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Sedimentary organic matter accumulation provinces in the Santos Basin, SW Atlantic: insights from multiple bulk proxies

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Abstract

The organic matter (OM) of surface sediments (0–2 cm) from the Santos Basin was characterized by employing elemental (C, N, P), isotopic ($\delta^{13}C$ and $\delta^{15}N$), biochemical (total carbohydrates, proteins, and lipids), and pigment (chlorophyll-*a* and phaeopigments) proxies, as part of the Santos Project – The Santos Basin Regional Environmental Characterization (PCR-BS) – coordinated by PETROBRAS. Samples were collected in a regional scale throughout the entire basin, including 11 isobaths from eight cross-margin transects ranging from 25 to 2,400 m water depths, and 12 additional samples obtained from the São Paulo Plateau. The aims of this study were two-fold, namely, to identify the major oceanographic and depositional processes driving OM transport and accumulation and to provide information on OM origin and availability to benthic secondary producers. The multiple employed proxies and the use of machine learning allowed to categorize distinct OM accumulation provinces displaying two major regional features: shallow sediments (< 50-m isobaths) are OM-poor (TOC < 2.0 mg g^{-1}) but the available amount is readily hydrolysable, and mid-shelf deposits (75-m and 100-m isobaths) throughout the entire basin present muddy and OM-rich sediments (TOC ranging from 7.0 to 14.0 mg g^{-1}) seemingly available to heterotrophs. Similarly, relatively OM-rich deposits are found at depths ranging from 700 to 1,300-m isobaths throughout the basin, but their composition and nutritional value (as suggested by biochemical indicators) are not related to mid-shelf deposits. The general overview of OM accumulation and composition provided herein validated the selected organic geochemical proxies for benthic ecology studies at the shelf and deep ocean sectors of the studied region.

Keywords: Organic matter, Cross-margin sediment transects, Bulk properties, Isotopic composition, Biopolymeric carbon

INTRODUCTION

Continental margins are characterized by complex physical-biogeochemical interactions and are responsible for mediating the transfer of energy, organisms, and both natural and anthropogenic materials between continents and the open ocean (Levin and Sibuet, 2012). Continental margin ecosystems provide globally relevant key functions, goods, and services, although the footprint of multiple human activities, *i.e.,* fisheries, aquaculture, navigation, eutrophication, fossil, and renewable energy exploitation, among others, are increasing threats to the biodiversity and sustainable use of these habitats (Liu et al., 2010). These concerns have induced the development of integrated ecosystem-based tools in the management of alterations caused by natural variability, climate change, and human interventions, as already proposed, for example, for the North Sea (Piet et al., 2019) and the Mediterranean (Manea et al., 2020).

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One major feature of related ecosystems is their role in the sources, fates, and exchanges of organic matter (OM) in the ocean margin. For instance, high primary production rates induced by nutrient availability derived from upwelling events, continental runoff, and ground-water discharges result in over 90% of the global carbon stored in continental margin sediments, even though these regions represent only circa 11% of the total ocean area (Hedges and Keil, 1995; Jahnke, 2009; Bauer et al., 2013). In addition, the balance between OM production/import and respiration in the water column and the rates of sinking organic particle velocities and sediment remineralization coupled with hydrodynamics and sedimentological characteristics define the ultimate amount and quality of OM provided to deep-sea (> 200 m water column depths) benthic organisms (Pusceddu et al., 2009; Abballe and Chivas, 2017; Bao et al., 2018; Ausín et al., 2021; Fanelli et al., 2021).

Concerning the Brazilian continental margin, knowledge on OM sources, nature, transport, and accumulation of in surface sediments has increased in the last two decades (Yoshinaga et al., 2008; Oliveira et al., 2013; Alves et al., 2014; Carreira et al., 2015; Lourenço, Magalhães, et al., 2017 a; Magalhães et al., 2017; Ceccopieri et al., 2018; Cordeiro et al., 2018; 2019; Ramos et al., 2019; Mahiques et al., 2020; 2021, 2022). However, most assessments are either limited by the areal coverage, focusing on shallow (< 200 m water depth) or deep (200–3,000 m water depths) portions of the margin, with few exceptions (Yoshinaga et al., 2008; Ceccopieri et al., 2018, 2019; Cordeiro et al., 2018). In this context, we present herein a multi-proxy OM characterization, including elemental (C, N, and P), isotopic ($\delta^{13}C$ and $\delta^{15}N$), biochemical (total hydrolysable carbohydrates, proteins, and amino acids), and phytopigment indicators. The dataset comprises top core (0-2 cm) sediments collected from nine cross-shelf transects (25 – 2,400 m water depths) along the Santos Basin, on the South-eastern Brazilian continental margin.

The regional overview of bulk sedimentary OM properties provided herein aims to contribute to a better understanding of the environmental processes that define the nature and type of OM that accumulates in heterogeneous sedimentary habitats that occur along bathymetric gradients in the study region. The obtained dataset can also shed light on processes driving OM bioavailability to deep-sea benthic organisms, adding to the ongoing efforts represented by the Regional Environmental Characterization of the Santos Basin (PCR-BS) project towards implementing an integrated ecosystem-based approach in the management of future multiple uses of the Brazilian continental margin portion represented by the Santos Basin (Moreira et al., 2023).

METHODS

Study area and regional setting

The Santos Basin (Figure 1) comprises approximately 350,000 km^2 and is located on the south-eastern Brazilian continental margin, which extends from Cabo de Santa Marta, in the south (28° 30' S, 49° 00' W), to Cabo Frio, in the north (23° 00' S, 42° 00' W). The continental shelf at the Santos Basin varies from 73 to 231 km, with a slope declivity between 1:656 and 1:1333 and shelf break located between 120 m and 180 m water depths (Mahiques et al., 2004). The northern portion of the shelf, in the vicinity of Cabo Frio, is narrower compared with the south portion. Both sectors are marked by crossed narrow and long channels that connect shallow areas to the shelf break and favor sediment transport to deeper regions. The most evident ones are the Cabo Frio, Rio de Janeiro, Ilha Grande, Cananéia, and Itajaí channels (Zembruscki, 1979; Ferreira et al., 2020).

The water circulation pattern on the southeastern Brazilian margin is mainly controlled by the flow of the Brazil Current (BC) towards the south (Soutelino et al., 2013), which transports Tropical Waters (TW) from the tropical South Atlantic in its upper levels and South Atlantic Central Waters (SACW) between 200 and 700 m water depths (Campos et al., 2000). The Brazil Current meandering and eddies are observed in the study region in response to morphology and bathymetric variations along the coast, inducing SACW intrusion onto the mid-shelf and shelf-break (Silveira and Patchineelam, 1996; Campos et al., 2000). Particularly in the northernmost limit of the Santos Basin, in the Cabo Frio region, the sudden change in the shoreline orientation associated with prevailing NE winds are responsible for an intermittent surface SACW upwelling (Lorenzzetti and Gaeta, 1996; Campos et al., 2000; Castelao and Barth, 2006; Franchito et al., 2008).

The SACW intrusions, both near the coast and/or in the mid-shelf and shelf break, increase nutrient concentrations and enhance primary production rates to > 0.5 g C m^{-2} d^{-1} (Brandini et al., 2018 and references therein), promoting a biomass export to the adjacent oligotrophic (< 0.04 g C m^{-2} d^{-1}) continental shelf and open waters dominated by warm TW (Marone et al., 2010; Brandini et al., 2018).

Sediment transport and accumulation dynamics vary along the Santos Basin shelf according to the bottom topography and hydrodynamic fronts, such as the BC meandering, resulting in a complex and heterogeneous array of sedimentary provinces over the shelf and slope (Mahiques et al., 2004; Ferreira et al., 2020). Sedimentation rates are strongly associated to primary productivity, continental inputs, and the BC flow, varying from ca 5 mm kyr^{-1} on the shelf break of the northern portion of the basin up to 660 mm kyr^{-1} in the vicinity of the Cabo Frio upwelling system (Lourenço, Magalhães, et al., 2017). The input of terrigenous materials by the drainage basin is less important in the northern portion, with contributions from small rivers and Guanabara Bay (Carreira et al., 2012; Albuquerque et al., 2014), and eventually from the Paraíba do Sul river, whose discharge occurs outside the limits of the Santos Basin (Souza et al., 2010). On the other hand, inputs of local coastal systems in the southern portion, like the Itajaí River and the Babitonga, Paranaguá, and Santos bays (Lourenço, Martins, et al., 2017; Santos et al., 2020), as well as the more distant contribution from the Patos Lagoon and the La Plata River, are all relevant nutrient, sediment, and contaminant sources to the shelf (Burone et al., 2013; Ferreira et al., 2020; Bícego et al., 2021).

