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TITLE OF THE Ph.D. THESIS

Effects of anthropogenic and natural disturbance on sedimentary organic matter quantity, nutritional quality and degradation in river, lagoon, and coastal marine ecosystems

Scientific Disciplinary Sector

BIO/07 Ecology

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I. Abstract

Ecological disturbance can play a fundamental role in regulating habitats' structure and biodiversity. Most aquatic ecosystems are generally characterized by medium-to-short timescales of natural variability and strong interspecific interactions and, somehow, appear to be more sensitive to disturbances than terrestrial ecosystems. This holds true particularly for marine coastal areas and lagoons, important transition zones between freshwater and marine ecosystems, where anthropogenic and natural disturbance and their ecological consequences are more tangible. The ecological consequences of natural variability at broad spatial scales are currently difficult to be evaluated, most often because of the lack of properly replicated studies, the generally disturbance-specific nature of most studies, and of the lack of comparisons across sources of disturbance and across ecosystems. Changes in the trophic status of aquatic ecosystems and biogeochemical cycling are recurrently being reported due to either natural or anthropogenic drivers, including climate change. In this context, my doctoral thesis aimed at investigating the ecological effects of selected anthropogenic and natural disturbances on sedimentary organic matter quantity, biochemical composition, nutritional quality, and C degradation rates, with the aim of exploring the magnitude and direction of changes in the benthic trophic status of river, lagoon, and coastal marine ecosystems through experiments carried out with in the field or in mesocosms.

First, I investigated the possible effects on benthic trophic status of bottom trawling, one of the major anthropogenic disturbances to which marine benthic ecosystems are subjected worldwide. The study was conducted within an area located in the Bornholm Basin (Baltic Sea) in sites putatively representative of null, low, and medium trawling intensities down to 4 cm depth. Almost consistently across the entire sediment column, untrawled sediments were characterized by organic loads far higher than those in trawled ones, reflecting the typical removal of sediments caused by trawling gears observed in other seas and water depths. Such activity can impoverish the amount of bioavailable food for benthic detritus feeders, but the quantitative "oligotrophication" effect of bottom trawling could be, at least partially, counteracted by an invariant food availability (i.e., lability) of the remining organic substrates. Nevertheless, my results confirm the capacity of bottom trawling, whatever the intensity level, to mix the sediment layers, ultimately causing an overall homogenization of the trophic status across the whole sediment column and altering benthic ecosystem functioning. In conclusion, I infer that bottom trawling can severely modify the benthic trophic status of the Bornholm Basin.

Then, I focused on marine heatwaves (MHWs), since their intensification caused by climate change is expected to increasingly affect coastal biogeochemistry. I investigated MHWs' effects on sediment organic matter (OM) in a nearshore locality (NW Sardinia, Mediterranean Sea) receiving an artificial warm water plume generating temperature anomalies of 1.5-5.0 °C. Sediments were collected before and after 3 and 11 weeks from the initial plume release. Both MHWs influenced sedimentary OM features, with

major effects associated with the highest temperature anomaly after 3 weeks. The autotrophic and lipid contents decreased with time, suggesting a drop in the nutritional quality of OM, along with a slowdown of its turnover mediated by extracellular enzymes, suggesting a decreased ecosystem functioning. I contend that MHWs' intensification will affect not only species and communities but will also alter sediment biogeochemistry and, possibly, the energy transfer towards higher trophic levels.

I also tested in mesocosm the possible use of the Mediterranean deposit-feeder sea cucumber *Holothuria tubulosa* (Gmelin, 1788) as a bioreactor to counteract benthic eutrophication under different scenarios of sea warming due to climate change. I investigated changes in OM features in oligo-mesotrophic and meso-eutrophic sediments and in feces of *H. tubulosa* under different temperatures, either after specimens' acclimation (at 14, 17, 20, 23, 26, 29 °C) or after heat snaps (at 26 and 29 °C). In both experiments, OM features differed significantly between sediments and feces at almost all temperatures and in both types of sediment, and feces resulted always organically enriched when compared with sediments. *H. tubulosa* specimens showed a high adaptability to higher temperatures (i.e., 26 and 29°C), maintaining its bioreactor capacity at all temperatures, irrespectively of the available food. This suggests that this species could be used to mitigate benthic eutrophication under different climate change scenarios, but also that a calibration of sea cucumbers' use as eutrophication bioremediators should be scaled on the initial sediments' trophic conditions. I pinpoint that further studies are required to clarify if this species can adapt in the long term to persistent and more frequent marine heat waves.

Moreover, I investigated the effects of intense rainfall and freshwater flash-flood events, among the major threats for coastal ecosystems worldwide, which are becoming increasingly frequent and catastrophic due to current climate change especially in the Mediterranean Basin. I conducted two experiments, one in the field following natural rainfall events, and one in mesocosm simulating a major flooding on lagoon sediments, both aimed at investigating the effects of such events on sediments biogeochemistry of a river and a coastal lagoon. The results of such experiments pinpoint that these events can have differential consequences on quantity, biochemical composition, nutritional quality, and degradation processes of sedimentary OM in either rivers or coastal lagoons. The observed consequences, their direction and amplitude vary, at times inexplicably, according to the magnitude of the rainfall and, possibly, with the exposure time of the impacted sediments to flooding events, with consequences, when observed, that can last also during the entire recovery of natural salinity values.

Finally, I carried out a metanalysis aimed at identifying the strongest effect of each selected disturbance on the tested variables and the most threatening disturbance (among those investigated in my thesis) on sedimentary OM stocks and degradation rates. I conclude that bottom trawling represents, overall, the most threatening source of disturbance on the sedimentary biogeochemistry of aquatic ecosystems.

II. Thesis outline

This thesis, aiming to explore magnitude and direction of changes in the benthic trophic status of river, lagoon and coastal marine ecosystems caused by natural and anthropogenic disturbances, is divided in six chapters. The thesis begins with a general introduction followed by four chapters structured as self-standing scientific papers and closes with a chapter illustrating, through a metanalytical approach, the comparison of the magnitude of the investigated disturbances effects on sedimentary OM features.

In **Chapter 1**, I review the concept of disturbance in ecology focusing on coastal aquatic ecosystems. Here I also provide science-based cues to support the choice of focusing my thesis on the effects of disturbance on the sedimentary trophic status and functioning of different aquatic ecosystems.

In **Chapter 2**, I report the results of a multiyear field correlative survey aimed at investigating the effects of bottom trawling, one of the most recurrent and severe anthropogenic disturbance in coastal seas worldwide, on the benthic trophic status of the Bornholm Basin (Baltic Sea) in the uppermost 4 cm of the sedimentary column.

In **Chapter 3**, I illustrate the results of a manipulative experiment in the field aimed at investigating the effects of marine heatwaves, a recurrent and increasingly frequent manifestation of current climate change, on OM features in sediments receiving an artificial warm water plume, generating temperature anomalies of 1.5–5.0°C.

In **Chapter 4**, I report the results of mesocosm manipulative experiments aimed at exploring the possible use of the Mediterranean deposit-feeder sea cucumber *Holothuria tubulosa* (Gmelin, 1788), a strong bioturbator and bioreactor, as a possible candidate to counteract natural and anthropogenic benthic eutrophication in different scenarios of sea warming and heat snaps, under either meso-eutrophic or oligo-mesotrophic statuses.

In **Chapter 5**, I report the results of a field survey aimed at investigating the effects of different rainfalls on the sedimentary biogeochemistry of a river and a coastal lagoon (SW Sardinia, Mediterranean Sea). In the same chapter, I also illustrate the results of a manipulative experiment in mesocosm aimed at assessing the effects of a simulated major flooding event and its short-term recovery on lagoon sediment biogeochemistry.

In **Chapter 6**, I illustrate the results of a meta-analysis exercise aimed at comparing the effect magnitude of each disturbance source investigated in Chapters 2-5 on sedimentary OM features and assessing a rank of their consequences.

1. Introduction and aims of the thesis

1.1 Concept, definition, and role of disturbance in ecology

Ecological disturbance, defined differently over the years, can be still generally defined as an abrupt, unpredictable, and spatially and temporally discrete event (of physical, chemical, or biological as well as natural or anthropogenic nature) that can directly or indirectly change organization and dynamics of habitats and communities (Sousa, 1984; Pickett and White, 1985; Petraitis et al., 1989; Mackey and Currie, 2001; Walker and Del Moral, 2003; Battisti et al., 2016; Newman, 2019).

Ecological disturbance (hereafter disturbance) can be an abiotic (e.g., resources availability, environmental variables) or biotic (e.g., predation, competition, biological invasions) factor that can destabilize nature at all hierarchical levels of the ecological organization (Hobbs and Huenneke, 1992; Sommer et al., 1993; Freedman, 1995; Mackey and Currie, 2001; Walker, 2012), thus representing a fundamental aspect in natural selection even at evolutionary scales (Krebs, 2001).

Disturbance *per se* can also play a fundamental role in regulating habitats' structure along with biodiversity (in terms of either species richness and equitability) (Randall Hughes et al., 2007; Schaffner, 2010; Murphy and Romanuk, 2012; Willig and Presley, 2017; Burton et al., 2020). Indeed, biodiversity can be (positively and negatively) affected or maintained by a certain threshold of disturbance, specifically known as the *intermediate disturbance*, which is still the most valid instant-in-time descriptor for the non-equilibrium of species biodiversity and, occasionally, comprehends combined phenomena (Connell, 1978; Wilson, 1990; Reynolds et al., 1993; Crandall et al., 2003; Roxburgh et al., 2004).

According to the *Intermediate Disturbance Hypothesis* (IDH), the response of biodiversity to disturbance can be assumed to be unimodal, with highest biodiversity occurring at intermediate levels of disturbance (Connell, 1978; Mouillot et al., 2013; Willig and Presley, 2017). At low levels of disturbance, biodiversity is kept low by resources availability (caused by constant environments) and, thus, by competition, whereas a "sufficient" disturbance or "disturbance regime" (i.e., persistent in a certain time, uniform or patchy, with or without post-disturbance residuals) can facilitate the increase and maintenance in species diversity in the long term. Further, once reached the tipping point, excessive disturbance becomes intolerable for most species but a few highly tolerant ones, thus causing the overall reduction in the species diversity either in the short or the long term (Sheil and Burslem, 2013; Fox, 2013; Mouritsen et al., 2018; Marriner et al., 2022). Moreover, prolonged disturbance is quite probable to cause irreversible habitat changes and an overall reduction of the remnant species' resilience (Bêche, 2009; Hillebrand and Kunze, 2020).

1.2 The role of disturbance in aquatic ecosystems

Most aquatic ecosystems, possibly with exception of the hadal ocean, are generally characterized by medium-to-short timescales of natural variability, strong interspecific interactions and, somehow, appear to be more sensitive to disturbances than terrestrial ecosystems (Murphy and Romanuk, 2012). This holds true particularly for marine coastal areas and lagoons, important transition zones between freshwater and marine ecosystems, where natural and anthropogenic disturbance and their ecological consequences are more tangible (Kron, 2013; Golla et al., 2020; Hossain et al., 2020).

Besides natural disturbances, in the last 50 years, because of the growing human populations in coastal areas and the related increase of marine resources demand (Cardillo et al., 2004; Mora, 2008), the coastal oceans, along with coastal lagoons and tributary rivers are experiencing the exacerbation of current climate change manifestations, which, moreover, will increase in the near future and will become even more intense, frequent and long-lasting (Ummenhofer and Meehl, 2016; Abd-Elgawad et al., 2019; Yao et al., 2021). Not by chance, in fact, climate change is progressively threatening, likely irreversibly, most aquatic ecosystems, with significant risks for the global biogeochemical cycles (Pörtner et al., 2022). In this regard, climate change (boosted by greenhouse gases emissions; Dai, 2011; Morant et al., 2020) is quickly and worryingly affecting the duration, magnitude, frequency, timing, dynamics, and spatial extent of a multitude of disturbance sources (Leigh, 2013).

Among these, particular attention is currently being given to many different sources of disturbance impacting aquatic ecosystems. Impacts generated by human activities have been determining severe consequences for the maintenance of global bio-geosphere integrity (Steffen et al., 2007). Despite the huge conservation efforts and the continuous implementation of environmental management measures aimed at preserving marine ecosystems' abilities to produce goods and services needed for human wellbeing, the aquatic ecosystems are experiencing unprecedented rates of change: these pervade all hierarchical levels of ecological organization, from individuals to populations, from communities to ecosystems (Butchart et al., 2010). For example, a considerable portion of the global ocean (>40 %) is exposed to multiple stressors (Halpern et al., 2008) and the Mediterranean Sea alone is under siege because of pollution, destructive fishing practices (e.g., bottom trawling), overfishing, aquaculture, spread of invasive species, eutrophication, oil and gas operations, offshore renewable energy search and development, coastal engineering, and development, besides climate change (Micheli et al., 2013).

Aquatic ecosystems are ever-increasingly threatened by sea level rising, marine heat waves and global warming (Halpern et al., 2008; Perkins et al., 2012; Wernberg et al., 2016; Oliver et al., 2018; Garrabou et al., 2022; Pörtner et al., 2022; Kauppi and Villnäs, 2022), glaciers' melting (Verdugo et al., 2022), ocean acidification (Doney et al., 2009; Kroeker et al., 2013; Wallace et al., 2014; Yuan et al., 2015; Hoegh-Guldberg et al., 2017), salinization (Kaushal et al., 2005; Ali et al., 2022 and citations therein), but also increasingly frequent occurrence of extreme or episodic weather events such as drought (Sheffiel and Wood, 2008; Dai, 2011; Murphy and Romanuk, 2012), hurricanes (Gardner et al., 2005; Jentsch et al., 2007; Simmons et al., 2021; Schaffer et al., 2022; Li et al., 2023), rainfall, river runoff, and flooding (Crandall et al., 2003; Poff, 2003; Islam et al., 2016; Talbot et al., 2018).

In addition to these, there is an ample and increasingly growing array of anthropogenic disturbances able to impair aquatic ecosystems, including (not exhaustively): bottom trawling (Hiddink et al., 2019) and aquaculture (Kalantzis and Karakassis, 2006; Holmer, 2010) in marine ecosystems, water extraction along with hydropower generation (Robson et al., 2013), coastal and offshore infrastructures (e.g., oil platforms and wind turbine parks offshore; Galpasoro et al., 2022; Punzo et al., 2017), invasive alien species (Murphy and Romanuk, 2012; Simberloff et al., 2013; Pusceddu et al., 2016; Rizzo et al., 2017; Palmas et al., 2019; Geraldi et al., 2020), and many others.

Despite these efforts, the evaluation of the ecological consequences of disturbance on aquatic ecosystems is still far from being fully achieved. This limitation is most often due to the lack of properly replicated studies and their generally disturbance-specific nature, and often to the lack of comparisons across sources of disturbance and across ecosystems (Buma, 2021).

1.3 Sedimentary organic matter as a proxy for benthic trophic status and eutrophication

The excessive nutrients enrichment in coastal waters causing an accelerated growth of algae and plants (Murphy and Romanuk, 2012) is a well-studied phenomenon known as eutrophication. A dated definition says that eutrophication represents *"an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned"* (OSPAR, 2003). The main sources of eutrophication have been identified with agrozootechnical activities (i.e., fertilizers and animals), aquaculture facilities (i.e., mollusks, fish), lack or malfunction of systems for the correct treatment of wastewaters (especially in poor areas of the world and in tourist spots during high-season vacancies) (Pusceddu et al., 2007; Nixon, 2009; Malone and Newton, 2020).

In the last 20 years, one of the recurrent focusses of aquatic ecology has been the question: *"How does anthropogenic nutrient enrichment cause change in the structure or function of nearshore coastal ecosystems?"* (Cloern, 2001). Till the 90ies of last century, most investigations aimed at addressing that question used inorganic nutrient concentrations as the sole pre-requisite causing eutrophication, in terms of increased algal biomass (Zurlini et al., 1996; Vollenweider et al., 1998). Since then, our conceptual model (*sensu* Cloern, 2001) of eutrophication has shifted towards models that consider the supply of organic C to the system (Nixon, 1995). In a strictly ecological perspective, such an approach appears ideal: in fact, it does consider not only the increase in algal biomass, but, more correctly and properly, the whole of organic substrates, including the detrital ones (i.e., at large, the whole food for consumers, in a "trophic-dynamic" perspective; Lindeman, 1942) (Grall and Chauvaud, 2002). Indeed, inputs of organic matter to aquatic ecosystems are the triggering mechanism leading to the ecological consequences of eutrophication: oxygen reduction and hypoxia up to mortality of marine organisms and biodiversity loss (Cloern, 2001). The approach proposed by Nixon, however, is difficult to apply on a large scale due to the high operative and technical costs of estimating, exhaustively, all C fluxes to the system.

The sediments of aquatic ecosystems have the advantage of being able to also record water column processes and are the ultimate depository for the accumulation of either autochthonous or allochthonous C inputs (Fabiano et al., 1995; Dell'Anno et al., 2002; Pusceddu et al., 2009). Accordingly, core records of sediment organic C, as well as specific biochemical markers (biogenic silica and lipids), have been used to describe long-term trends of increasing inputs of algal derived organic matter (Zimmerman and Canuel, 2000). More recent changes in the benthic trophic status (and thus in eutrophication) can be detected by means of an analysis of organic C inventories (Cornwell et al., 1996; Emeis et al., 2000; Farías, 2003). However, it must be considered that sedimentary organic matter in aquatic ecosystems is composed of a complex array of molecules exhibiting different levels of bioavailability for consumers, ranging from labile (i.e., immediately digestible) to highly refractory (recalcitrant to decomposition) (Pusceddu et al., 2003). Accordingly, due to the preeminently conservative and refractory nature of total organic C, changes in the trophic status (and thus eutrophication) of the sediments in aquatic ecosystems can be more evident in terms of organic matter biochemical composition (Pusceddu et al., 2007; 2009; 2011) rather than using the total organic C contents.

1.4 Aims of the thesis

This thesis aimed at investigating the effects of selected natural and anthropogenic disturbances on the quantity, biochemical composition, nutritional quality, C degradation rates and turnover time of sedimentary organic matter in river, lagoon, and coastal marine environments.

More specifically, I tested the null hypothesis by which the quantity (in terms of biopolymeric C contents), biochemical composition (in terms of protein, carbohydrate, lipid, and phytopigment contents), nutritional quality (i.e., algal fraction of biopolymeric C and/or the protein to carbohydrate ratio), potential C degradation rates (estimated from rates of extracellular enzymatic activities), and C turnover time of sedimentary organic matter do not vary:

I. across levels of bottom trawling intensities (i.e., null, low, medium) in the coastal Baltic Sea (**Chapter 2**)

II. under the influence of marine heatwaves causing different temperature anomalies in the coastal Mediterranean Sea (**Chapter 3**)

III. between ambient sediments and feces of the Mediterranean deposit-feeder sea cucumber *Holothuria tubulosa* (Gmelin, 1788): i) acclimated under different temperatures and ii) exposed to heat snaps, to evaluate the use of *H. tubulosa* as a possible bioreactor to counteract benthic eutrophication under different scenarios of sea warming (**Chapter 4**)

IV. before *vs.* after rainfall events with different intensity in natural Mediterranean alluvial plain, including a river and a coastal lagoon (**Chapter 5**)

V. before *vs.* after a simulated (in mesocosm) flooding event and the subsequent recovery in Mediterranean lagoon sediments (**Chapter 5**)

Finally, in the concluding **Chapter 6**, using a metanalytical approach based on estimates of the effect magnitude metric, I tried to identify: i) which among organic matter content, nutritional quality, and degradation is more affected by each of a selected array of sources of disturbance and ii) which among the different sources of disturbance is more threatening, separately, organic matter content, nutritional quality, and degradation.

For this doctoral thesis, I had the great opportunity to collaborate with Prof. Clare Bradshaw (University of Stockholm, **Chapter 2**), Prof. Giulia Ceccherelli (University of Sassari, **Chapter 3**), Dr. Pere Puig and Dr. Albert Palanques (Institute of Marine Sciences - ICM-CSIC - of Barcelona, **Chapter 5**).

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2. Effects of bottom trawling disturbance on quantity, biochemical composition, nutritional quality, and C turnover of sedimentary organic matter in a coastal marine environment

2.1 Introduction

Bottom trawling, a commonly widespread fishing technique, is one of the major anthropogenic disturbances to which marine benthic ecosystems are subjected worldwide (Kaiser et al., 2002; Olsgard et al., 2008; Oberle et al., 2016; Hiddink et al., 2017; González-García et al., 2022). The trawl doors leave pronounced deep marks on the seabed by scraping the substrate and their activity can cause a turbulent bottom reworking and, subsequently, induce resuspension and flattering of the fine, soft, and sandy-muddy sediments (Smith et al., 2000, 2003; Ferré et al., 2008; Martín et al., 2014; Mérillet et al., 2018; Mengual et al., 2019; Jac et al., 2022). Bottom trawling can physically modify the sediment characteristics (Bhagirathan et al., 2010; Paradis et al., 2017, 2018a-b, 2021b; Daly et al., 2018; Arjona-Camas et al., 2021; Nielsen et al., 2023) homogenizing and smoothing the seafloor, and reducing its original complexity even in the deep seascape (Puig et al., 2012). Moreover, it can alter sea bottom biogeochemistry (Pusceddu et al., 2005a; Bhagirathan et al., 2010; van de Velde et al., 2018; Paradis et al., 2021a; Morys et al., 2021), at times enhancing eutrophication of coastal ecosystems (Polymenakou et al., 2005; Ferguson et al., 2020) or further impoverishing oligotrophic deep sea beds (Pusceddu et al., 2014a), impairing benthic habitats and communities (McLaverty et al., 2020; Nielsen et al., 2023), especially those in soft-muddy sands (Kaiser et al., 2006) and ecosystem functioning (Pusceddu et al., 2005a; Pusceddu et al., 2014a).

The extent of the impacts of bottom trawling on benthic ecosystem are not consistent across areas, regions, and oceans. In fact, the frequency and intensity of fishing activities vary among several habitat types affecting differently the seabed (Kaiser et al., 2002), and their actual impact can be even controversial (Rice, 2000; Løkkeborg, 2007). To partly bypass biases affecting the possibility to assess properly trawling impacts, tools such as high-resolution vessel track reconstruction, the calculation of the spatial distribution of bottom trawling intensity and associated sediment resuspension (Oberle et al., 2016) have been recently used as proxies for evaluating levels of bottom trawling disturbance.

Bottom trawling signs of disturbance were found highly evident on the seafloor of the Baltic Sea (ICES, 2018). This is a large, shallow, semi-enclosed, brackish sea, which nowadays is considered one of the main coastal areas worldwide where the combination of both anthropogenic eutrophication and overfishing are heavily impairing its ecosystem structure and function (Möllmann et al., 2009, 2012; Aps and Lassen, 2010; Eero et al., 2011; Gustafsson et al., 2012; Popov, 2017; ICES, 2018; Reusch et al., 2018), particularly in the Bornholm Basin. The Bornholm basin is among the deepest basins in the south-central Baltic Sea, characterized by seasonal fluctuations driven by a strong physical-chemical stratification (i.e., temperature, salinity, and oxygen gradients), showing a high ecologic importance for the entire Baltic Sea, especially for the benthic fauna (Pedersen, 1977; Krauss and Brügge, 1991; Schulz et al., 2012; Stigebrandt, 2017; Szymczycha et al., 2019). Nevertheless, in the Bornholm Basin, more than 80 % of the seabed is disturbed by several human activities (HELCOM, 2022), of which bottom trawling represents the most thriving and widespread fishing activity (Bager et al., 2007; ICES, 2018), particularly for cods (Voss et al., 2003; Pierce et al., 2017). Moreover, the surficial sediments of the Bornholm Basin are mainly soft and muddy (Stigebrandt, 2017; **Figure S1**), which could locally contribute to a bottom trawling-induced eutrophication effect.

Gray et al. (2006, 2007) and Løkkeborg (2007) critically discussed about the difficulty to monitor and assess the impact of bottom trawling on soft-sediments and to provide a correct overall interpretation. That is due to a lack of uniformity in the measurement units of trawling impact or because of sampling designs often biased by the lack of real controls or inadequate spatial replicates. Consequently, the possible effects of different trawling intensity on benthic trophic status (i.e., quantity, biochemical composition, and nutritional quality of sedimentary organic matter) are still under-investigated.

To provide new insights into this topic, we tested the null hypothesis by which the quantity, biochemical composition (in terms of protein, carbohydrate, lipid, and phytopigment contents), nutritional quality (i.e., algal fraction of biopolymeric C and the protein to carbohydrate ratio), and potential degradation rates (expressed as rates of extracellular enzymatic degradation of proteins and carbohydrates) of organic matter (OM) in coastal marine sediments do not vary across different intensities of bottom trawling.

2.2 Materials and Methods

2.2.1 Study area and sampling strategy

The study was conducted over two years (May 15-23, 2019, and May 25-30, 2020) during two oceanographic campaigns carried out by the University of Stockholm within an area located between the Bornholm Island and Simrishamn, on the Swedish coast (**Figure 1**).

A total of 8 sites were investigated at each of which bottom water temperature (1 m.a.b.), salinity, and oxygen concentration were measured by multiparametric probes (**Table 1**). Fishing intensity for the initial selection of sites was obtained from Swedish fishing vessels ≥ 12 m equipped with GPS-based Vessel Monitoring System (VMS) coupled with logbooks information on vessel size and gear used (González-García et al., 2022). Moreover, Swept Area Ratio (SAR) was calculated following the approach of Eigaard et al. (2017) on a 250 x 250 m grid over the period 2016-2019. This led to the identification of twelve sites of different estimated levels of trawling impact. More in details, we considered sites: D-F representative of null trawling conditions, B-G-J as low trawling, and C-H-I as medium trawling (**Figure 1**, **Table 1**). In each site, sediment samples were collected using a multicorer (9 cm internal diameter, 60 cm total length), obtaining four independent samples from stations D, F, B, C and three independent samples from stations G, J, H, I. Once on board, the sediment was vertically sliced into four layers (0-1, 1-2, 2-3, 3-4 cm), and stored in plastic ziplock bags at -20 °C until analyses.

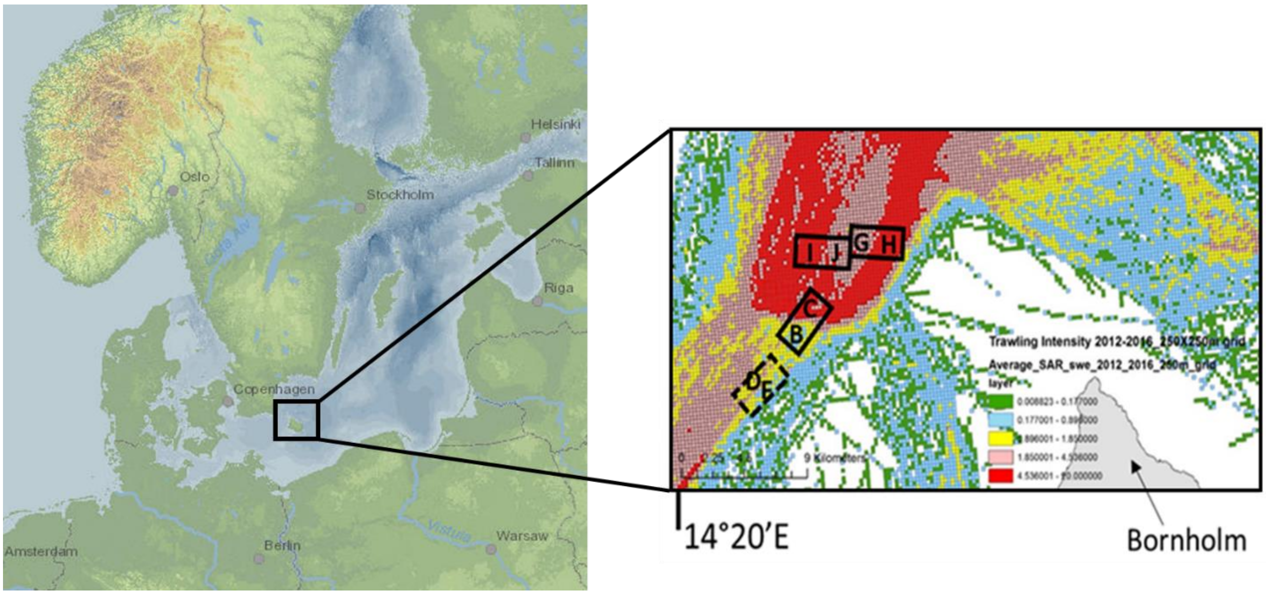


Figure 1. Study area and location of the eight stations in the Bornholm Basin.

Table 1. Temperature, salinity, and dissolved O_2 concentrations measured at 0.5 - 1 m above the sea bottom in the Bornholm Basin. Trawling effort was estimated as the average data of swept area ratio (SAR) between 2016 and 2019 (see the text for further details).

Site	Date of sampling	Latitude (N)	Longitude (E)	Depth (m)	Temperature (°C)	Salinity	O_2 (mL L^{-1})	Trawling (SAR)
D	21 May 2019	55.3069	14.4371	47	7.88	14.84	5.82	0.00
F	23 May 2019	55.3050	14.4405	47	7.94	13.89	6.03	0.00
B	19 May 2019	55.3330	14.4415	51	7.83	15.51	5.65	0.08
G	25 May 2020	55.3980	14.5145	58	6.52	12.89	3.65	1.11
J	27 May 2020	55.3910	14.4911	53	6.15	9.52	5.25	1.35
C	20 May 2019	55.3488	14.4495	49	7.65	16.23	5.32	2.01
H	26 May 2020	55.4052	14.5536	62	6.58	14.05	3.19	2.51
I	27 May 2020	55.3838	14.4612	55	6.11	8.99	5.54	3.81

2.2.2 Biochemical composition and freshness of sedimentary organic matter

Protein, carbohydrate, and lipid contents were determined spectrophotometrically based on the protocols detailed in Danovaro (2010). More specifically, proteins were determined according to Lowry et al. (1951), as modified by Hartree (1972) and Rice (1982), using the Folin-Ciocalteau reagent in a basic environment and expressed as bovine serum albumin equivalents. The procedure proposed by Gerchakov and Hatcher (1972), based on the phenol and concentrated sulfuric acid reaction with saccharides, was used to determine carbohydrates, then expressed as D (+) Glucose equivalents. Lipids, after extraction in chloroform: methanol (1:1, vol:vol) (Bligh and Dyer, 1959), and evaporation in a dry hot bath at 80 to 100°C for 20 min, were determined after the sulfuric acid carbonization procedure (Marsh and Weinstein, 1966) and expressed as tripalmitin equivalents. For each biochemical assay, blanks were obtained using precalcinated sediments (450°C for 4 h). Protein, carbohydrate, and lipid concentrations were converted into C equivalents using the conversion factors 0.49, 0.40, and 0.75 mgC mg^{-1}, respectively, obtained from the C contents of the respective standard molecules (albumin, glucose and tripalmitin, respectively), and their sum was reported as the biopolymeric C (BPC) (Fabiano et al., 1995).

Chlorophyll-a (Chl-a) and phaeopigment analyses were carried out according to Danovaro (2010). Pigments were extracted (overnight at 4°C in the dark) from triplicate 0.1 g sediment subsamples using 5 mL of 90 % acetone as the extractant. Extracts were analyzed fluorometrically (430 nm excitation and 665 nm emission wavelengths) to estimate Chl-a, and, after acidification with 200 µl 0.1N HCl, phaeopigment concentrations. Total phytopigment was defined as the sum of Chl-a and phaeopigment concentrations and, once converted into C equivalents using 40 µgC µg^{-1} as a conversion factor, I calculate the algal fraction of BPC as the percentage of phytopigment-to-BPC concentrations, used to estimate the fraction of the organic material of autotrophic origin including either the living (Chl-a) and senescent/detrital (phaeopigment) fractions (Danovaro, 2010; Pusceddu et al. 2010, 2014b). I chose the algal fraction of BPC and the protein to carbohydrate ratio as descriptors of the aging and nutritional quality and enrichment of the sedimentary OM (Dell'Anno et al., 2002; Pusceddu et al. 2009, 2010).

