suggests that microbial ecology remains poorly studied in the SAO, compared to other oligotrophic ocean basins.

Considering that the simple numerical counting of the absolute number of selected articles can lead to erroneous conclusions (Kumari, 2006), we quantified these contributions by calculating an Activity Index (AI). Initially suggested by Frame (1977) and used in several scientometric assessments (Brusoni and Geuna, 2003; Thomaidis et al., 2000; Kumari, 2006), AI normalizes the publication profile, i.e. one region in relation to the others.

For a cross-comparison between the five major subtropical gyres (North Atlantic Gyre, North Pacific Gyre, South Pacific Gyre, Indian Ocean Gyre and the South Atlantic Gyre), we calculate the AI as follows:

Activity Index (AI) of given gyre g :

$$\frac{\{\text{Number of Publications in marine microbial ecology (MME) in gyre } g \text{ in year } y / \text{Total number of publications for all years for that gyre}\}}{\{\text{Total MME in all gyres output in year } y / \text{Total MME in all gyres output for all years}\}}$$

AI = 1 indicates that the number of research studies on the gyre is relatively equal to the other gyres.

AI > 1 indicates that research efforts on the gyre are higher than average compared to the others gyres

AI < 1 indicates lower research efforts on the gyre compared to the global output.

Despite the absolute number of publications pointing to a greater research effort in the North Atlantic Gyre and North Pacific Gyre (Table S3), the calculated AI showed a great effort in the SASG in some periods over the last twenty years in comparison with the other gyres in the southern hemisphere, mainly between 2015 and 2017 (Figure 4).

In addition, we present the main information from these 38 studies in the SASG and a database of available data on microbial parameters and environmental variables (Table S2). We also compiled the sampling stations where each study was conducted, in an attempt to provide an overview of the studied area in the oligotrophic SAO (Figure 6).

STATISTICAL ANALYSES

In order to explore general patterns of microbial parameters in the SASG, initially we applied multivariate analysis techniques to the current data (log-transformed) available for those listed by sampling station (Table S2). Principal component analysis (PCA) was applied for microbial plankton abundance (heterotrophic and autotrophic bacteria, eukaryotic picoplankton) and environmental variables (temperature, salinity and inorganic nutrients), to investigate the main environmental drivers of microbial abundance.

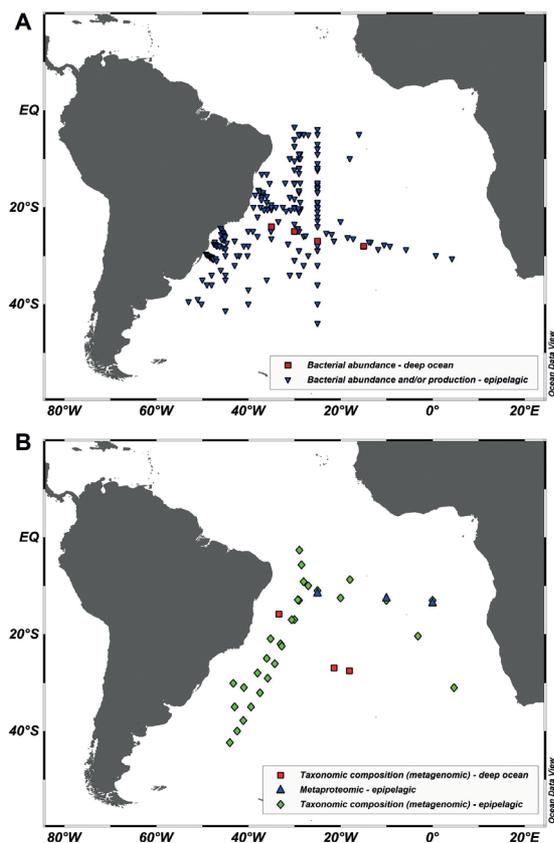


Figure 6. Sampling stations from studies conducted in the SASG and SWAO, extracted from the 38 publications resulting from our review. (A) Studies involving bacterial abundance by flow cytometry/epifluorescence microscopy and/or bacterial production by radioisotopic methods, and/or metabolic state by O₂ method; green triangles indicate studies conducted in the epipelagic zone, red squares indicate studies conducted in the deep ocean. (B) Studies involving metagenomic or metaproteomic analyses (taxonomic or functional composition); green circles indicate metagenomic analyses in the epipelagic zone, blue triangles indicate metaproteomic analyses in the epipelagic zone, and red squares indicate metagenomic analyses in the deep ocean.

Additionally, we performed descriptive statistics using box plots and ANOVA tests to visualize and analyze differences in microbial abundances between regions, seasons and depths. Pearson's correlations and simple linear regressions were also applied to measure the linear dependences between normalized biotic and abiotic data.

For all analyses, the dataset was classified according to the sampling location, SWAO or SASG (western and eastern boundaries of the SASG, or central gyre) and its respective layer (euphotic zone, depth maximum chlorophyll (DCM), or mesopelagic zone).

The PCA analyses were performed using the FactoMinerR package available in R statistical software version 3.6.3 (Lê et al., 2008). The box plots, followed by an ANOVA test, as well as the Pearson's correlation coefficient and the linear regressions, were performed using the software PAST version 4.02 (Hammer et al., 2001).