The modern surface sediment in the Santos Basin region is composed of very thin siliciclastic sands and silts with variations in clay and calcium carbonate contents in distinct Santos Basin sectors (Mahiques et al., 2004, 2010). One relevant Santos Basin characteristic comprises the occurrence of mud-depocenters at shallow waters, both along the 100 m isobath and in the mid-slope (Ferreira et al., 2020; Timoszczuk et al., 2021). The occurrence and distribution of mud belts and carbonate sediments are being detailed in the ongoing PCR-BS project (Figueiredo et al., 2023).

Sampling

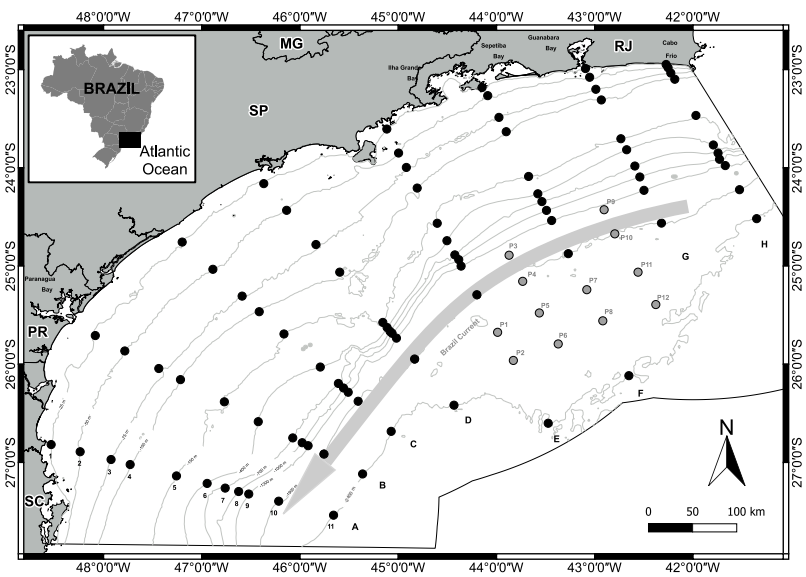

Figure 1. Location of the surface (0-2 cm) sediment sampling stations throughout the Santos Basin. Each transect (A, B, C, D, E, F, G, and H) is divided by isobaths as follows: #1, 25 m; #2, 50 m; #3, 75 m; #4, 100 m; #5, 150 m; #6, 400 m; #7, 700 m; #8, 1000 m; #9, 1300 m; #10, 1900 m; and #11, 2400 m, indicated by black dots. Samples from the São Paulo plateau are labelled as P1 to P12 (gray dots), excluding P3, P9, and P10 (slope samples), and are also represented by samples at 2400 m (#11) in all transects.

A total of 100 sampling stations were defined in the Santos Basin Regional Characterization Project (PCR-BS) conducted by CENPES/PETROBRAS. A total of 87 stations are distributed along eight cross-margin transects (A, B, C, D, E, F, G, and H) and 11 isobaths (25, 50, 75, 100, 150, 400, 700, 1,000, 1,300, 1,900, and 2,400 m), in addition to 19 sampling stations located in the São Paulo Plateau (Figure 1). One replicate was collected in each of three independent deployments of the sampling device in each station, totaling 289 samples. Undisturbed sediments were collected with an adapted Van Veen sampler at the continental shelf stations (and at station A07) and with a box corer at the slope stations (Moreira et al., 2023).

Total organic carbon (TOC), total nitrogen (TN), $\delta^{13}C$ and $\delta^{15}N$

The collected sediments were freeze-dried and weighed (10 ± 5.0 mg) directly in silver capsules. Carbonates were removed by acid treatment (HCl 0.1 M) and heating (100 °C/6 h). The TOC, TN, $\delta^{13}C$, and $\delta^{15}N$ variables of the bulk OM were determined in the same analytical run using an elemental analyzer equipped with a thermal conductivity detector (Flash 2000 model OEA) coupled to an isotope ratio mass spectrometer (Delta V IRMS, Thermo). Two pulses of the reference gas N_2 were introduced at the beginning of each analysis and two pulses of the reference gas CO_2 were introduced at the end. Both reference gases were previously calibrated with the IAEA USGS40 standard material ($\delta^{13}C$ = −26.39 ± 0.04‰, $\delta^{15}N$ = −4.52 ± 0.06‰). The $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) were calculated according to the value of the second pulse of the correspondent reference gas. The quantification of TOC (%) and TN (%) contents was based on a calibration curve employing an acetanilide standard (C = 71.09%, N = 10.36%). All values (TOC, TN, $\delta^{13}C$, and $\delta^{15}N$) are reported as the average of at least two replicate analyses of each sample.

An intermediate amount of the acetanilide standard (0.150 ± 0.020 mg) was analyzed as unknown samples throughout the sequence for every 12 injections (or 6 samples) for calibration monitoring. The same was performed for the IAEA USGS40 standard (0.200 ± 0.050 mg) every 6 injections (or 3 samples). Instrumental accuracy was verified using the NIST 1941B standard (Organics in Marine Sediments).

Determination of total and organic phosphorus

Phosphorus speciation was performed following sample digestion employing a spectrophotometric method (Aspila et al., 1976). For the total phosphorus determinations, freeze-dried sediment aliquots (0.5 to 1.5 g ± 0.1 g) were combusted in a muffle furnace at 550 °C for 2 h, followed by residue extraction with 1 M HCl for 12 h at room temperature. After centrifugation (3000 rpm for 10 min), 2 mL of the supernatants were diluted with water to 50 mL and a blue molybdenum complex was developed (Grasshoff et al., 1999) for spectrophotometric determinations at 800 nm. The P-PO_4 concentrations, in μg g^{-1}, were calculated based on a calibration curve of a potassium dihydrogen phosphate [KH_2PO_4] solution. Inorganic phosphorus contents were determined in another sediment aliquot following the same procedure for total phosphorus, excluding the thermal treatment. Organic phosphorus levels were calculated by the difference between total and inorganic phosphorus.

Determination of chlorophyll-*a* and phaeopigments

The chlorophyll-*a* (chl-*a*) and phaeopigments (phaeo) contents of the sediment samples were determined according to the spectrophotometric method proposed by Lorenzen (1967). The freeze-dried sediments were first weighed into Falcon tubes (1.0 g ± 0.010 g) and 10 mL of a 90% acetone solution were added. The tubes were then vigorously shaken by vortexing, ultrasonicated for 3 minutes, and stored overnight at 4 °C in the dark. Following centrifugation (3000 rpm for 10 minutes), the supernatant absorbances were determined at the wavelengths of 750 and 665 nm against a blank consisting of 90% acetone. For phaeopigment determinations, the acetone extracts were acidified directly in a spectrophotometer cuvette with 2 drops of 0.1 N HCl, and the absorbances were then determined again at 750 and 665 nm (Parsons et al., 1984). The chlorophyll-*a* and phaeopigments contents were calculated according to Equations 1 and 2, respectively.

$$chlorophyll - a\ (\mu g\ g^{-1}) = \frac{11.9 \times 2.43[(abs_{665} - abs_{750}) - (abs_{665acid} - abs_{750acid})] \times 10}{sample\ mass\ (g)}$$

(Equation 1)

$$phaeopigments - a\ (\mu g\ g^{-1}) = \frac{11.9 \times 2.43[1.7x(abs_{665} - abs_{750}) - (abs_{665acid} - abs_{750acid})] \times 10}{sample\ mass\ (g)}$$

(Equation 2)

Determination of total proteins, carbohydrates, and lipids

The protein content of the sediment samples was determined according to Bradford (1976) and Hartree (1972), modified by Rice (1982). The colorimetric method allows for the reaction of proteins with racemic tartrate and the Folin-Ciocalteu reactive in a basic environment (pH 10). The reaction provides a stable blue coloration whose intensity is proportional to the protein concentration in the reaction solution (Danovaro, 2010). Results were quantified through a calibration curve of bovine serum albumin (BSA) equivalents.