2.2.3 Extracellular enzymatic activities, C degradation rates and turnover times

OM degradation rates were estimated from aminopeptidase and β-glucosidase activities, determined by the cleavage of artificial fluorogenic substrates (L-leucine-4-methylcoumarinyl-7-amide, for aminopeptidase; 4-methylumbelliferone-D-glucopyranoside, for β-glucosidase) at saturating concentrations (Danovaro, 2010; Bianchelli et al., 2020). Extracellular enzymatic activities were measured after the addition of 100 µl of substrate to 1 ml of a slurry prepared using 1:1 volume of filtered (0.2 µm) and sterile seawater and sediment (substrate final concentration 200 µM) (Danovaro, 2010; Bianchelli et al., 2020). Substrate incubations were performed in the dark at in situ temperature for 1 h. After incubation, the slurries were centrifuged (3000 rpm, 5 min) and supernatants were analyzed fluorometrically (at 380 nm excitation and 440 nm emission for aminopeptidase; at 365 nm excitation and 455 nm emission for β-glucosidase) (Danovaro, 2010; Bianchelli et al., 2020). Data were normalized to sediment dry weight (60°C, 24 h) and reported as nanomole of hydrolyzed substrate released per g of sediment dry weight h^{-1}. Protease and glucosidase activities were converted into C equivalents using 72 as a conversion factor (estimated from the C content of the fluorescent component released after reaction with the enzymes) and their sum, reported as the potential C degradation rate (μgC g^{-1} h^{-1}). The turnover times (per day) of the whole protein and carbohydrate pools were calculated as the inverse number of the ratios of the hourly C degradation rates (once multiplied by 24) and the whole protein and carbohydrate C contents in the sediment. Although these estimates are only potential (maximum) rates of protein and carbohydrate turnover, they are considered good proxies of ecosystem functioning (Pusceddu et al., 2014b).

2.2.4 Statistical analyses

To test the null hypothesis by which OM quantity, biochemical composition (in terms of protein, carbohydrate, lipid, and phytopigment contents), nutritional quality, C degradation rates and turnover times do not vary across different bottom trawling intensities, permutational multivariate analyses of variance (PERMANOVA) (Anderson, 2001) were carried out in either the uni- or multi-variate context with two fixed and orthogonal factors: trawling intensity (3 levels: null, low and medium) and sediment layer (4 levels: 0-1; 1-2; 2-3; 3-4). Considering the ample variability in both OM sedimentary loads and enzymatic activities in the sediments of the study area (see also **Figure S2** and **Figure S3**), we used arrays of stations as described above as true replicates for each of the levels of trawling intensity. Briefly: D-F as spatial replicates for null trawling conditions, B-G-J for low trawling, and C-H-I for medium trawling. For each station all data obtained by the analyses of sediments obtained from independent deployments of the multicorer were used as replicates as well.

PERMANOVA is a semiparametric method described as a geometric partitioning of multivariate variation in the space of a chosen dissimilarity measure according to a given ANOVA design, with p-values obtained using appropriate distribution-free permutation techniques. Since PERMANOVA on one response variable using Euclidean distance yields the classical univariate F statistic, PERMANOVA can also be used to perform univariate ANOVA, but where p values are obtained by permutation (Anderson and Millar, 2004), thus avoiding the assumption of normality (Anderson, 2014).

The analyses were carried out on Euclidean distance-based resemblance matrixes obtained from previously normalized data, using 999 random permutations of the appropriate units. When significant differences were observed, pairwise tests were also carried out to ascertain patterns of differences between coupled sites and among layers. Multivariate differences in OM biochemical composition (in terms of protein, carbohydrate, lipid and total phytopigment contents) were visualized with a biplot after a canonical analysis of the principal coordinates (CAP). CAP allows identification of an axis through the multivariate cloud of points that is best at separating the groups. The motivation for the CAP routine arose as sometimes there are real differences among a priori groups in multivariate space that cannot be easily seen in an unconstrained ordination (as in PCA or MDS plots) (Anderson et al., 2008).

PERMANOVA and CAP tests were carried out through the software PRIMER 6+, using the included routine package PERMANOVA (Anderson et al., 2008).

2.3 Results

Data on the OM quantity, nutritional quality, enzymatic activities, C degradation rates and turnover in the sediments exposed to null, low, and medium intensity of bottom trawling are reported in **Table S1**.

Sedimentary contents of almost all classes of organic compounds, along with biochemical composition, enzymatic activities (with exception of aminopeptidase and C degradation) were affected by the Trawling × Layer interaction (**Table 2**). Results of the pairwise tests carried out to ascertain differences in the quantity, nutritional quality, biochemical composition, and degradation rate and turnover time of OM between pairs of sediment layers (0-1, 1-2, 2-3, 3-4 cm) at each level of bottom trawling intensity are reported in **Table S2**.

2.3.1 Quantity and biochemical composition of sedimentary organic matter

The results of the post-hoc tests ascertaining differences and their entity among levels of bottom trawling intensity in each sediment layer are illustrated in **Figure 2**.

Overall, OM contents were significantly higher in untrawled sites than in trawled sites, independently of the trawling intensity. More in details, in the top 1st cm sediment layer OM contents showed values in the untrawled sites ca. 3-5 times significantly higher than those exposed to either low or medium bottom trawling intensity, which, instead did not differed one each other. The pattern of OM contents across sites becomes less evident with increasing depth in the sediment (**Figure 3A-E**).

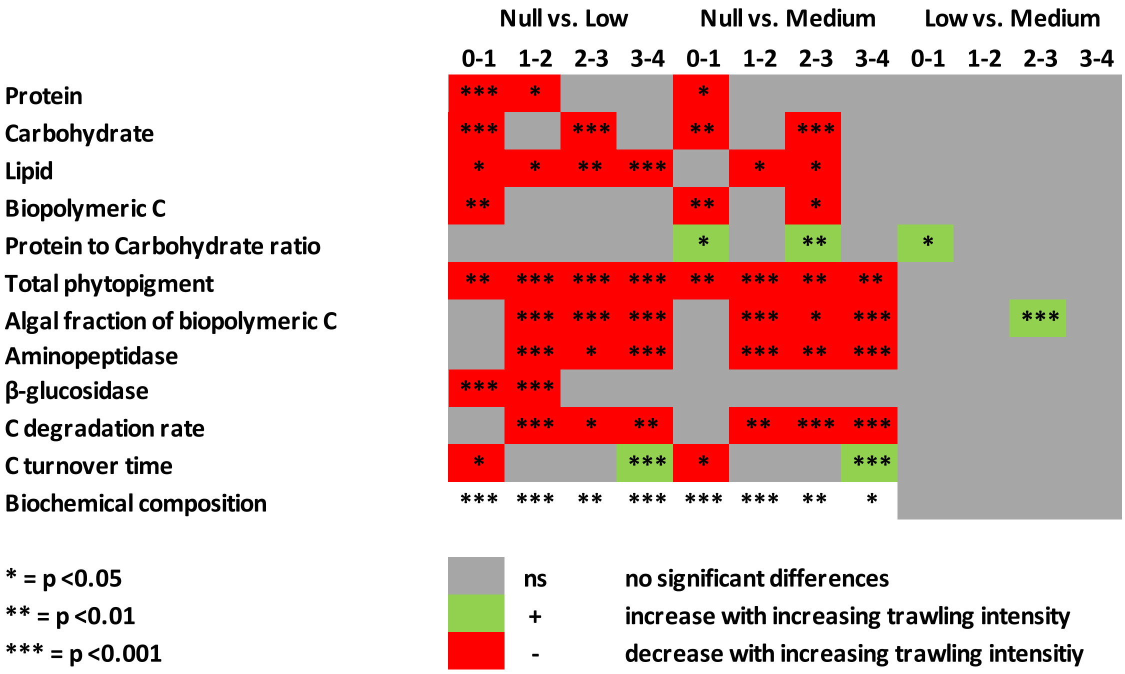
Values of the protein to carbohydrate ratio did not differ significantly between the untrawled sites and those exposed to low intensity of bottom trawling, whereas values in the untrawled sites resulted lower than those in sites exposed to medium levels of bottom trawling intensity only in the top 1st cm layer (by ca. 1.5 times) and in the 2-3 cm one (by ca. 2 times) (**Figure 3F**).

In the top 1st cm of the sediment, the algal fraction of biopolymeric C did not differ among sites with different levels of bottom trawling intensity, whereas in the deeper sediment layer of the untrawled site it was up to 3 times higher than that in the two other trawling conditions (**Figure 3G**).

Table 2. Results of the PERMANOVA carried out to investigate differences in the quantity, biochemical composition, nutritional quality, and degradations rates of organic matter among levels of bottom trawling intensity (TR; null, low, medium) and among sediment layers (LA; 0-1, 1-2, 2-3, 3-4 cm). df = degrees of freedom; MS = mean square; Pseudo-F = F statistic; P(MC) = probability level after Monte Carlo simulation (** = $p < 0.01$; * = $p < 0.05$; ns = not significant). Reported is also the percentage of variation explained by each factor (% EV), their interaction and residual (i.e., unexplained) variance.

Variable	Source	df	MS	Pseudo-F	P(MC)	% EV
Protein	TR	2	1.585	3.826	*	5
	LA	3	0.569	1.373	ns	1
	TR × LA	6	1.072	2.589	*	12
	Residual	124	0.414			82
Carbohydrate	TR	2	1.077	9.846	**	12
	LA	3	1.080	9.874	**	16
	TR × LA	6	0.387	3.538	**	13
	Residual	124	0.109			59
Lipid	TR	2	11.283	13.687	**	22
	LA	3	0.611	0.742	ns	0
	TR × LA	6	0.109	0.132	ns	0
	Residual	124	0.824			78
Biopolymeric C	TR	2	1.748	10.609	**	15
	LA	3	0.883	5.361	**	9
	TR × LA	6	0.385	2.336	*	8
	Residual	124	0.165			68
Protein to Carbohydrate ratio	TR	2	2.684	2.759	ns	3
	LA	3	3.763	3.868	*	7
	TR × LA	6	2.580	2.652	*	12
	Residual	124	0.973			78
Phytopigment	TR	2	16.943	58.691	**	46
	LA	3	3.471	12.024	**	12
	TR × LA	6	0.837	2.901	*	6
	Residual	124	0.289			36
Algal fraction of BPC	TR	2	15.633	37.080	**	37
	LA	3	2.013	4.775	**	5
	TR × LA	6	1.547	3.668	**	11
	Residual	124	0.422			46
Biochemical composition	TR	2	46.430	24.369	**	29
	LA	3	10.854	5.696	**	8
	TR × LA	6	5.292	2.778	**	9
	Residual	124	1.905			55
Aminopeptidase activity	TR	2	4.807	11.664	**	12
	LA	3	9.448	22.928	**	35
	TR × LA	6	0.606	1.471	ns	2
	Residual	124	0.412			51

β-glucosidase activity	TR	2	3.049	6.294	**	6
	LA	3	13.605	28.091	**	40
	TR × LA	6	1.271	2.624	*	7
	Residual	124	0.484			48
C degradation rates	TR	2	4.890	12.528	**	13
	LA	3	9.911	25.394	**	37
	TR × LA	6	0.582	1.491	ns	2
	Residual	124	0.390			49
C turnover time	TR	2	0.568	0.881	ns	0
	LA	3	11.857	18.402	**	32
	TR × LA	6	1.791	2.780	*	9
	Residual	124	0.644			59



	Null vs. Low				Null vs. Medium				Low vs. Medium			
	0-1	1-2	2-3	3-4	0-1	1-2	2-3	3-4	0-1	1-2	2-3	3-4
Protein	🟥 ***	🟥 *	▨	▨	🟥 *	▨	▨	▨	▨	▨	▨	▨
Carbohydrate	🟥 ***	▨	🟥 ***	▨	🟥 **	▨	🟥 ***	▨	▨	▨	▨	▨
Lipid	🟥 *	🟥 *	🟥 **	🟥 ***	▨	🟥 *	🟥 *	▨	▨	▨	▨	▨
Biopolymeric C	🟥 **	▨	▨	▨	🟥 **	▨	🟥 *	▨	▨	▨	▨	▨
Protein to Carbohydrate ratio	▨	▨	▨	▨	🟩 *	▨	🟩 **	▨	🟩 *	▨	▨	▨
Total phytopigment	🟥 **	🟥 ***	🟥 ***	🟥 ***	🟥 **	🟥 ***	🟥 **	🟥 **	▨	▨	▨	▨
Algal fraction of biopolymeric C	▨	🟥 ***	🟥 ***	🟥 ***	▨	🟥 ***	🟥 *	🟥 ***	▨	▨	🟩 ***	▨
Aminopeptidase	▨	🟥 ***	🟥 *	🟥 ***	▨	🟥 ***	🟥 **	🟥 ***	▨	▨	▨	▨
β-glucosidase	🟥 ***	🟥 ***	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
C degradation rate	▨	🟥 ***	🟥 *	🟥 **	▨	🟥 **	🟥 ***	🟥 ***	▨	▨	▨	▨
C turnover time	🟥 *	▨	▨	🟩 ***	🟥 *	▨	▨	🟩 ***	▨	▨	▨	▨
Biochemical composition	***	***	**	***	***	***	**	*	▨	▨	▨	▨

* = p <0.05	▨	ns	no significant differences
** = p <0.01	🟩	+	increase with increasing trawling intensity
*** = p <0.001	🟥	-	decrease with increasing trawling intensitiy

Figure 2. Results of the pairwise tests carried out to assess differences in quantity, biochemical composition, nutritional quality, extracellular enzymatic activity, C turnover times of sedimentary organic matter between pairs of bottom trawling intensity levels in each of the four sediment layers.

In all sediment layers the biochemical composition of trawled sediments, independently of the level of bottom trawling intensity, was generally homogeneous, whereas the biochemical composition of untrawled sediments, at all sediment depths, was clearly different from that of both trawled sites, with differences mostly explained by OM contents generally higher in untrawled sites than in those exposed to either low or medium intensity of bottom trawling (**Figure 4**).

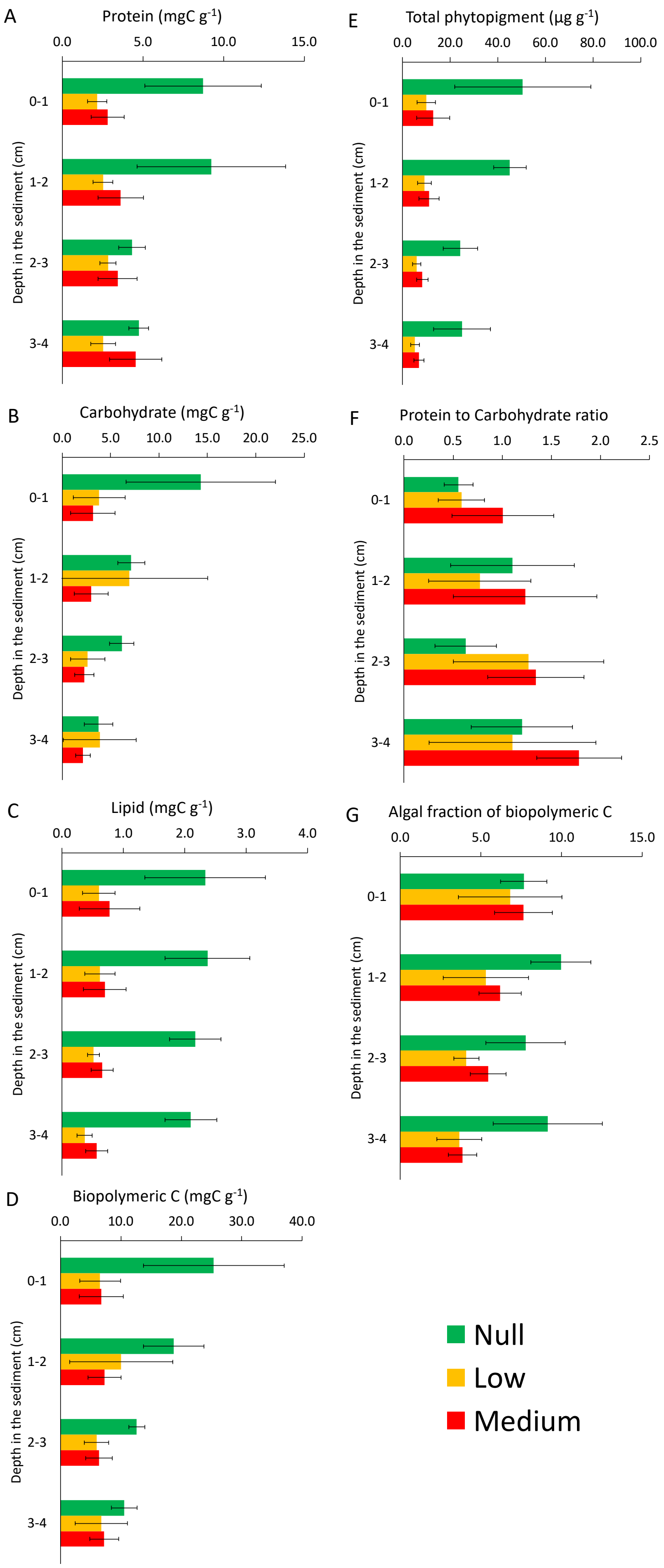


Figure 3. Vertical distribution (0-4 cm) of protein (A), carbohydrate (B), lipid (C), biopolymeric C (D), total phytopigment (E) contents and protein to carbohydrate ratio (F) and algal fraction of biopolymeric C (G) in the sediments exposed to null, low, and medium intensity of bottom trawling. The error bars indicate the standard deviation among replicates (n=3-4).

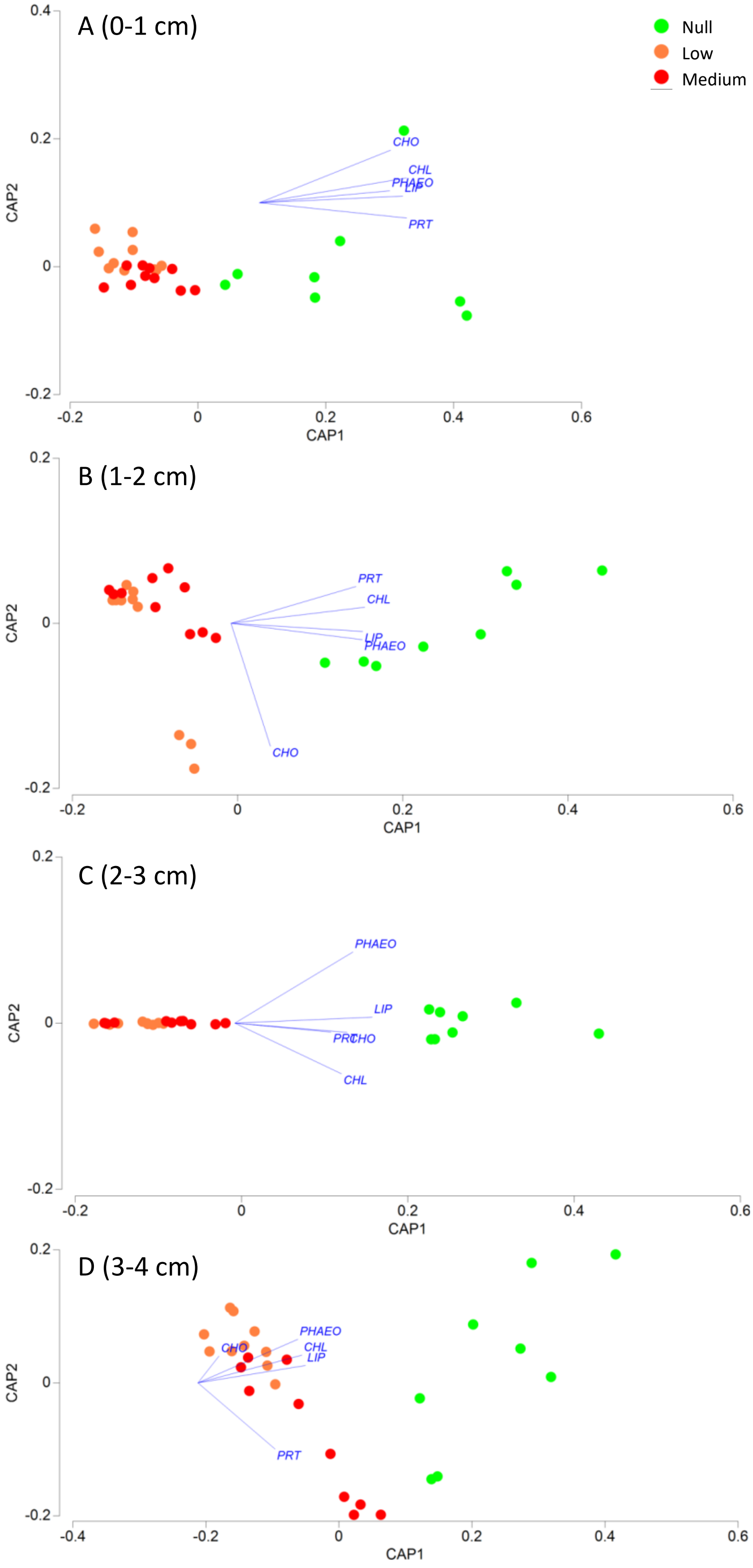


Figure 4. Biplots obtained after CAP showing differences in the biochemical composition of sedimentary organic matter among sites exposed to null, low, and medium intensity of bottom trawling in layers 0-1 cm (A), 1-2 cm (B), 2-3 cm (C), 3-4 cm (D). PRT = protein; CHO = carbohydrate; LIP = lipid; CHL = chlorophyll-a; PHAEO = phaeopigment. Vectors are proportional to the explained differences.

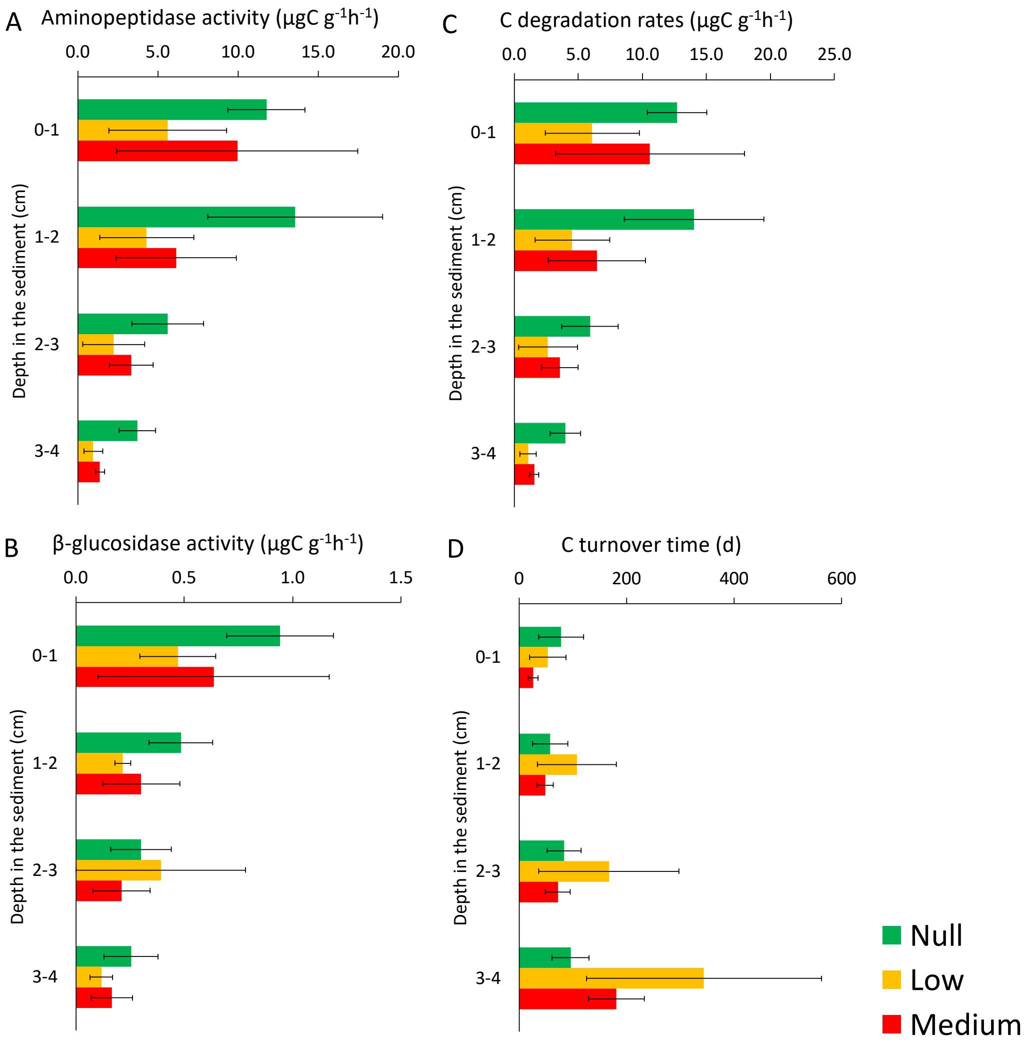


Figure 5. Vertical distribution (0-4 cm) of aminopeptidase (A) and b-glucosidase (B) activities and C degradation rates (C) and turnover time (D) in the sediments exposed to null, low, and medium intensity of bottom trawling. The error bars indicate the standard deviation among replicates (n=3-4).

2.3.2 Enzymatic activities and C degradation and turnover time

Aminopeptidase activity was not significantly affected by the Trawling × Layer interaction, but, in almost all sediment layers, with exception of the surficial (0-1 cm) layer showed significant differences between untrawled sediments and the trawled ones, being in the former 2-4 times higher than in the latter; in all sediments, aminopeptidase activity decreased, though not significantly in each sediment layer, with increasing depth in the sediment (**Figure 5A**).

β-glucosidase activity showed differences only between the uppermost 2 cm of the sediment of untrawled sediments and the same layers of sediments exposed to low intensity of bottom trawling, resulting in the former 1-2 times higher than in the latter; variations of β-glucosidase activity with depth in the sediment were more pronounced in untrawled sites than in the trawled ones (**Figure 5B**).

C degradation rates were not affected by the Trawling × Layer interaction but differed among sites with different bottom trawling intensities in all sediment layers, but the uppermost one; at each level of trawling intensity, C degradation rates in the uppermost 2 cm were generally 1-2 times higher than those in the deeper sediment layers (**Figure 5C**).

C turnover time in the uppermost 1st cm of the untrawled sites was ca. 1-3 times significantly higher than that in the trawled ones. This pattern is reversed in the deepest sediment layer, where the highest C turnover time (i.e., more than 300 d) occurred in the sites characterized by low levels of bottom trawling intensity; at both trawled sites C turnover time increased significantly with depth in the sediment, whereas in the untrawled sites remained almost constant across the entire sediment column (**Figure 5D**).

2.4 Discussion

2.4.1 Effects of bottom trawling on sedimentary OM quantity, composition, and nutritional quality

Bottom trawling is one of the major anthropogenic disturbances to which marine benthic ecosystems are subjected worldwide (Kaiser et al., 2002; Olsgard et al., 2008; Oberle et al., 2016; Eigaard et al., 2017; Hiddink et al., 2017; González-García et al., 2022), especially in shallow and semi-enclosed basins such as the Baltic Sea. Although it is still difficult to disentangle bottom trawling impacts from those caused by other environmental disturbances, it is well established that this typology of fishery can alter sea bottom biogeochemistry (Pusceddu et al., 2005a; Bhagirathan et al., 2010; van de Velde et al., 2018; Paradis et al., 2021a; Morys et al., 2021). Nevertheless, the results of studies carried out to ascertain the effects of bottom trawling on sedimentary OM are still controversial. Some studies, in fact, indicate that bottom trawling can enhance the consequences of coastal eutrophication (Polymenakou et al., 2005; Ferguson et al., 2020). Other studies revealed that bottom trawling can depauperate sedimentary organic loads, especially after chronic trawling disturbance, with largest effects observed especially in the deep sea (Pusceddu et al., 2014).

The Bornholm Basin (Baltic Sea), also because of its semi-enclosed and persistent stratified water column, is exposed to a complex array of physical disturbances that, besides anthropogenic activities, can influence trophodynamic processes at different spatial scales (Schulz et al., 2012). This condition limits the disentanglement of the consequences generated by bottom trawling from those caused by other natural or anthropogenic disturbances. This holds true also because of the peculiar dominance of finer silt sediment particles in the Baltic Sea, which, being more easily resuspended and transported by local hydrological phenomena (Zhurbas et al., 2010; Porz et al., 2022), could be transported across the entire basin, independently from the presence of bottom trawling, thus possibly masking its effects. Moreover, trawling impact in natural ecosystems is difficult to assess mainly because of the lack of real untrawled areas worldwide, which can be used as control. This, in fact, has caused a heated debate in the recent literature regarding the possible correct sampling and monitoring methodologies in the study of sediment disturbance caused by bottom trawling (see Gray et al., 2006, 2007 and Løkkeborg, 2007). In addition, it has been highlighted that some fishing gears can physically impact the seabed well beyond the first 3 centimeters of the sedimentary column (Depestele et al., 2019).

In this chapter, I investigated the quantity, biochemical composition, nutritional quality, C degradation rates and turnover time of OM in sediments subjected to different bottom trawling intensities, including sediments untrawled since at least 4 years and a set of two assets exposed to low and medium intensity of bottom trawling, respectively, each represented by 3-4 true spatial replicates. The study was conducted over two different years and, to limit seasonal biases, sampling was carried out in the same month of the two years (namely May 2019 and May 2020).

Here, I show that the sediments of all investigated sites, independently of the trawling intensity, were characterized by biopolymeric C contents higher than 3.0 mgC g^{-1} and an algal fraction lower than 12 %, which, according to the classification proposed by Pusceddu et al. (2009, 2011), can be ranked from eutrophic (i.e., B, J, I) to hypereutrophic (i.e., D, F, G, C, H). Not surprisingly, such values resemble more closely those observed in coastal lagoons or other highly eutrophic coastal areas such as the N Adriatic Sea and the Thermaikos Gulf, both theaters of intensive, basin-wide, and chronic disturbance caused by bottom trawling (Micheli et al., 2013; Pusceddu et al., 2005a).

I report here that, almost consistently across the entire sediment column with exception of the deepest (3-4 cm) layer, untrawled sediments of the Bornholm Basin were characterized by OM and phytopigment contents far higher than those in both trawled ones. This result reflects the typical removal of sediments caused by trawling gears observed in other seas and water depths (Bhagirathan et al., 2010; Pusceddu et al., 2014). Notably, the difference between trawled and untrawled sediments below 3 cm depth were much smoother than above, according to the average depth of gears penetration reported by Hiddink et al. (2017) and adopted also by Sala et al. (2021) to estimate CO_2 emissions caused by bottom trawling activities worldwide. The pattern observed in the Bornholm Basin, however, is not recurring in other settings. For instance, Pusceddu et al. (2005a) found that in the Thermaikos Gulf (Eastern Mediterranean Sea) biopolymeric C contents in the sediment increased immediately after the initiation of trawling activity, most likely because of mixing of OM richer deep sediment layers. In deeper sediments, Paradis et al. (2019) observed higher OM contents in superficial (i.e., top 1st cm) untrawled bottoms at ca. 500 m depth which, however, were related to recent inputs of fresh OM from the upper water column. Altogether, these results confirm that the effects of bottom trawling on OM sediment contents can be highly variable depending not only on the extent, duration, and intensity of trawling activities, but also on the general and contextualized conditions of the investigated areas. Nonetheless, the results of this study allow identifying bottom trawling as an anthropogenic disturbance able to impoverish the amount of biopolymeric C and phytopigments, that is the amount of bioavailable food for benthic detritus feeders (Pusceddu et al., 2009).