CURRENT KNOWLEDGE IN THE OPEN OCEAN OF SOUTH ATLANTIC SUBTROPICAL GYRE (SASG)

The pioneer studies concerning microbial ecology in the open ocean of the SASG date only from the last two decades (Figure 4 and Table S3). The first investigations in this region were based on a series of cruises of the Atlantic Meridional Transect (AMT) program which had as one of its main goals to advance understanding of the picoplankton community structure and function over a wide latitudinal range. Focusing on the role of the oceans in the global carbon cycle, these early studies compared the microbial composition (Zubkov et al., 1998; 2000a; 2000c) and heterotrophic bacterial production (Zubkov et al., 2000b) in the North and South Atlantic over a meridional transect from 50°N to 50°S. The composition of the picoplankton community, including heterotrophic bacteria, eukaryotic picophytoplankton, *Synechococcus* and *Prochlorococcus*, indicated that this area could be grouped into five large regions, presently called the North Atlantic Drift Province (NADR), North Atlantic Tropical Gyre (NATL), Western Tropical Atlantic (WTRA), South Atlantic Subtropical Gyre (SASG) and the South Subtropical Convergence (SSTC). These results also indicated that heterotrophic bacterial production is lowest in the North and South Atlantic gyres, tending to be highest where the phototrophic biomass is highest.

Over the same transect (AMT), González et al. (2002) investigated microbial metabolism using direct measurements of GPP:R. This study also showed that the region of the South Atlantic Gyre is less productive than the other Atlantic provinces, tending to a net heterotrophic balance (GPP<R). Hoppe et al. (2002) also investigated the net community metabolism of the South Atlantic Subtropical Gyre in an expanded meridional transect, from 53°N to 65°S. Their results were consistent with previous studies in this region, showing that the South Atlantic was

in net heterotrophic balance. In contrast, the study conducted by Serret et al. (2002) in the central SASG showed a prevalence of net autotrophy in this region ($GPP > R$), suggesting the existence of different trophic dynamics in similarly unproductive planktonic communities. Both studies also found that the ratio between BP and primary production was closely related to the meridional profile of water temperature, with a predominance of net heterotrophy in the warm oligotrophic regions.

Later studies also focused on the microbial metabolism underlined the influence of the regional and temporal variability of nutrient inputs on biomass and heterotrophic bacterial production (Hoppe et al., 2006; Vázquez-Domínguez et al., 2008; Zubkov et al., 2008; Gasol et al., 2009; Gist et al., 2009; Otero-Ferrer et al., 2018), reinforcing a global dependence of bacterial activity and production on the nutrient supply.

Focusing on the first predictions of organic matter inputs of atmospheric origin into the oceans (Dentener 2006; Duce 2008), two relevant studies involving microcosm experiments were conducted over a wide latitudinal transect in the Atlantic Ocean, including the central SASG (26°N to 29° S). Martínez-García et al. (2010) in an attempt to determine general patterns in the connection between the type of organic matter input, the initial biotic and abiotic conditions and the interactions between microbial compartments, showed that bacterioplankton responses were much higher than phytoplankton responses when both inorganic and organic nutrients were supplied, thus potentially driving the microbial community towards heterotrophy. Teira et al. (2010) investigating changes in the relative abundance of the bacterioplankton community composition at the level of major taxonomic groups observed that distinct groups responded differently to nutrient additions: SAR11 and Bacteroidetes responded negatively to organic and mixed additions, while *Roseobacter* spp. and Gammaproteobacteria responded positively. A clear increasing gradient from north to south in the magnitude of the Gammaproteobacteria response to organic inputs (containing carbon and nitrogen) suggested a strong link between bacterial community composition and carbon cycling in the oligotrophic ocean.

Other studies focusing on bacterial metabolic activities have assigned temperature as the main driver

of bacterial production. Hill et al. (2011) suggested that bacterial activity is a sensitive marker of gyre boundaries and distinct water masses. Mazuecos et al. (2015) through derived PGE indicated a strong correlation between R and PGE across a temperature gradient, suggesting that temperature variability in the mesopelagic zone plays a significant role in the remineralization of organic matter.

Evans et al. (2015) based on experimental results of light-enhanced uptake of methionine, leucine and ATP by *Prochlorococcus* and SAR11, provided new evidence of bacterial metabolism, indicating that photoheterotrophy is characteristic of dominant bacterioplankton populations in the global oligotrophic ocean and a widespread biological process in the SASG.

Still focusing on the metabolic state of subtropical oligotrophic gyres, Serret et al. (2015) and Tilstone et al. (2015) provided important contributions to the discussion that is still ongoing. To test the assumption that no regional differences exist in either the P:R relationship or the metabolic balance through the oligotrophic ocean, Serret et al. (2015) compiled vertical profiles of the *in-vitro* GPP, R and net community production (NCP) measurements made during the Atlantic Meridional Transect (AMT) cruises from 2000 through 2012. Based on the differences in both GPP and R, the authors demonstrated the existence of systematic differences in the metabolic state of the oligotrophic North and South Atlantic gyres, reinforcing the previously suggested theory that the oligotrophic ocean is neither auto- nor heterotrophic, but functionally diverse. With the main goal of deriving accurate estimates of net community production, Tilstone et al. (2015) compared a number of empirical approaches using primary production data from satellites with *in-vitro* measurements of changes in dissolved O₂ concentrations in the North and South Atlantic Ocean. Their results showed that the South Atlantic Subtropical Gyre fluctuated from being net autotrophic in austral spring-summer, to net heterotrophic in austral autumn-winter, indicating that recent decadal trends suggest that the SASG is becoming a more important source of CO₂.