Total carbohydrate contents were determined according to Dubois et al. (1956), optimized for sediments by Gerchacov and Hatcher (1972) following minor modifications. This colorimetric assay is based on the reaction between sugars and phenol in the presence of concentrated sulfuric acid (Danovaro, 2010). Absorbances were determined by spectrophotometry at 485 and 600 nm and the results quantified in D-glucose equivalents using a calibration curve.

The total lipid concentrations in marine sediment samples were determined according to Bligh and Dyer (1959) and Marsh and Weinstein (1966), slightly modified (Danovaro, 2010). The results were determined by spectrophotometry at 375 nm and quantified through a calibration curve comprising tripalmitin equivalents.

Determination of biopolymeric carbon (BPC)

To estimate carbohydrate, protein, and lipid TOC contributions, those results were converted into carbon equivalents by applying the respective coefficients of 0.40, 0.49, and 0.75 μg C μg^{-1} obtained from the relative standards analyses (Fabiano et al., 1995). The sum of the C equivalents of the three main biochemical organic compound classes is reported as biopolymeric carbon (BPC) (Fabiano et al., 1995; Pusceddu et al., 2009; Danovaro, 2010).

Statistical analysis

To map variable associations and how they are structured at the Santos Basin, a self-organizing map (SOM) (Kohonen, 1990, 2001) was applied. The SOM comprises a neural network method built by an unsupervised learning process based on data similarity comparisons within a space continuum, with increasing applications in environmental studies due to its ability to extract information from multi-dimensional and complex datasets (Chon, 2011).

The SOM input nodes included the chlorophyll-a, phaeopigments, total organic carbon (TOC), molar carbon/nitrogen ratio, stable isotopic ($\delta^{13}C$ and $\delta^{15}N$), organic phosphorus, total phosphorus, biopolymeric carbon (BPC), TOC/BPC ratio, and protein/carbohydrates ratio values. All variables were normalized to a mean 0 and standard deviation 1 distribution (Z-score). The SOM model was applied employing the following parameters, according to the guidelines reported by Fonseca and Vieira, (2023): 500 interactions, a learning rate decrease from 0.05 to 0.001, Euclidean distances, and bubble neighborhood function. The output layer comprised 49 nodes in a 7 × 7 cells grid. After calculating the SOM, the network was submitted to a hierarchical clustering algorithm to reduce the number of classes employing Ward's linkage method. The number of clusters was determined through an elbow plot.

RESULTS AND DISCUSSION

The Santos Basin geographically corresponds to the region also known as the South Brazil Bight (SBB). The general hydrodynamic, sedimentologic, and oceanographic features observed at the SBB and their biogeochemical cycle, sediment accumulation, and ecological functioning implications in both local and regional scales have been studied over the last decades. These include the investigation of frontal system effects on ecological and biogeochemical process in the present (e.g. Brandini et al., 2018; Ferreira et al., 2020; Mahiques et al., 2020; Calil et al., 2021), as well as those that took place over

geological time-scales (e.g. Dauner et al., 2019; Ferreira et al., 2020; Nagai et al., 2020; Bícego et al., 2021). More recently, evidence of the presence and accumulation of contaminants in shelf and offshore sediments is increasing (e.g. Santos et al., 2020; Timoszczuk et al., 2021).

Herein, we provide further knowledge on the sedimentary OM in the Santos Basin by integrating information on TOC, TN, C/N ratios, and $\delta^{13}C$ and $\delta^{15}N$ contents of surface sediments covering a range of latitudes, comprising nine transects from 23°S to 28°S, and a wide bathymetric gradient, with stations ranging from shallow waters (25 m depth) to the deep ocean (2,900 m), including the São Paulo Plateau. We also provide the first large scale regional assessment of the biochemical OM composition and the corresponding biopolymeric carbon (BPC) distribution, since previous information of BPC in the SBB focused on an inner shelf region near the São Sebastião Island (Venturini, 2007).

Elemental (C, N, and P) and isotopic ($\delta^{13}C$ and $\delta^{15}N$) OM sediment compositions

Total organic carbon (TOC) content averaged 6.55 ± 3.27 mg g^{-1} (range 0.312–15.5 mg g^{-1}; Table S1, Supplementary Material). Spatial TOC distribution among shelf isobaths (25–150 m) is marked by lower inner-shelf values (25 and 50 m isobaths), contrasting with the higher values detected at the mid-shelf (100 m and less intense at 75 m in depth; Figure 2-A). Slope (400–1,900 m) concentrations were generally higher compared with the shelf, presenting secondary maxima at the 700 m, 1,000 m, and 1,300 m isobaths, with median TOC concentrations of 8.19 mg g^{-1}, 8.97 mg g^{-1}, and 9.53 mg g^{-1}, respectively. Concerning the deeper stations (2,400-m isobath), TOC concentrations were low (median of 4.34 mg g^{-1}), whereas values over most of the samples in the São Paulo Plateau (2,140–2,260 m, indicated by an asterisk in Figure 2-A) were close to 5.0 mg g^{-1}, with similar values also observed at 150 m and 400 m depths.

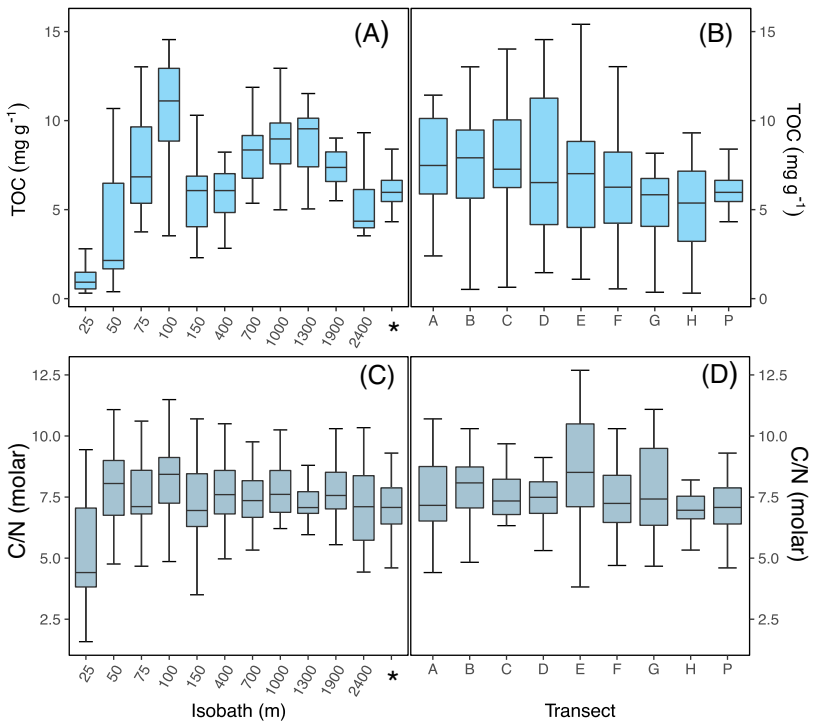

Figure 2. Distribution of TOC (upper panel) and C/N molar ratios (lower panel) according to sampling isobaths (A-C) and transects (B-D) in Santos Basin core-top sediments (n = 289 samples). Legend: horizontal bar = median value; box = interquartile range; whisker = highest and lowest values within 1.5 times the interquartile range. The asterisks (*) in (A) and (C) represent samples ranging from 2,140 m to 2,260 m in the São Paulo Plateau. Transect P includes all samples shown as gray dots in Figure 1 (see legend for details).

When comparing TOC concentrations among the transects, in addition to the expected wide concentration variations due to the inclusion of shallow and deep-ocean samples across the margin, a trend of relatively higher concentrations at transects A-C in comparison with transects G-H was detected. Figure 2-A also indicates a relatively low TOC content variation in the P transect, including 9 samples from 2,140-m to 2,260-m isobaths in the São Paulo Plateau and in three other samples in the slope not pertaining to the A–H transects.