I report here also that trawled sediments were generally characterized by invariant values of the protein to carbohydrate ratio in all sediment layers and of the algal fraction of biopolymeric C, at least within the top 2 cm of the sediment column. These results, indicate that, while bottom trawling determined an impoverishment of the sea bottom, it has minor or null effects on the nutritional quality of organic substrates, ultimately indicating that the "oligotrophication" effect of bottom trawling could be, at least partially, counteracted by an invariant food availability of the remining organic substrates. According to the *optimal foraging theory* (Stephens and Krebs, 1986), which foresees that consumers tend to optimize energy intake by means of a trade-off between the quantity of energy necessary for survival and that spent to acquire it, ultimately dependent upon its nutritional quality, these results would predict that a model deposit feeder in the trawled sediments of the Bornholm Basin would be obliged to ingest a larger amount of sediment than that needed in untrawled sediments.

The results of the CAP also allowed me to highlight that bottom trawling, independently of the intensity level and in all sediment layers, caused a clear homogenization of the biochemical composition among replicates, whereas the OM biochemical composition in untrawled sediments was characterized by a much wider variability. This result confirms further the capacity of bottom trawling, whatever the intensity level, to mix the sediment layers, ultimately causing an overall homogenization of the trophic status across the whole sediment layers (at least down to 4 cm depth). Notably the biochemical composition of trawled sediments was also largely different from that in untrawled sediments. Since the trophic status of marine sediments is the result of the combination of OM quantity, biochemical composition, and nutritional quality (Pusceddu et al., 2009; 2011), the results of this study highlight that bottom trawling, whatever the intensity level, can severely modify the trophic status of the Bornholm Basin sediments.

2.4.2 Effects of bottom trawling on sedimentary OM degradation and turnover time

Sedimentary C mobilization mediated by extracellular enzymes and turnover time are commonly used as proxy of ecosystem functioning in variably disturbed sediments (Pusceddu et al. 2009, 2014a; Soru et al., 2022). Here, I report that aminopeptidase activities in the top 1st cm of the sediment column of untrawled bottoms did not differ from those in sediments exposed to both low and medium intensity of bottom trawling, whereas values in deeper sediment layers of untrawled bottoms were consistently higher than those in both trawled sediments. Instead, β-glucosidase activities in untrawled sediments were significantly higher than those in sediments exposed to low bottom trawling intensity only in the top 2 cm of the sedimentary column. C degradation rates followed the pattern of aminopeptidase activity. On the one hand, these results suggest that both protein and carbohydrate degradation in the uppermost layer of trawled sediments were unaffected by bottom trawling. In contrast, deeper sediment layers (i.e., those below the uppermost 1st cm of the water column) of disturbed sediments, whatever the intensity level of bottom trawling, showed C degradation rates lower than those in untrawled bottoms. Altogether, these results would suggest that the effects of bottom trawling on benthic ecosystem functioning (in terms of C degradation) could have been masked in the uppermost layer of the sediment by local uncontrolled factors, ultimately causing its impairment only in the deeper layers of the sediment. This result is in contrast with previous studies, conducted in other seas or in mesocosm, which observed the stimulation of C degradation immediately after real or simulated bottom trawling (Polymenakou et al., 2005; Pusceddu et al., 2005). Such discrepancy between my results and the previously ones is hard to explain, but it could be related to the different approaches used: a control-impact one in the Bornholm study *vs.* a Before-After in the others. Nonetheless, it is noticeable that the top layer of both trawled sediments was characterized by C turnover time lower than that in untrawled bottoms, determined actually by lower OM loads and invariant C degradation rates in the former.

Ultimately, although this study represents just a small piece of the complex mosaic of the effects of bottom trawling on marine sediment biogeochemistry worldwide, my results pinpoint that bottom trawling fisheries, by severely influencing sea bottom biodiversity and ecosystem integrity (Kaiser et al., 2002; Hiddink et al., 2006; de Juan et al., 2013; Merillet et al., 2018; Nielsen et al., 2023), are among the most threatening anthropogenic activities influencing either stocks or degradation rates of organic C in marine sediments, with potential implications on global biogeochemical cycles (Sala et al., 2021).

2.5 Conclusions

Bottom trawling is one of the major anthropogenic disturbances to which marine benthic ecosystems are subjected worldwide that can alter sea bottom biogeochemistry and ecosystem functioning. In the Bornholm Basin it represents the most thriving and widespread fishing activity. The results of this study, almost consistently across the entire sediment column, pinpoint that untrawled sediments were characterized by organic loads far higher than those in trawled ones, reflecting the typical removal of sediments caused by trawling gears observed in other seas and water depths. Such activity can impoverish the amount of bioavailable food for benthic detritus feeders, but the "oligotrophication" effect of bottom trawling could be, at least partially, counteracted by an invariant food availability of the remining organic substrates. Nevertheless, my results confirm the capacity of bottom trawling, whatever the intensity level, to mix the sediment layers, ultimately causing an overall homogenization of the trophic status across the whole sediment column and altering benthic ecosystem functioning (i.e., lower C turnover time in top layer of the trawled sites). In conclusion, I infer that bottom trawling can severely modify the benthic trophic status of the Bornholm Basin.

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2.7 Supplementary tables

Table S1. Sedimentary organic matter protein (PRT), carbohydrate (CHO), lipid (LIP), biopolymeric C (BPC), phytopigments (PHY) contents, aminopeptidase (LEU) and β-glucosidase (BETA), activity, C degradation rates (CDR) and turnover time (CTT) in the sediments exposed to null, low, and medium intensity of bottom trawling. Reported are standard deviations (n=3-4).

Site	**Layer**	**PRT**	**CHO**	**LIP**	**BPC**	**PHY**	**LEU**	**BETA**	**CDR**	**CTT**
	cm	**mgC g^{-1}**	**mgC g^{-1}**	**mgC g^{-1}**	**mgC g^{-1}**	**µg g^{-1}**	**µgC g^{-1} h^{-1}**	**µgC g^{-1} h^{-1}**	**µgC g^{-1} h^{-1}**	**days**
D	0-1	5.91 ± 0.92	7.35 ± 0.75	1.49 ± 0.28	14.75 ± 1.85	24.99 ± 3.59	12.47 ± 2.83	0.72 ± 0.04	13.19 ± 2.83	42.58 ± 4.69
	1-2	5.49 ± 1.47	7.04 ± 1.56	2.04 ± 0.27	14.56 ± 2.03	40.19 ± 6.41	17.20 ± 5.58	0.45 ± 0.12	17.65 ± 5.60	32.11 ± 12.18
	2-3	3.81 ± 0.36	6.52 ± 0.54	1.86 ± 0.01	12.20 ± 0.78	21.36 ± 7.75	7.21 ± 1.84	0.20 ± 0.08	7.41 ± 1.90	62.03 ± 20.61
	3-4	4.45 ± 0.18	2.5 ± 0.77	1.73 ± 0.11	8.68 ± 0.61	16.18 ± 7.62	3.80 ± 1.28	0.17 ± 0.08	3.98 ± 1.32	77.92 ± 21.80
F	0-1	11.55 ± 2.92	21.26 ± 3.15	3.17 ± 0.54	35.98 ± 3.53	75.97 ± 12.84	11.08 ± 2.02	1.16 ± 0.10	12.24 ± 1.99	114.23 ± 24.01
	1-2	13.01 ± 3.13	7.22 ± 1.46	2.70 ± 0.86	22.93 ± 2.78	49.88 ± 2.39	9.92 ± 1.74	0.52 ± 0.18	10.44 ± 1.74	83.76 ± 24.17
	2-3	4.84 ± 0.88	5.76 ± 1.76	2.48 ± 0.39	13.08 ± 1.74	27.26 ± 6.29	4.01 ± 1.15	0.40 ± 0.10	4.41 ± 1.23	104.63 ± 26.43
	3-4	5.03 ± 0.78	4.99 ± 0.66	2.47 ± 0.19	12.49 ± 0.73	33.82 ± 8.16	3.65 ± 1.15	0.34 ± 0.11	3.98 ± 1.24	113.58 ± 37.29
B	0-1	1.67 ± 0.43	2.29 ± 0.29	0.37 ± 0.05	4.33 ± 0.60	11.03 ± 0.47	5.24 ± 1.29	0.66 ± 0.02	5.90 ± 1.30	29.46 ± 9.42
	1-2	2.36 ± 0.79	1.83 ± 0.51	0.49 ± 0.13	4.69 ± 1.08	8.41 ± 0.95	4.34 ± 1.10	0.22 ± 0.05	4.56 ± 1.07	40.77 ± 15.38
	2-3	3.02 ± 0.55	2.26 ± 0.23	0.52 ± 0.12	5.80 ± 0.67	6.79 ± 1.10	1.72 ± 0.22	0.22 ± 0.02	1.94 ± 0.24	115.15 ± 23.56
	3-4	2.45 ± 0.56	2.29 ± 0.8	0.45 ± 0.06	5.19 ± 0.37	6.21 ± 0.68	1.40 ± 0.12	0.17 ± 0.03	1.57 ± 0.10	126.03 ± 11.76
G	0-1	2.86 ± 0.26	7.57 ± 1.37	0.91 ± 0.25	11.34 ± 1.41	13.77 ± 1.74	10.23 ± 0.90	0.34 ± 0.08	10.57 ± 0.94	41.42 ± 7.11
	1-2	2.78 ± 0.18	18.61 ± 1.43	0.92 ± 0.20	22.31 ± 1.49	12.96 ± 0.68	7.72 ± 0.93	0.21 ± 0.01	7.94 ± 0.93	112.75 ± 6.19
	2-3	2.91 ± 0.35	4.82 ± 1.45	0.56 ± 0.02	8.30 ± 1.40	7.02 ± 0.92	4.84 ± 0.75	0.95 ± 0.02	5.78 ± 0.76	56.36 ± 12.86
	3-4	3.21 ± 0.95	9.01 ± 2.00	0.39 ± 0.02	12.61 ± 2.74	6.32 ± 1.03	1.20 ± 0.31	0.10 ± 0.02	1.30 ± 0.30	414.35 ± 169.22
J	0-1	2.11 ± 0.08	2.08 ± 0.08	0.60 ± 0.08	4.78 ± 0.08	4.85 ± 0.65	1.52 ± 0.48	0.34 ± 0.07	1.86 ± 0.42	97.55 ± 25.19
	1-2	2.48 ± 0.74	2.02 ± 0.27	0.47 ± 0.09	4.97 ± 0.73	6.55 ± 1.36	0.83 ± 0.27	0.21 ± 0.05	1.04 ± 0.30	192.01 ± 64.78
	2-3	2.52 ± 0.54	0.93 ± 0.09	0.45 ± 0.10	3.89 ± 0.61	3.76 ± 0.11	0.35 ± 0.01	0.06 ± 0.01	0.41 ± 0.00	346.91 ± 55.48
	3-4	2.00 ± 0.40	0.83 ± 0.25	0.23 ± 0.13	3.06 ± 0.46	2.79 ± 0.83	0.15 ± 0.01	0.06 ± 0.00	0.21 ± 0.01	563.75 ± 103.73
C	0-1	2.77 ± 0.74	2.51 ± 0.31	0.54 ± 0.12	5.82 ± 1.14	12.96 ± 1.94	5.18 ± 0.72	1.20 ± 0.32	6.38 ± 0.63	34.68 ± 6.74
	1-2	4.49 ± 1.65	2.02 ± 0.36	0.61 ± 0.13	7.12 ± 1.56	11.48 ± 1.96	4.71 ± 0.74	0.45 ± 0.13	5.16 ± 0.66	52.80 ± 12.50
	2-3	3.90 ± 1.19	2.27 ± 0.73	0.74 ± 0.08	6.91 ± 1.75	10.41 ± 0.96	3.75 ± 1.23	0.35 ± 0.08	4.10 ± 1.25	69.69 ± 33.20
	3-4	6.31 ± 0.25	2.62 ± 0.62	0.68 ± 0.09	9.61 ± 0.97	8.00 ± 1.04	1.57 ± 0.41	0.27 ± 0.01	1.84 ± 0.42	210.04 ± 53.75
H	0-1	3.86 ± 0.82	6.33 ± 0.10	1.47 ± 0.11	11.66 ± 0.67	20.81 ± 4.64	20.62 ± 2.93	0.41 ± 0.11	21.03 ± 2.85	20.51 ± 3.68
	1-2	3.85 ± 0.53	5.45 ± 0.59	1.15 ± 0.10	10.45 ± 1.17	15.83 ± 1.12	11.23 ± 2.03	0.31 ± 0.04	11.54 ± 2.07	34.00 ± 4.71
	2-3	4.19 ± 0.57	3.36 ± 0.29	0.77 ± 0.11	8.32 ± 0.54	8.61 ± 0.62	4.34 ± 0.69	0.17 ± 0.03	4.51 ± 0.68	71.01 ± 12.95
	3-4	4.01 ± 0.49	2.38 ± 0.43	0.63 ± 0.16	7.02 ± 0.35	7.81 ± 2.03	1.22 ± 0.04	0.11 ± 0.04	1.34 ± 0.07	199.69 ± 1.40
I	0-1	1.83 ± 0.25	0.91 ± 0.06	0.39 ± 0.08	3.13 ± 0.14	4.84 ± 0.04	5.65 ± 0.79	0.11 ± 0.03	5.77 ± 0.77	20.15 ± 4.04
	1-2	2.24 ± 0.08	1.78 ± 0.14	0.36 ± 0.11	4.38 ± 0.27	6.08 ± 0.16	2.99 ± 0.78	0.09 ± 0.01	3.08 ± 0.77	57.44 ± 18.19
	2-3	2.05 ± 0.19	1.25 ± 0.40	0.41 ± 0.02	3.71 ± 0.34	5.29 ± 0.54	1.78 ± 0.38	0.08 ± 0.02	1.86 ± 0.38	76.83 ± 22.23
	3-4	2.76 ± 0.23	1.26 ± 0.04	0.35 ± 0.06	4.36 ± 0.17	4.28 ± 0.89	1.31 ± 0.15	0.08 ± 0.02	1.39 ± 0.15	121.87 ± 19.50

Table S2. Results of the pairwise tests carried out to ascertain differences in the quantity, nutritional quality, biochemical composition, and degradation rate and turnover time of organic matter between pairs of sediment layers (0-1, 1-2, 2-3, 3-4 cm) at each level of bottom trawling intensity. t = statistic t; P(MC) = probability level after Monte Carlo simulation; * = p <0.05; ** = p <0.01; *** = p <0.001; ns = not significant.

Variable	Trawling	Groups	t	P(MC)
Protein	**Null**	0-1 vs. 1-2	0.248	ns
		0-1 vs. 2-3	3.359	**
		0-1 vs. 3-4	3.080	**
		1-2 vs. 2-3	2.972	*
		1-2 vs. 3-4	2.742	*
		2-3 vs. 3-4	1.136	ns
	Low	0-1 vs. 1-2	0.880	ns
		0-1 vs. 2-3	1.498	ns
		0-1 vs. 3-4	0.782	ns
		1-2 vs. 2-3	0.818	ns
		1-2 vs. 3-4	0.125	ns
		2-3 vs. 3-4	0.929	ns
	Medium	0-1 vs. 1-2	0.778	ns
		0-1 vs. 2-3	0.193	ns
		0-1 vs. 3-4	0.759	ns
		1-2 vs. 2-3	1.110	ns
		1-2 vs. 3-4	0.224	ns
		2-3 vs. 3-4	1.348	ns
Carbohydrate	**Null**	0-1 vs. 1-2	2.581	*
		0-1 vs. 2-3	2.946	*
		0-1 vs. 3-4	3.792	**
		1-2 vs. 2-3	1.475	ns
		1-2 vs. 3-4	4.676	**
		2-3 vs. 3-4	3.459	**
	Low	0-1 vs. 1-2	0.937	ns
		0-1 vs. 2-3	2.403	*
		0-1 vs. 3-4	1.104	ns
		1-2 vs. 2-3	2.281	*
		1-2 vs. 3-4	1.624	ns
		2-3 vs. 3-4	1.037	ns
	Medium	0-1 vs. 1-2	0.190	ns
		0-1 vs. 2-3	1.534	ns
		0-1 vs. 3-4	1.309	ns
		1-2 vs. 2-3	1.599	ns
		1-2 vs. 3-4	1.401	ns
		2-3 vs. 3-4	0.244	ns
Lipid	**Null**	0-1 vs. 1-2	0.090	ns
		0-1 vs. 2-3	0.423	ns
		0-1 vs. 3-4	0.615	ns
		1-2 vs. 2-3	0.694	ns
		1-2 vs. 3-4	0.947	ns

		2-3 vs. 3-4	0.346	ns
	Low	0-1 vs. 1-2	0.027	ns
		0-1 vs. 2-3	0.519	ns
		0-1 vs. 3-4	1.352	ns
		1-2 vs. 2-3	0.576	ns
		1-2 vs. 3-4	1.475	ns
		2-3 vs. 3-4	0.959	ns
	Medium	0-1 vs. 1-2	0.379	ns
		0-1 vs. 2-3	0.679	ns
		0-1 vs. 3-4	0.500	ns
		1-2 vs. 2-3	0.300	ns
		1-2 vs. 3-4	0.137	ns
		2-3 vs. 3-4	0.144	ns
Biopolymeric C	**Null**	0-1 vs. 1-2	1.477	ns
		0-1 vs. 2-3	3.072	**
		0-1 vs. 3-4	3.533	**
		1-2 vs. 2-3	3.333	**
		1-2 vs. 3-4	4.241	***
		2-3 vs. 3-4	2.312	*
	Low	0-1 vs. 1-2	1.035	ns
		0-1 vs. 2-3	0.067	ns
		0-1 vs. 3-4	0.434	ns
		1-2 vs. 2-3	0.930	ns
		1-2 vs. 3-4	1.446	ns
		2-3 vs. 3-4	0.473	ns
	Medium	0-1 vs. 1-2	0.348	ns
		0-1 vs. 2-3	0.903	ns
		0-1 vs. 3-4	0.395	ns
		1-2 vs. 2-3	1.265	ns
		1-2 vs. 3-4	0.780	ns
		2-3 vs. 3-4	0.686	ns
Protein to Carbohydrate ratio	**Null**	0-1 vs. 1-2	2.405	*
		0-1 vs. 2-3	0.589	ns
		0-1 vs. 3-4	3.388	**
		1-2 vs. 2-3	1.920	ns
		1-2 vs. 3-4	0.331	ns
		2-3 vs. 3-4	2.675	*
	Low	0-1 vs. 1-2	1.672	ns
		0-1 vs. 2-3	2.474	*
		0-1 vs. 3-4	2.650	*
		1-2 vs. 2-3	2.129	*
		1-2 vs. 3-4	1.861	ns
		2-3 vs. 3-4	1.130	ns
	Medium	0-1 vs. 1-2	0.764	ns
		0-1 vs. 2-3	1.751	ns
		0-1 vs. 3-4	3.129	**
		1-2 vs. 2-3	0.677	ns

		1-2 vs. 3-4	1.903	ns
		2-3 vs. 3-4	1.495	ns
Phytopigment	**Null**	0-1 vs. 1-2	0.523	ns
		0-1 vs. 2-3	2.508	*
		0-1 vs. 3-4	2.325	*
		1-2 vs. 2-3	5.877	***
		1-2 vs. 3-4	4.121	**
		2-3 vs. 3-4	0.139	ns
	Low	0-1 vs. 1-2	0.730	ns
		0-1 vs. 2-3	1.603	ns
		0-1 vs. 3-4	1.981	ns
		1-2 vs. 2-3	1.122	ns
		1-2 vs. 3-4	1.610	ns
		2-3 vs. 3-4	0.418	ns
	Medium	0-1 vs. 1-2	0.894	ns
		0-1 vs. 2-3	1.517	ns
		0-1 vs. 3-4	1.932	ns
		1-2 vs. 2-3	0.825	ns
		1-2 vs. 3-4	1.374	ns
		2-3 vs. 3-4	0.529	ns
Algal fraction of BPC	**Null**	0-1 vs. 1-2	2.760	*
		0-1 vs. 2-3	0.110	ns
		0-1 vs. 3-4	1.138	ns
		1-2 vs. 2-3	2.004	ns
		1-2 vs. 3-4	0.602	ns
		2-3 vs. 3-4	0.924	ns
	Low	0-1 vs. 1-2	1.654	ns
		0-1 vs. 2-3	3.347	**
		0-1 vs. 3-4	2.951	**
		1-2 vs. 2-3	1.561	ns
		1-2 vs. 3-4	1.275	ns
		2-3 vs. 3-4	0.113	ns
	Medium	0-1 vs. 1-2	2.483	*
		0-1 vs. 2-3	2.720	*
		0-1 vs. 3-4	5.407	***
		1-2 vs. 2-3	0.085	ns
		1-2 vs. 3-4	2.852	*
		2-3 vs. 3-4	3.649	**
Biochemical composition	**Null**	0-1 vs. 1-2	1.179	ns
		0-1 vs. 2-3	2.982	*
		0-1 vs. 3-4	3.025	**
		1-2 vs. 2-3	3.557	**
		1-2 vs. 3-4	3.417	**
		2-3 vs. 3-4	0.613	ns
	Low	0-1 vs. 1-2	0.569	ns
		0-1 vs. 2-3	1.226	ns
		0-1 vs. 3-4	1.392	ns

		1-2 vs. 2-3	0.992	ns
		1-2 vs. 3-4	1.263	ns
		2-3 vs. 3-4	0.900	ns
	Medium	0-1 vs. 1-2	0.591	ns
		0-1 vs. 2-3	0.955	ns
		0-1 vs. 3-4	1.015	ns
		1-2 vs. 2-3	0.731	ns
		1-2 vs. 3-4	0.605	ns
		2-3 vs. 3-4	0.479	ns
Aminopeptidase activity	**Null**	0-1 vs. 1-2	0.848	ns
		0-1 vs. 2-3	5.330	***
		0-1 vs. 3-4	8.590	***
		1-2 vs. 2-3	3.816	**
		1-2 vs. 3-4	4.993	***
		2-3 vs. 3-4	2.138	*
	Low	0-1 vs. 1-2	1.743	ns
		0-1 vs. 2-3	2.675	*
		0-1 vs. 3-4	3.312	**
		1-2 vs. 2-3	2.124	ns
		1-2 vs. 3-4	3.989	***
		2-3 vs. 3-4	2.067	*
	Medium	0-1 vs. 1-2	1.309	ns
		0-1 vs. 2-3	2.610	*
		0-1 vs. 3-4	3.418	**
		1-2 vs. 2-3	2.154	*
		1-2 vs. 3-4	3.791	**
		2-3 vs. 3-4	3.125	**
β-glucosidase activity	**Null**	0-1 vs. 1-2	4.519	**
		0-1 vs. 2-3	6.403	***
		0-1 vs. 3-4	7.038	***
		1-2 vs. 2-3	2.573	*
		1-2 vs. 3-4	3.386	**
		2-3 vs. 3-4	0.694	ns
	Low	0-1 vs. 1-2	4.578	***
		0-1 vs. 2-3	1.108	ns
		0-1 vs. 3-4	6.364	***
		1-2 vs. 2-3	1.170	ns
		1-2 vs. 3-4	2.618	*
		2-3 vs. 3-4	2.147	*
	Medium	0-1 vs. 1-2	2.083	ns
		0-1 vs. 2-3	2.763	*
		0-1 vs. 3-4	3.209	***
		1-2 vs. 2-3	1.366	ns
		1-2 vs. 3-4	2.394	*
		2-3 vs. 3-4	1.025	ns
C degradation	**Null**	0-1 vs. 1-2	0.635	ns
		0-1 vs. 2-3	6.037	***

		0-1 vs. 3-4	9.473	***
		1-2 vs. 2-3	3.925	**
		1-2 vs. 3-4	5.113	***
		2-3 vs. 3-4	2.196	ns
	Low	0-1 vs. 1-2	1.825	ns
		0-1 vs. 2-3	2.696	*
		0-1 vs. 3-4	3.430	**
		1-2 vs. 2-3	1.924	ns
		1-2 vs. 3-4	4.013	***
		2-3 vs. 3-4	2.152	ns
	Medium	0-1 vs. 1-2	1.456	ns
		0-1 vs. 2-3	2.835	**
		0-1 vs. 3-4	3.689	**
		1-2 vs. 2-3	2.240	*
		1-2 vs. 3-4	3.941	***
		2-3 vs. 3-4	3.280	**
C turnover time	**Null**	0-1 vs. 1-2	1.238	ns
		0-1 vs. 2-3	0.794	ns
		0-1 vs. 3-4	1.548	ns
		1-2 vs. 2-3	1.848	ns
		1-2 vs. 3-4	2.330	*
		2-3 vs. 3-4	0.940	ns
	Low	0-1 vs. 1-2	2.420	*
		0-1 vs. 2-3	4.290	**
		0-1 vs. 3-4	5.522	***
		1-2 vs. 2-3	1.413	ns
		1-2 vs. 3-4	2.600	*
		2-3 vs. 3-4	2.146	*
	Medium	0-1 vs. 1-2	2.416	*
		0-1 vs. 2-3	3.423	**
		0-1 vs. 3-4	4.932	**
		1-2 vs. 2-3	1.488	ns
		1-2 vs. 3-4	4.278	***
		2-3 vs. 3-4	3.202	**

2.8 Supplementary figures

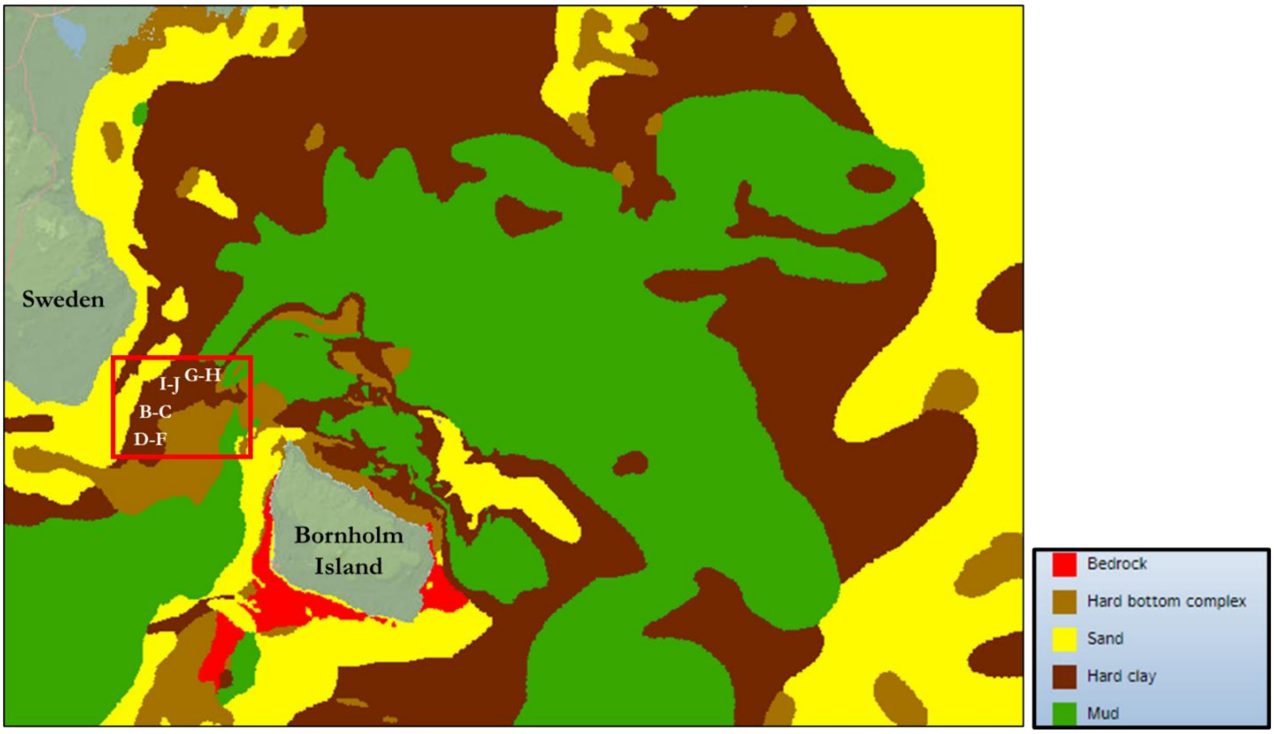

Figure S1. Sediment types in the Bornholm Basin and around Bornholm Island (Baltic Sea). In the red square the eight sampled stations are reported. Map from HELCOM Map and Data Service (2022).

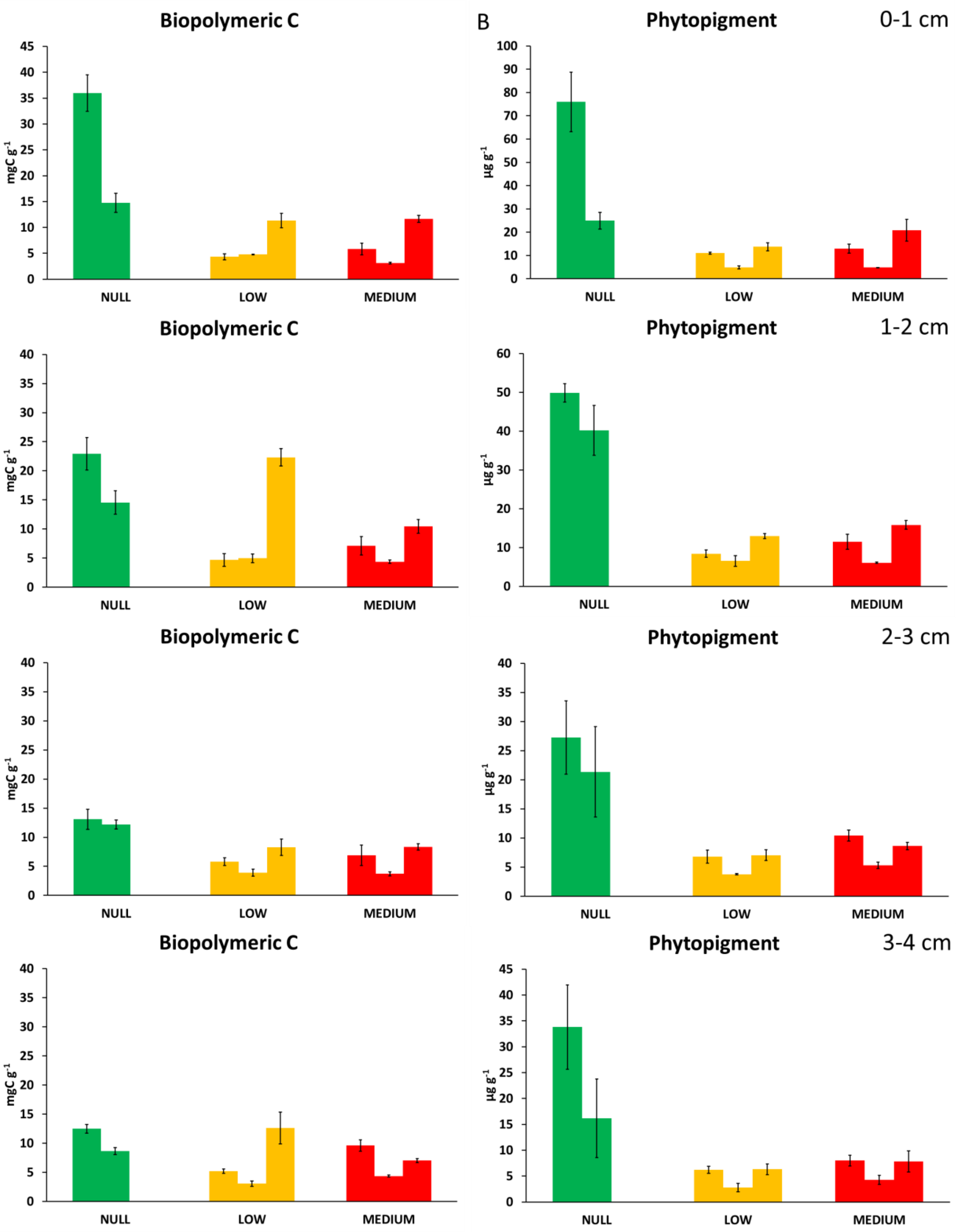

Figure S2: Biopolymeric C (A) and phytopigment (B) contents in the sediments of the sites exposed to null, low, and medium intensity of bottom trawling in the four sediment layers. The error bars indicate the standard deviation (n=3-4).