Finally, in terms of deriving the metabolic state of SASG, García-Martín et al. (2017) conducted direct measurements of bacterial respiration along north to south transects (50.45°N to 44.33°S), including the

dissolved O₂ concentration. Results from this study showed a significantly higher cell-specific bacterial respiration in the temperate and upwelling regions compared with oligotrophic gyres, including the SASG, which suggests that bacterial carbon turnover is slower in this province. The study also showed that the bacterial contribution to depth-integrated community respiration varied widely within provinces (4–77%), suggesting that the proportion of total community respiration attributable to bacteria is similar in the different oceanographic regions studied.

Concerning “-omics” analyses, the first studies using genomic approaches in the SASG have agreed with these previous studies, also showing different patterns of latitudinal distribution of the taxonomic composition and diversity of the bacterial community. Schattenhofer et al. (2009) and Morris et al. (2012) showed that SAR11 bacterioplankters are less abundant in the South Atlantic than in the North Atlantic, and that the biomass of *Prochlorococcus* reached its peak in this region. Morris et al. (2010), conducted the first and unique metaproteomic study in the SASG and indicated that the microbial community structure also shifted along the nutrient concentration gradients, from the open ocean to the coast. Swan et al. (2014) were the first to investigate the genomic and metabolic diversity of Thaumarchaeota, one of the most abundant and cosmopolitan chemotrophs within the global dark ocean. This study, however, included only one sampling station in the mesopelagic zone of the SASG.

Important contributions for current knowledge of microbial structure, diversity, function, activity and biogeographical patterns in the SASG were provided by the Tara Oceans Project, Malaspina Circumnavigation Expedition and the GEOTRACES cruises. Metagenomic data from these global expeditions are available from Sunagawa et al. (2015), Tully et al. (2017), Biller et al. (2018) and Sanz-Saéz et al. (2020).

Overall, these studies assessed patterns of diversity and marine stratifying factors of the microbial community composition from different depths, generating unprecedented amounts of environmental sequencing data and making it possible to address the question of how microbial communities respond to global environmental variations. The Malaspina

Expedition, in turn, provided an unprecedented assessment of the state of the dark ocean, allowing for the first global assessment of bacterial abundance in mesopelagic and bathypelagic waters of the main ocean basins. Data on the bacterial abundance in the SASG deep ocean are available from Pernice et al. (2015). Tully et al. (2017) reconstructed microbial genomes from these metagenomics samples, presenting 360 additional draft genomes from the SAO samples for Bacteria and Archaea.

In light of the need to understand the current and future configuration of marine microbes in the global ocean, recent studies examined the ecological mechanisms that shape these microorganisms in surface waters on a planetary scale. From samples obtained from the Tara Ocean Project and the Malaspina Expedition, Logares et al. (2020) identified patterns using amplicon sequencing data, suggesting that the surface water picoplankton may not show a single response to global change, and that perhaps prokaryotes will display more pronounced changes in their community structure as a response to temperature increase than picoeukaryotes.

Finally, the most recent study on SASG analyzed a collection of 31 metagenomes including the epipelagic, mesopelagic and bathypelagic zones (Coutinho et al., 2021). By revealing that many of these genomes are derived from poorly characterized taxa of Bacteria and Archaea, these results reinforce the lesser attention that the SASG microbiome has received compared to other regions of the global ocean.

CURRENT KNOWLEDGE OF THE SOUTHWEST ATLANTIC OCEAN (SWAO)

Microbial communities in the Southwest Atlantic Ocean (SWAO) off Brazil are beginning to be investigated, but studies conducted in the southwestern portion of the SAO, especially on the inner shelf of the South Brazilian Bight (SBB), have also provided evidence of the dependence of bacterial production on the nutrient supply, the coupling of heterotrophic bacteria and primary producers, as well as the temporal variability in the metabolic state of open oligotrophic waters.

The SWAO is one of the most oligotrophic areas in the entire ocean (Alves Junior et al., 2015); the waters on the Brazilian continental shelf, which

are predominantly autotrophic, are fertilized during spring and summer by the onshore intrusion of the oceanic South Atlantic Central Water (SACW), a cold and nutrient-rich water mass (Castro et al., 2006).

The first study concerning bacterial dynamics in the SWAO (Andrade et al., 2004) was conducted over a broad area of the Atlantic Ocean, from São Tomé Cape (ST Cape; 22°S, 41°W) to the Salvador coast (13°S, 38°W), and off the Brazilian oceanic islands Trindade and Martim Vaz (20°S, 29°W). The authors conducted a preliminary characterization of bacterioplankton in this region and evaluated the heterotrophic bacterial abundance and production over this large spatial scale. Their results addressed the relationship of bacteria distribution and activity to upwelling phenomena in this area of the southwestern Atlantic Ocean.

In the same region, Andrade et al. (2007) investigated the distribution pattern of the high nucleic acid content (HNA) and low nucleic acid content (LNA) bacteria, and their relationship to physical and chemical features. Their results showed the distribution of these groups in two different oceanic provinces, from coastal waters to the open ocean: HNA bacteria were dominant in warmer waters with riverine nutrient inputs, and LNA bacteria were the dominant cells in regions with higher nitrate levels.