A high and significant correlation between TOC and TN ($r = 0.89$; $p < 0.05$; $n = 289$) was detected, indicating a common source for both elements. This validates any inference of an OM nature based on the C/N ratio (Meyers, 1997). The molar C/N ratio of Santos Basin sediments ranged between 3.34 and 9.90, with an overall mean (n = 289) of 7.56 ± 1.57 (Table S1; Supplementary Material). The very low median C/N value for the 25 m-deep samples may consist of a bias caused by calculating a ratio between very low – close to their respective limits of quantification – C and N contents in those samples. Concerning the other isobaths, including the São Paulo Plateau samples (indicated by an asterisk in Figure 2-A, see legend for details), the median C/N (molar) ratio values lie between 7.5 and 8.0. Regarding transect distribution, only transect E exhibited a somewhat distinct median and range for the C/N ratio values compared with the other transects (Figure 2-D).

The total phosphorus (TP) distribution is marked by low concentrations (medians below 250 μg g^{-1}) along the 25 m, 50 m, and 75 m isobaths, whereas at water depths from 150 m to 1900 m similar concentrations were observed, with a median around 550-600 μg g^{-1} (Figure 3-A). Regarding the transects (Figure 3-B), TP concentrations exhibit wide variations, reflecting low shelf sample concentrations, although no marked differences in median values among the transects were observed. The organic phosphorus (OP) concentration averaged 240 ± 139 μg g^{-1} and represented 53.2 ±17.7 % of the total phosphorus content. A relatively wide variation in OP concentrations was observed, ranging from non-detected to 670 μg g^{-1} (Table S1; Supplementary Material). The isobath OP distribution (Figure 3-C) resembles those observed for TP and TOC, i.e., lowest concentration detected at the 25-depth isobath, and marked increases – although less intense for OP compared to TOC – in the mid-shelf (100 m isobath) and mid-slope (at the 1000 m isobath). In fact, a slight but significant correlation was detected between OP and TOC ($r = 0.41$; $p < 0.05$) when considering the entire dataset ($n = 248$). The OP contents also exhibited a similar distribution compared to TOC when data were compared according to sampling transects (Figure 3-D), characterized by relatively higher concentrations in the southern transects (A to D) in comparison to the northern ones (D-H).

The TOC-bulk $\delta^{13}C$ ranged from −19.9 to −24.8 ‰, with an overall mean of −21.4 ± 0.78‰ (Table S1; Supplementary Material). A slight depletion of the bulk OM ^{13}C contents was observed for samples collected along the 25-m isobath, with 25% of them presenting $\delta^{13}C$ below −23.0‰ (Figure 4-A). Following from the inner to the shelf-break, a slight $\delta^{13}C$ increase was noted, but still with relatively wide variations noted for a particular isobath. On the other hand, the $\delta^{13}C$ contents of the slope and deep-ocean samples were similar and less variable, with values around −21.0‰. Regarding the transects (Figure 4-B), ^{13}C-isotopically enriched OM occurs preferentially in the southern transects (A-C) – in addition to the samples from the P transect (which includes nine samples from the São Paulo Plateau) –, whereas in the northern transects (F-H), OM is relatively more depleted in ^{13}C (Figure 4-B).

Nitrogen isotopes were below the limit of detection in 11% of the 289 analyzed samples, particularly in the samples obtained at the 25-m isobath (Table S1; Supplementary Material). Considering the remaining sampling stations, $\delta^{15}N$ values varied between 3.70 and 11.90‰, with an overall mean of 6.57 ± 2.70‰ (Figure 4-C). No marked variation in $\delta^{15}N$ was observed for either the isobaths or the transects (Figure 4-D.)

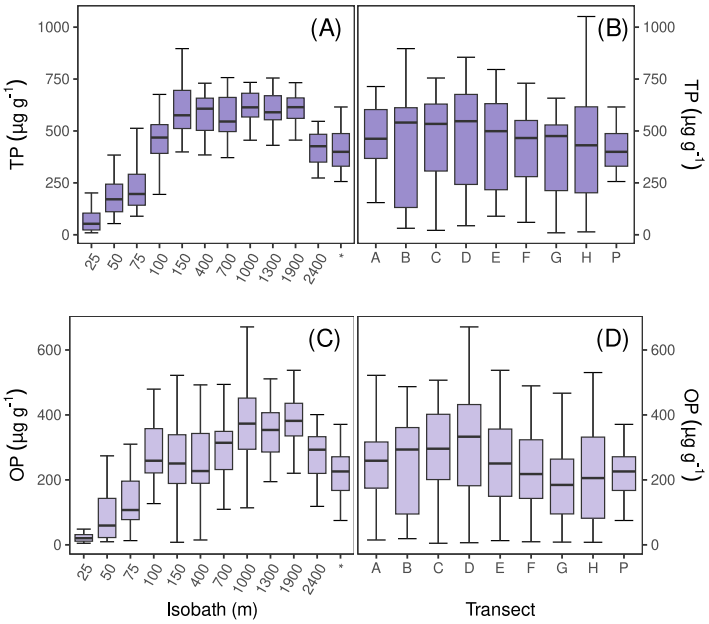

Figure 3. Distribution of total phosphorus (upper panel) and organic phosphorus (lower panel) according to sampling isobaths (A-C) and transects (B-D) in Santos Basin core-top sediments (n = 289 samples). Legend: horizontal bar = median value; box = interquartile range; whisker = highest and lowest values within 1.5 times the interquartile range. The asterisks (*) in (A) and (C) represent samples ranging from 2,140 m to 2.260 m in the São Paulo Plateau. Transect P includes all samples shown as gray dots in Figure 1 (see legend for details).

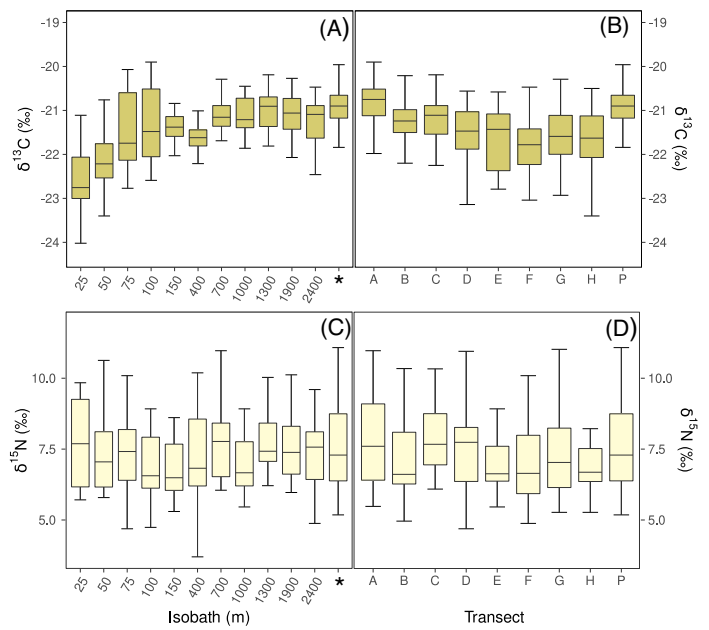

Figure 4. Distribution of $\delta^{13}C$ (upper panel) and $\delta^{15}N$ (lower panel) according to sampling isobaths (A-C) and transects (B-D) in Santos Basin core-top sediments (n = 289 samples). Legend: horizontal bar = median value; box = interquartile range; whisker = highest and lowest values within 1.5 times the interquartile range. The asterisks (*) in (A) and (C) represent samples ranging from 2,140 m to 2,260 m in the São Paulo Plateau. Transect P includes all samples shown as gray dots in Figure 1 (see legend for details).

Pigments (chlorophyll-a and phaeopigments) and biochemical organic matter composition (CHO, PRT, LIP, and Biopolymeric Carbon)

Chlorophyll-*a* concentration averaged 0.68 ± 0.64 μg g^{-1} (from non-detected to 4.18 μg g^{-1}), whereas phaeopigments were comparatively present at *circa* one-order of magnitude higher concentrations, averaging 4.87 ± 5.14 μg g^{-1} and ranging from non-detected to 33.4 μg g^{-1} (Table S1 – Supplementary material). The high standard deviations over the mean chl-*a* and phaeo concentrations are due to steep concentration gradient from the mid-shelf to deep-ocean stations (Figure 5-A and C). Stations located at the 75 and 100 m shelf isobaths are enriched in pigments, and depleted at 25 m, particularly for the phaeopigments, which is similar to the observed TOC pattern (Figure 2). However, in contrast to TOC, pigment concentrations were generally low in the slope and in the deep ocean, including at the São Paulo Plateau. In addition, water depth is more important concerning pigment distribution than latitudinal variation, as suggested by the relatively similar median concentrations of both chl-*a* and phaeo when the samples are grouped along the southern A-C to the northern F-H transects (Figure 5-B and D). In this figure, it is also interesting to highlight the consistent low pigment contents of samples on the P transect, which includes nine samples from the São Paulo plateau ranging from 2,140 m to 2,260 m.