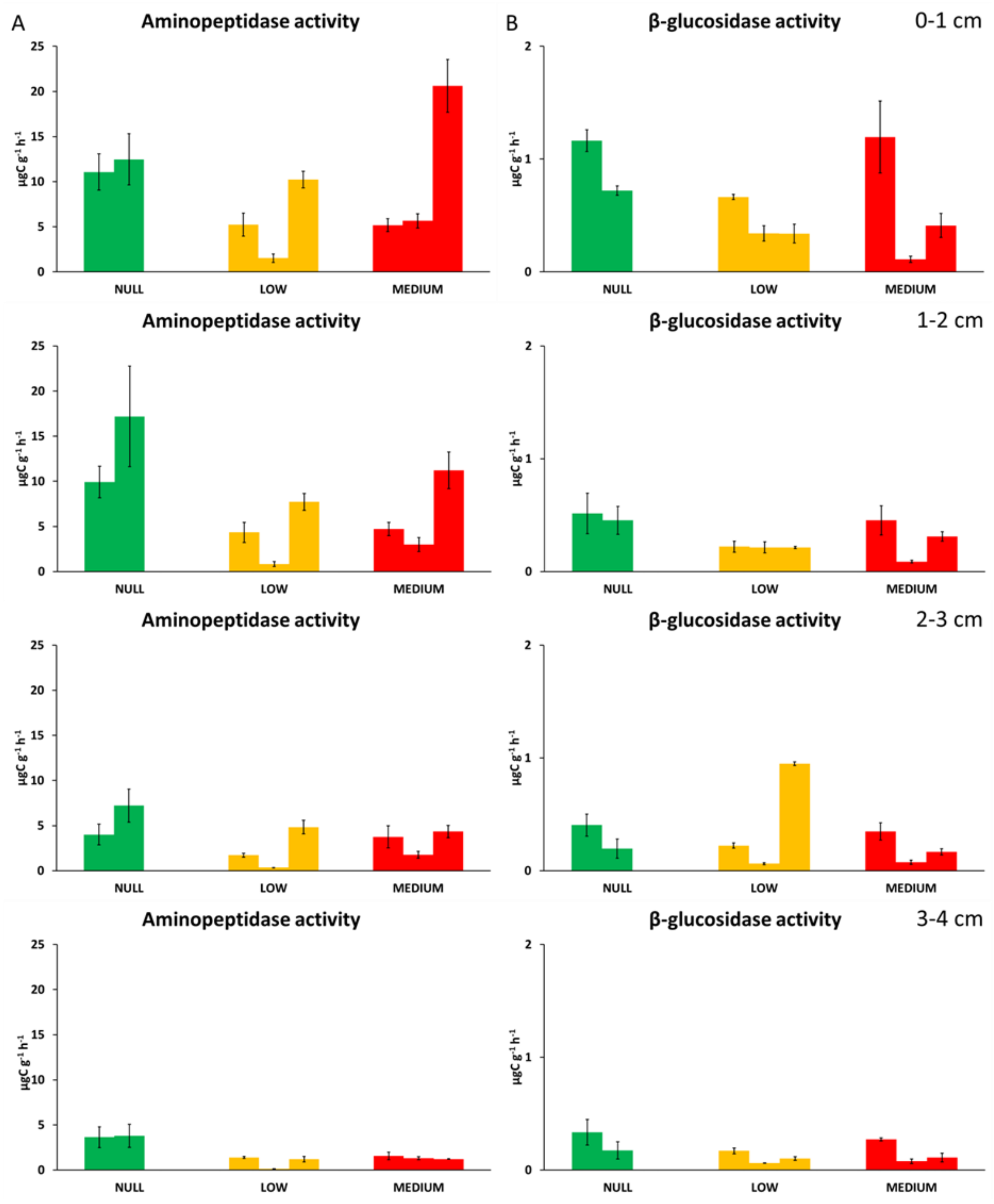

Figure S3: Aminopeptidase (A) and β-glucosidase activities (B) in the sediments of the sites exposed to null, low, and medium intensity of bottom trawling in the four sediment layers. The error bars indicate the standard deviation (n=3-4).

3. Effects of field simulated marine heatwaves on sedimentary organic matter quantity, biochemical composition, and degradation rates

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3.1 Introduction

Anthropogenic global warming is rapidly emerging as a major threat to ecosystems worldwide (Hughes et al., 2018; Hoegh-Guldberg et al., 2018). Marine heatwaves (hereafter MHWs), as discrete but persistent (>5 days) positive (2-4 °C) anomalies in sea surface temperatures (SST) (Hobday et al., 2016), are one of the most concerning and ubiquitous manifestations of global warming (Frölicher et al., 2018). Marine warmth anomalies have become increasingly frequent in the last century (Oliver et al., 2019), and episodes that have occurred across the last 20 years have caused severe biological, ecological, and economic consequences (Benedetti-Cecchi et al., 2021). Recent projections indicate that such a surge in the frequency of MHWs could persevere for the whole current century (Oliver et al., 2019) as the consequence of the persisting global ocean warming. Over the last two decades, several MHWs have been recorded globally (Hobday et al., 2016; Sen Gupta et al., 2020), including in the Mediterranean Sea (Kuglitsch et al., 2010; Darmaraki et al., 2019a-b). Being a miniature, shallow and warm ocean more prone to climate change than the open oceans (Lejeusne et al., 2010), the annual mean SST of the Mediterranean basin is expected to increase from +1.5 °C to +3 °C by the end of the 21st century, fostering MHWs' occurrence (Darmaraki et al., 2019a; Jacox et al., 2022).

MHWs can have severe impacts on marine ecosystems (Hughes et al., 2018; Wernberg et al., 2013, 2016; Cavole et al., 2016; Oliver et al., 2017; Smale et al., 2017). Most of the knowledge accumulated regarding the effects of heatwaves on marine ecosystems derived from studies that assessed mass mortality, abundance reduction and changes in community structures of macro-and mega-benthos populations *in situ* (Wernberg et al., 2013; Cerrano et al., 2000; Garrabou et al., 2001, 2009; Coma et al., 2009; Marbà et al., 2010; Rivetti et al., 2014; Brodeur et al., 2019). Other studies carried out manipulative experiments on different marine communities and organisms to examine their response to warming (Sorte et al., 2010; Smale et al., 2011, 2015; Samuels et al., 2021; Stipcich et al., 2022). Nevertheless, little research effort has been made to understand how temperature rise associated with MHWs could affect the nutrient and C cycling in coastal sediments (Sanz-Lázaro et al., 2015; Alsterberg et al., 2012), and to date, little, if any, attention has been given to the effects of MHWs on organic matter gross biochemical composition and degradation rates in coastal sediments. Since about half of annual marine C burial takes place in shallow water ecosystems (Duarte et al., 2005), and temperature affects the rates of any (bio)chemical reaction, we predict that MHWs will affect C stocks and degradation rates in coastal marine ecosystems.

In order to provide new insights into this knowledge gap, we investigated short-term changes in quantity, biochemical composition, and degradation rates of organic matter in coastal sediments exposed to two simulated marine heatwaves, one simulating ocean temperatures corresponding to the future low emission scenario (MT) and one corresponding to a high emission scenario (HT) (Abd-Elgawad et al., 2019), generated by the cooling systems of an electric generation plant located in North-Western Sardinia (Mediterranean Sea). More specifically, we tested the null hypothesis by which sedimentary organic matter quantity, biochemical composition (in terms of protein, carbohydrate, lipid and phytopigment contents), and potential degradation rates (expressed as rates of extracellular enzymatic degradation of proteins and carbohydrates) are not influenced by MHWs' occurrence and intensity (in terms of temperature anomaly).

3.2 Materials and Methods

3.2.1 Study area and sampling strategy

This study was conducted during the summer of 2020 in North-Western Sardinia (Italy, Western Mediterranean), in front of the Fiume Santo thermoelectric plant (40.85° N, 8.30° E), set up in the 1960s to supply electricity to Sardinia. The plant consists of two coal-fired units, each with a nominal power of 320 MW. To cool the whole system, seawater is continuously taken from the sea at 1 km from the coast and released back into the shore (12–24 m^3 s^{-1}) about 6-8 °C warmer (Power Plant Water, PPW). This creates a marked seawater temperature gradient in the vicinity of the discharge point which is conceivably moderated by season and weather conditions. The historical occurrence, duration, and intensity of MHWs occurring in the last 20 years in the study area have been characterized previously (Stipcich et al., 2022). To run our experiment, we profited from ca. 5 weeks of inactivity of the power plant.

In order to evaluate the effects of the simulated MHWs on the sedimentary organic matter attributes, three sites were identified: (i) a "Control Temperature" site (CTRL), unaffected by PPW; (ii) a "Medium Temperature" site (MT) with PPW largely mixed with the natural seawater, with a mean surface T positive delta (compared to the control site) of ca. 2 °C; and a "High Temperature" site (HT) with PPW minimally mixed with the natural water, with a mean surface T positive delta (compared to the control site) of ca. 6 °C. Sediment sampling was carried out on July 14 (T_0, before the power plant ignition and the consequent injection of warm water at sea), August 3 (T_1, 3 weeks after the beginning of warm water injection), and October 1, 2020 (T_2, 11 weeks after the beginning of warm water injection). Samples collected after 3 and 11 weeks from the power plant ignition (T_1 and T_2, respectively) were assumed to represent medium- and long-term duration steps of the simulated MHWs. Six independent replicated sediment cores were collected at ca. 3 m of depth at each station and sampling date using plexiglass corers (4.7 cm internal diameter) operated manually by SCUBA divers. The top first cm of each sediment core was stored in Petri dishes at -20 °C until analyses.

Temporal variations in sea bottom temperature (at ca. 3 m of depth) were similar at the three study sites, with a clear increase in T_1 (after 3 weeks from the initial plume injection) and a partial recovery in T_2 (after 11 weeks; **Figure S1A**). The simulated MHWs resembled either current or future scenarios according to Hobday et al. (2016): the magnitude of the two T anomalies corresponded to conditions observed in the study area during the 2000-2009 decade (+1.4-1.8 °C) and those expected in the future under the worst prediction scenarios (+4.5-5.1 °C) (Stipcich et al., 2022) (**Figure S1B**).

3.2.2 Biochemical composition of sedimentary organic matter

Chlorophyll-a (Chl-a) and phaeopigment analyses were carried out according to Danovaro (2010). Pigments were extracted (overnight at 4 °C in the dark) from triplicate superficial (0-1 cm) 0.1 g sediment subsamples using 5 mL of 90 % acetone as extractant. Extracts were analyzed fluorometrically (430 nm excitation and 665 nm emission wavelengths) to estimate Chl-a, and, after acidification with 200 µL 0.1N HCl, phaeopigment concentrations. Total phytopigment was defined as the sum of Chl-a and phaeopigment concentrations and, once converted into C equivalents using 40 µg C µg phytopigment^{-1} as a conversion factor, utilized to estimate the fraction of organic material of autotrophic origin (Pusceddu et al., 2010). Although the C:Chl-a can vary from 10 to 100 (on average 35 for phytoplankton), we used the conversion factor proposed in Pusceddu et al. (2010) to allow comparison with other studies carried out in a variety of shallow coastal aquatic environments (Pusceddu et al., 2009).

Protein, carbohydrate, and lipid contents were determined spectrophotometrically according to the protocols detailed in Danovaro (2010). More specifically, proteins were determined according to Lowry et al. (1951), as modified by Hartree (1972) and Rice (1982), using the Folin-Ciocalteau reagent in a basic environment and expressed as bovine serum albumin equivalents. The procedure proposed by Gerchakov and Hatcher (1972), based on the phenol and concentrated sulfuric acid reaction with saccharides, was used to determine carbohydrates, then expressed as D (+) Glucose equivalents. Lipids, after extraction in chloroform: methanol (1:1, vol:vol) (Bligh and Dyer, 1959), and evaporation in a dry hot bath at 80 to 100 °C for 20 min, were determined after the sulfuric acid carbonization procedure (Marsh and Weinstein, 1966) and expressed as tripalmitin equivalents. For each biochemical assay, blanks were obtained using pre-calcinated sediments (450 °C for 4 h). Protein, carbohydrate, and lipid concentrations were converted into C equivalents using the conversion factors 0.49, 0.40, and 0.75 mgC mg^{-1}, respectively, obtained from the C contents of the respective standard molecules (albumin, glucose and tripalmitin, respectively), and their sum was reported as the biopolymeric C (BPC) (Fabiano et al., 1995).

To assess the variations in the relative contribution of the basic organic matter's biochemical components (C equivalents of protein, carbohydrate, lipid, and phytopigment sedimentary contents) between the three temperature treatments, an index of biochemical diversity (IBD) was calculated as follows:

$$\mathrm{IBD} = 1 - (b^2{}_1 + b^2{}_2 + b^2{}_3 + \ldots b^2{}_n)$$

where b is the relative contribution of each biochemical compound (i.e., protein, carbohydrate, lipid and phytopigment carbon equivalents) to the cumulative sum of total biopolymeric carbon (BPC) and phytopigment C loads, and n is the number of biochemical compounds. Since IBD has a rank inversely related to biochemical homogeneity, we calculated the IBD-1 value; for n = 4 compounds, as in this study, the IBD-1 index ranges from 0 (minimum homogeneity) to 0.75 (maximum homogeneity).

3.2.3 Extracellular enzymatic activities, C degradation rates and turnover

Organic matter degradation rates were estimated from aminopeptidase and β-glucosidase activities, determined by the cleavage of fluorogenic substrates (L-leucine-4-methylcoumarinyl-7-amide, for aminopeptidase; 4-methylumbelliferone-D-glucopyranoside, for β-glucosidase) at saturating concentrations (Bianchelli et al., 2020). Extracellular enzymatic activities were measured after the addition of 150 µL of substrate to 1mL of a slurry prepared using 1:1 volume of filtered (0.2 µm) and sterile seawater and sediment (substrate final concentration 200 µM) (Bianchelli et al., 2020). Substrate incubations were performed in the dark at *in situ* temperature for 1 h. After incubation, the slurries were centrifuged (3000 rpm, 5 min) and supernatants were analyzed fluorometrically (at 365 nm excitation, 455 nm emission for β-glucosidase, and 380 nm excitation, 440 nm emission for aminopeptidase) (Bianchelli et al., 2020). Data were normalized to sediment dry weight (60 °C, 24 h) and reported as nanomole of substrate released per g of sediment dry weight h^{-1}. Protease and glucosidase activities were converted into C equivalents using 72 as a conversion factor (estimated from the C content of the fluorescent component released after reaction with the enzymes) and their sum, reported as the potential C degradation rate (µgC g^{-1} h^{-1}). The turnovers (per day) of the whole protein and carbohydrate pools were calculated as the ratios of the hourly C degradation rates (once multiplied by 24) and the whole protein and carbohydrate C contents in the sediment (Pusceddu et al., 2014). Although these estimates are only potential (maximum) rates of protein and carbohydrate turnover, they are considered good proxies of ecosystem functioning (Pusceddu et al., 2014).

3.2.4 Effect size

In order to visualize the magnitude of the reported effects on organic matter quantity, biochemical composition and diversity, and degradation rates in a standardized unambiguous metric regardless of the initial differences among sites, the forest plot representation was used based on the effect magnitude metric. The effect magnitude quantifies the results of an experiment as the log-proportional change between the mean (X) of treatment (T) and a control (C) group, as follows:

$$R_i = \ln (XT_i/XC_i)$$

In this study, R_i is the log-response ratio for the variable i, and XT_i and XC_i are the mean values of the metric for the heated (MT or HT) and control (CTRL) sites, respectively.

3.2.5 Statistical analyses

In order to test the null hypothesis by which variations in organic matter quantity, biochemical composition (in terms of protein, carbohydrate, lipid, and phytopigment contents), degradation rates and turn-over time among treatments and sampling times, permutational multivariate analyses of variance (PERMANOVA) (Anderson, 2001) were carried out in either the uni- or multi-variate context with two fixed and orthogonal factors: treatment (Control "CTRL", Medium Temperature "MT" and High Temperature "HT" anomaly) and time (T_0, T_1 and T_2). PERMANOVA is a semiparametric method described as a geometric partitioning of multivariate variation in the space of a chosen dissimilarity measure according to a given ANOVA design, with p-values obtained using appropriate distribution-free permutation techniques. Since PERMANOVA on one response variable using Euclidean distance yields the classical univariate F statistic, PERMANOVA can also be used to perform univariate ANOVA, but where p values are obtained by permutation (Anderson and Millar, 2004), thus avoiding the assumption of normality (Anderson, 2014).

The analyses were carried out on Euclidean distance-based resemblance matrixes obtained from untransformed data, using 999 random permutations of the appropriate units. When significant differences were observed, pairwise tests were also carried out to ascertain patterns of differences among treatments and/or sampling times. Multivariate differences in organic matter biochemical composition (in terms of protein, carbohydrate, lipid and phytopigment contents) were visualized with a biplot after a canonical analysis of the principal coordinates (CAP). CAP allows identification of an axis through the multivariate cloud of points that is best at separating the groups. The motivation for the CAP routine arose as sometimes there are real differences among a priori groups in multivariate space that cannot be easily seen in an unconstrained ordination (as in PCA or MDS plots) (Anderson et al., 2008). To quantify the homogeneity of dispersion among the data, a PERMDISP test was also carried out. Differences in the magnitude of the simulated heatwave effect among sampling times were determined after post-hoc pairwise tests. PERMANOVA, CAP and PERMDISP tests were carried out through the software PRIMER 6+, using the included routine package PERMANOVA (Anderson et al., 2008).

3.3 Results

3.3.1. Effects of the thermal anomalies on organic matter quantity and biochemical composition

Differences in organic matter quantity and biochemical composition largely depended on the interaction between treatments and sampling times (with exceptions for lipid and chlorophyll-a contents, and the autotrophic fraction of biopolymeric C; **Table 1**). Post-hoc tests were carried out to ascertain separately: (i) differences among treatments before (T_0) and after (T_1 and T_2) PPW injection, and (ii) differences among sampling times in each treatment.

Before the injection of PPW, protein, carbohydrate, lipid, biopolymeric C and total phytopigment sedimentary contents differed significantly among treatments (**Table S1A**), with much higher contents (up to seven times) in the HT site and lower in the CTRL (**Figure 1A-G**), whereas organic matter biochemical composition was rather similar among treatments (**Table S1A**), with protein being the dominant class (40-58% of biopolymeric C), followed by lipids (36–58%) and carbohydrates. Quantitative differences in protein, carbohydrate, lipid and biopolymeric C contents among treatments were generally preserved in T_1 (3 weeks after PPW injection) and T_2 (11 weeks after), whereas differences in phytopigment contents weakened from T_1 to T_2 (**Figure 1E-G**). The autotrophic fraction of biopolymeric C, a proxy of sediment organic matter nutritional quality, was about two-fold higher in CTRL sediments than in MT and HT during the entire study period (**Figure 1H**).

In order to visualize the effects of thermal anomalies on organic matter sedimentary contents, once having subtracted the differences among treatments at T_0, we plotted the effect size for protein, carbohydrate, lipid, biopolymeric C, total phytopigment and autotrophic fraction of BPC against values in the CTRL treatment in T_1 and T_2 (**Figure 2**). After 3 weeks (T_1), the injection of PPW determined a clear and significant increase in the sedimentary contents of all the investigated classes of organic compounds when compared to those in CTRL (**Table 2**), with effect sizes consistently larger in HT than in MT (**Figure 2A-E**). After 11 weeks from the initial PPW injection (T_2), though organic matter contents in both MT and HT remained significantly higher than those in CTRL (**Table 2**), the positive effect of the thermal anomaly smothered this, when compared to that in T_1, for all classes of organic compounds, apart from phytopigments, whose contents returned close to those encountered in CTRL (**Figure 2A-E**). The effect of the thermal anomaly on the autotrophic fraction of biopolymeric C in MT was null in T_1 and negative in T_2, and negative in both T_1 and T_2 in HT; in both sampling times, the effect was consistently more negative in HT than in MT (**Figure 2F**).

Table 1. Results of PERMANOVA testing for differences in sedimentary OM contents and biochemical composition among treatments (Tr: CTRL, MT, HT) and sampling times (Ti: T_0, T_1, T_2). Df = degrees of freedom; MS = mean square; F = statistic F; P (MC) = probability level after Monte Carlo simulations and 999 permutations; ** = $p < 0.01$; * = $p < 0.05$; ns = not significant. Reported is also the percentage of variation explained (EV %) by each factor, their interaction and residual (Res; unexplained) variance.

Variable	Source	Df	MS	F	P (MC)	EV (%)
Protein	Tr	2	1.705	29.0	**	50.9
	Ti	2	0.149	2.5	ns	2.8
	Tr *x* Ti	4	0.205	3.5	*	13.6
	Res	45	0.058			32.6
Carbohydrate	Tr	2	1.149	11.8	**	26.1
	Ti	2	0.414	4.3	*	7.9
	Tr *x* Ti	4	0.403	4.2	**	22.7
	Res	45	0.097			43.3
Lipid	Tr	2	0.186	9.8	**	28.0
	Ti	2	0.040	2.1	ns	3.6
	Tr *x* Ti	4	0.042	2.2	ns	11.5
	Res	45	0.019			56.9
Chlorophyll-a	Tr	2	1.479	2.9	ns	7.6
	Ti	2	0.740	1.4	ns	1.8
	Tr *x* Ti	4	1.237	2.4	ns	17.1
	Res	45	0.516			73.5
Phaeopigment	Tr	2	8.211	16.231	**	28.4
	Ti	2	2.724	5.385	**	8.2
	Tr *x* Ti	4	3.216	6.358	**	29.9
	Res	45	0.506			33.5
Total phytopigment	Tr	2	16.554	12.5	**	27.3
	Ti	2	5.575	4.2	*	7.6
	Tr *x* Ti	4	5.499	4.2	**	22.5
	Res	45	1.322			42.6
Biopolymeric C	Tr	2	7.861	23.8	**	44.1
	Ti	2	0.794	2.4	ns	2.7
	Tr *x* Ti	4	1.372	4.2	**	18.3
	Res	45	0.330			34.8
Autotrophic fraction of biopolymeric C	Tr	2	0.064	16.9	**	37.1
	Ti	2	0.021	5.5	**	10.6
	Tr *x* Ti	4	0.009	2.5	ns	10.4
	Res	45	0.004			41.9
OM biochemical composition	Tr	2	12.731	10.6	**	24.2
	Ti	2	4.068	3.4	*	6.0
	Tr *x* Ti	4	5.103	4.3	**	24.6
	Res	45	1.196			45.2

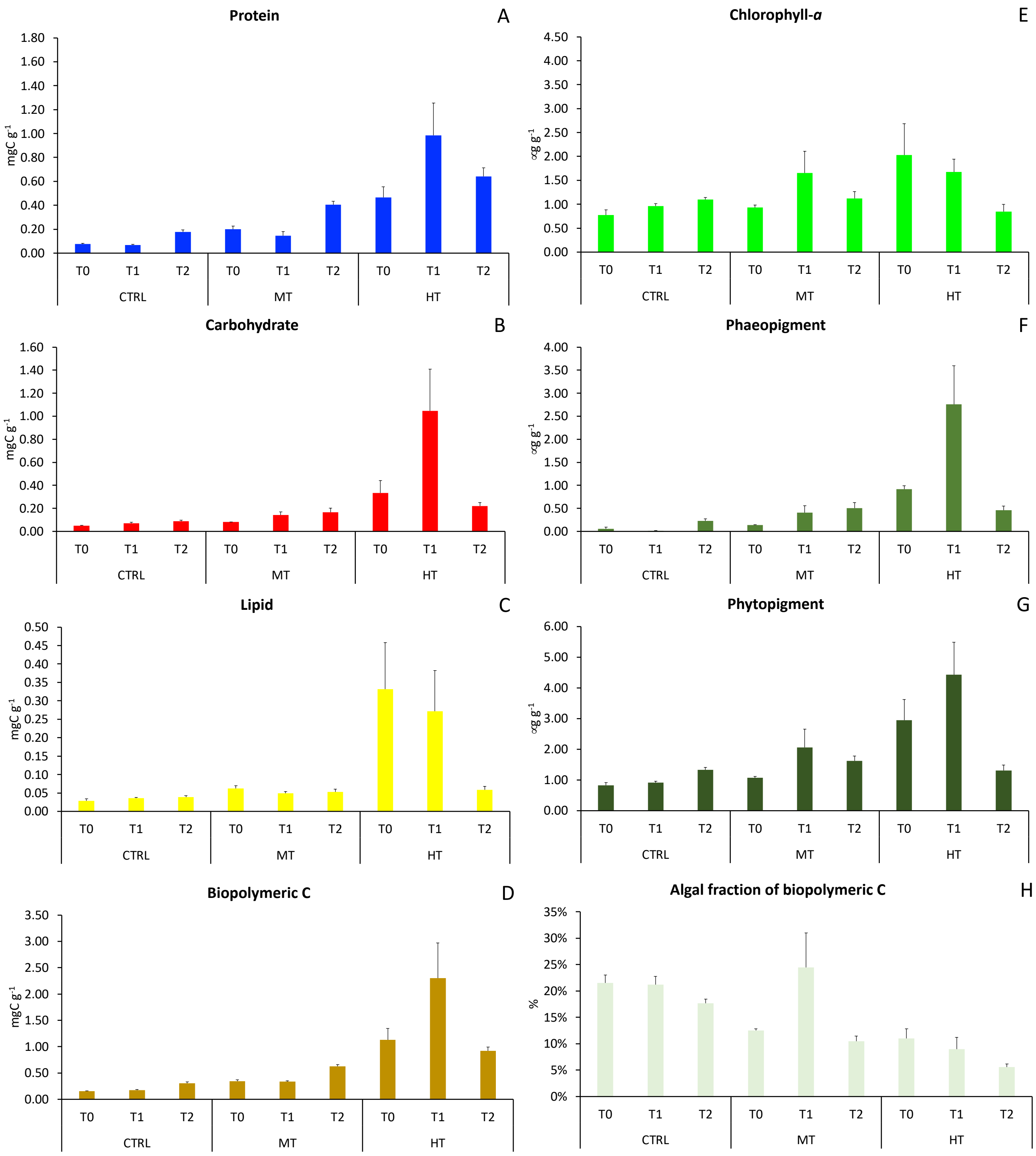

Figure 1. Changes in organic matter sedimentary contents in the three sampling sites at each sampling time: (A) protein, (B) carbohydrate, (C) lipid, (D) biopolymeric C, (E) chlorophyll-a, (F) phaeopigment, (G) total phytopigment, and (H) autotrophic fraction of biopolymeric C. CTRL = control; MT = medium temperature anomaly; HT = high temperature anomaly. T_0 = before PPW injection; T_1 = after 3 weeks from PPW injection; T_2 = after 11 weeks from PPW injection. Error bars are standard errors (n = 6).

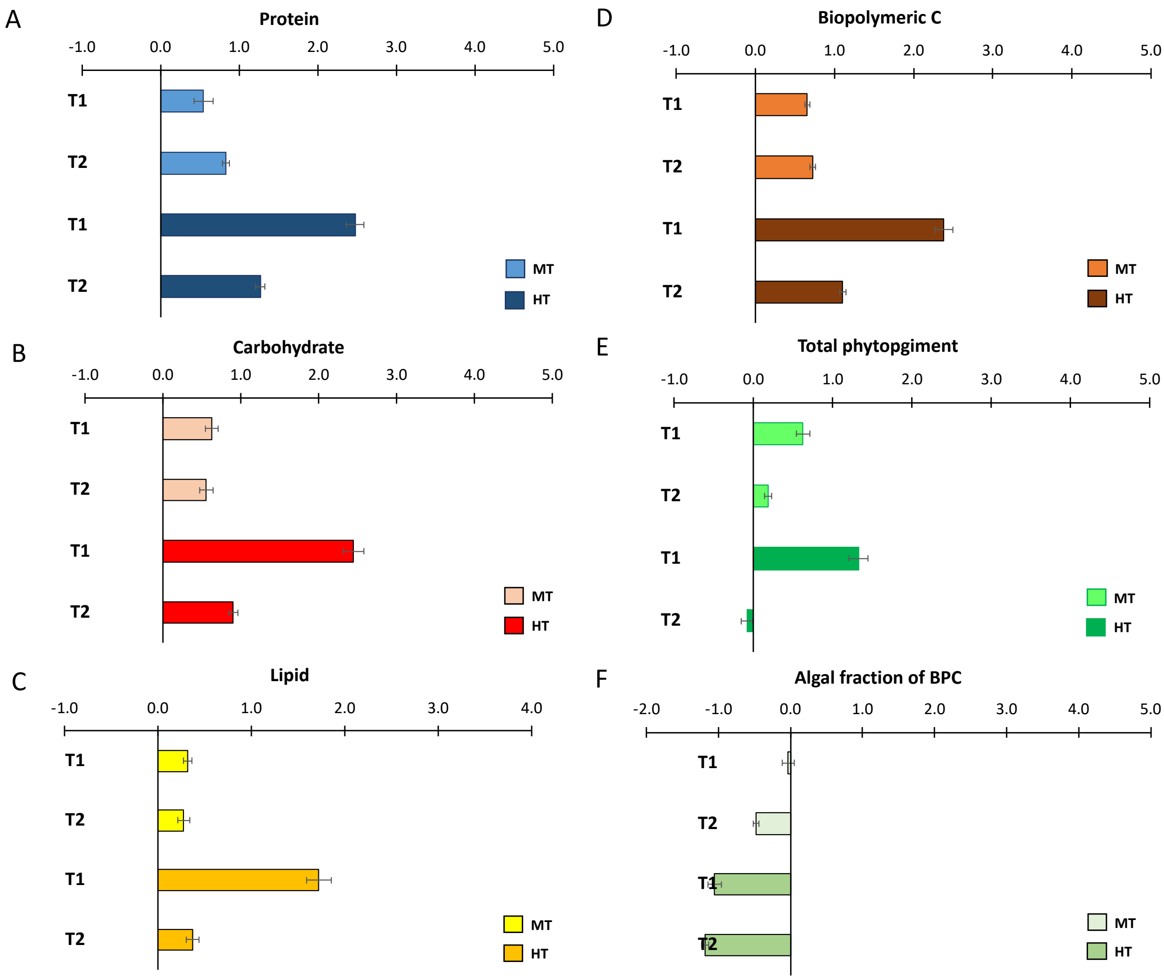

Figure 2. Size effects of temperature anomalies generated by the simulated heat wave on OM sedimentary contents: (A) protein, (B) carbohydrate, (C) lipid, (D) biopolymeric C, (E) total phytopigment, and (F) the autotrophic fraction of biopolymeric C. CTRL = control; MT = medium temperature anomaly; HT = high temperature anomaly. T_1 = after 3 weeks from PPW injection; T_2 = after 11 weeks from PPW injection. Error bars are standard errors (n = 6).

Table 2. Results of the post-hoc tests assessing differences in the effect size of the thermal anomaly in MT and HT between pairs of sampling times. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant.

Anomaly	Contrast	Protein	Carbohydrate	Lipid	Phytopigment	Biopolymeric C
MT	$T_0 x T_1$	**	ns	**	**	**
	$T_0 x T_2$	*	ns	**	*	*
	$T_1 x T_2$	*	ns	ns	**	ns
HT	$T_0 x T_1$	**	**	*	ns	**
	$T_0 x T_2$	**	***	***	***	***
	$T_1 x T_2$	***	***	***	***	***

The results of the two-way PERMANOVA test revealed a significant effect of the interaction between treatment and sampling time on organic matter biochemical composition (**Table 1**). The biplot made after the CAP analysis (**Figure 3**) reveals that organic matter biochemical composition in CTRL and MT remained relatively homogeneous during the entire study period.