To assess the distribution of pico- and nanoplankton communities in different water masses on the inner shelf of the South Brazilian Bight (SBB), Ribeiro et al. (2016a; 2016b) determined the abundance of heterotrophic bacteria, *Prochlorococcus*, *Synechococcus* and autotrophic pico- and nanoeukaryotes along three transects extending from 23°S to 31°S and 39°W to 49°W. The results obtained from this study agree with observations in other marine areas, where *Synechococcus* and autotrophic eukaryotes dominate the mesotrophic waters, while *Prochlorococcus* dominates in oligotrophic areas. Using the same approach, Bergo et al. (2017) assessed the influence of a summer intrusion of the South Atlantic Central Water (SACW) on the spatial and vertical dynamics of plankton abundance and carbon biomass across environmental gradients. Regarding the importance of each group to carbon biomass partitioning, the authors showed that the dominance of heterotrophic bacteria is governed by upwelling conditions, while the relative contribution of each phytoplankton group

is more evenly distributed when the SACW is not as influential.

Finally, we highlight the first metagenomic study conducted in the SWAO, which covered the Abrolhos Bank and the Campos Basin (from -08° to -23° latitude), both in the southwestern Atlantic (Alves Junior et al., 2015). This study was the first attempt, and unique until now, to characterize the taxonomic and functional community diversity along the depth continuum and different water masses in the SWAO and to compare this diversity with the microbial community diversity of the global ocean. The results showed that the SWAO has a significant proportion of endemic genetic diversity and a unique microbial signature. SWAO microbial communities share genes with the global ocean in at least 70 cellular functions; however, more than a third of reported genome sequences comprise a unique gene pool in the global ocean. This interesting study suggests that the SWAO is maybe a hotspot of microbial diversity.

DISCUSSION

Despite the facilities provided by the method used in our search for scientific articles, it is possible that some restrictions in this database have resulted in an underestimation of our results concerning studies on marine microbial ecology. Thus, we must consider our result (n=758) as a rough estimate of the total scientific publications over these years, which in turn allowed us to address the methodological trends and the main conceptual advances in this field over the last five decades, regarding oligotrophic pelagic ecosystems.

These same facilities allowed us to easily access publications that have at least one sampling station in the region of interest (SASG/SWAO), and compile the number of publications in each of the five main subtropical gyres used to calculate the AI (Figure 5).

Despite the last two decades of experimental and field observations, four central issues have hampered efforts to effectively improve knowledge of the mechanisms that shape microbiological and biogeochemical processes in this region: 1) the controversy over its metabolic balance/state, 2) the main drivers of biogeographic patterns in the microbial community composition, 3) lack of knowledge about taxonomic composition, 4) spatial and temporal undersampling, especially in deep layers.

Since the balance between net autotrophic and heterotrophic production by direct measures of GPP and R was the most investigated topic in the SASG, the spatial distribution and temporal variability of the sampling, as well as differences due to the use of different methods, may explain the lack of agreement on its metabolic state.

Concerning the differences in spatial distribution, the first investigations conducted in the SASG already suggested the existence of different trophic dynamics within the gyre. Gonzales et al. (2002) and Hoppe et al. (2002) investigating the western boundary of the SASG showed a net heterotrophic balance ($R > GPP$), while Serret et al. (2002) investigating the central SASG showed a prevalence of net autotrophy ($GPP > R$). A series of other studies have also reported differences in the metabolic state of the SASG, including seasonal trends (Vasquez-Dominguez et al., 2008; Zubkov et al., 2008; Gasol et al., 2009; Serret et al., 2015; Tilstone et al., 2015). Overall, these studies reinforce the existence of regional and temporal variability in the trophic dynamics of the gyre, suggesting that the influence of nutrient inputs may be more important than temperature in the regulation of bacterial growth characteristics on a large spatial scale.

Later studies focusing on bacterial metabolic activities, however, attributed temperature as the main driver of biomass and heterotrophic bacterial production (Hill et al., 2011; Mazuecos et al., 2015; Logares et al., 2020). The geographic pattern of the net community metabolism was also attributed to the functional diversity of the SASG pelagic ecosystem, highlighting to the relative importance of local vs. allochthonous sources of organic matter. Since the responses of heterotrophs and autotrophs to nutrient inputs are different (Martínez-García et al., 2010; Teira et al., 2010), it is suggested that different processes control bacterial dynamics in oligotrophic waters.

Over the last five years, the advancement of metagenomic studies in the SASG, as well access to the taxonomic and genomic content of global ocean microbial communities (Sunagawa et al., 2015; Tully et al., 2017; Biller et al., 2018; Logares et al., 2020; Sans-Saéz et al., 2020; Coutinho et al., 2021), have provided new evidence of the diversity and functional patterns of this region. This new evidence has also emerged with new questions, suggesting that the geographic pattern of net community metabolism

may be related to the functional diversity of each ocean basin.

This variability between regions and years is expected due to differences in the physicochemical characteristics and microbial community composition. In an attempt to find general patterns in the SASG that would illuminate some of these questions, we conducted statistical analysis using microbial abundance and environmental parameters of data currently available (Table S2). These analyses allowed us to explore some of these issues and provide support to answer the following questions: Are there any geographic, seasonal and/or depth patterns in the abundance and diversity of microbial plankton subgroups in the oligotrophic SAO (SASG/SWAO)? Are there different patterns of these variables within the gyre (SASG)? What are the main environmental drivers shaping these microbial parameters in these regions?