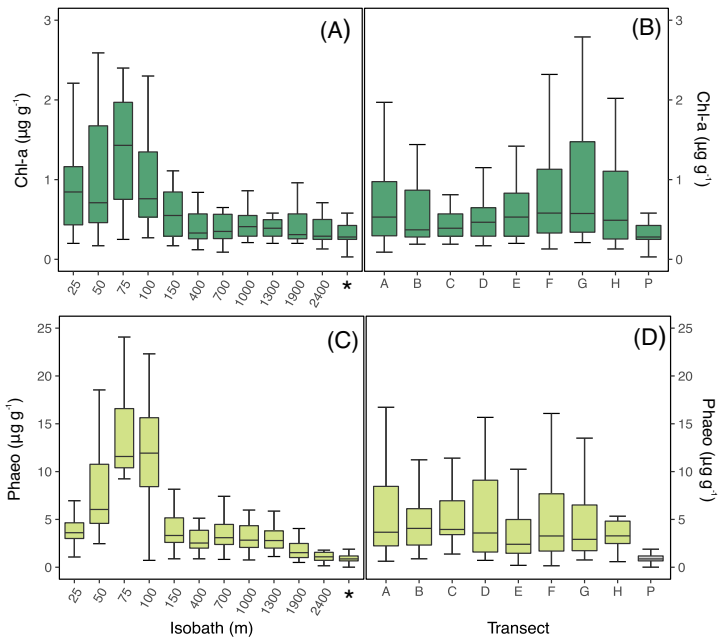

Figure 5. Distribution of chlorophyll-*a* (upper panel) and phaeopigments (lower panel) according to sampling isobaths (A-C) and transects (B-D) in Santos Basin core-top sediments (n = 289 samples). Legend: horizontal bar = median value; box = interquartile range; whisker = highest and lowest values within 1.5 times the interquartile range. The asterisks (*) in (A) and (C) represent samples ranging from 2,140 m to 2,260 m in the São Paulo Plateau. Transect P includes all samples shown as gray dots in Figure 1 (see legend for details).

The biochemical sedimentary organic matter classes in sediment were expressed in terms of total carbohydrate (CHO), total protein (PRT), and total lipid (LIP) concentrations (Table S1 – Supplementary Material). Considering the entire dataset (n = 289), CHO (1.24 ± 0.76 mg g^{-1}) was the most abundant biopolymer, followed closely by PRT (1.02 ± 0.57 mg g^{-1}), while LIP (0.32 ± 0.25 mg g^{-1}) was a minor component. Some variations were noted along the bathymetric gradient, with a trend of relatively higher PRT and LIP contributions in shallow shelf areas (25–50-m isobaths) and an increasing CHO contribution from mid-shelf (75 m depth) to the deep ocean,

including São Paulo Plateau samples (Figure 6-A). The same figure indicates only minor variations in the relative proportions of CHO, PRT, and LIP when comparing the cross-margin transects (A-H).

The biopolymeric carbon (BPC), represented by the normalized sum of CHO, PRT, and LIP, concentrations, follows a trend along the bathymetric gradient similar to TOC. First, the BPC concentration is very low over the shelf at the 25-m isobath, reaching its maximum at the 100-m isobath (Figure 6-B). Second, the mid-slope samples (700–1,300-m isobaths) exhibit a second maxima in BPC concentrations, with values within the 1,900 and 2,400-m isobaths. This latter isobath, in the São Paulo Plateau, has similar BPC concentrations to other depths (2,140 m to 2,260 m, the latter presenting similar concentrations to the São Paulo Plateau samples (represented by an asterisk in Figure 6). Contrary to the CHO, PRT, and LIP distributions by transect, a relative increase in BPC concentrations in transects A, B, and C were noted when compared with G-H transects (Figure 6-B).

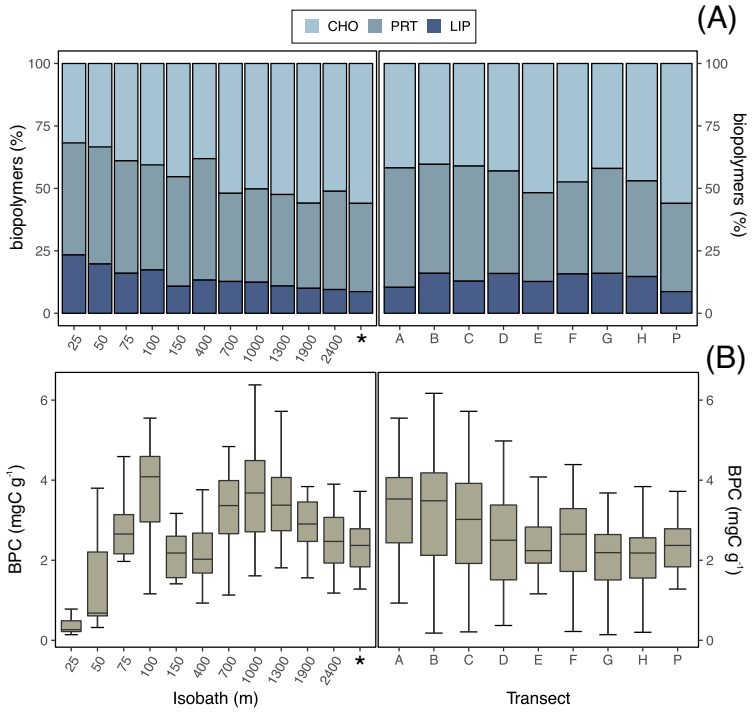

Figure 6. Biochemical organic matter composition (upper panel), in terms of (A) total carbohydrates (CHO), total proteins (PRT) and total lipids (LIP), and (B) the resulting biopolymeric carbon (BPC) in core-top sediments (n = 289) from the Santos Basin. Legend for (B): horizontal bar = median value; box = interquartile range; whisker = highest and lowest values within 1.5 times the interquartile range. The asterisks (*) in (A) and (C) represent samples ranging from 2,140 m to 2,260 m in the São Paulo Plateau. Transect P includes all samples shown as gray dots in Figure 1 (see legend for details).

Bulk properties of OM on regional scale in the Santos Basin

Information regarding the Santos Basin sedimentary organic matter characterization, including information on sources, transport, and accumulation patterns, is relatively scarce. The first comprehensive regional OM characterization in the Santos Basin was published by Mahiques et al. (2004). Based on a regional bulk OM composition dataset (TOC, TN, C/N ratio, $\delta^{13}C$, and $\delta^{15}N$), grain size, and carbonate contents of shelf and upper slope sediments, these authors proposed an SBB division in two sectors, southern and northern, divided by the São Sebastião Island, which corresponds to proximity to our transect E, at the 25 m isobath (Figure 1).

Organic matter dynamics in the SBB's southern sector is related to high primary production rates supported by continental runoff nutrients transported

northwards from the La Plata River by the Subtropical Shelf Front (SSF), (Piola et al., 2000) and by meso-scale hydrodynamics processes (Campos et al., 2000; Silveira et al., 2008). These forcings result in the deposition of OM-rich and muddy sediments along the 100-m isobath between longitudes 45°–47° 30' W (Mahiques et al., 2004). On the other hand, an overall predominance of nutrient-poor and warm waters is observed in the northern SBB sector, with complex sediment and patchy OM-rich sediment distributions. The oligotrophic northern SBB condition is seasonally (spring-summer) disrupted by the input of cold and nutrient-rich waters into the photic layer by the Cabo Frio Upwelling Front (CFUF), (Brandini et al., 2018), resulting in OM accumulation near Cabo Frio and extending south-west along the 100-m isobath (Mahiques et al., 2004).

These regional sedimentary SBB OM accumulation patterns depicted by Mahiques et al. (2004) were validated by several local benthic and pelagic shelf and/or upper slope studies (e.g. Burone et al., 2013; Lourenço, Martins, et al., 2017; Mahiques et al., 2017; Ramos et al., 2019; Ferreira et al., 2020; Tura and Brandini, 2020; Yamashita et al., 2020; Bícego et al., 2021), as well as by combining satellite images and pigment data (Oliveira et al., 2021).