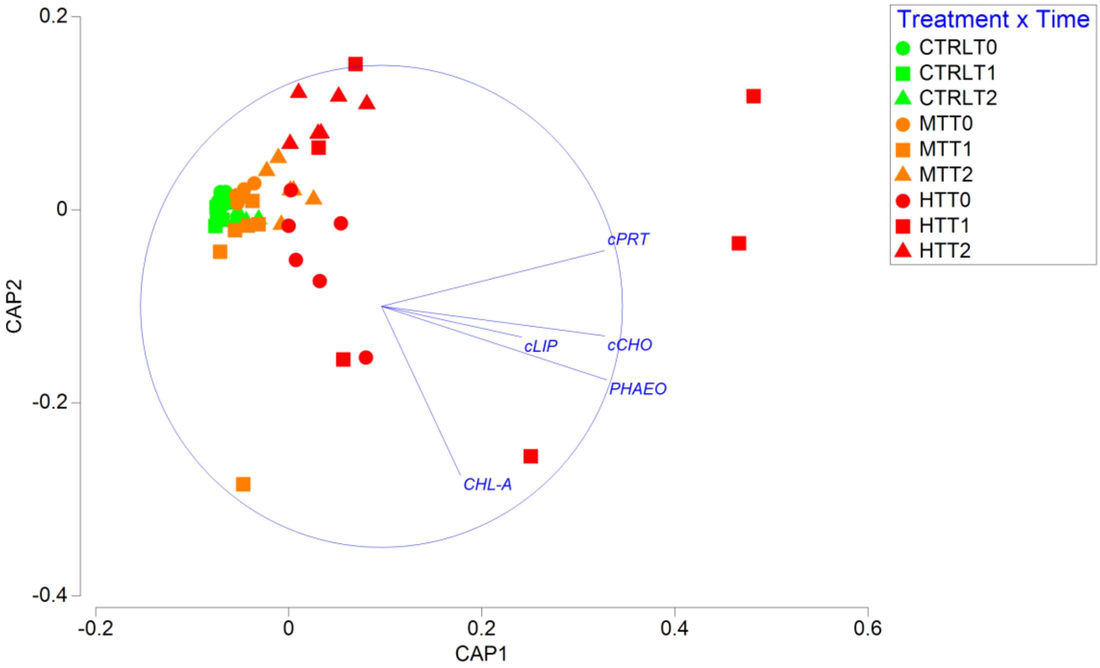

Figure 3. Biplot obtained after CAP analysis showing differences in the biochemical composition of sedimentary organic matter among treatments and sampling times. cPRT = protein; cCHO = carbohydrate; cLIP = lipid; Chl-a = chlorophyll-a; Phaeo = phaeopigment. CTRL = control; MT = intermediate temperature anomaly; HT = high temperature anomaly. T_0 = before PPW injection; T_1 = after 3 weeks from PPW injection; T_2 = after 11 weeks from PPW injection.

Notably, organic matter composition in HT after 3 weeks from PPW injection was largely different from that in all other treatments and sampling times, then, in T_2 (after 11 weeks from PPW injection) returned to resemble the composition observed in T_0 and T_1. As corroborated by the PERMDISP test, differences among replicates, a proxy for compositional heterogeneity, remained low in CTRL during the entire study period, whereas in both MT and HT it increased in T_1 and then decreased again in T_2 (**Figure S2**). Overall, the effect of the thermal anomaly on the biochemical diversity index was consistently negative in both T_1 and T_2, and increased with time in both treatments, indicating a progressive increase in organic matter heterogeneity (**Figure S3A**), mostly associated with an increase in the protein fraction at the expense of the lipid one (**Figure S3B**).

3.3.2. Sedimentary organic matter degradation rates

Extracellular enzymatic activities were characterized by a significant effect of the simulated heatwaves but, except for protein, carbohydrate and C turnover times, differences among treatments did not vary with time (**Table 3**; **Figure 4A-C**).

Table 3. Results of PERMANOVA testing for differences in extracellular enzymatic activities, C degradation rates and turnover time among treatments (Tr: CTRL, MT, and HT) and sampling times (Ti: T_0, T_1, and T_2). Df = degrees of freedom; MS = mean square; F = statistic F; P (MC) = probability level after Monte Carlo simulations and 999 permutations; *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; ns = not significant. Reported is also the percentage of variation explained (EV %) by each factor, their interaction and residual (Res; unexplained) variance.

Variable	Source	Df	MS	F	P (MC)	EV (%)
Aminopeptidase	Tr	2	4.760	13.7	**	35.1
	Ti	2	1.071	3.1	ns	5.7
	Tr *x* Ti	4	0.744	2.1	ns	9.5
	Res	45	0.347			49.7
β-glucosidase	Tr	2	0.184	14.7	**	34.6
	Ti	2	0.030	2.4	ns	3.6
	Tr *x* Ti	4	0.039	3.1	*	16.3
	Res	45	0.013			45.6
C degradation rate	Tr	2	6.744	15.0	**	38.1
	Ti	2	0.927	2.1	ns	2.9
	Tr *x* Ti	4	0.998	2.2	ns	9.9
	Res	45	0.451			49.1
Protein turnover time	Tr	2	490.2	11.6	**	30.4
	Ti	2	178.7	4.2	*	9.2
	Tr *x* Ti	4	85.5	2.0	ns	8.8
	Res	45	42.3			51.6
Carbohydrate turnover time	Tr	2	3842.8	6.9	**	18.2
	Ti	2	5574.0	10.0	**	27.8
	Tr *x* Ti	4	438.2	0.8	ns	0.0
	Res	45	559.7			54.0
C turnover time	Tr	2	905.4	19.6	**	34.9
	Ti	2	424.1	9.2	**	15.3
	Tr *x* Ti	4	178.1	3.9	*	16.1
	Res	45	46.1			33.7

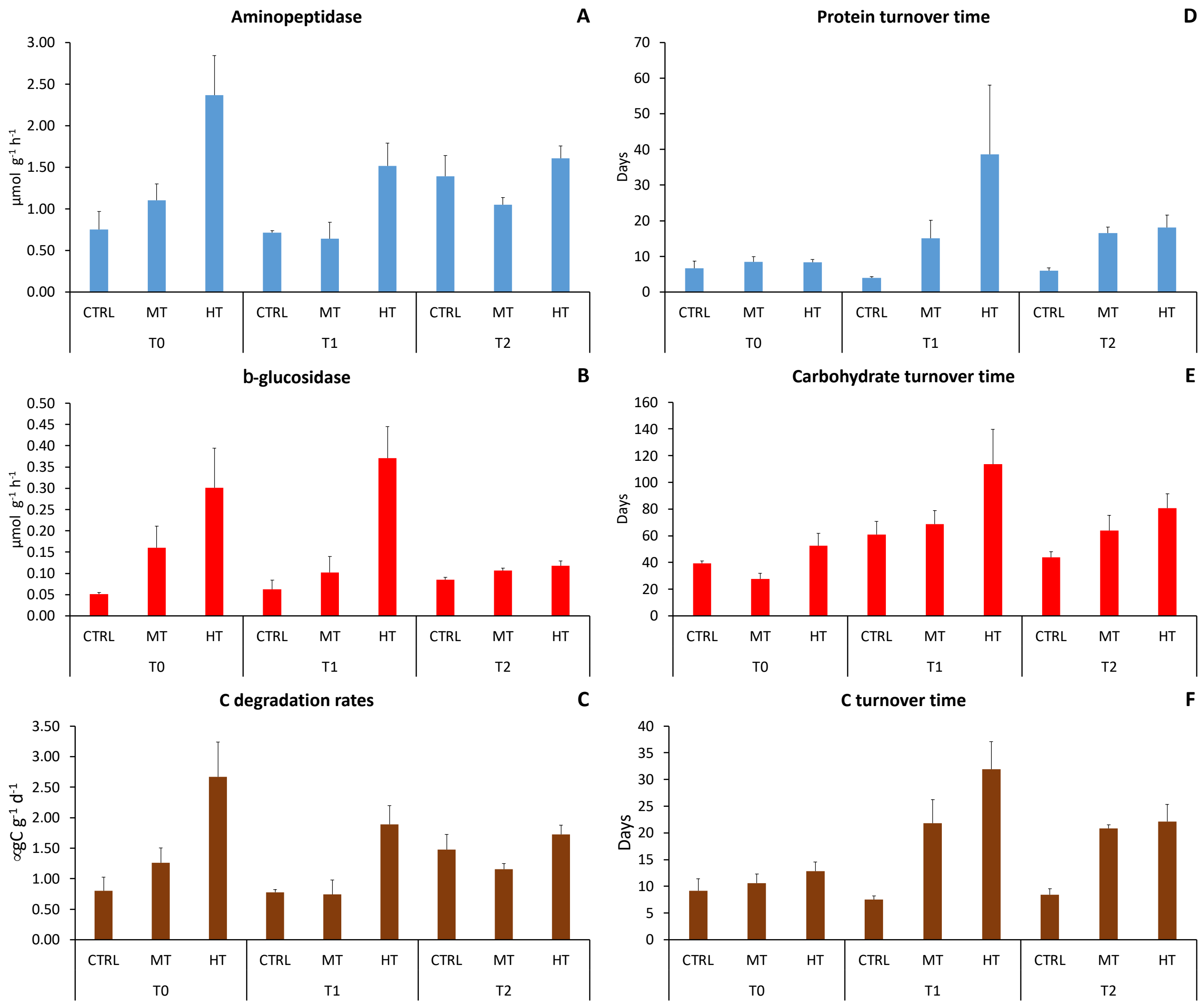

Figure 4. Changes in extracellular enzymatic (A) aminopeptidase, (B) β-glucosidase activities, (C) C degradation rates, (D) protein turnover time, (E) carbohydrate turnover time, and (F) C turnover time in the three sampling sites at each sampling time. CTRL = control; MT = medium temperature anomaly; HT = high temperature anomaly. T_0 = before PPW injection; T_1 = after 3 weeks from PPW injection; T_2 = after 11 weeks from PPW injection.

Before (T_0) and after 3 weeks (T_1) from PPW injection, aminopeptidase and β-glucosidase activities at HT were ca. 2-3 times higher than those in the MT and the control site. After 11 weeks from PPW injection (T_2), such differences weakened (**Table S2 A-B**), with values in HT ca. 1-1.5 times higher than those in CTRL and MT. Aminopeptidase activity and C degradation rates remained constant between T_0 and T_1 and increased in T_2 at CTRL, decreased in T_1 and increased again in T_2 at MT, and decreased in T_1 and stabilized in T_2 at HT. β-glucosidase activity slightly increased over time in CTRL, decreased in T_1 and stabilized in T_2 at MT, whereas in HT it remained constant between T_0 and T_1 and strongly decreased in T_2. Turnover time of proteins, carbohydrates and C increased in both MT (ca. 1.5 times) and HT (ca. 8 times) in T_1 then slightly recovered in T_2 (**Figure 4D-F**).

The effects of the temperature anomalies on aminopeptidase activities differed among treatments, being negative at MT and positive at HT (**Figure 5A**), whilst they were consistently positive on β-glucosidase activity in both treatments, much higher in T_1 at HT than in all other cases (**Figure 5B**). Like aminopeptidase activity, C degradation rates were negatively affected by the simulated heatwave at MT and were stimulated at HT, with a size effect in T_1 much higher than that in T_2 (**Figure 5C**). C turnover time increased similarly in MT and HT (**Figure 5D**).

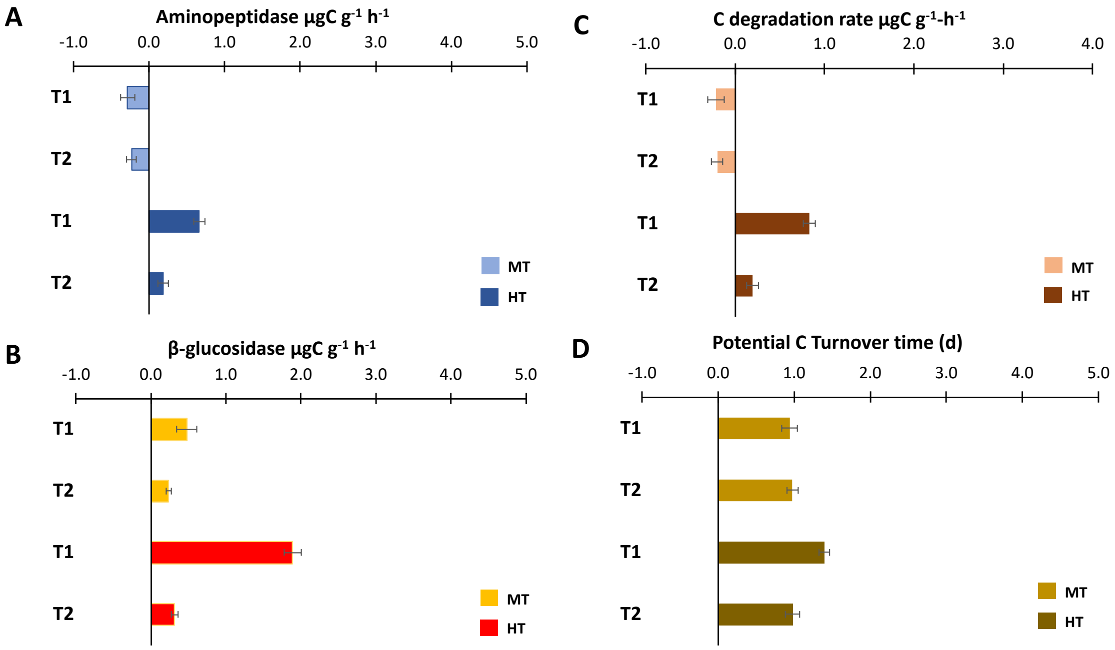

Figure 5. Size effects of temperature anomalies generated by the simulated heat wave on extra-cellular enzymatic (A) aminopeptidase and (B) β-glucosidase activities, on (C) C degradation rates and (D) C potential turnover time. MT = medium temperature anomaly; HT = high temperature anomaly. T_0 = before PPW injection; T_1 = after 3 weeks from PPW injection; T_2 = after 11 weeks from PPW injection.

3.4 Discussion

3.4.1. MHWs effects on sedimentary organic matter quantity, biochemical composition, and nutritional quality

Mean ocean surface temperature has increased by approximately 0.13 °C per decade over the past 100 years due to the massive heat adsorption by the oceans in response to the global warming caused by the increase of greenhouse gases in the atmosphere (Levitus et al., 2005; Harley et al., 2006). The consequences of oceans warming are multiple and affect both physical-chemical and biological features of the world's oceans (Sanz-Lázaro et al., 2015; Harley et al., 2006). Evidence that global warming is leading to progressively more frequent and intense MHWs is accumulating (Frölicher et al., 2018; Jacox et al., 2022), along with proofs of MHW impacts on marine species, habitats, and communities (Garrabou et al., 2009; Marbà et al., 2010; Oliver et al., 2018; Traving et al., 2021). Nonetheless, the effects of MHWs on sedimentary organic matter contents, composition and degradation rates are, to the best of our knowledge, so far to be assessed. Quantity and biochemical composition (in terms of proteins, carbohydrates, and lipids) of sedimentary organic matter are commonly used as proxies of the trophic state of coastal marine sediments (Dell'Anno et al., 2002; Bianchelli et al., 2016). While increasing biopolymeric C contents can be interpreted as an increase in the overall food availability for benthic consumers, variations in its biochemical composition and the autotrophic fraction of biopolymeric C influence its nutritional quality (Pusceddu et al., 2009).

We show here that persistent MHWs (up to 11 weeks), irrespectively of the generated T anomaly, can lead to a consistent increase in sedimentary contents of all classes of organic compounds, when compared to the reference site uninfluenced by the MHW, with the highest T anomaly effect size larger than that of the intermediate one. This result would indicate that, at least in the short-term, MHWs can cause a localized increase in the whole amount of food for benthic consumers. We also report that, after 3 weeks from the initial release of the PPW plume, phytopigment sedimentary contents increased, irrespectively of the T anomaly level, but dropped down to levels observed before PPW injection after 11 weeks. While the positive response of phytopigments in the shorter term could be due to an increased microphytobenthos production stimulated by increased temperature range (Finkel et al., 2010) and rising C incorporation rates (Lewandowska et al., 2012), the prolonged exposition to the MHWs at week 11 could have caused a severe cellular stress. This hypothesis is corroborated by the observed positive effects of both MHWs on sedimentary lipid contents, which could have been caused by the increase in the lipid production of benthic microalgae in response to rising temperature and oligotrophication (Novak et al., 2019). Our hypothesis is also corroborated by previous studies showing that the effect of heatwaves on marine phytoplankton (and, thus, conceivably, on microphytobenthos) depends on the intensity of the heatwave (Remy et al., 2017), and that more intense heatwaves usually result in increased mortality (Samuels et al., 2021). These results, thus, suggest that, under a sort of negative feedback path, more persistent and more intense MHWs will not only cause a general oligotrophication of the surface ocean (because of enhanced nutrient limitation), but will also impair the survivorship of microphytobenthos, thus ultimately impairing C sequestration processes in nearshore sediments. Moreover, in the longer term (at week 11), we observed a general decrease in the autotrophic fraction of biopolymeric C, again with an effect size caused by the highest T anomaly more negative than that caused by the lowest one. Phytopigments in shallow coastal sediments are a proxy of the amount of organic matter produced by photosynthesis (Dell'Anno et al., 2002) and their contribution to biopolymeric C is proportional to the bio-digestible (labile) fraction of biopolymeric C (Pusceddu et al., 2003, 2009). The observed decrease in the autotrophic fraction of biopolymeric C is indicative of a progressive depletion of sedimentary organic matter nutritional quality, which is also corroborated by the decrease in the high-energy lipid fraction of biopolymeric C along with a general progressive decrease in organic matter biochemical diversity. Thus, our results indicate that persistent and prolonged MHWs, besides their direct effects on benthic fauna and communities' survivorship (Pansch et al., 2018), could indirectly influence their ecological performance by altering the nutritional quality of the available food, with larger negative consequences associated with the most severe MHWs.

Recent modelling exercises showed that, at the end of the 21st century, a warmer Mediterranean Sea could be characterized by an overall expansion of P-limitation and a 10% reduction in phytoplankton net primary productivity (Richon et al., 2019), according to a predicted trophic attenuation of temperate seas with increasing sea temperature (Chust et al., 2014). Conceivably, the ecological consequences of this climate change-related oligotrophication of shallow areas of the Mediterranean Sea (Solidoro et al., 2009) could be locally exacerbated during prolonged events of MHWs. Although we have not investigated the responses of benthic fauna to the simulated MHWs, we could infer that, according to the *optimal foraging theory* (Stephens and Krebs, 1986), these persistent and prolonged events of thermal anomaly associated with MHWs could also affect the benthic community trophic structure, favoring species with high thermal tolerance and a preference for high quantities of nutritionally poor organic matter over species with low thermal tolerance and a preference for low quantities of nutritionally rich food. Moreover, our results confirm previous contentions, by which increasing frequency of more intense heatwaves could impair community resilience to withstand subsequent heatwaves (Sorte et al., 2010; Remy et al., 2017; Pansch et al., 2018).

3.4.2. MHWs' effects on organic C degradation rates

Food availability for benthic consumers depends not only on organic matter quantity, biochemical composition, and nutritional quality, but also on the rates at which complex organic matter is made progressively more prone to consumer assimilation through microbial activities. This step is crucial for the degradation of marine sedimentary organic matter, which is generally dominated by large and relatively refractory polymeric molecules (Pusceddu et al., 2009) and, thus, must undergo extracellular enzymatic hydrolysis to become nutritionally available for higher trophic levels. C degradation rates mediated by extracellular enzymes are influenced by temperature, so that rates of biogeochemical processes generally increase with increasing temperatures (Li et al., 2019; Arndt et al., 2013). Studies investigating the effects of temperature on extracellular enzymes in marine sediments have generally dealt with seasonal and geographical variability patterns (Christian et al., 1995; Patel et al., 2000; Arnosti and Jørgensen, 2003). To the best of our knowledge, our study is the first ever providing insights on the effects of MHWs and associated T anomalies on C degradation rates mediated by extracellular enzymatic activities, which have been repeatedly used as a proxy of benthic ecosystem functioning (Pusceddu et al., 2014; Franzo et al., 2019).

We report here that, although both simulated MHWs caused an overall slowdown of C turnover (i.e., an increased C turnover time), the one generating a narrower T anomaly caused a different response of aminopeptidase and β-glucosidase activities, with the former depressed and the latter stimulated. Instead, the most severe MHW, associated with a larger T anomaly, determined a positive response of both activities, more relevant in the short-term, then attenuating in the long-term. These results indicate that the extent of the generated T anomaly is a crucial parameter of MHWs influencing differently the microbial-mediated C degradation. Nonetheless, our results suggest that MHWs, according to our initial hypothesis, can exert significant effects on the rates of C degradation, apparently enhancing ecosystem functioning. Heterotrophic microbes, through the microbial loop, are the most important nutrient flywheel in marine food webs (Azam et al., 1983). According to the size–reactivity model, microbes selectively degrade high-molecular-weight molecules (Amon and Benner, 1996; Benner and Amon, 2015), as these compounds are generally too large to be transported across cell membranes (Weiss et al., 1991). Therefore, microbial extracellular enzymatic activity is the rate-limiting step in the degradation of organic matter in the oceans (Thomson et al., 2017). Our results, therefore, indicate that persistent MHWs, especially if generating T anomalies above 1.5 °C, can stimulate extracellular enzymatic activities and thus C degradation rates, causing a potential rise in the efficiency of energy transfer to higher trophic levels. This result agrees with the observed progressive decrease in the nutritional value of sedimentary organic matter and would suggest that MHWs can have severe effects on the whole trophic status of marine coastal sediments and, by cascade, on benthic trophic webs. However, the transfer of energy towards higher trophic levels is also a combination of changes in substrate quantity and rates of microbially mediated degradation. When combining the observed increase in substrate availability (proteins and carbohydrates) with the rise in their degradation rates mediated by enzymes under the larger T anomaly, we observed that, overall, the potential C turnover time increases, leading, ultimately, to a slowdown of benthic ecosystem functioning. This effect attenuates over time (i.e., at week 11 from PPW injection), possibly suggesting a sort of resilience of microbial activities, while the T anomaly persists. We cannot, however, exclude that this apparent recovery is due also to other mechanisms. For instance, the increase of total phytopigment contents at T_1 under the largest T anomaly could have stimulated the degradation also of refractory buried C by self-priming (van Nugteren et al., 2009) ultimately causing a decrease in biopolymeric C contents in the longer term.

3.5 Conclusions

The Mediterranean Sea, a semi-enclosed and relatively shallow basin, is one of the world regions most vulnerable to climate change (Lionello and Scarascia, 2018), with a projected sea surface warming rate approximately 3-4 times higher than the global ocean (Juza et al., 2022, and citations therein). Coastal aquatic ecosystems are among the most geochemically and biologically active areas of the biosphere and play a considerable role in the global biogeochemical cycles and, at the same time, they are among the most extensive and important carbon (C) reservoirs on the planet (Atwood et al., 2020). Our results indicate that benthic trophic status (in terms of organic matter quantity, composition, and nutritional quality) and ecosystem functioning (in terms of C degradation rates) of even very shallow nearshore marine sediments can be severely impaired by prolonged MHWs, with larger impacts associated with higher T anomalies. Based on these results, we can anticipate that the increase in frequency, intensity, and duration of MHWs, foreseen to cause abrupt ocean transitions in the coming decades (Benedetti-Cecchi, 2021; Jacox et al., 2022) will cause not only direct effects on species and communities, hence overall threatening benthic biodiversity (Cerrano et al., 2000; Garrabou et al., 2009; Juza et al., 2022; Verdura et al., 2019), but also provoke indirect effects by altering C biogeochemistry and the efficiency of energy transfer towards higher trophic levels.

3.6 References

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3.7 Supplementary material

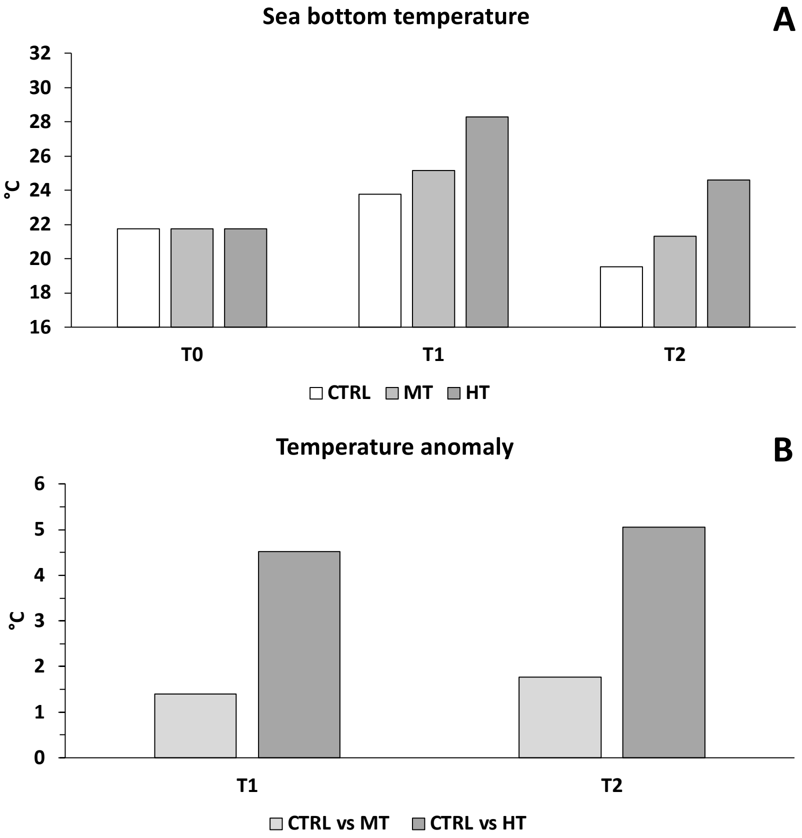

Figure S1. Temporal variations in (A) bottom temperature (°C) in the study sites, and (B) temperature anomaly (°C) at the MT and HT sites after the injection of Power Plant Water (PPW).

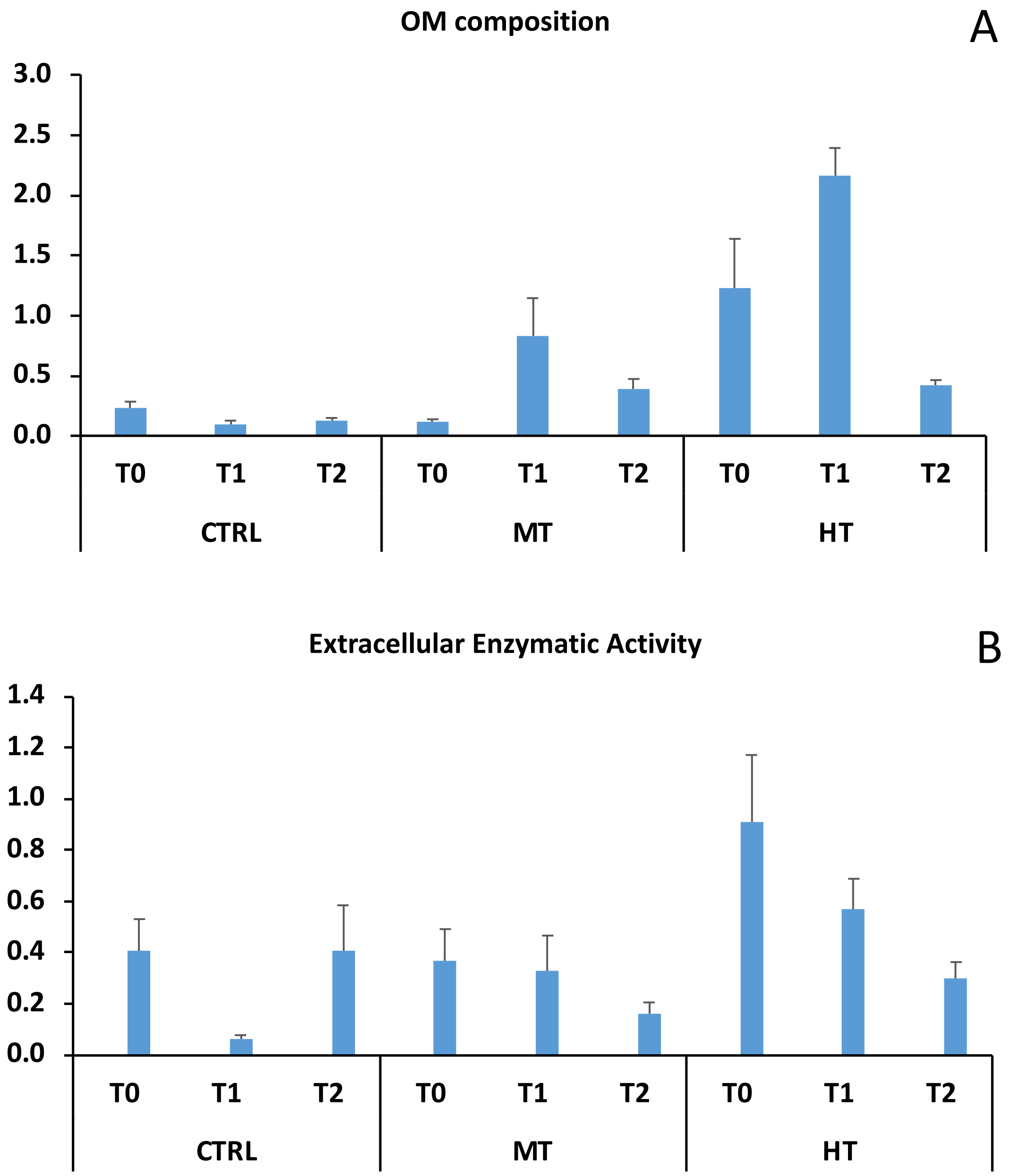

Figure S2. Output of the of the homogeneity of dispersion analysis (PERMDISP) on (A) sedimentary organic matter biochemical composition, and (B) enzymatic activities among treatments and times.

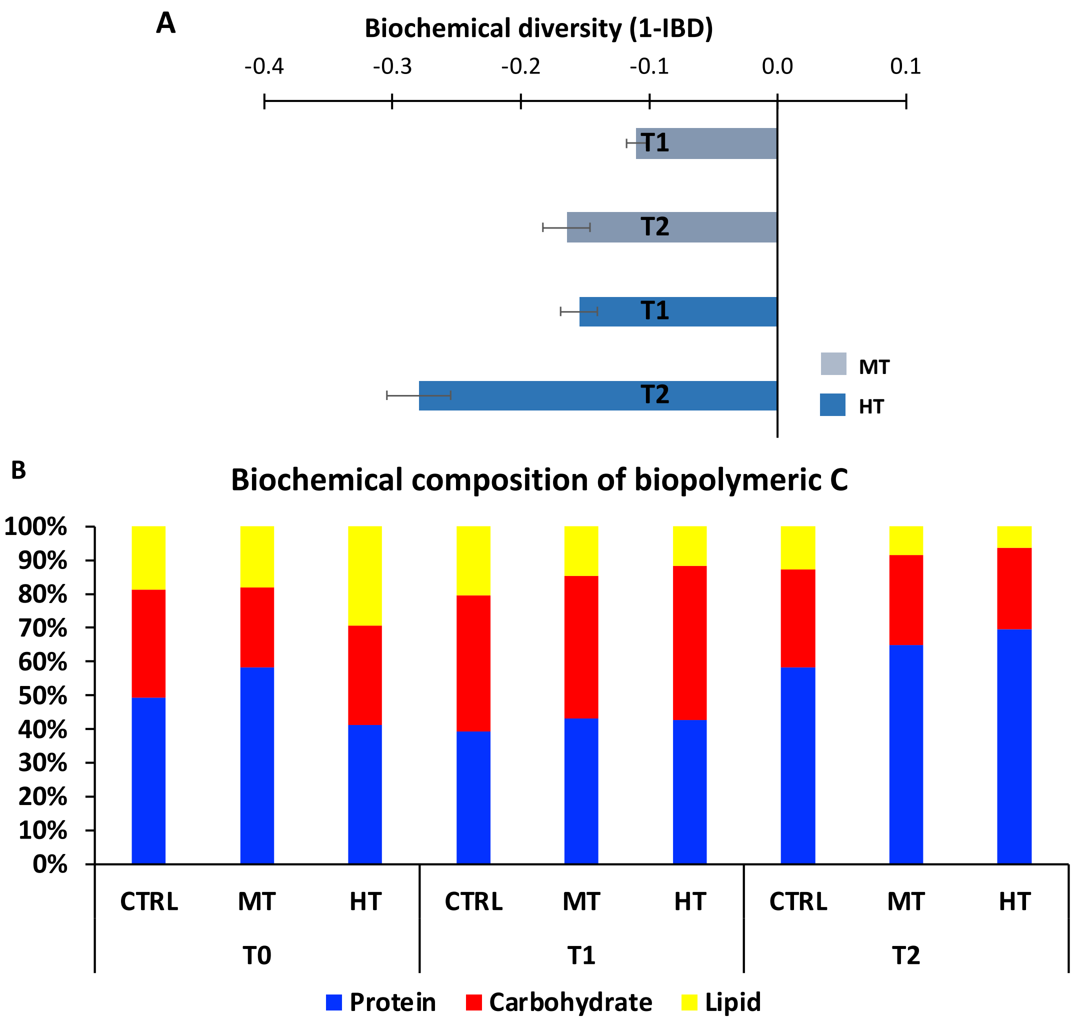

Figure S3. Changes in the biochemical composition of sedimentary organic matter. (A) Size effects of temperature anomalies generated by the simulated heat wave on the index of biochemical diversity (IBD). Since IBD has a rank inversely related with biochemical homogeneity, IBD-1 values were used to calculate the effect size. MT: intermediate temperature anomaly; HT = high temperature anomaly. T_1 = after 3 weeks from PPW injection; T_2 = after 11 weeks from PPW injection. Error bars are standard errors (n=6). (B) Changes in the relative (%) importance of protein, carbohydrate, and lipid contents in the biopolymeric C.