The first PCA analysis ($n = 334$) applied to both regions of oligotrophic SAO (SASG/SWAO) indicated that the first two components are sufficient to explain 90.6% of the total variance of microbial and environmental variables (Figure 7A). PC1 explained 57.9% of the data variability, while PC2 explained 32.7% of the variability. The highest contributions to PC1 were eukaryotic picoplankton, PO_4 and NO_2^- , while the highest contributions to PC2 were temperature, heterotrophic bacteria and NO_3^- . These results showed a strong relationship between heterotrophic bacterial abundance and temperature, and between autotrophic picoplankton and NO_3^- concentration in both regions. The biplot graph also showed the grouping of samples according to the spatial (SASG vs. SWAO) and vertical distribution. For samples from mesopelagic zones, it is possible to notice similarities for both regions. For the euphotic zone of the SWAO, no spatial distribution pattern was observed.

To further explore spatial patterns in the SASG, we applied an additional PCA ($n = 170$) including only the different areas of the gyre (central (SASG), western boundary (WB-SASG) and eastern boundary (EB-SASG)). For this PCA analysis (Figure 7B), the two components explained 99% of the total variance. PC1 explained 76% of the variability, while PC2 explained 23%. Heterotrophic bacteria, salinity and PO_4 were the major contributors to PC1, while nitrate and eukaryotic picoplankton were the main contributors to PC2. This biplot indicated a clear vertical and geographic pattern, showing that in the euphotic zone

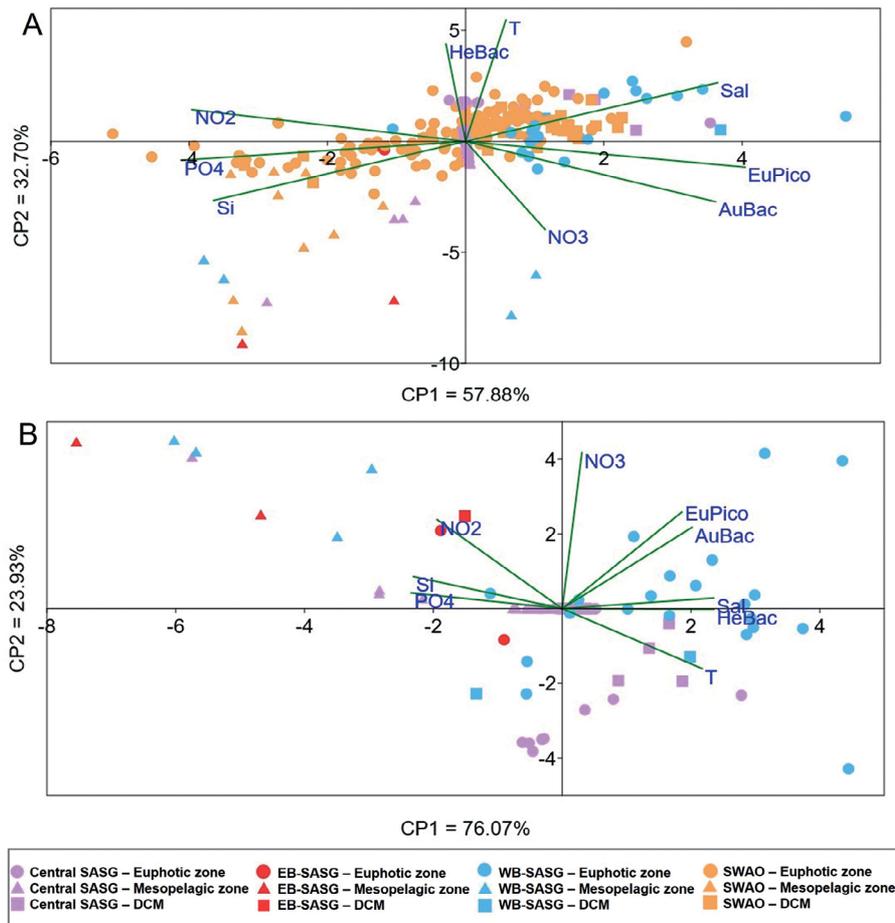


Figure 7. Biplots of the principal component analysis (PCA) of microbial abundances from flow cytometry (heterotrophic bacteria (HeBac), autotrophic bacteria (AuBac), eukaryotic picoplankton (EuPico)) and environmental parameters (temperature (T), Salinity (Sal), Phosphate (PO₄), Nitrate (NO₃), Nitrite (NO₂) and Silicate (Si)) for the oligotrophic regions of SAO. Circles represent the data in the euphotic zone, squares represent the samples from the depth of chlorophyll maximum (DCM), and triangles represent the samples in the mesopelagic zone. The size of the lines indicates the contribution of the components. Different colors represent the different regions: (lilac) central SASG, (red) eastern boundary of the SASG (EB-SASG), (blue) western boundary of the SASG (WB-SASG), (orange) the oligotrophic SWAO. (A) PCA applied for SASG and SWAO. (B) PCA applied only for the different regions in the SASG.

of the western boundary of the SASG, autotrophic picoplankton and NO₃ are positively correlated, while heterotrophic bacteria are more connected with the temperature in the euphotic zone of the central and western boundary of the SASG.