The TOC sediment distribution verified herein (Figure 7) is consistent with the influence of frontal systems on SBB biogeochemical processes, as highlighted in the aforementioned studies. A belt of relatively OM-rich sediments (TOC ranging from 7.0 to 14.0 mg g^{-1}) on the shelf (25 to 150-m isobaths, or #1 to #4 in each transect of Figure 7) is noted, extending not only along the 100-m isobath both in the southern (transects A-D) and northern (transects F-H) sectors, but also at the 75-m isobath in transects A and B and near São Sebastião Island (transect E, station 2, at 50-m isobath).

Interestingly, concerning regional TOC distribution, a second belt of OM-enriched sediment occurs in the lower slope (1,000 and 1,300-m isobaths) only in the southern sector of the Santos Basin (transects A to D; Figure 7). A similar OM-enriched sediment distribution on the slope was also observed at Campos Basin, in the vicinity of Cabo Frio (23° S), where high TOC contents (> 0.9 to 16 mg g^{-1}) were detected in lithoclastic mud depocenters at depths ranging from the 400 to 1,300-m isobaths (Cordeiro et al., 2018). This Campos Basin feature seems to result from the influence of Brazil Current eddies and meanders near Cabo Frio that laterally transport the OM produced in the water column near the shelf-break, although the exact mechanism is still unclear (Marone et al., 2010; Oliveira et al., 2013). At the SBB, an increase in TOC in the mid-shelf may also be explained by a similar lateral OM transport process produced in shelf waters. Further analyses of other indicators, like lipid biomarkers and radiocarbon, are necessary to confirm or refute this hypothesis.

The bulk carbon and nitrogen stable isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) and the C/N ratio provide clues on the origin of sedimentary OM (e.g. Macko et al., 1994; Meyers, 1997). Previous data concerning these proxies confirmed the autochthonous OM origin in muddy mid-shelf sediments (100-m isobath) in both the SBB's northern and southern sectors, as well as the presence of ^{13}C depleted OM in sediments close to the continent near the São Sebastião Island, suggesting terrigenous inputs to shallow waters (Mahiques et al., 2004). This pattern is further detailed by our results (Figure 8).

The by-plot of $\delta^{13}C$ and C/N (Figure 8-A) separating the southern (transects A–D) and northern (transects F-H) sectors revealed two distinct features in the shelf stations (< 150-m isobaths): in the northern sector, sediment exhibited relatively ^{13}C depleted OM within a narrow range of values ($\delta^{13}C$ between −21.8‰ and −22.5‰), indicating mixture of marine and terrigenous/riverine inputs of OM (Meyers, 1994) throughout the shelf. In the southern sector, on the other hand, only the very shallow stations (25 to 50-m isobaths) have ^{13}C depleted OM, but with more negative $\delta^{13}C$ values (between −22.5‰ and −23.3‰) compared with the northern sector, whereas stations from 75 to 150-m isobaths have enriched ^{13}C values ($\delta^{13}C$ from −21.3‰ to −20.2‰), suggesting an increased deposition of marine-derived OM in the mid- to outer shelf in the southern sector. These features suggest that the potential influence of continental input to the

southern sector, including discharges from local rivers and bays as well as the potential influence of the Sub-Tropical Shelf front that brings waters from La Plata River to the SBB inner shelf (Piola et al., 2000; Brandini et al., 2018) are limited to the inner shelf and/or are 'diluted' by autochthonous residues in the outer shelf stations. Similarly, in coastal sediments located between 21° S and 23° S, in the Campos Basin, only shallow stations nearby river discharges (25–50-m isobaths) exhibited a $\delta^{13}C$ average value of −24.6‰ (Cordeiro et al., 2018), suggesting that in both basins the continental input of OM has limited competence to export continentally-derived OM to deep sediments.

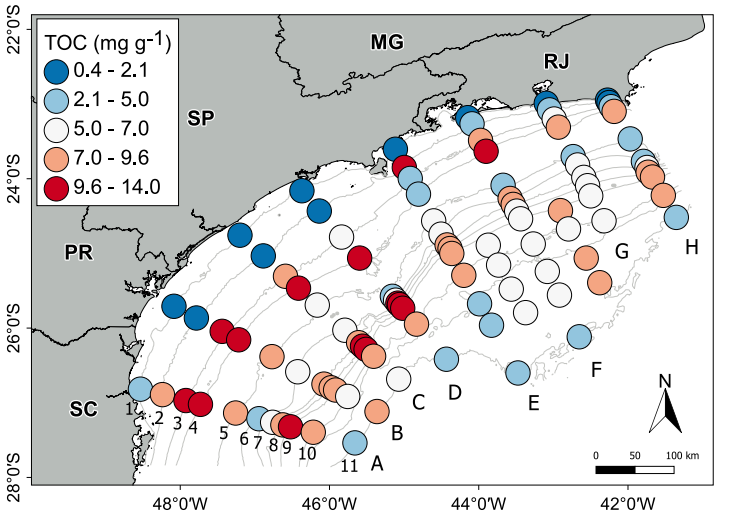

Figure 7. Spatial TOC distribution in core-top sediments from cross-margin Santos Basin transects. The concentration at each station represents the average value of three independent replicates. Refer to Figure 1 for the depth correspondence of each isobath code (from #1 to #11 in the figure) and for the identification of the São Paulo plateau samples.

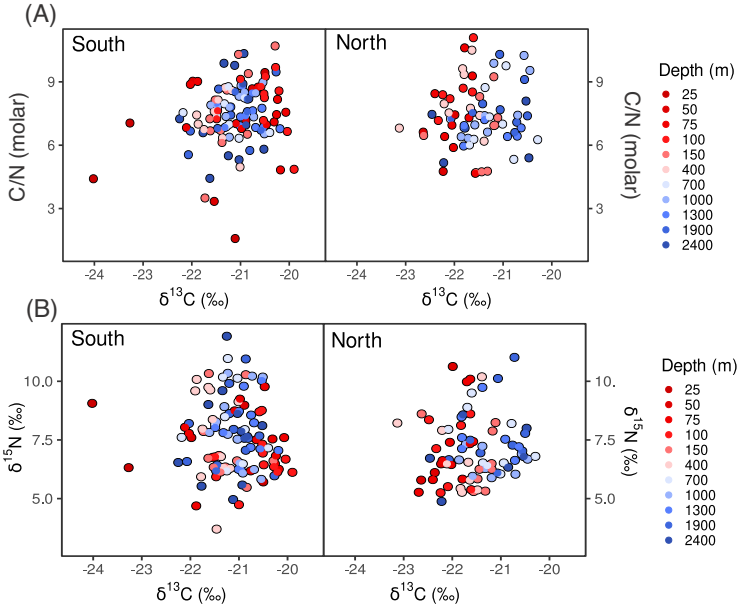

Figure 8. Biplots of $\delta^{13}C$ against the C/N ratio (A) and against $\delta^{15}N$ (B) considering all surface sediment samples split into southern (transects A-D) and northern (transects F-G) Santos Basin. Samples from transect E (the limit between the two sectors, Mahiques et al., 2004 1172) and from the "P" group (which includes samples from de São Paulo plateau) were not considered.

The C/N molar ratio (Figure 8-A) and $\delta^{15}N$ (Figure 8-B) data did not contribute to sample separation according to depth or geographical localization. The observed C/N ratio values (C/N = 7.56 ± 1.58, range 3.34–12.7) are typically found in autochthonous OM and are similar to values observed for sediments from the adjacent Campos Basins (Cordeiro et al., 2018). We did not, however, detect the elevated C/N values > 20 in coastal sediments southward to 25° S as reported by Mahiques et al. (2004), probably because this study collected many samples from shallow waters along the coast. Regarding $\delta^{15}N$ values (Figure 8-B), the average value of 6.6 ± 2.7‰ for samples from the shelf to the deep ocean is very similar to that previously observed for the SBB, with an $\delta^{15}N$ average of 6.1 ± 0.7 in the northern sector and 6.3 ± 0.7 in the southern sector (Mahiques et al., 2004) (1172). Even if not contributing to resolve OM sources, note that the sediments are relatively enriched in ^{15}N in comparison with zooplankton in the same region, whose $\delta^{15}N$ varies from 3.9 ± 2.0‰ to 5.6 ± 1.3‰ in animals belonging to different taxonomic groups collected from the shelf-break and from 0.3 ± 1.3‰ to 3.6 ± 1.5‰ in animals collected offshore (Troina et al., 2020). The $\delta^{15}N$ signature offset between zooplankton in the water column and sedimentary OM illustrates the complexity of employing nitrogen isotopes for sedimentary OM fingerprinting in the marine realm (Mayer et al., 1988; Altabet, 2007; Riddle et al., 2022).