Table S1A. Results of the pairwise comparisons testing for differences among treatments in sedimentary organic matter quantity and biochemical composition separately for each sampling time. CTRL = control; MT = medium anomaly; HT = highest anomaly. T_0 = before PPW injection; T_1 = 3 weeks after PPW injection; T_2 = 11 weeks after PPW injection. t = statistic t; P(MC) = probability level after Monte Carlo simulations; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant.

Variable	**Time**	**Contrast**	**t**	**P(MC)**
Protein	T_0	*CTRL x MT*	5.237	**
		CTRL x HT	4.426	**
		MT x HT	2.876	*
	T_1	*CTRL x MT*	2.148	ns
		CTRL x HT	3.424	*
		MT x HT	3.111	*
	T_2	*CTRL x MT*	6.781	**
		CTRL x HT	6.205	**
		MT x HT	2.996	*
Carbohydrate	T_0	*CTRL x MT*	5.976	**
		CTRL x HT	2.635	*
		MT x HT	2.327	*
	T_1	*CTRL x MT*	2.421	*
		CTRL x HT	2.703	*
		MT x HT	2.497	*
	T_2	*CTRL x MT*	2.108	**
		CTRL x HT	4.585	**
		MT x HT	1.216	*
Lipid	T_0	*CTRL x MT*	3.124	*
		CTRL x HT	2.402	*
		MT x HT	2.149	ns
	T_1	*CTRL x MT*	1.937	ns
		CTRL x HT	2.090	ns
		MT x HT	1.998	ns
	T_2	*CTRL x MT*	2.200	ns
		CTRL x HT	2.320	*
		MT x HT	0.287	ns
Chlorophyll-*a*	T_0	*CTRL x MT*	1.319	ns
		CTRL x HT	1.899	ns
		MT x HT	1.676	ns
	T_1	*CTRL x MT*	1.491	ns
		CTRL x HT	2.646	*
		MT x HT	0.047	ns
	T_2	*CTRL x MT*	0.135	ns
		CTRL x HT	1.598	ns
		MT x HT	1.312	ns

Phaeopigment	T_0	*CTRL x MT*	2.361	*
		CTRL x HT	1.809	ns
		MT x HT	1.728	ns
	T_1	*CTRL x MT*	2.661	*
		CTRL x HT	3.268	*
		MT x HT	2.748	*
	T_2	*CTRL x MT*	2.130	ns
		CTRL x HT	2.272	*
		MT x HT	0.311	ns
Total phytopigment	T_0	*CTRL x MT*	2.479	*
		CTRL x HT	1.876	ns
		MT x HT	1.734	ns
	T_1	*CTRL x MT*	1.940	ns
		CTRL x HT	3.338	**
		MT x HT	1.963	ns
	T_2	*CTRL x MT*	1.732	ns
		CTRL x HT	0.142	ns
		MT x HT	1.341	ns
Algal fraction of biopolymeric C	T_0	*CTRL x MT*	5.692	**
		CTRL x HT	2.324	ns
		MT x HT	0.150	ns
	T_1	*CTRL x MT*	0.450	ns
		CTRL x HT	4.700	**
		MT x HT	2.267	*
	T_2	*CTRL x MT*	5.662	**
		CTRL x HT	13.027	**
		MT x HT	4.045	**
Biopolymeric C	T_0	*CTRL x MT*	7.940	**
		CTRL x HT	4.614	**
		MT x HT	3.693	**
	T_1	*CTRL x MT*	8.552	**
		CTRL x HT	3.200	*
		MT x HT	2.959	*
	T_2	*CTRL x MT*	7.494	**
		CTRL x HT	7.990	**
		MT x HT	3.678	**
OM biochemical composition	T_0	*CTRL x MT*	1.755	ns
		CTRL x HT	1.870	ns
		MT x HT	1.732	ns
	T_1	*CTRL x MT*	1.646	ns
		CTRL x HT	3.156	*
		MT x HT	2.401	*
	T_2	*CTRL x MT*	1.807	*
		CTRL x HT	2.883	**
		MT x HT	1.345	ns

Table S1B. Results of the pairwise test comparison testing for differences in sedimentary organic matter quantity and composition between pairs of sampling time in each of the treatments. CTRL = control; MT = medium anomaly; HT = highest anomaly. T_0 = before PPW injection; T_1 = 3 weeks after PPW injection; T_2 = 11 weeks after PPW injection. t = statistic t; P(MC) = probability level after Monte Carlo simulations; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant.

Variable	**Term**	**Groups**	**t**	**P(MC)**
Protein	CTRL	$T_0 \times T_1$	0.785	ns
		$T_0 \times T_2$	5.805	**
		$T_1 \times T_2$	6.160	**
	MT	$T_0 \times T_1$	1.323	ns
		$T_0 \times T_2$	5.465	**
		$T_1 \times T_2$	5.724	**
	HT	$T_0 \times T_1$	1.848	ns
		$T_0 \times T_2$	1.533	ns
		$T_1 \times T_2$	1.248	ns
Carbohydrate	CTRL	$T_0 \times T_1$	2.236	ns
		$T_0 \times T_2$	3.558	*
		$T_1 \times T_2$	1.534	ns
	MT	$T_0 \times T_1$	2.028	ns
		$T_0 \times T_2$	2.315	*
		$T_1 \times T_2$	0.544	ns
	HT	$T_0 \times T_1$	1.894	ns
		$T_0 \times T_2$	1.007	ns
		$T_1 \times T_2$	2.278	*
Lipid	CTRL	$T_0 \times T_1$	1.784	ns
		$T_0 \times T_2$	1.213	ns
		$T_1 \times T_2$	0.791	ns
	MT	$T_0 \times T_1$	1.074	ns
		$T_0 \times T_2$	0.447	ns
		$T_1 \times T_2$	0.565	ns
	HT	$T_0 \times T_1$	0.368	ns
		$T_0 \times T_2$	2.161	ns
		$T_1 \times T_2$	1.918	ns
Chlorophyll-*a*	CTRL	$T_0 \times T_1$	1.570	ns
		$T_0 \times T_2$	2.825	*
		$T_1 \times T_2$	2.188	*
	MT	$T_0 \times T_1$	1.556	ns
		$T_0 \times T_2$	1.271	ns
		$T_1 \times T_2$	1.097	ns
	HT	$T_0 \times T_1$	0.503	ns
		$T_0 \times T_2$	1.760	ns
		$T_1 \times T_2$	2.697	*

Phaeopigment	CTRL	T_0 x T_1	1.206	ns
		T_0 x T_2	3.229	**
		T_1 x T_2	5.182	**
	MT	T_0 x T_1	1.787	ns
		T_0 x T_2	3.010	*
		T_1 x T_2	0.494	ns
	HT	T_0 x T_1	0.582	ns
		T_0 x T_2	1.423	ns
		T_1 x T_2	2.718	*
Total phytopigment	CTRL	T_0 x T_1	0.845	ns
		T_0 x T_2	4.382	**
		T_1 x T_2	4.785	**
	MT	T_0 x T_1	1.670	ns
		T_0 x T_2	3.520	*
		T_1 x T_2	0.714	ns
	HT	T_0 x T_1	0.217	ns
		T_0 x T_2	1.587	ns
		T_1 x T_2	2.923	*
Algal fraction of biopolymeric C	CTRL	T_0 x T_1	0.076	ns
		T_0 x T_2	2.137	ns
		T_1 x T_2	2.042	ns
	MT	T_0 x T_1	1.822	ns
		T_0 x T_2	1.801	ns
		T_1 x T_2	2.106	ns
	HT	T_0 x T_1	1.052	ns
		T_0 x T_2	2.181	ns
		T_1 x T_2	1.415	ns
Biopolymeric C	CTRL	T_0 x T_1	1.320	ns
		T_0 x T_2	5.492	**
		T_1 x T_2	4.531	**
	MT	T_0 x T_1	0.388	ns
		T_0 x T_2	6.907	**
		T_1 x T_2	7.893	**
	HT	T_0 x T_1	1.681	ns
		T_0 x T_2	0.939	ns
		T_1 x T_2	2.068	ns
OM biochemical composition	CTRL	T_0 x T_1	1.545	ns
		T_0 x T_2	2.985	**
		T_1 x T_2	3.552	**
	MT	T_0 x T_1	1.579	ns
		T_0 x T_2	2.329	*
		T_1 x T_2	1.144	ns
	HT	T_0 x T_1	0.771	ns
		T_0 x T_2	1.532	ns
		T_1 x T_2	2.572	*

Table S2A. Results of the pairwise tests assessing differences in extracellular enzymatic activities, protein, carbohydrate and C degradation rates and turnover times among treatments separately at each sampling time. CTRL = control; MT = medium anomaly; HT = highest anomaly. T_0 = before PPW injection; T_1 = 3 weeks after PPW injection; T_2 = 11 weeks after PPW injection. t = statistic t; P(MC) = probability level after Monte Carlo simulations; * = p < 0.05; ** = p < 0.01; *** = p < 0.001; ns = not significant.

Variable	**Term**	**Groups**	**t**	**P(MC)**
Aminopeptidase	T_0	*CTRL x MT*	1.180	ns
		CTRL x HT	3.092	*
		MT x HT	2.464	*
	T_1	*CTRL x MT*	0.369	ns
		CTRL x HT	2.960	**
		MT x HT	2.618	*
	T_2	*CTRL x MT*	1.251	ns
		CTRL x HT	0.740	ns
		MT x HT	3.252	**
β-glucosidase	T_0	*CTRL x MT*	2.186	ns
		CTRL x HT	2.656	*
		MT x HT	1.313	ns
	T_1	*CTRL x MT*	0.925	ns
		CTRL x HT	4.016	**
		MT x HT	3.241	*
	T_2	*CTRL x MT*	2.591	*
		CTRL x HT	2.524	*
		MT x HT	0.777	ns
C degradation rate	T_0	*CTRL x MT*	1.389	ns
		CTRL x HT	3.074	**
		MT x HT	2.287	*
	T_1	*CTRL x MT*	0.133	ns
		CTRL x HT	3.602	**
		MT x HT	2.970	*
	T_2	*CTRL x MT*	1.178	ns
		CTRL x HT	0.847	ns
		MT x HT	3.206	*
Potential protein turnover time (d)	T_0	*CTRL x MT*	0.740	ns
		CTRL x HT	0.834	ns
		MT x HT	0.043	ns
	T_1	*CTRL x MT*	2.204	*
		CTRL x HT	3.879	**
		MT x HT	0.735	ns
	T_2	*CTRL x MT*	5.801	**
		CTRL x HT	3.428	**
		MT x HT	0.403	ns

Potential carbohydrate turnover time (d)	T_0	*CTRL x MT*	2.512	*
		CTRL x HT	1.427	ns
		MT x HT	2.487	*
	T_1	*CTRL x MT*	0.525	ns
		CTRL x HT	1.691	ns
		MT x HT	1.285	ns
	T_2	*CTRL x MT*	5.801	**
		CTRL x HT	3.428	**
		MT x HT	0.403	ns
C turnover time (d)	T_0	*CTRL x MT*	0.509	ns
		CTRL x HT	1.330	ns
		MT x HT	0.951	ns
	T_1	*CTRL x MT*	3.207	*
		CTRL x HT	4.686	*
		MT x HT	1.486	ns
	T_2	*CTRL x MT*	9.089	**
		CTRL x HT	4.005	**
		MT x HT	0.394	ns

Table S2B. Results of the pairwise test comparison testing for differences in in extracellular enzymatic activities, protein, carbohydrate and C degradation rates and turnover times between pairs of sampling times separately for each treatment. CTRL = control; MT = intermediate anomaly; HT = high anomaly. T_0 = before PPW injection; T_1 = after 3 weeks from PPW injection; T_2= after 11 weeks from PPW injection. t = statistic t; P(MC) = probability level after Monte Carlo simulations; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant.

Variable	Term	Groups	t	P(MC)
Aminopeptidase	CTRL	T_0 x T_1	0.158	ns
		T_0 x T_2	1.894	ns
		T_1 x T_2	2.626	*
	MT	T_0 x T_1	1.635	ns
		T_0 x T_2	0.223	ns
		T_1 x T_2	1.906	ns
	HT	T_0 x T_1	1.557	ns
		T_0 x T_2	1.533	ns
		T_1 x T_2	0.287	ns
β-glucosidase	CTRL	T_0 x T_1	0.610	ns
		T_0 x T_2	4.583	**
		T_1 x T_2	0.960	ns
	MT	T_0 x T_1	0.905	ns
		T_0 x T_2	1.022	ns
		T_1 x T_2	0.132	ns
	HT	T_0 x T_1	0.587	ns
		T_0 x T_2	1.918	ns
		T_1 x T_2	3.395	*
C degradation rate	CTRL	T_0 x T_1	0.110	ns
		T_0 x T_2	1.994	ns
		T_1 x T_2	2.719	*
	MT	T_0 x T_1	1.524	ns
		T_0 x T_2	0.396	ns
		T_1 x T_2	1.644	ns
	HT	T_0 x T_1	1.212	ns
		T_0 x T_2	1.616	ns
		T_1 x T_2	0.487	ns
Potential protein turnover time	CTRL	T_0 x T_1	1.265	ns
		T_0 x T_2	0.284	ns
		T_1 x T_2	2.243	*
	MT	T_0 x T_1	1.265	ns
		T_0 x T_2	3.685	*
		T_1 x T_2	0.285	ns
	HT	T_0 x T_1	2.772	*
		T_0 x T_2	2.768	*
		T_1 x T_2	0.320	ns

Potential carbohydrate turnover time	CTRL	T_0 x T_1	2.157	ns
		T_0 x T_2	1.049	ns
		T_1 x T_2	1.607	ns
	MT	T_0 x T_1	3.658	*
		T_0 x T_2	3.008	*
		T_1 x T_2	0.296	ns
	HT	T_0 x T_1	2.173	ns
		T_0 x T_2	2.008	ns
		T_1 x T_2	0.657	ns
C turnover time	CTRL	T_0 x T_1	0.698	ns
		T_0 x T_2	0.300	ns
		T_1 x T_2	0.656	ns
	MT	T_0 x T_1	2.383	*
		T_0 x T_2	5.501	**
		T_1 x T_2	0.231	ns
	HT	T_0 x T_1	3.518	**
		T_0 x T_2	2.551	*
		T_1 x T_2	1.612	ns

4. Effects of the sea cucumber *Holothuria tubulosa* (Gmelin, 1788) on the trophic status of coastal sediments under different temperature regimes and after marine heat snaps

4.1 Introduction

The increase in heat content, persisting sea surface temperatures (SSTs) anomalies, and the increasing frequency, intensity, and duration of marine heatwaves, are among the major manifestations of global warming in oceans worldwide (Levitus et al., 2005; Möller et al., 2008; Kumar et al., 2009; Hobday et al. 2016; Li et al., 2019; Darmaraki et al., 2019a-b; Ibrahim et al., 2021). All these conditions negatively and long-windedly compromise the integrity, structure, and functioning of marine ecosystems worldwide, which are indeed expected to worsen under any predicted scenario of climate change (Bates et al., 2014; Hobday et al., 2016; Alawad et al., 2020; Masson-Delmotte et al., 2021; Oliver et al., 2021; IPCC, 2022).

The Mediterranean Sea is one of the regions where the strongest warming is expected (Hoegh-Guldberg et al., 2018), especially in coastal waters (Juza et al., 2022). This basin represents a hotspot for global warming where SSTs have increased by ~1.54 ± 0.09°C in the last five decades and anomalies of up to 3.59 ± 0.08°C by 2100 (under the worst-case warming and overshooting IPCC scenario; IPCC 2021), with consequently increasing risks in the foreseeable future (Hilmi et al., 2022; Marriner et al., 2022). The negative effects of these phenomena on marine biogeochemistry and communities of this region have already been assessed and represent a possible future scenario of what could happen globally (Danovaro et al., 2001; Lejeusne et al., 2010; Ben Rais Lasram et al., 2010; Rivetti et al., 2014; Garcias-Bonet et al., 2019; George et al., 2019; Pagès et al., 2020; Chimienti et al., 2021; Cossarini et al., 2021; Gentilucci et al, 2021; Sarà et al. 2021; Soru et al., 2022).

At the same time, eutrophication is another climate-induced and exacerbated disturbance (Lloret et al., 2008; Nixon, 2009) which occurs locally in the Mediterranean coastal surface waters where the greatest input of nutrients derives from a variety of anthropogenic activities (Karydis et al., 2012; Viaroli et al., 2015; Tsikoti et al., 2021; Akcay et al., 2022). For instance, eutrophication largely occurs near aquaculture facilities (Tsiaras et al., 2022), exacerbated by the accumulation of waste feeds and thus organic matter (OM) in the sediments, consequently affecting sedimentary biogeochemistry, benthic micro-, meio- and macrofauna communities (Danovaro et al., 2003; Holmer et al., 2005; Pusceddu et al., 2007; David et al., 2009; Mirto et al., 2010; Dimitriou et al., 2017; González-Gaya et al., 2022).

In this context, deposit-feeders sea cucumbers are important elements of the benthic biodiversity being able to intercept and transform surplus OM coming from human activities such as aquaculture (Pasquini et al., 2023) thanks to their feeding behavior. They are among the most effective seafloor bioturbators, and their digestive system can be considered a true bioreactor where nutritive elements from ingested OM can be quickly assimilated (Jumars, 2000; Amaro et al., 2010; Purcell et al., 2016; Senff et al., 2022; Pasquini et al., 2023). Also, these animals can grow faster in mariculture-impacted sites where protein-enriched feed is abundant (Dumalan et al., 2019; Robinson et al., 2019) and have recently begun to be used as bioremediators in polycultures and Integrated Multi-Trophic Aquaculture (IMTA) systems with promising results (Slater et al., 2007; Nelson et al., 2012; MacDonald et al., 2013; Tolon et al., 2017; Cutajar et al., 2022). Sea cucumbers are ectothermic and osmo-conformers (Coteur et al., 2004) although increasing water temperatures may negatively affect their metabolic machinery and other functional traits such as, for example, those traits involved in the immune response (Wang et al., 2008; Parisi et al., 2021; Jobson et al., 2021). Besides this, some holothuroids (i.e., *Holothuria scabra*) have shown tolerance and adaptability to this kind of stress after an initial disturbance in energy balance due to the increase in temperature (Kühnhold et al., 2017, 2019) as well as other environmental variations such as chronic salinity fluctuations (Yuan et al., 2010; Bai et al., 2015).

The Mediterranean cucumber *Holothuria tubulosa* (Gmelin, 1788) in particular, is among the most active deposit-feeders able to modify sedimentary organic features (Amon et al., 1991a-b; İşgören-Emiroğlu et al., 2007a-b; Neofitou et al., 2019; Grosso et al., 2020; Pasquini et al., 2021). This species can tolerate a wide range of physicochemical stress (İşgören-Emiroğlu et al., 2007a) and juveniles easily survive under controlled conditions up to at least 30°C (Günay et al., 2015). Their elevated functional plasticity makes them as ideal candidates for benthic remediation (Pasquini et al., 2023) under increasing organic enrichment due to the eutrophication or direct influence of human activities and contextual increasing temperatures due to climate warming.

Thus, here with the aim to increase our understating about the effectiveness of sea cucumbers as bioreactors to counteract benthic eutrophication under different scenarios of sea warming, I designed a suite of experiments to test the null hypothesis by which, under different trophic status conditions, sedimentary OM content and biochemical composition (as expressed as protein, carbohydrate and lipid concentrations) should not vary between ambient sediments and feces of *H. tubulosa* i) acclimated under different temperatures or ii) exposed to a sudden temperature increment as a consequence of a heat snap. Under the same experimental conditions, I also investigated changes in feces production rates. Such an information is crucial when assessing the effects of environmental change – due to the interaction between local (e.g., organic enrichment) and global (e.g., increasing temperature) drivers – on the ability of organisms to adapt their behavior to changing conditions and when designing management measures to increase the use of deposit-feeders in integrated practices such as IMTA.

4.2 Materials and Methods

4.2.1 Sediment sampling

Sediments were collected in two sites (5-10 m depth): one located near a mariculture plant in the Gulf of Oristano (Western Sardinia, Mediterranean Sea), characterized by muddy sediments, and one, located in the Gulf of Teulada (Southern Sardinia, Mediterranean Sea), characterized by sandy-mud sediments and nearby meadows of the endemic seagrass *Posidonia oceanica* (Delile, 1813). The two sites were previously ranked as meso-eutrophic (Oristano) and oligo-mesotrophic (Teulada) (Pasquini et al., 2023), according to what was measured in situ in terms of mean biopolymeric carbon (C) contents (Pusceddu et al., 2009, 2011). The upper part (2 cm) of surface sediments were scraped by scuba divers in December 2020 and put in 50 ml Falcon-type tubes. Sediments collected from each site were mixed, homogenized, and stored into sterile 250 ml jars at -20°C until mesocosms preparation.

4.2.2 Sea cucumber holding tanks

Specimens of *H. tubulosa* (mean wet weight 108.8 ± 35.3 g) were collected in the same sites as for the sediments. All specimens were kept, under *in situ* temperature (14°C) and running seawater, in two 350-L tanks (one per sediment type, each with 1 cm-thick layer of the original sediment) at the experimental aquaculture facility of the University of Cagliari (SW Sardinia, Italy), till the initiation of the acclimation phase (see below).

4.2.3 Experimental set-up

A schematic representation of the experimental set-up is reported in **Figure S1**. Six 350-L tanks were filled with seawater and equipped with heaters, thermostats, and thermometers to control and maintain temperature at the desired values. Each of these tanks contained smaller 150-L tanks in which sea cucumbers were acclimated (see below for details) and then starved prior to the feeding and feces production experiments. The large thermally stable 350-L tanks were also used to host the small 6-L tanks used during the feeding and feces collection phases.

Two separated experiments, namely Experiment 1 (hereafter Exp 1) and Experiment 2 (hereafter Exp 2) were carried out. In Exp 1 sea cucumbers, before the feeding and feces production phases, were gradually (0.5°C per day till the chosen temperature) acclimated to 14, 17, 20, 23, 26, 29°C, with 14°C representing the minimum temperature faced by *H. tubulosa* specimens in winter (Coulon et al., 1993) as well as the minimum average sea surface winter temperature in the Mediterranean Sea between 2003 and 2019 (Ghosh et al., 2021; García-Monteiro et al., 2022). In Exp 2, sea cucumbers were first acclimated at 23°C (minimum average SST in summer in the SW Mediterranean Sea between 2010 and 2019; Pansini et al., 2021), and then, during the feeding and feces production phases, suddenly exposed to thermal shocks at 26 and 29°C.

In both experiments and for both sediment types (n=2; meso-eutrophic vs. oligo-mesotrophic), replicate 6-L tanks (n=3) were prepared per each temperature (n=36 for Exp 1, n=18 for Exp 2). During acclimation sea cucumbers were fed with algae paste and maintained at salinity and dissolved oxygen constant levels (36.5 and above 6 mg L^{-1}, respectively). During acclimation, ½ of the tank volume was replaced every 3 days, using seawater with a temperature equal to that reached at the day of water exchange. Once all established temperatures were achieved, 3 sea cucumbers per each experimental temperature and sediment type (for a total of 36 acclimated specimens for Exp 1 and a total of 18 specimens in for Exp 2) were translocated in thermally preconditioned 150-L tanks and starved without sediments for 72h (time required to completely empty the sea cucumbers intestine; Massin et al., 1976). During starvation, sea cucumbers were placed on a 1-cm mesh net to let feces sinking on the tank bottom and, thus, avoid coprophagia. During the feeding phase, replicate (n=3) 6-L mesocosms per each experimental temperature and sediment typology (for a total of 36 tanks for Exp 1 and 18 tanks for Exp 2) were filled with a 1-cm thick layer of original sediment and thermally preconditioned sea water (1:20 v/v). One sea cucumber was then placed in each 6-L tank (gently aerated to avoid water stratification and ensure adequate oxygenation) and left to feed on sediment for 12 hours. At the end of the feeding phase, sediments were collected and immediately stored at -20 °C till the analyses. After the feeding phase, all sea cucumbers were translocated in separate thermally stable empty (i.e., without sediment) 6-L tanks and feces were collected every 6-8 hours for the subsequent 72h. Feces produced by each specimen were stored in 10-mL PPE tubes at -20°C, until analysis. During both experiments, no specimen died, and, after the experiments, all individuals were then relocated at the original sampling site.

4.2.4 Quantity and biochemical composition of OM in sediments and feces

Protein, carbohydrate and lipid contents of sediments and holothuroid feces were determined spectrophotometrically according to the protocols detailed in Danovaro (2010). More in details, protein contents were determined according to Lowry et al. (1951), as modified by Hartree (1972) and Rice (1982), using the Folin-Ciocalteau reagent in a basic environment and expressed as bovine serum albumin equivalents. The procedure proposed by Gerchakov and Hatcher (1972), based on the phenol and concentrated sulfuric acid reaction with saccharides, was used to determine carbohydrates, then expressed as D (+) Glucose equivalents. Lipids, after extraction in chloroform: methanol (1:1, vol:vol; Bligh et al., 1959), and evaporation in a dry hot bath at 100 °C for 20 min, were determined after the sulfuric acid carbonization procedure (Marsh et al., 1966) and expressed as tripalmitin equivalents. For each biochemical assay, blanks were obtained using pre-calcinated (450 °C for 4 h) sediments or feces. All the analyses were performed in triplicate, with about 1 g of sediment or feces per replicate. Protein, carbohydrate, and lipid concentrations were converted into C equivalents using the conversion factors 0.49, 0.40, and 0.75 mgC mg^{-1}, respectively, obtained from the C contents of the respective standard molecules (albumin, glucose and tripalmitin, respectively), and their sum was reported as the biopolymeric C (BPC, Fabiano et al., 1995).

4.2.5 Effects magnitude

To compare the magnitude of the OM enrichment of holothuroids feces at different thermic regimes (Exp 1) and after heat snaps (Exp 2), in both meso-eutrophic and oligo-mesotrophic conditions, forest plot representations were done based on the ln–response ratio metric calculated as follows:

$$R_i = \ln (F_i/S_i)$$

where, F_i and S_i are organic matter contents of feces and sediments, respectively, per each specimen at the different experimental temperature (Exp 1: 14, 17, 20, 23, 26, 29°C; Exp 2: 23, 26, 29°C).

4.2.6 Statistical analyses

Non-parametric permutational analysis of variance (PERMANOVA; Anderson, 2001, 2017) were performed to test for differences in OM quantity and biochemical composition (in terms of protein, carbohydrate, and lipid contents) between sediment and feces across sites and temperatures, followed by post-hoc tests in the case of significant effects ($p<0.05$). For both experiments (Exp 1, Exp 2), the design included three fixed orthogonal factors: Site (S; meso-eutrophic vs. oligo-mesotrophic), Matrix (M; sediments vs. feces) and Temperature (T; 14, 17, 20, 23, 26, 29°C and 23, 26, 29°C for Exp 1 and Exp 2, respectively), with $n=3$ for the combination of factors. PERMANOVA tests were conducted on Euclidean distance-based resemblance matrices obtained from previously normalized data, using 999 random permutations of the appropriate units and the p-values were obtained after Monte Carlo simulation. PERMANOVA on one response variable using Euclidean distance yields the classical univariate F statistic and can be used, as in this study, to do univariate ANOVA where p values are obtained by permutation (Anderson and Millar, 2004), thus avoiding the assumption of normality (Anderson et al., 2008). Multivariate differences in OM biochemical composition (in terms of protein, carbohydrate, lipid) between sediment and feces under different experimental assets (separately for the two trophic status conditions) were visualized with biplots obtained after a canonical analysis of the principal coordinates (CAP) (Anderson et al., 2003). CAP allows identifying an axis through the multivariate cloud of points that is best at separating *a priori* groups (Anderson et al., 2008). All the statistical analyses were performed using the routines included in the PRIMER 6+ software (Anderson et al. 2008).

4.3 Results

4.3.1 Quantity and biochemical composition of sediment and holothuroid feces at different temperatures

Protein, carbohydrate, lipid, and BPC contents of sediment and feces during Exp 1 are provided in **Table S1**. Sedimentary contents of all classes of organic compounds were characterized by a significant effect of the M×S×T interaction (**Table 1**).

In meso-eutrophic conditions feces were from 2 to 13 times significantly richer in BPC than the relative ambient sediment at all temperatures, except at 14 and 29°C (**Table 2**; **Figure 1A**). Protein and lipid contents were significantly higher in feces than in the sediment only at 20°C (ca. 15 times for proteins, 58 times for lipids) and 26°C (3 times for proteins, 8 times for lipids) (**Table 2**; **Figure S2 A-C**). Feces carbohydrate content was significantly higher than that in the sediment at all temperatures (from 3 to 26 times), except at 14 and 29°C (**Table 2**, **Figure S2B**). During Exp 1 under meso-eutrophic conditions, the OM biochemical composition differed significantly between feces and meso-eutrophic sediments at all temperatures, except at 14 and 29°C (**Figure 1B**). Such differences varied across temperatures. More in details, at 17, 23 and 26°C feces were characterized by protein (by 20-77%) and carbohydrate (by 36-66%) contributions to BPC lower and higher, respectively, than in the sediment. At 20°C feces, when compared to the corresponding sediment, were characterized by higher protein (64 and 71% in sediment and feces, respectively), higher lipid (6 and 20%) and lower carbohydrate (30 and 9%) contributions to BPC (**Figure 1B**). At the lowest temperatures (14 and 17°C) feces and sediments were characterized by relatively similar biochemical composition, whereas the largest differences occurred at 20°C. At 23-29°C differences persisted but appeared relatively less marked than those at 20°C (**Figure 1C**).

In oligo-mesotrophic conditions, feces were from 2 to 5 times significantly richer in BPC than the relative ambient sediments at all temperatures (**Figure 1D**). Feces protein content was from 2 to 4 times significantly higher than that in the sediment at all temperatures, except at 17 and 23°C (**Table 2**; **Figure S2D**). Feces carbohydrate content was 2-3 times significantly higher than that in the sediment at all temperatures, except 23 and 29°C (**Table 2**; **Figure S2E**). Feces lipid content was from 4 to 15 times significantly higher than that in the sediment at all temperatures, except at 17°C (**Table 2**; **Figure S2F**). During Exp 1 under oligo-mesotrophic conditions, the OM biochemical composition differed significantly between feces and the corresponding sediment at all temperatures, except at 17°C (**Table 2**). Such differences were due to lipid contributions to BPC in feces generally higher than those in the corresponding sediment at all temperatures, but at 29°C, accompanied by lower carbohydrate contributions to BPC at 14 and 20°C (**Figure 1E**). Differences in the OM biochemical composition between feces and the corresponding sediment persisted at all temperatures, with differences at the lowest temperatures (14 and 17°C) less marked than those at the higher ones (20-29°C) (**Figure 1F**).