The analysis of the box plots, followed by verification of the result of the ANOVA test, both performed to investigate the differences in microbial abundance between regions, seasons and depths, indicated significantly higher abundance (p -value<0.001) of heterotrophic bacteria in the SWAO region compared to the SASG (Figure 8A), but lower abundance of autotrophic bacteria when compared to the western boundary of the SASG (WB-SASG) (Figure 8D). When contrasting microbial abundance between seasons,

heterotrophic bacteria showed a more homogeneous distribution, whereas autotrophic bacteria were significantly (p -value<0.001) higher in autumn than in spring or summer (Figure 8E). Interestingly, the abundance of eukaryotic picophytoplankton did not show significant differences between seasons or between study regions (Figure 8G and H). Regarding depth, significant differences (p -value<0.01) in microbial abundance were found along the water column, being significantly lower in the mesopelagic zones than in the DCM and euphotic zones (Figure 8C, F and I).

In addition, the analysis of Pearson's correlation coefficients (r) (Table 1) showed a strong positive correlation between temperature and heterotrophic

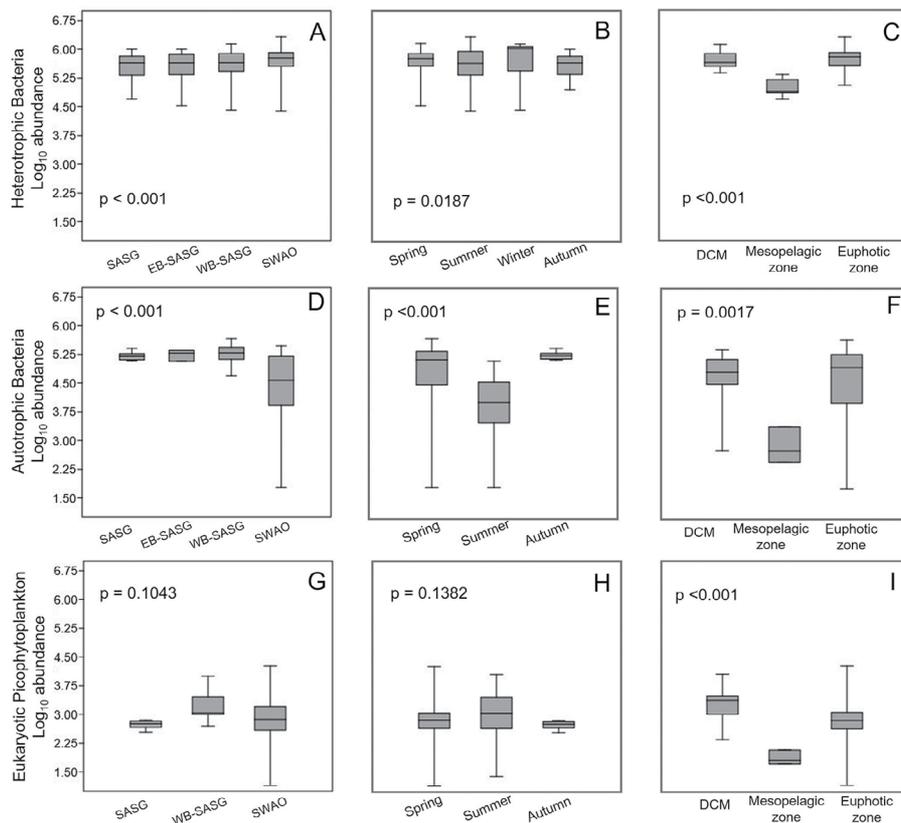


Figure 8. Box plots of microbial abundance data from flow cytometry (Log₁₀ abundance): (A-C) Heterotrophic bacteria, (D-F) Autotrophic bacteria, (G-I) Eukaryotic picophytoplankton. Data are grouped according to study region (central SASG (SASG), eastern boundary of the SASG (EB-SASG), western boundary of the SASG (WB-SASG), and SWAO), season (spring, summer, autumn and winter) and pelagic zone (DCM, mesopelagic and euphotic zone). The p-values obtained in the ANOVA tests are indicated in the graphs, for each data group.

Table 1. Pearson's correlation coefficient (r) between microbial abundances from flow cytometry (heterotrophic bacteria (HeBac), autotrophic bacteria (AuBac), eukaryotic picoplankton (EuPico)) and environmental parameters (Temperature (T), Salinity (Sal), Nitrate (NO₃), Nitrite (NO₂), Phosphate (PO₄), Silicate (Si), and Chlorophyll fluorescence (Flu)).

	HeBac	AuBac	EuPico	Depth	T	Sal	NO ₃	NO ₂	PO ₄	Si	Flu
HeBac	1										
AuBac	0.512	1									
EuPico	0.534	0.466	1								
Depth	-0.654	-0.518	-0.197	1							
T	0.714	0.511	0.190	-0.646	1						
Sal	0.210	0.121	-0.050	-0.264	0.634	1					
NO₃	-0.635	-0.666	-0.232	0.661	-0.787	-0.486	1				
NO₂	0.291	-0.082	0.204	-0.036	0.185	0.017	0.110	1			
PO₄	-0.356	-0.336	-0.217	0.457	-0.604	-0.528	0.629	0.066	1		
Si	-0.295	-0.117	0.193	0.414	-0.643	-0.660	0.598	-0.048	0.800	1	
Flu	0.255	0.258	0.569	0.168	0.017	0.017	-0.070	0.347	-0.068	0.126	1

bacterial abundance ($r = 0.71$) and a moderate positive correlation with the abundance of autotrophic bacteria ($r = 0.51$), which were corroborated by linear

regressions (Figure 9). Depth was negatively correlated with heterotrophic ($r = -0.65$) and autotrophic ($r = -0.52$) bacterial abundance, which was also revealed