Bioavailable OM according to pigments and biopolymeric carbon

Marine sediment OM accumulation is a relevant process from a biogeochemical perspective, since it relates to ocean carbon degradation and burial rates (Farrington, 1987; Summons, 1993; Hedges and Keil, 1995). On the other hand, from an ecological viewpoint, this process is essential to deliver food to sustain heterotrophic benthic organisms (Danovaro, 2010). In this sense, both the amount and the quality (i.e*.,* its nutritional value) are considered benthic trophic structure and energy flow drivers (Campanyà-Llovet et al., 2017).

Many indicators allow for bioavailable OM fraction estimates to heterotrophs in sediments. These include hydrolysable amino acids (Pruski et al., 2019), phytosterols, fatty acids (Oliveira et al., 2013), and chloroplastic pigment (Vezzulli and Fabiano, 2006) concentrations, as well as biochemical marine sediment composition (Fabiano and Danovaro, 1994; Pusceddu et al., 2009). The latter is represented by the amount of total carbohydrates, proteins, and lipids, which are then converted by specific factors to the Biopolymeric Carbon index (BPC) (Dell'Anno et al., 2002).

Herein, we considered pigments (chlorophyll-*a* and phaeopigments) and BPC contents to evaluate the nutritional status of sedimentary SBB OM. Initially, a weak, albeit significant correlation ($r = 0.27$; $p < 0.05$) was observed between total pigments (*i.e.,* chl-*a* plus phaeo) and BPC for the entire dataset ($n = 289$). In addition, a higher correlation between TOC and BPC ($r = 0.69$; $p < 0.05$) was noted compared with that between chl-*a* + phaeo and TOC ($r = 0.46$; $p < 0.05$). These values suggest the influence of distinct factors affecting pigments and BPC distribution. In fact, both chl-*a* and phaeopigments were present at higher concentrations in shelf samples, and a similarity with OM-enriched sediments in the shelf was observed particularly for the determined phaeopigments (see previous discussion). Pigment concentrations were low and relatively similar from the shelf-break stations (150-m isobath) to the deep ocean (2,400-m isobath), with few exceptions (Figure 9-A/B, for chlorophyll-*a* and phaeopigments, respectively).

Other studies concerning sediments close to the shoreline of the state of São Paulo reported higher concentrations than those obtained herein. For instance, Venturini (2007) reported average chlorophyll-*a* concentrations from 2.22 to 14.8 μg g^{-1}, and phaeopigments from 2.04 to 40.2 μg g^{-1}, while Quitana et al. (2015) reported chlorophyll-*a* from 1 to 21 μg g^{-1}, and Rodrigues Alves et al. (2014) found chlorophyll-*a* from 1.5 ± 2.1 μg g^{-1} to 7.8 ± 2.1 μg g^{-1} and phaeopigments ranging from 8.2 ± 2.8 to 48.9 ± 19.8 μg g^{-1}. All these values are higher than our data, which integrate a wide range of water depths (25 to 2,400 m).

The relatively high chlorophyll-*a* and phaeopigment concentrations detected in the shelf sediments in the studied region reflects OM 'freshness' in the bottom of shallow waters. Even considering the low TOC

contents observed in sediments at the 25 and 50-m isobaths along the entire basin (Figure 7), these characteristics may favor local benthic organisms. However, considering that pigments degrade faster than biopolymers during OM transport down the water column and in the upper sediment layer (Wakeham and Canuel, 2006), pigments may underestimate the actual nutritional OM values in slope and deep ocean sediments.

Regarding the BPC (Figure 9-C), the distribution over the shelf (#1 to #5 in Figure 9-C) resembles both the phaeopigment and TOC distributions, indicating that BPC and phaeopigments provide similar information regarding potential OM availability in shallow SBB waters. In slope sediments, however, particularly between the 700 and 1,900-m isobaths (#7 to #10 in Figure 9-C) of transects A, B, and C, the BPC was present at a high concentration range (BPC = 3.7 to 4.8 mg C g^{-1}). Thus, in the mid- to lower slope the OM still presents nutritional value to benthic organisms. However, in most of these samples the PRT:CHO ratio (Figure 9-D) lies close or below the 1.0 threshold, inverting the general biopolymeric partition of PRT > CHO > LIP in 'fresh' OM (Fabiano and Danovaro, 1994). This result indicates the OM has already been altered by microbial degradation, since proteins are more labile than carbohydrates (Dell'anno et al., 2008; Pusceddu et al., 2009, 2011; Pruski et al., 2019), so its nutritional value should be evaluated cautiously and must consider other chemical and biological parameters.

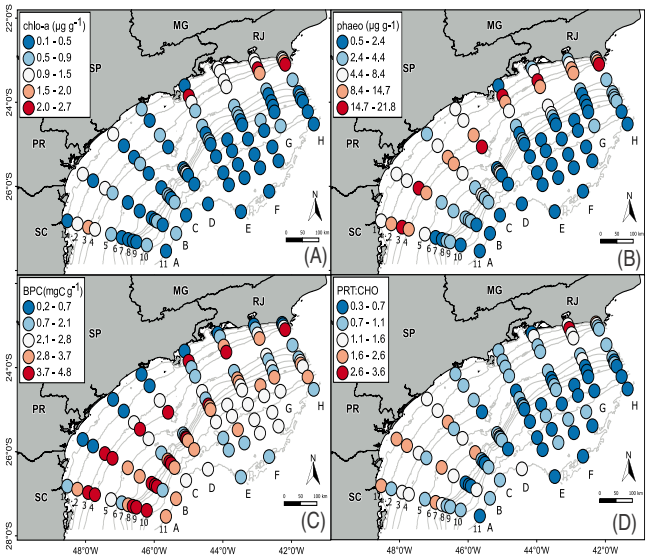

Figure 9. Distribution of chlorophyll-*a* (A), phaeopigments (B), biopolymeric carbon (C), and total protein to total carbohydrates ratios (D) in surface sediments. The value of each station represents the average of three independent replicates. Refer to Figure 1 for the depth correspondence of each isobath code (from #1 to #11 in the figure) and for the identification of the São Paulo plateau samples.

Self-Organizing Map (SOM) Based on Bulk OM Proxies

To deal with the multiple evidence provided by the proxies considered herein, a neural network method known as SOM (self-organizing maps) was applied to perform further inferences on the amount and quality of OM that accumulates in sedimentary provinces under the influence of bathymetry and distinct oceanographic forcings at the SBB. The network indicated quantization and topographic errors of 0.765 and 0.05, respectively. The analysis indicated that TOC, C/N, P-total, P-org, $\delta^{13}C$, $\delta^{15}N$, and BPC were associated with each other in the upper left corner of the plot, while chl-*a*, phaeo, and the ratios BPC/TOC and PRT/CHO were clustered in the opposite directions (Figure 10-A). The within-clusters sum of squares performed on the SOM indicated no clear threshold and five groups were responsible for nearly half of the dataset variability (Figure 10-B). These five groups are associated

as follows: group 1 (dark blue) is influenced by TOC, BPC, and pigment data; group 2 (light blue) contains few samples and is influenced only by pigment content; group 3 (white), the smallest group, is influenced by the both the PRT/CHO ratio and chl-*a* data; group 4 (orange) is the largest one, influenced by isotopes and the BPC/TOC ratio; and group 5 (red), the second largest, is influenced by both the elemental and isotopic proxies determined herein.