Table 1. Results of the PERMANOVA tests carried out to investigate differences during Exp 1 in the quantity and biochemical composition of organic matter between the two matrices (M; sediments vs. feces) subjected to different temperatures (T; 14, 17, 20, 23, 26, 29°C) in the two sites (S; meso-eutrophic- vs. oligo-mesotrophic). df = degrees of freedom; MS = mean square; Pseudo-F = F statistic; P (MC) = probability level after Monte Carlo simulations (** = $p < 0.01$; * = $p < 0.05$; ns = not significant); % EV = percentage of explained variance.

Variable	Source	df	MS	Pseudo-F	P(MC)	% EV
Protein	Matrix	1	6.814	39.389	**	6.2
	Site	1	15.809	91.381	**	14.5
	Temperature	5	2.606	15.064	**	6.8
	M × S	1	5.399	31.208	**	9.7
	S × T	5	2.651	15.324	**	13.8
	M × T	5	2.740	15.840	**	14.3
	M × S × T	5	2.756	15.930	**	28.8
	Residual	48	0.173			5.8
Carbohydrate	Matrix	1	13.526	80.224	**	18.1
	Site	1	24.514	145.400	**	33.0
	Temperature	5	0.373	2.214	ns	0.8
	M × S	1	9.660	57.294	**	25.8
	S × T	5	0.259	1.536	ns	0.7
	M × T	5	0.843	4.997	**	5.5
	M × S × T	5	0.647	3.840	**	7.8
	Residual	48	0.169			8.2
Lipid	Matrix	1	7.448	33.924	**	6.6
	Site	1	10.878	49.547	**	9.7
	Temperature	5	2.726	12.415	**	6.8
	M × S	1	4.968	22.630	**	8.6
	S × T	5	2.697	12.285	**	13.5
	M × T	5	3.210	14.621	**	16.3
	M × S × T	5	3.079	14.026	**	31.2
	Residual	48	0.220			7.2
Biopolymeric C	Matrix	1	10.641	64.988	**	10.1
	Site	1	21.458	131.040	**	20.6
	Temperature	5	1.882	11.493	**	5.0
	M × S	1	7.961	48.619	**	15.1
	S × T	5	1.857	11.342	**	9.8
	M × T	5	2.129	13.000	**	11.4
	M × S × T	5	2.073	12.658	**	22.2
	Residual	48	0.164			5.7
Biochemical composition	Matrix	1	27.788	49.520	**	9.4
	Site	1	51.201	91.244	**	17.4
	Temperature	5	5.705	10.167	**	5.3
	M × S	1	20.027	35.690	**	13.4
	S × T	5	5.607	9.993	**	10.4
	M × T	5	6.793	12.105	**	12.8
	M × S × T	5	6.483	11.552	**	24.4
	Residual	48	0.561			6.9

Table 2. Results of the pairwise tests carried out to assess differences in contents of protein, carbohydrate, lipid, biopolymeric C, and biochemical composition between matrixes (sediments vs. feces) in meso-eutrophic and oligo-mesotrophic conditions, at six temperatures (14, 17, 20, 23, 26, 29°C) during Exp 1. t = statistic t; P(MC) = probability level after Monte Carlo simulation; * = p <0.05; ** = p <0.01; *** = p <0.001; ns = not significant.

Variable	Temperature °C	t	P(MC)	t	P(MC)
		Meso-Eutrophic		Oligotrophic	
Protein	14	1.862	ns	4.099	*
	17	1.135	ns	0.764	ns
	20	4.614	*	2.741	*
	23	1.217	ns	2.603	ns
	26	4.195	*	11.389	***
	29	1.401	ns	6.745	**
Carbohydrate	14	2.409	ns	4.28	*
	17	3.632	*	7.28	**
	20	19.215	***	2.828	*
	23	7.308	**	2.773	ns
	26	2.975	*	2.874	*
	29	2.371	ns	2.705	ns
Lipid	14	0.668	ns	4.795	*
	17	2.48	ns	1.194	ns
	20	4.205	*	4.857	**
	23	2.744	ns	4.778	**
	26	3.158	*	4.705	*
	29	0.431	ns	2.665	*
Biopolymeric C	14	2.243	ns	6.41	**
	17	3.086	*	3.057	*
	20	4.893	**	24.477	**
	23	4.306	*	3.333	*
	26	3.766	*	5.495	**
	29	1.908	ns	3.929	*
Biochemical composition	14	2.069	ns	4.641	**
	17	2.746	*	1.636	ns
	20	4.446	*	3.416	**
	23	6.538	**	3.006	*
	26	3.12	*	3.63	**
	29	2.243	ns	2.946	*

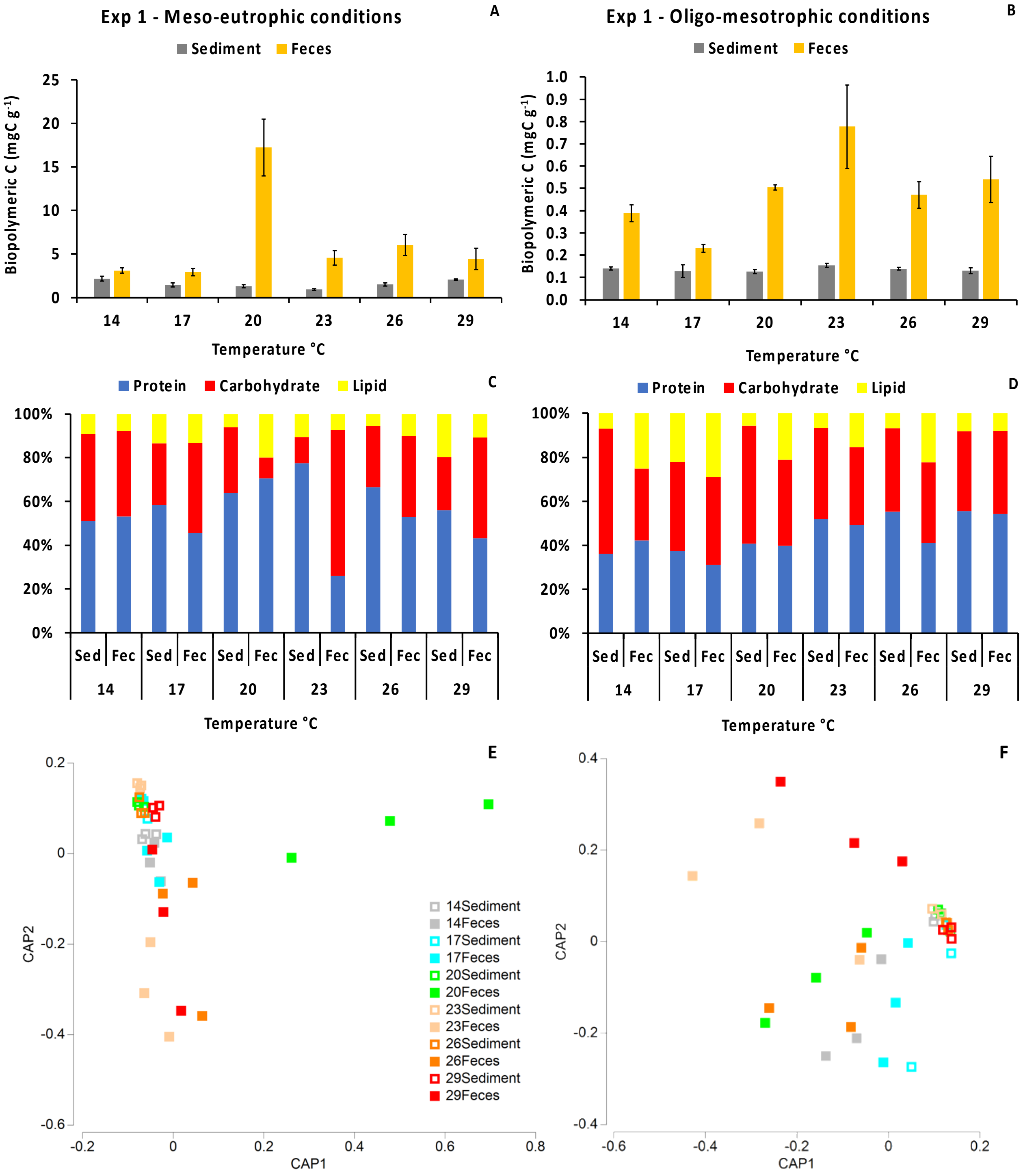

Figure 1. Changes in BPC contents (A-B) and protein, carbohydrate, and lipid percentage contributions to BPC (C-D) in sediment (Sed) and feces (Fec) at the six acclimation temperatures (14, 17, 20, 23, 26, 29 °C) in meso-eutrophic (left panel) and oligo-mesotrophic (right panel) conditions during Exp 1. Error bars indicate the standard errors (n = 3). Reported are also the biplots obtained after CAP analysis illustrating differences in OM biochemical composition in sediments and feces during Exp 1 (E) and Exp 2 (F). PRT = proteins; CHO = carbohydrates; LIP = lipids.

4.3.2 Effects of heat snaps on quantity and biochemical composition of sediment and feces

Protein, carbohydrate, lipid, and biopolymeric C contents of sediment and feces during Exp 2 are reported in **Table S2**. Sedimentary contents of all classes of organic compounds were characterized by a significant effect of the M×S×T interaction (**Table 3**).

In meso-eutrophic conditions, feces were from 2 to 5 times significantly richer in BPC than the relative ambient sediment at all temperatures (**Table 4**; **Figure 2A**). While protein contents did not differ significantly between feces and sediments at all temperatures (**Table 4**; **Figure S3A**), carbohydrate content was from 2 to 26 times significantly higher in feces than in the corresponding sediment at all temperatures (**Table 4**; **Figure S3B**). Lipid content was from 3 to 8 times significantly higher in feces than in the corresponding sediment only after the sudden exposure of sea cucumbers at 26 and 29°C (**Table 4**; **Figure S3C**). During Exp 2, the OM biochemical composition differed significantly between feces and the corresponding sediments at all temperatures (**Figure 2B**). Such differences were consistent at all temperatures, with feces depauperated (by 32-66%) in protein and enriched (by 24-82%) in carbohydrate fractions, when compared with the sediment, at all temperatures. Feces produced by sea cucumbers acclimated at 23°C were characterized by lipid contributions to the BPC that were 30% lower than the corresponding sediment, whereas feces produced by sea cucumbers suddenly exposed to heat snaps at 26 and 29°C lipid contributions of feces were 51 and 37% higher than those in the sediment, respectively. During Exp 2, differences in the OM biochemical composition between feces and the corresponding sediment persisted at all temperatures, with differences at the highest temperature (29°C) less marked than those at the lower ones (23-26°C) (**Figure 2C**).

In oligo-mesotrophic conditions and at all temperatures, feces were from 3 to 31 times significantly richer in BPC than the relative ambient sediment (**Table 4**; **Figure 2D**). Protein content was higher in feces than in the corresponding sediment only at 26 and 29°C (3 and 53 times, respectively) (**Table 4**; **Figure S3D**), whereas carbohydrate content was 4 times significantly higher in feces than in the corresponding sediment only at 26°C (**Table 4**; **Figure S3E**). Lipid content was from 8 to 87 times higher in feces than in the corresponding sediment at all temperatures (**Table 4**; **Figure S3F**). During Exp 2, the OM biochemical composition differed significantly between feces and oligo-mesotrophic sediments at all temperatures (**Figure 2E**). Such differences varied across temperatures. More in details, at 23 and 26°C feces were characterized by (5 and 21 %, respectively) lower protein and higher lipid (57 and 56%, respectively) contribution. On the contrary, at 29°C feces were characterized by 39% higher protein and by 28% lower lipid contributions. Feces were characterized by 16% higher carbohydrate contribution at 26 °C and 15-69% lower at 23 and 29°C, respectively. Differences in the OM biochemical composition of feces and the corresponding sediment persisted at all temperatures, with differences at the highest temperature (29°C) more marked than those at the lower ones (23-26°C) (**Figure 2F**).

Table 3. Results of the PERMANOVA carried out to investigate differences during Exp 2 in the quantity and biochemical composition of organic matter between the two matrices (M; sediments vs. feces) subjected to different temperatures (T; 23, 26, 29°C) in the two sites (S; meso-eutrophic and oligo-mesotrophic), df = degrees of freedom; MS = mean square; Pseudo-F = F statistic; P (MC) = probability level after Monte Carlo simulation (** = $p < 0.01$; * = $p < 0.05$; ns = not significant); % EV = percentage of explained variance.

Variable	Source	df	MS	Pseudo-F	P(MC)	% EV
Protein	Matrix	1	1.217	44.592	**	14.8
	Site	1	0.396	14.502	**	4.6
	Temperature	2	0.219	8.042	**	3.6
	M × S	1	0.146	5.360	**	3.0
	S × T	2	0.617	22.612	**	22.0
	M × T	2	0.242	8.865	**	8.0
	M × S × T	2	0.537	19.678	**	38.0
	Residual	24	0.027			6.1
Carbohydrate	Matrix	1	15.730	156.680	**	23.1
	Site	1	17.385	173.170	**	25.6
	Temperature	2	0.331	3.299	*	0.5
	M × S	1	9.827	97.884	**	28.8
	S × T	2	0.734	7.315	**	2.8
	M × T	2	0.939	9.353	**	3.7
	M × S × T	2	1.538	15.321	**	12.8
	Residual	24	0.100			2.7
Lipid	Matrix	1	1.035	72.008	**	38.2
	Site	1	0.235	16.334	**	8.2
	Temperature	2	0.067	4.687	**	3.0
	M × S	1	0.028	1.914	ns	1.0
	S × T	2	0.111	7.756	**	10.9
	M × T	2	0.035	2.465	ns	2.4
	M × S × T	2	0.133	9.284	**	26.7
	Residual	24	0.014			9.7
Biopolymeric C	Matrix	1	3.964	102.930	**	27.5
	Site	1	2.631	68.328	**	18.2
	Temperature	2	0.050	1.299	ns	0.1
	M × S	1	0.363	9.434	**	4.5
	S × T	2	0.675	17.529	**	13.4
	M × T	2	0.013	0.340	ns	0.0
	M × S × T	2	0.788	20.463	**	31.5
	Residual	24	0.039			4.9
Biochemical composition	Matrix	1	17.981	126.580	**	22.8
	Site	1	18.015	126.820	**	22.8
	Temperature	2	0.618	4.350	**	0.9
	M × S	1	10.001	70.400	**	25.2
	S × T	2	1.463	10.298	**	5.1
	M × T	2	1.216	8.562	**	4.1
	M × S × T	2	2.209	15.547	**	15.8
	Residual	24	0.142			3.3

Table 4. Results of the pairwise tests carried out to assess differences in contents of protein, carbohydrate, lipid, biopolymeric C, and biochemical composition between matrixes (sediments vs. feces) in meso-eutrophic and oligo-mesotrophic conditions at each temperature (23, 26, 29°C) during Exp 2. t = statistic t; P(MC) = probability level after Monte Carlo simulation; * = p <0.05; ** = p <0.01; *** = p <0.001; ns = not significant.

Variable	**Temperature °C**	**t**	**P(MC)**	**t**	**P(MC)**
		Meso-Eutrophic		**Oligotrophic**	
Protein	23	1.217	ns	2.603	ns
	26	1.802	ns	4.860	**
	29	0.714	ns	16.943	***
Carbohydrate	23	7.308	**	2.773	ns
	26	7.808	**	3.378	*
	29	5.692	**	2.767	ns
Lipid	23	2.744	ns	4.778	*
	26	3.385	*	5.567	**
	29	2.836	*	10.554	***
Biopolymeric C	23	4.306	*	3.333	*
	26	4.130	*	4.753	*
	29	7.015	**	14.526	***
Biochemical composition	23	6.538	**	3.006	*
	26	6.490	**	3.589	*
	29	4.268	**	7.847	***

4.3.3 Magnitude of feces organic enrichment

In Exp 1 under oligo-mesotrophic conditions, BPC enrichment of holothuroid feces below 20°C was ca. 1.7 times higher than that at lower temperatures (14-17°C), whereas, under meso-eutrophic conditions, it showed a quasi-unimodal distribution with a peak at 20°C (**Figure 3A**). BPC enrichment of feces in oligo-mesotrophic conditions was higher than that in meso-eutrophic conditions at 14 and 29°C, lower at 20°C, and similar at all other temperatures. Under oligo-mesotrophic conditions protein enrichment of feces did not vary among treatments, the lipid one was lowest at 17° and 29°C, and the carbohydrate one slightly increased with temperature, resulting at 29°C ca. 3 times higher than that at 14°C (**Figure 3B-D**). Under meso-eutrophic conditions protein, lipid and carbohydrate enrichment showed quasi-unimodal distributions across treatments, with the highest values at 20°C for protein and lipid, and at 23°C for carbohydrate. Protein and lipid enrichment of feces in oligo-mesotrophic conditions is higher than that in meso-eutrophic ones at 14, 23, and 29°C, while the opposite is observed at 20°C. Carbohydrate enrichment in oligo-mesotrophic conditions is generally lower than or equal to that in meso-eutrophic conditions at all temperatures.

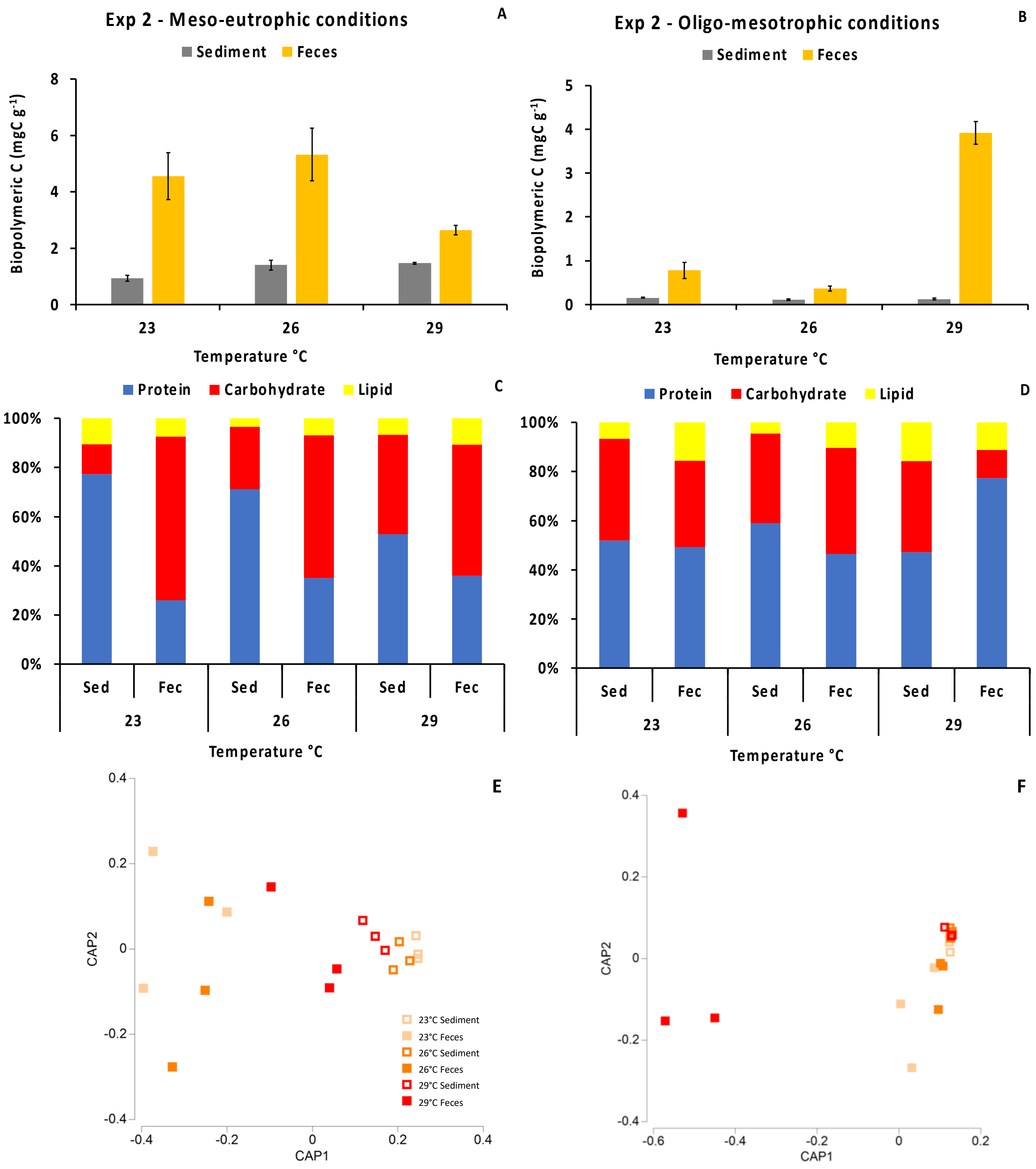

Figure 2. Changes in BPC content (A-B) and protein, carbohydrate, and lipid contributions to BPC (C-D) in sediment (S) and feces (F) at the three temperatures (23, 26, 29 °C) in meso-eutrophic (left panel) and oligo-mesotrophic (right panel) conditions during Exp 2. Error bars indicate the standard error (n = 3). Reported is also the biplot obtained after CAP analysis illustrating differences in the OM biochemical composition among of sediments and feces at the different experimental temperatures. PRT = proteins; CHO = carbohydrates; LIP = lipids.

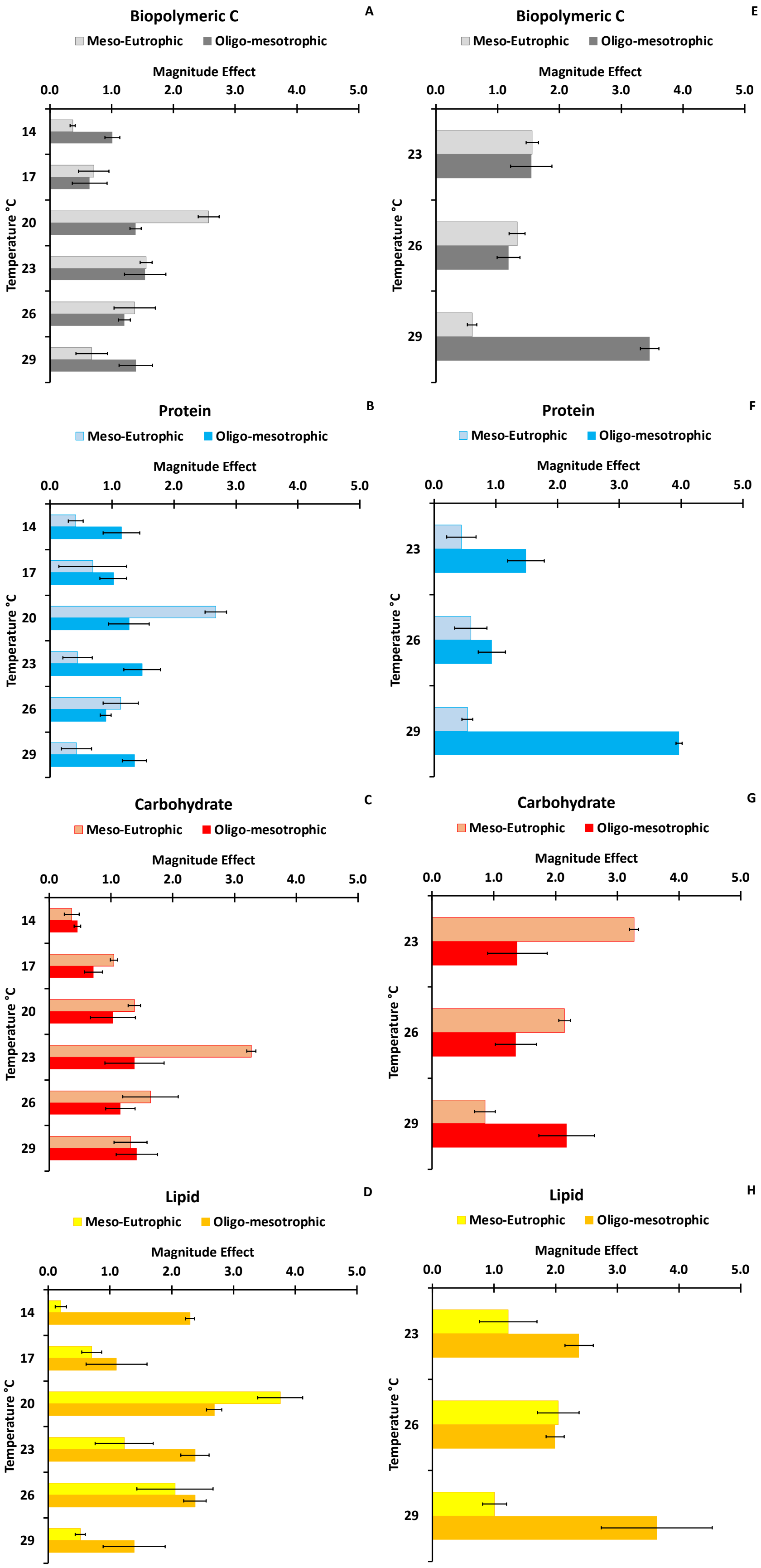

Figure 3. Magnitude of the effects of different temperatures (A-D) and heat snaps (E-H) on the organic enrichment of holothuroid feces in the meso-eutrophic and oligo-mesotrophic conditions. Error bars indicate the standard error (n = 3).

In Exp 2, BPC enrichment of holothuroid feces in meso-eutrophic conditions after the heat snap at 29°C was about half those measured at 23°C and after the heat snap at 26°C, whereas in oligo-mesotrophic conditions the BPC enrichment of feces at 29°C was ca. 2.2-2.9 times those measured at 23°C and after the heat snap at 26°C, respectively (**Figure 3E**). BPC enrichment at 23°C and after the heat snap at 26°C did not vary between trophic conditions, whereas at 29°C BPC enrichment under oligo-mesotrophic conditions was ca. 6 times higher than that measured under meso-eutrophic conditions. In oligo-mesotrophic conditions, protein, carbohydrate, and lipid enrichment of feces after the heat snap at 29°C were higher than (or equal to) those at 23°C and after the heat snap at 26°C (**Figure 3F-H**). In meso-eutrophic conditions, protein enrichment of feces did not vary among treatments, whereas lipid and carbohydrate enrichment decreased after the heat snap at 29°C. In oligo-mesotrophic conditions protein, lipid and carbohydrate enrichment were higher after the heat snap at 29°C. Protein and lipid enrichments of holothuroid feces under oligo-mesotrophic conditions were generally higher than or equal to that under meso-eutrophic conditions. The same applies to carbohydrate enrichment after the heat snap at 29°C, whereas at 23°C and after the heat snap at 26°C values under meso-eutrophic conditions were higher than those under oligo-mesotrophic ones.

4.4 Discussion

Eutrophication is among the most recurrent phenomena of coastal seas degradation worldwide (Cloern, 2001; Pusceddu et al., 2009, Le Moal et al., 2019). Eutrophication, mostly caused by increased inorganic nutrient inputs from inland agriculture and industries, can be also generated by the accumulation, over sustainable thresholds, of OM produced by activities at sea (Pusceddu et al., 2009), like mariculture (Pusceddu et al., 2007, Holmer et al., 2008; Sarà et al., 2011). Integrated Multi-Trophic Aquaculture (IMTA), by potentially transforming mariculture potential wastes (e.g., uneaten food and fish feces) into food sources for other reared species (Slater and Carton, 2007, 2009; Zamora and Jeffs, 2012; Lamprianidou et al., 2015; Shpigel et al., 2018), could thus also help to mitigate the impacts of marine aquaculture on the benthic trophic status. In this regard, holothuroids, being potentially able to modify quantity and composition of sedimentary OM and use fish farm waste in IMTA (Cutajar et al., 2022) could represent a reliable tool to modify benthic trophic status (Pasquini et al., 2023).

Sea water warming due to climate change will likely modify the nutritional physiology of marine organisms (Brierley et al., 2009; Pörtner et al., 2010; Bartolini et al., 2013; Poloczanska et al., 2016; Manríquez et al., 2021; Shahjahan et al., 2022), including *Holothuria tubulosa*, one of the most common sea cucumbers of the shallow Mediterranean Sea (Koukouras et al., 2007; Pasquini et al., 2021). Based on these assumptions, I investigated the capacity of *H. tubulosa* to influence sedimentary OM pools under different temperature regimes and once exposed to sudden heat snaps.

4.4.1 Effects of acclimated sea cucumbers feces on the sediment trophic status

At all temperatures and in both trophic conditions (in terms of BPC loads), the feces produced by the acclimated specimens of *H. tubulosa* were characterized by OM contents significantly higher than those of the corresponding sediments (**Figure 1**). Such enrichment is a well-known feature of this and other holothuroids. For example, Amon et al. (1991) and Mercier et al. (1999) reported that holothuroids, under natural T regimes, produce C- and N-enriched feces. Such organic enrichment of feces is, most likely, the result of OM concentration in the initial digestive tract (i.e., the esophagus) (Pasquini et al., 2023) and of the selection of food particles from the original sediment (e.g., by chemo-selection; Mezali et al., 2013; Schneider et al., 2013; Lee et al., 2018; Pasquini et al., 2021). Organic enrichment of holothuroids feces can also be due to the peculiar process of compression and packing of feces within an organic mucus before excretion (Ward-Rainey et al., 1996; Mercier et al., 1999). Moreover, it must be also considered that, during the feces transit in the intestine, the ingested sediment is mixed with its digestive fluids and bacterial flora, which could further contribute to enrich feces with additional organic C pools (Amon et al., 1991; Roberts et al., 2000; MacTavish et al., 2012; Boncagni et al., 2019; Yamazaki et al., 2019).

I report here also that the magnitude of BPC enrichment of feces varies between trophic conditions and across temperatures. BPC enrichment under meso-eutrophic conditions resulted larger than that under oligo-mesotrophic conditions only at 20°C, whereas at all other temperatures, especially at the highest ones, the enrichment is equal to or lower than that under oligo-mesotrophic conditions (**Figure 3**). This result suggests that, even under the highest temperature regimes and in all trophic conditions, the rate of organic enrichment of sediments due to the release of holothuroid feces could be similar. This, in principle, would let me conclude that the role of holothuroids in influencing the organic loads of marine coastal sediments could not be affected by future sea warming. On the other hand, the overall organic enrichment of feces, irrespectively of trophic conditions, temperature, and degree of the heat snap, could also lead me to conclude, unexpectedly, that this species could act as a sort of flywheel of benthic eutrophication. Nonetheless, the overall trophic status of incoherent sediments is determined not only by organic loads but also by their biochemical composition (nutritional quality) (Pusceddu et al., 2009). In this regard, previous studies reported that deep-sea holothuroids preferably ingest large quantities of labile organic material, thus influencing the overall trophic conditions of deep-sea sediments (Witbaard et al., 2001; Amaro et al., 2010). Based on the richer protein and lipid contents of holothuroids feces when compared to the source sediment (**Figure S2**) and since proteins are more rapidly digested than carbohydrates and lipids are energy-rich compounds (Pusceddu et al., 2003), our results lead me to conclude that *H. tubulosa*, besides increasing the overall quantity of the sedimentary organic loads, can also enhance its nutritional quality. In this sense, holothuroids feces would represent a "fresh" source of OM available for benthic consumers and, as such, could foster and accelerate the energy transfer to the higher trophic levels, partially counteracting the accumulation of organic C due to the release of feces.