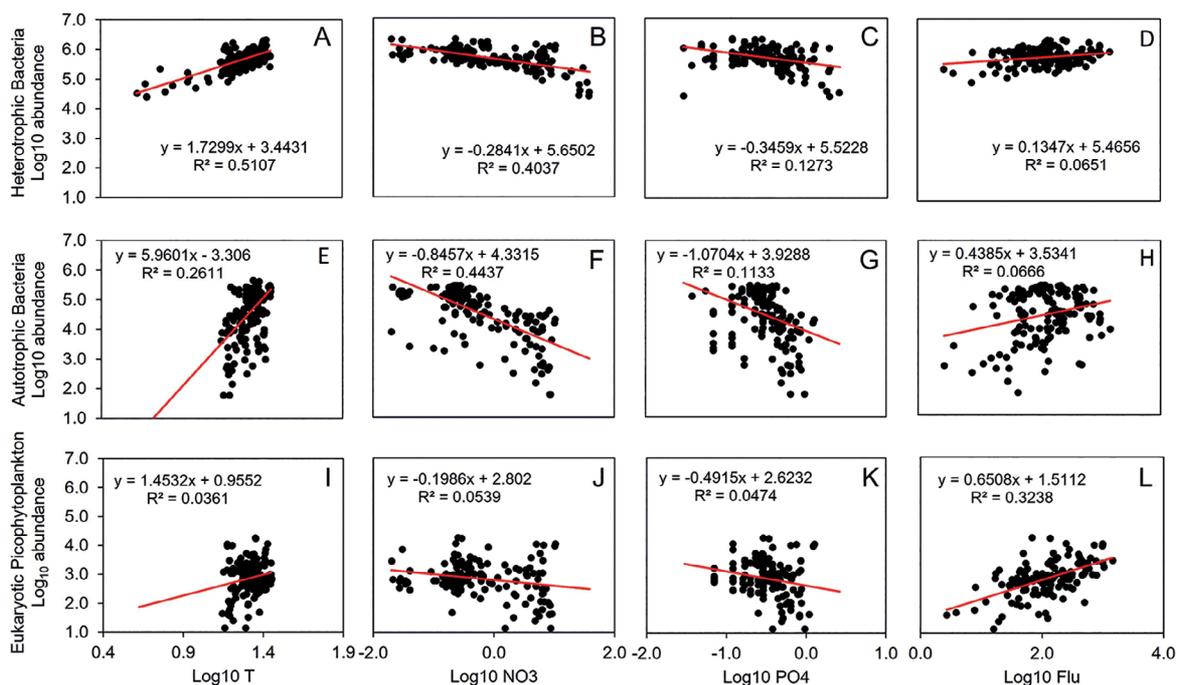


Figure 9. Linear regressions between microbial abundance data from flow cytometry and environmental parameters (temperature (T), nutrients (NO₃ and PO₄) and chlorophyll fluorescence (Flu)): (A-D) Heterotrophic bacteria, (E-H) Autotrophic bacteria, and (I-L) Eukaryotic picophytoplankton.

by box plot analyses. Nutrient concentrations, mainly NO₃ and PO₄, also showed negative correlations with bacterial abundance. This trend was especially observed for autotrophic bacteria *versus* nitrate (Figure 9F). The abundance of eukaryotic picophytoplankton showed a positive correlation with chlorophyll fluorescence ($r = 0.57$), which was corroborated by the linear regression (Figure 9L).

Overall, these analyses support biogeographic patterns of microbial communities in different areas of oligotrophic SAO (SASG vs. SWAO), between two areas within the gyre itself (i.e., marginal vs. central), as well as differences between seasons and depths. They also indicate the relative importance of different local vs. allochthonous sources of organic matter on microbial structure, suggesting that SASG is predominantly autotrophic, acting as CO₂ sink.

At an ocean-wide scale, different environmental conditions regulate the growth characteristics of picoplankton. While the growth of autotrophic bacteria and eukaryotic picophytoplankton are supported by nutrients, the growth of heterotrophic bacteria is partially supported by organic compounds produced by phytoplankton cells and remineralization of

inorganic nutrients (Azam et al., 1983). Thus, due the coupling between phytoplankton and heterotrophic bacteria, changes in the microbial community are expected along a nutrient gradient and under different environmental conditions. This explains the highest abundance of heterotrophic bacteria in SWAO compared to SASG, and the highest abundance of autotrophic bacteria in the SASG.

The growth of heterotrophic bacteria in SWAO benefits from increased organic matter derived from phytoplankton communities and allochthonous sources (Susini-Ribeiro et al., 2013, Brandini et al., 2014). The growth of autotrophic bacteria in WB-SASG is influenced by the seasonal (spring-summer) intrusion of the nutrient-rich South Atlantic Central Water (SACW) along the adjacent continental shelf of SWAO (Castro et al., 2006, Ribeiro 2016b, Bergo et al., 2017), which promotes fertilization of the euphotic layer through a shift from regenerated to new production (Metzler et al., 1997). In the most central SASG and eastern boundary, the highest abundance of autotrophic bacteria is supported by *Prochlorococcus*, which dominate in more oligotrophic areas (Partensky et al., 1999). Regarding the differences between seasons,

the lower abundance of autotrophic bacteria in spring and summer can also be explained by the upwelling phenomena on the SWAO continental shelf (Ribeiro et al., 2016b, Bergo et al., 2017), which favors the growth of larger phytoplankton cells. With respect to eukaryotic picophytoplankton, their ability to move in the water column in response to different light and nutrient gradients to improve their optimal growth conditions (Raven, 1998) may explain their positive correlation with chlorophyll fluorescence.