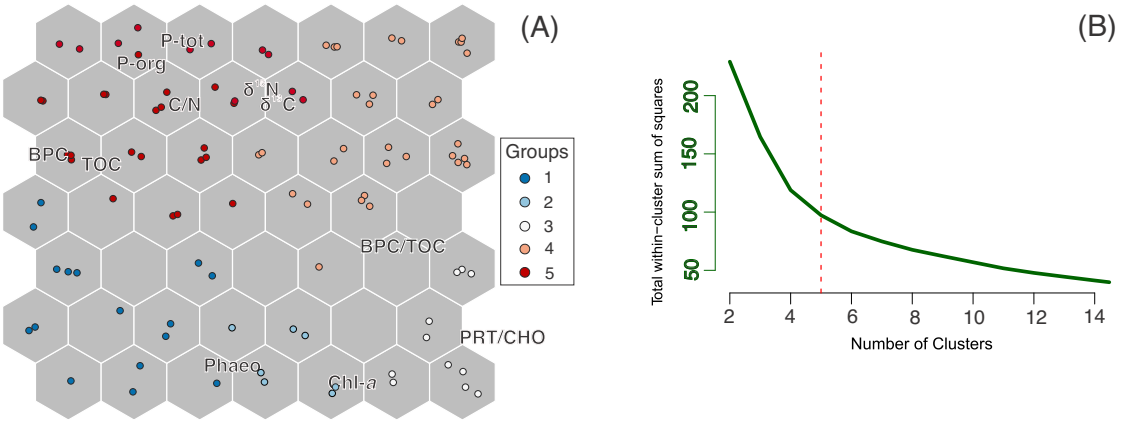

Figure 10. (A) Two-dimensional mapping type obtained by the SOM algorithm for bulk OM data of core-top sediment from Santos Basin cross-margin transects and the São Paulo plateau samples. Each colored dot represents the mean concentration of the three replicates from each station, including total organic carbon (TOC), molar C/N ratio (C/N), biopolymeric carbon (BPC), total P (P-tot), organic P (P-org), carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) compositions, chlorophyll-*a* (chl-*a*), phaeopigments (phaeo), biopolymeric carbon to total carbon ratios (BPC/TOC), and total protein to total carbohydrate ratios (PRT/CHO); (B) Groups 1–5, defined according to the Cluster Analysis and Ward's linkage method.

When plotting the five clusters on the map, it becomes evident that they are geographically and bathymetrically structured (Figure 11), and the following OM accumulation provinces can be proposed for the SBB:

- Group 1 (dark blue) is formed by all mid-shelf samples along the entire basin containing high TOC contents (see Figure 7) and muddy sediments (Figueiredo et al., 2023). Concerning the SOM map (Figure 10), BPC, TOC, and phaeo were the closest variables to this group. This is consistent with the pair-wise correlation between these variables discussed previously. Sediments from group 1, therefore, accumulate OM in relatively high amounts and exhibit relatively good nutritional potential.
- Group 2 (light blue) contains only seven samples, directly influenced in the SOM map by pigment deposition. All these samples are from the shelf and contain intermediate TOC contents between 2.1 and 7.0 mg g^{-1}. It is possible that benthic organisms may be favored by this OM nature in this province, although this is not a recurrent SBB feature.
- Group 3 (white) is formed only by samples from both the 25 and 5-m isobaths at transects B, C, D, and H, as well as the 25-m isobath at transects E, F, and G. These shallow samples are also poor in TOC – also presenting the coarsest sediments according to Figueiredo et al. (2023) – indicating low OM accumulation. However, as this group is defined by chl-*a* and PRT/CHO associated with labile OM fractions (Pusceddu et al., 2009; Campanyà-Llovet et al., 2017), even in low amounts, the OM presents a high nutritional value and may favor some opportunistic species.
- Group 4 (orange) is the second largest group, containing many samples from the São Paulo plateau and almost all transects at the 2,500-m isobath (#11 in Figure 11), as well as mid-slope of transects G and H and random samples in the southern portion

(transects A-D). Most samples in this group exhibit intermediate TOC values between 5.0 and 7.0 mg g^{-1}. The most representative variables defining this group are isotopic OM composition ($\delta^{13}C$ and $\delta^{15}N$) and the BPC/TOC ratio. Since no other variable is more directly involved with this group, the current information available does not allow for inferences on how the OM can be used by benthic organisms.

– Group 5 (red) is formed by most of the samples from the mid- to low-slope (700 to 1,300-m isobaths or #7 to #10 in Figure 11) in the southern and some of the northern sectors transects. This group is defined by bulk OM properties, namely the elemental (C, N, P), isotopic ($\delta^{13}C$ and $\delta^{15}N$), and biochemical (BPC) composition, but not by pigments. As discussed previously, this bathymetric region presents muddy sediment relatively enriched in OM whose origin is still unknown. All the proxies considered herein have no 'power' to resolve whether this OM was laterally advected or if it was produced in the photic zone offshore and directly transported to the sediment. The former hypothesis, as detailed previously, is supported by the absence of labile pigments, indicating some degree of bacterial transformation. However, we cannot rule out the less plausible mechanism of efficient direct export from the water column of primary/secondary-produced OM, despite the fact that there is no indication of any relevant hydrodynamic forcing that would inject nutrients in offshore oligotrophic waters overlying the mid-to lower SBB slope (Brandini et al., 2018;Calil et al., 2021). Further analyses, including radiocarbon OM aging in selected top-cores are under way to clarify the OM origin at the SBB slope.

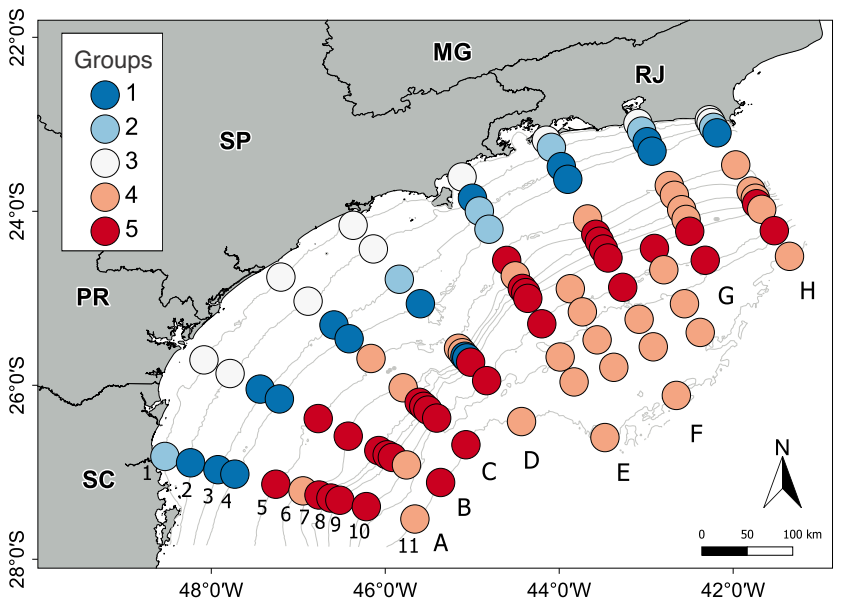

Figure 11. Spatialization of the five groups determined after SOM clustering (see Figure 10)

CONCLUSION

The approach combining multi-proxies including OM elemental, isotopic, biochemical, and pigment analysis in top-core sediments, coupled with a machine learning statistical analysis allowed for the proposition of five main provinces of organic matter accumulating in the Santos Basin, also known as the South Brazil Bight. Sectorization of these provinces by bathymetric and oceanographic forcings already pointed out in the SBB can be envisaged. Aspects related to the amount and

potential nutritional relevance of sedimentary OM are highlighted in four of the five provinces. For instance, a low regional OM accumulation trend is noted for shallow stations (< 50 m isobaths), but low depths allow for the labile OM to reach the sediment surface. Moreover, muddy sediments in the mid-shelf, mainly along the 100-m isobath throughout the SBB, are enriched in OM, displaying great potential to be used by benthic organisms. Similarly, relatively OM-rich deposits are found at depths from 700 to 1,300-m isobaths, but their composition and nutritional value are not related to the mid-shelf deposits.

The general scenario of the amount and quality of OM accumulated in top-core sediments may be applied in assessments concerning ecological relations of benthic organisms in the shelf and deep ocean of the studied region.

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AUTHOR CONTRIBUTIONS

R.S.C.: Conceptualization; Investigation; Funding Acquisition; Project Administration; Formal Analysis; Writing – original draft; Writing – review & editing;

L.L., M.C.: Methodology, Validation; Investigation; Formal Analysis; Writing – original draft; Writing – review & editing;

L.R.: Methodology; Validation; Investigation;

D.M.: Methodology; Validation; Investigation; Writing – original draft

G.F., D.C.V.: Formal Analysis; Writing – review & Editing;

C.G.M.: Validation; Investigation; Formal Analysis; Writing – review & editing.

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