Since photosynthesis (GPP) and respiration (BP) mediate the exchange of CO₂ between the oceans and atmosphere (Duarte et al., 2013, Tilstone et al., 2015), this strong link between microbial plankton communities and environmental conditions may provide indication of their role in the carbon cycling in the oligotrophic ocean, and therefore be appropriate to monitor the effect of possible changes in these conditions at the microbial level (Hoppe, 2006). Thus, considering that temperature seems to affect more prokaryotic assemblages than picoeukaryotes, and that prokaryotes would present more pronounced changes in their community structure in response to temperature increase on a planetary scale (Logares, 2020), the need for improvements is clear for providing data in open ocean basins and poorly studied subtropical oligotrophic gyres, such as the SASG.

Previous studies also corroborate these statements, indicating shifts in the structure and diversity of microbial metabolism induced by the sea surface temperature warming and ocean acidification (Sarmiento et al., 2010; Morán et al., 2015; Huete-Stauffer et al., 2016, Louca et al., 2016). Experimental studies using microcosms have also revealed the different responses of food web components to warming, through changes in biomass, production, respiration, and lower growth efficiency (Hoppe et al., 2008; Wohlers et al., 2009; Sarmiento et al., 2010; Lara et al., 2013). Reductions in bacterial size, through genome sizes, have also proved to be a short-term response to increasing temperature in experimental incubations (Morán et al., 2015; Huete-Stauffer et al., 2016). Moreover, the composition of bacterioplankton community changed consistently in response to elevated CO₂ at the ultraoligotrophic center of the South Pacific Gyre (Allen et al., 2020).

In view of the direct and indirect evidence that indicate changes in tropical and subtropical

oligotrophic gyres (Hill et al., 2011; Hu et al., 2017; Zhang et al., 2017), a shift in the balance between autotrophic production and heterotrophic consumption of organic matter is expected. A community dominated by smaller cells, mainly heterotrophic bacteria, would favor respiration of carbon in the microbial loop and reduction of the rate of carbon sequestration. The consequences of these changes for ocean ecosystems can be summarized as a potential reduction in the transfer of organic matter to higher trophic levels, bacterial losses to their grazers (Wohlers et al., 2009) and weakening of the biological and microbial carbon pump, with a positive feedback to the rising atmospheric CO₂ (Evans et al., 2011; Morán et al., 2015; Zhang et al., 2017) and potential changes in carbon flow in the pelagic system.

All of these issues support the need to conduct large-scale cruises and long-term monitoring programs across the SASG, in an attempt to integrate data on bacterial production, primary production, microbial abundance, taxonomic and genetic diversity. In line with the debate over whether the oligotrophic ocean acts as a CO₂ sink or CO₂ source to the atmosphere, efforts including empirical, theoretical and modeling approaches would help provide data to effectively improve knowledge about the SASG and support more accurate predictions in a changing global ocean.

CONCLUSIONS

In light of the exponential rate of progress in marine microbial ecology in the last few decades, our attempt to address the state of the art on the South Atlantic Subtropical Gyre encouraged us to also explore the dynamics of conceptual and methodological advances related to carbon flows and the role of oligotrophic gyres in the global biogeochemical cycles. In this context, the production of a timeline and compilation of publications in a historical perspective allowed us to summarize the main conceptual and methodological milestones that led to the current high level of understanding of the key oceanic processes with regard to microbial communities and the biological carbon pump. Furthermore, our overview of microbial ecology in oligotrophic open ocean basins allowed us to reinforce that better knowledge of subtropical oligotrophic gyres is a central task to determine the magnitude and variability of carbon

exported from the surface to the deep ocean, as well as the exchange CO₂ between the atmosphere and the ocean.

In turn, our compilation of publications on marine microbial ecology in SASG helped to highlight that the oligotrophic SAO is still among the least known oceanic provinces. Despite growing research efforts in this region over the past two decades, the lack of knowledge about the metabolic state, the main drivers of biogeographic patterns and taxonomic composition, together with undersampling, have hampered efforts to effectively improve knowledge of this region.

Ultimately, our metadata analysis helped reveal biogeographic patterns of microbial communities in different areas of oligotrophic SAO, highlighting the relative importance of different local vs. allochthonous sources of organic matter for the coupling between phytoplankton and heterotrophic bacteria in this region, suggesting that SASG is predominantly autotrophic, acting as a CO₂ sink.

Since previous studies have pointed to temperature as a key variable for shaping microbial structure in open ocean basins, current evidence of climate change and ocean warming from the last IPCC reports challenges the idea that a shift in the balance between autotrophic production and heterotrophic consumption in SASG would favor a positive feedback for increasing atmospheric CO₂.

Therefore, research efforts concerning marine microbial ecology in open ocean basins and subtropical oligotrophic gyres have assumed new importance in predicting the implications of global change for the microbial plankton community, carbon flow in the pelagic system, and CO₂ exchanges between the ocean and atmosphere.

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AUTHOR CONTRIBUTIONS

L.R.F.: Data curation, Visualization, Writing - original draft, Writing - review & editing;

S.B.P.: Data curation, Writing - original draft; Statistical analyses

L.S.M., G.M., C.G.: Data curation

R.M.L.: Conceptualization, Supervision, Writing - review & editing;

C.N.S.: Conceptualization, Supervision, Writing - review & editing.

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