However, I also notice that the protein and lipid enrichment of feces (and thus the enhanced nutritional quality of the recipient sediment) differed in magnitude among temperatures and between trophic conditions. More in details, I observed that, overall, protein and lipid enrichment of feces in oligo-mesotrophic conditions is higher than that in meso-eutrophic ones at 14, 23, and 29°C, while the opposite is observed at 20°C. This result indicates that the effect of holothuroids on the most labile class of organic compounds (i.e., lipids and proteins) at the highest temperature (29°C) could be larger in oligo-mesotrophic than meso-eutrophic sediments. On the other hand, the magnitude of carbohydrate enrichment in meso-eutrophic conditions is generally equal or higher than that in oligo-mesotrophic conditions at all temperatures. Overall, these results let me conclude that the eventual use of holothuroids to condition the benthic trophic status, whatever the temperature regime, would be accurately calibrated according to the initial trophic status of the sediments, with particular attention not only to the bulk of organic C but also to the relative importance of the different classes of organic compounds, and, ultimately, to the sediment nutritional quality for deposit-feeders.

The dissimilar effect of *H. tubulosa* on sedimentary OM loads and their biochemical composition at 20°C is, however, difficult to explain. I notice here that the range of temperatures used in Exp 1 with acclimated specimens ideally also includes temperatures that, with exception of 29°C, *H. tubulosa* could normally face in the Mediterranean Sea during seasonal transitions. Indeed, although *H. tubulosa* is a continuous deposit-feeder (Bulteel et al., 1991; Pasquini et al., 2021), an increase in temperatures dictated by seasonality can induce a variation of its feeding activity and of the organic content and biochemical composition of their feces (Amon and Herndl, 1991a, 1991b; Coulon et al., 1993; Kuhnhold et al., 2019; Pasquini et al., 2023). While during the transition from winter to spring temperatures, concurrently to the development of the gonads, holothuroids increase their metabolism, later in summer, when spawning occurs, their metabolism decreases (Coulon et al., 1993; Despalatović et al., 2004; Kazanidis et al., 2014). This pattern well fits the observed largest OM enrichment (whatever the compound) of feces at 20°C (the mean spring SST in the Central-Western Mediterranean Sea; García-Monteiro et al., 2022). This result also corroborates contention by which, whichever is the ultimate effect of this holothuroid species on the benthic trophic status of coastal incoherent sediments, their use in the conditioning of the organic contents of sediments should be accurately calibrated according to temperature regimes (and season) and initial background trophic conditions (in terms of organic loads).

4.4.2 Effects of heat snap-exposed sea cucumbers feces on the benthic trophic status

I report here that, whichever the temperature and the background trophic conditions of the sediments, also the feces produced by *H. tubulosa* subjected to sudden heat snaps were characterized by an enrichment in almost all classes of organic compounds. This result is supported quite well by what observed in Exp 1 at the highest temperature regimes, but with different patterns both under the two different trophic status conditions and the temperature anomaly of the heat snap (+3°C and +6°C).

Under oligo-mesotrophic conditions, the magnitude of protein, carbohydrate, lipid, and BPC enrichment of feces did not vary between 23°C and after the heat snap at 26°C, whereas it burst after the heat snap at 29°C. Under meso-eutrophic conditions, instead, the magnitude of BPC enrichment decreased with the increase of the temperature anomaly, resulting lowest after the heat snap at 29°C. More in details, under meso-eutrophic conditions, holothuroids feces showed higher carbohydrate and lipid contents than those in the corresponding sediment after both heat snaps, but with a decreasing pattern in magnitude with the increasing anomaly of temperature. On the contrary, under oligo-mesotrophic conditions, feces showed higher protein and lipid contents than those in the corresponding sediment after both heat snaps, with magnitude increasing with increasing the temperature anomaly. Altogether, these results indicate that the occurrence of heat snaps, that are increasingly becoming more and more frequent in the Mediterranean Sea because of climate change ultimately fostering marine heat waves (Benedetti Cecchi, 2022), can provoke different consequences on the OM quantity and biochemical composition of holothuroid feces, which can be different as a function of basal trophic conditions. Moreover, my results also highlight that the higher the temperature anomaly of the heat snap, the larger the magnitude of the effects under oligo-mesotrophic conditions and the lower under meso-eutrophic conditions. These results indicate that heat snaps with a temperature anomaly of 6°C (like the one experienced by *H. tubulosa* suddenly passing from 23° to 29°C) can determine a larger organic enrichment of holothuroids feces feeding on organically poorer sediments. On the one hand, heat snaps hitting oligo-mesotrophic sediments could determine an increase in OM quantity mediated by the release of OM-richer feces by holothuroids. On the other hand, the exposure of holothuroids feeding on organically richer sediments to heat snaps with a 6°C temperature anomaly, could have only minor effects on the quantity of food available for benthic deposit-feeders. These results, ultimately, indicate that the rate of organic enrichment of sediments through the release of holothuroid feces could be more pronounced in oligo-mesotrophic conditions under the highest heat snaps. Prospectively, my results suggest that the occurrence of heat snaps with a temperature anomaly as high as 6°C can lead holothuroids to enhance, through the modification of their feces, sediment organic loads under oligo-mesotrophic conditions, but to attenuate the effects on the nutritional quality of the recipient sediments under meso-eutrophic conditions. Reasons for such differences are again difficult to explain but could both be traced by investigating changes in the metabolism of organisms in combination with the different stocks of available resources and temperature anomalies.

4.5 Conclusions

Overall, confirming previous studies (Pasquini et al., 2023), I conclude that *H. tubulosa* can influence the trophic status of marine coastal sediments by increasing OM loads and affecting their nutritional quality. Despite holothuroids remained only 12 hours in contact with the ambient sediment during the feeding phase, feces produced at different temperature regimes exhibited different OM quantity and biochemical composition in both experiments and under both trophic statuses. In my experiments, at least for some days, *H. tubulosa* specimens showed a high adaptability to higher temperatures (26 and 29°C) and a large "bioreactivity", that, however, varied across the two trophic statuses and sea warming scenarios. The general increase in OM contents of feces, however, was not consistent under different benthic trophic conditions and temperatures. Nevertheless, although we did not measure the actual OM removal (and assimilation) by *H. tubulosa*, my results allow me to suggest that this species could be used for conditioning the trophic status of marine coastal sediments and that, based on the increase in the nutritional quality of sediments through protein and lipid-enriched feces, they could have relevant effects on benthic trophic webs, by specifically enhancing the energy transfer towards higher trophic levels, thus accelerating the resilience of eutrophicated sediments. Nevertheless, I pinpoint here that further experimental studies are required to clarify if this species can adapt in the long term to persistent and more frequent marine heatwaves. Also, I acknowledge that their use as a bioremediators of eutrophicated sediments, albeit promising, needs further calibrations in terms of animal density modulated according to the sediment carrying capacity and adaptation capability to different temperature regimes and levels of heat snaps.

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4.7 Supplementary tables

Table S1. Protein, carbohydrate, lipid, and BPC contents (mean ± standard error, n=3) in sediments and feces at the six temperatures (14, 17, 20, 23, 26, 29°C) in meso-eutrophic (M-E) and oligo-mesotrophic (O-M) conditions during Exp 1.

Matrix	Trophic Status	T (°C)	Protein mg g^{-1}	Carbohydrates mg g^{-1}	Lipid mg g^{-1}	Biopolymeric C mgC g^{-1}
Sediment	**M-E**	**14**	2.30±0.49	2.08±0.05	0.27±0.06	2.16±0.28
		17	1.72±0.30	1.04±0.23	0.25±0.01	1.44±0.24
		20	1.69±0.26	0.95±0.06	0.11±0.03	1.29±0.17
		23	1.48±0.16	0.29±0.05	0.13±0.03	0.94±0.10
		26	2.03±0.25	1.06±0.19	0.11±0.03	1.50±0.20
		29	2.39±0.34	1.27±0.12	0.54±0.12	2.08±0.08
	O-M	**14**	0.10±0.01	0.20±0.01	0.01±0.00	0.14±0.01
		17	0.10±0.03	0.11±0.02	0.04±0.03	0.13±0.03
		20	0.10±0.01	0.17±0.02	0.01±0.00	0.13±0.01
		23	0.16±0.01	0.16±0.03	0.01±0.00	0.15±0.01
		26	0.16±0.01	0.13±0.01	0.01±0.00	0.14±0.01
		29	0.15±0.02	0.12±0.01	0.01±0.00	0.13±0.01
Feces	**M-E**	**14**	3.36±0.30	3.04±0.40	0.33±0.06	3.11±0.31
		17	2.82±0.92	2.88±0.45	0.52±0.11	2.93±0.42
		20	25.01±5.05	3.76±0.13	4.65±1.08	17.25±3.26
		23	2.53±0.85	7.45±0.98	0.45±0.11	4.56±0.83
		26	6.44±1.02	5.64±1.53	0.85±0.23	6.05±1.19
		29	3.82±0.96	5.25±1.68	0.61±0.12	4.43±1.23
	O-M	**14**	0.34±0.06	0.31±0.03	0.13±0.02	0.39±0.04
		17	0.15±0.06	0.23±0.00	0.09±0.02	0.23±0.02
		20	0.40±0.11	0.50±0.11	0.14±0.03	0.50±0.01
		23	0.80±0.24	0.68±0.18	0.15±0.03	0.78±0.19
		26	0.39±0.02	0.44±0.11	0.14±0.03	0.47±0.06
		29	0.58±0.06	0.53±0.15	0.06±0.02	0.54±0.10

Table S2. Protein, carbohydrate, lipid, and BPC contents (mean ± standard error, n=3) in sediments and feces at the three temperatures (23, 26, 29°C) in meso-eutrophic (M-E) and oligo-mesotrophic (O-M) conditions during Exp 2.

Matrix	Trophic Status	T (°C)	Protein mg g^{-1}	Carbohydrates mg g^{-1}	Lipid mg g^{-1}	Biopolymeric C mgC g^{-1}
Sediment	**M-E**	**23**	1.48±0.16	0.29±0.05	0.13±0.03	0.94±0.10
		26	2.03±0.23	0.91±0.19	0.06±0.00	1.41±0.18
		29	1.59±0.14	1.48±0.11	0.13±0.01	1.47±0.03
	O-M	**23**	0.16±0.01	0.16±0.03	0.01±0.00	0.15±0.01
		26	0.13±0.02	0.10±0.02	0.01±0.00	0.11±0.01
		29	0.12±0.01	0.11±0.01	0.03±0.02	0.12±0.02
Feces	**M-E**	**23**	2.53±0.85	7.45±0.98	0.45±0.11	4.56±0.83
		26	3.93±1.03	7.53±0.83	0.51±0.13	5.32±0.93
		29	1.99±0.54	3.49±0.33	0.38±0.09	2.65±0.17
	O-M	**23**	0.80±0.24	0.68±0.18	0.15±0.03	0.78±0.19
		26	0.34±0.04	0.40±0.09	0.05±0.01	0.36±0.05
		29	6.17±0.36	1.14±0.37	0.58±0.05	3.91±0.26

4.8 Supplementary figures

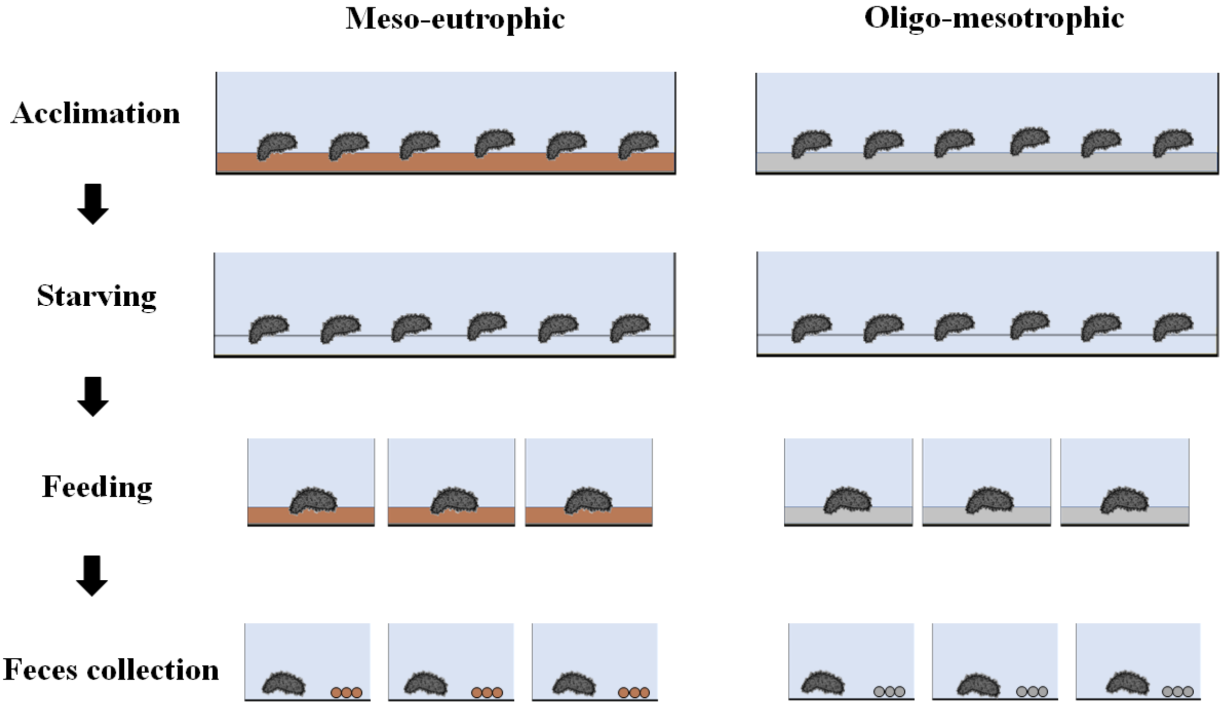

Figure S1. Schematic representation of the experimental set up in both meso-eutrophic and oligo-mesotrophic conditions. This scheme was applied to all the studied temperatures. In Exp 2 the Acclimation phase was carried out only for the 23°C.

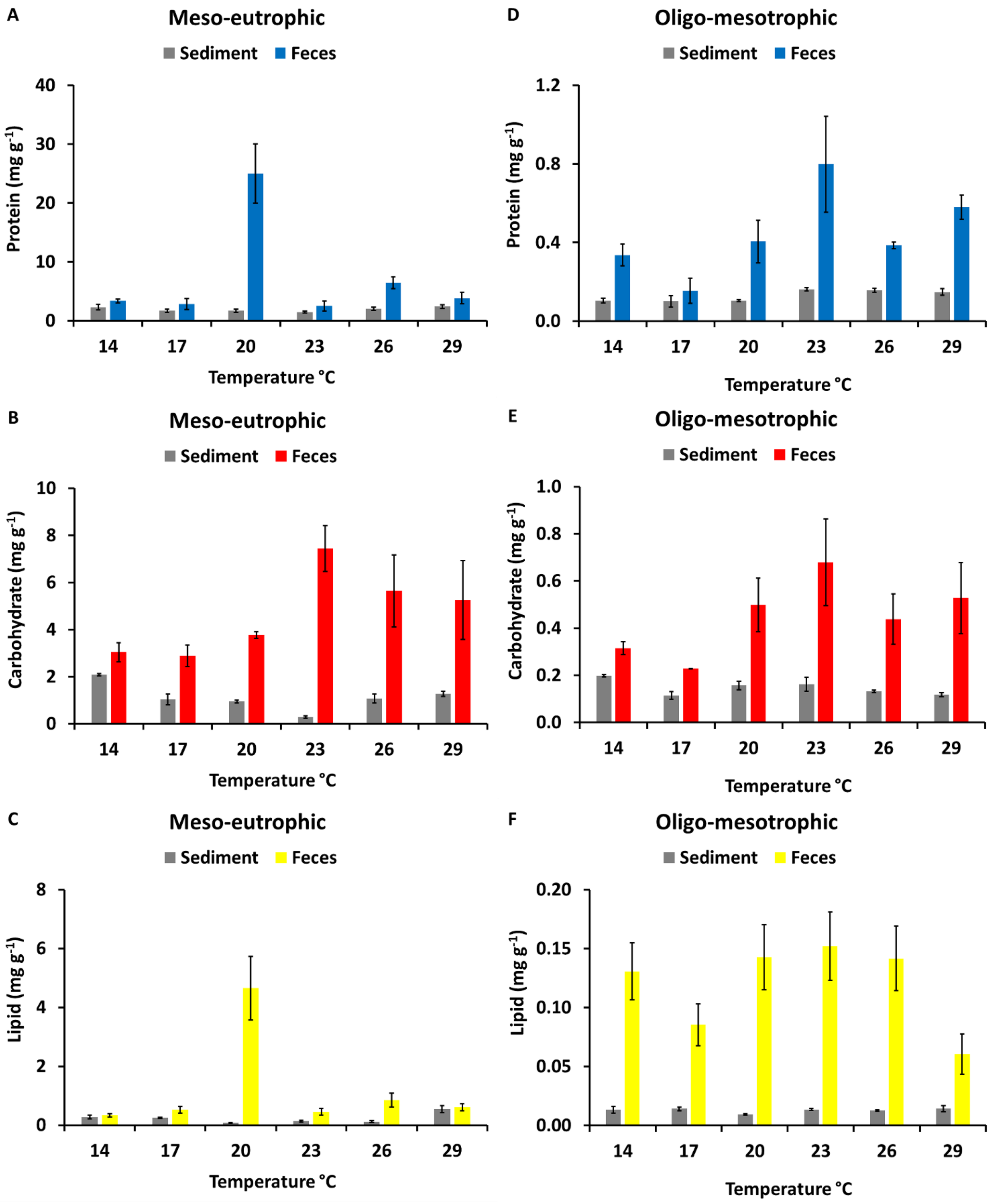

Figure S2. Variations related to Exp 1 in the concentration of proteins (A-D), carbohydrates (B-E), and lipids (C-F) in OM in sediments and feces at the six temperatures (14, 17, 20, 23, 26, 29°C) displayed separately for the two meso-eutrophic and oligo-mesotrophic conditions. Error bars indicate the standard error (n = 3).

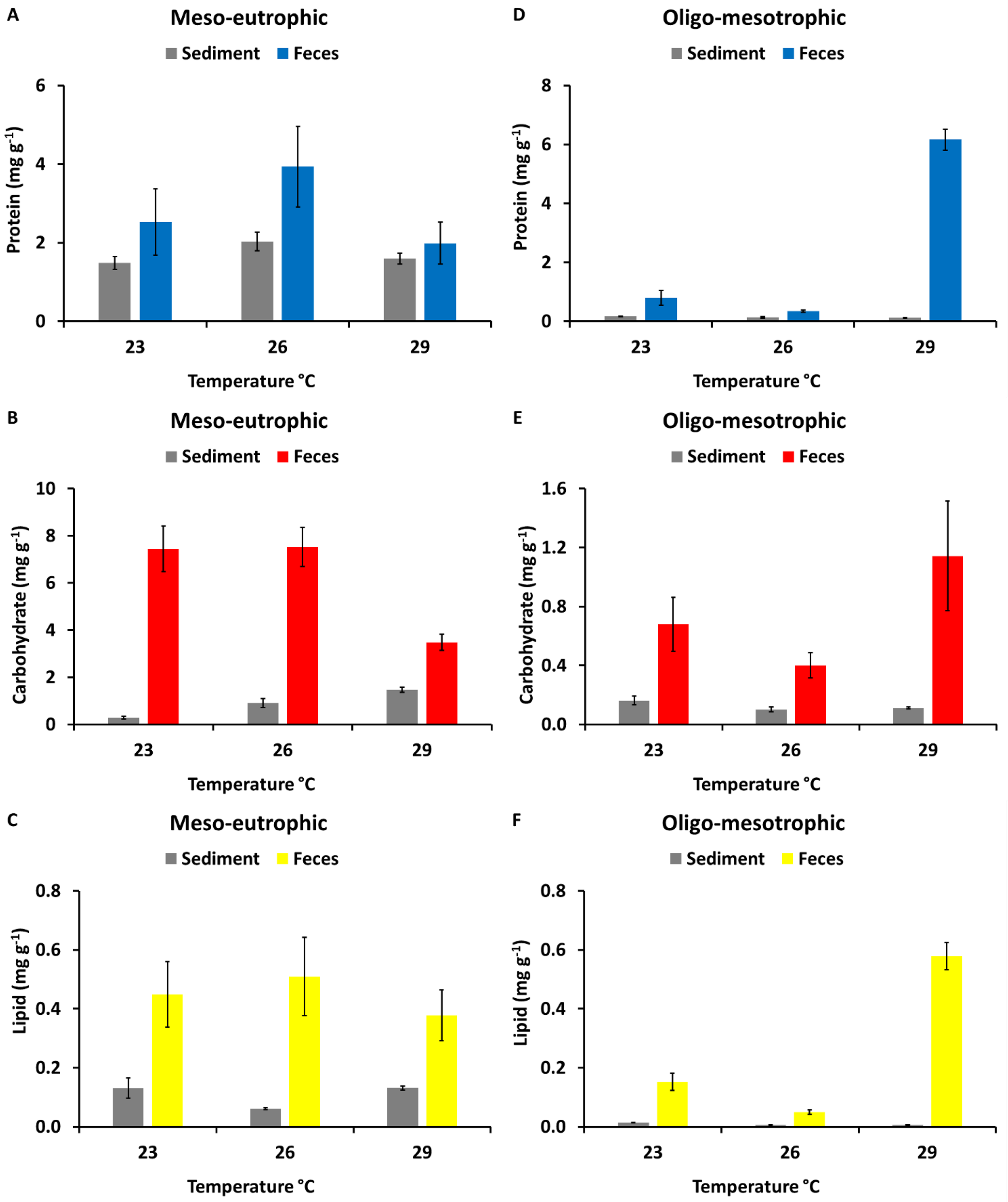

Figure S3. Variations related to Exp 2 in the concentration of proteins (A-D), carbohydrates (B-E), and lipids (C-F) in OM in sediments and feces at the three temperatures (23, 26, 29°C) displayed separately for the two meso-eutrophic and oligo-mesotrophic conditions. Error bars indicate the standard error (n = 3).

5. Effects of freshwater injections on river and lagoon sediments biogeochemistry: field and mesocosm experiments

5.1 Introduction

Extreme and intense rainfall and freshwater flash flood events, caused by episodically anomalous climatic conditions, are among the major threats for coastal ecosystems worldwide (Christensen and Christensen, 2003; Jentsch and Beierkuhnlein, 2008; Ummenhofer and Meehl, 2016). These phenomena are becoming increasingly frequent and their consequences progressively more catastrophic due to current climate change, with large consequences on ecosystem functioning and human lives (Jonkman, 2005; Jentsch and Beierkuhnlein, 2008; Wei et al., 2009; Alderman et al., 2012; Petrucci, 2022). In this regard, river flooding episodes and their impacts on European coastal ecosystems are expected to further increase by the end of the century, especially in the Mediterranean Basin (Kundzewicz et al., 2013; Dottori et al., 2020).

The Mediterranean is a semi-enclosed area and is considered an ideal hotspot to study anomalous climatic events by virtue of its peculiar pluviometric and ecological characteristics (Lutherbacher et al., 2006; Giorgi and Lionello, 2008; Hilmi et al., 2022). In this context, Mediterranean transitional aquatic ecosystems in general and, more specifically, wetlands and coastal lagoons, because of their position located between freshwater and the sea, are affected by several naturally ample hydrological fluctuations in temperature and salinity (Meredith et al., 2022). These ecosystems, hosting communities naturally adapted to large and sudden environmental fluctuations, could play therefore a crucial role in buffering the effects of climate-induced extreme events, such as flash floods on coastal aquatic ecosystems at large (Palumbo et al., 2020). Moreover, transitional aquatic systems, including coastal lagoons, provide important ecosystems services including flood control, shoreline stabilization, sediment and nutrient retention, local mitigation of climate change, water quality, biodiversity and biomass reservoirs, recreation and tourism, cultural values (Levin et al., 2000; Sousa et al., 2013; Newton et al., 2018; El Mahrad et al., 2020). At the same time, coastal lagoons, being characterized by the natural mixing of freshwater and seawater, whose importance depends on the natural fluctuations in freshwater inputs (in turn related to meteorological events) and seawater intrusion (depending on the width of the connection with the sea and its conditions) are hot spots of detrital C accumulation and degradation (Pusceddu e al., 2003, Danovaro and Pusceddu, 2007), being major agents of detrital organic matter (OM) redistribution for benthic consumers (Sarà, 2006).

To provide insights on this mostly under investigated topic, I conducted two experiments, one in the field following natural rainfall events, and one in mesocosm simulating a major flooding on lagoon sediments. More specifically, I tested the null hypothesis by which the quantity (in terms of biopolymeric C contents, total organic C and total N), elemental (C/N ratio), isotopic (i.e., $\delta^{13}C$ and $\delta^{15}N$) and biochemical (in terms of protein, carbohydrate, lipid, and phytopigment contents) composition, nutritional quality (i.e., algal fraction of biopolymeric C and the protein to carbohydrate ratio), potential degradation rates (expressed as rates of extracellular enzymatic degradation of proteins and carbohydrates), and turnover time of sedimentary OM do not vary after climate-induced flash floods, salinity fluctuations, and sediment slippage in a river-lagoon interconnected system. To do this, I investigated variations in OM features:

1) before *vs.* after rainfall events occurred in the sediments of a natural alluvial plain (river and lagoon) located in the South Sardinia
2) before *vs.* after a simulated (in mesocosm) flooding event and the subsequent recovery in lagoon sediments.

5.2 Materials and Methods

5.2.1 Sampling strategy and environmental parameters in the field

This study took place along the Capoterra alluvial plain (Southern Sardinia, Mediterranean Sea; **Figure 1**). In the Monte Arcosu Natural Park, the Gutturu Mannu and Guttureddu streams merge on the Santa Lucia River, which then inflows on the northern shore of the Capoterra lagoon. Sediment sampling was carried out before and after two anomalous rainfall events (defined below) between November 2019 and January 2020 in five stations. Three stations (namely: Fanebas, FAN; Is Pauceris, ISP; Santa Lucia, SLU) were located (downstream) along the Santa Lucia River. Other two stations were located along a putative salinity gradient in the Capoterra lagoon: an internal (INT) station, nearest to the Santa Lucia River mouth, and an external (EXT) station, nearest to the sea water inlet. In each river station three replicated sediment samples were collected by gently scraping the top 1st cm of the sediment, whereas in the lagoon stations three replicates were collected manually using plexiglass cores (4.7 cm internal diameter). The top 1st cm of each lagoon sediment core and the river samples were stored in Petri dishes at -20°C until the analysis. Moreover, in each station and before each sediment sampling, a SmarTROLL™ multiparametric handheld system (In-Situ Inc, USA) was used to measure temperature and salinity of surface and bottom water. Daily rainfall data (mm) were made available by the A.R.P.A.S. Geological Department of the Sardinia Region.

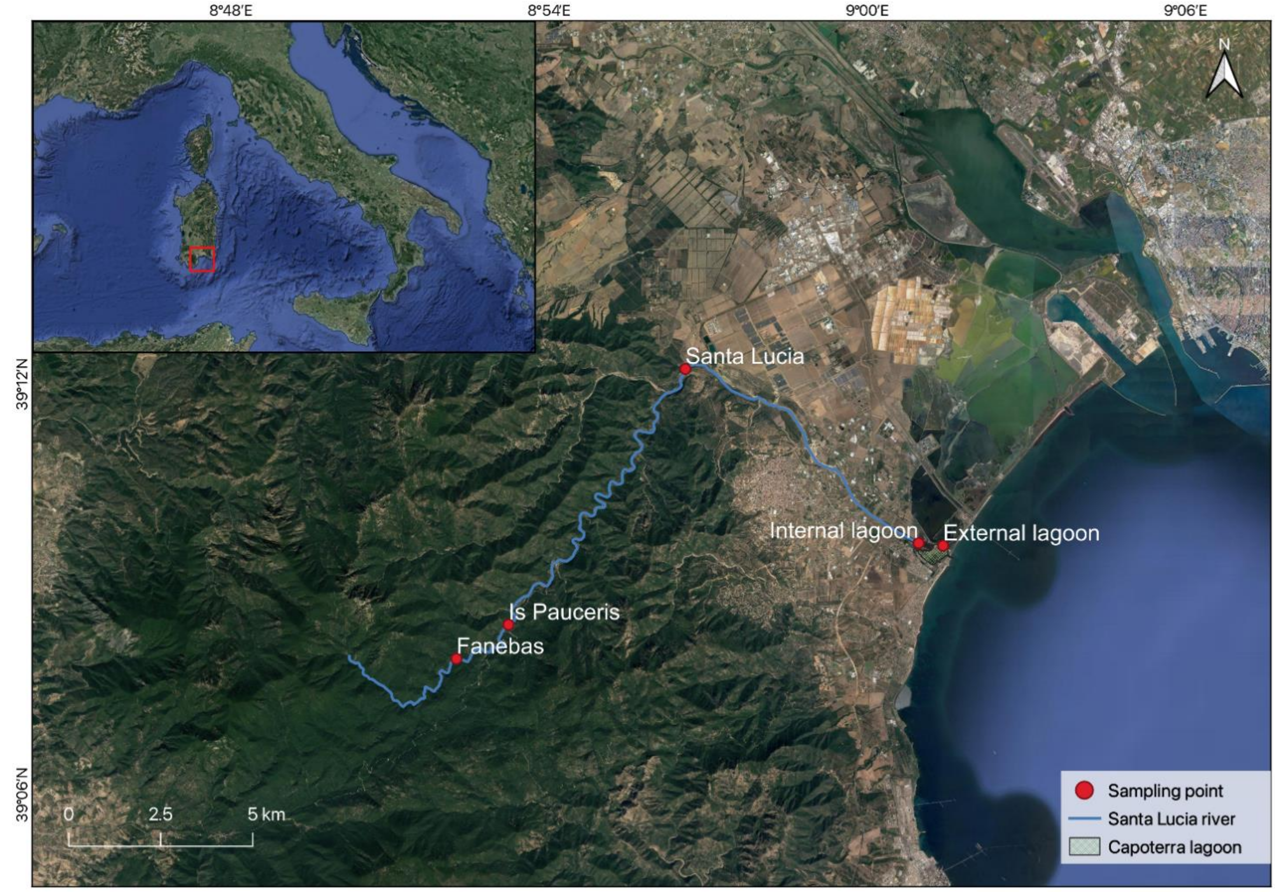

Figure 1. Study area and sampling station locations.

5.2.2 Mesocosms set-up

In July 2021, 150 L of freshwater were collected at the Gutturu Mannu stream along with ca. 20 kg of surface sediments (top 2 cm) from the Santa Gilla lagoon. At the same time, ca. 200 L of lagoon waters, pre-filtered and treated with skimmers and ozone, were taken from the aquaculture facility at the Santa Gilla Consortium. There, eight 6-L plexiglass tanks were filled with 1.5 L of previously sieved (Ø 1 mm) and homogenized sediments and 4.5 L lagoon water (1:3 v/v) (**Figure 2**). Each tank was gently aerated to ensure adequate oxygenation, avoiding any turbulent sediment resuspension. The eight mesocosms were positioned inside a large thermally stable 350-L tank (equipped with adjustable overflow drain) filled with water conditioned at a constant temperature of 24.5 ± 0.5 °C for the entire duration of the experiment. Mesocosms were set up 72 h before the start of the experiment.

As schematized in **Figure S1**, four tanks were subjected to consecutive flooding and recovery treatments (TR), and four untreated tanks served as references (CTRL). I first simulated the flooding (FL) by pouring a plume of freshwater (150 L) in all TR tanks. The freshwater was injected by means of a pump at a constant gentle flow (104 L/h) in all the TR tanks. Water was injected using a plastic tube placed at about half the height of each tank, to ensure mixing of the water, at the same time avoiding any turbulent sediment resuspension. FL took about 50 minutes to lower the salinity from 35 to 0 (OxyWiFi monitoring unit; Tecnos S.A.S.). After 24 hours of exposure to FL, three sediment samples were randomly taken from either two CTRL or two TR mesocosms, by gently scraping the sediment. I then simulated the recovery phase (RE) by replacing the freshwater with lagoon water (150 L) with the same salinity as that at the start of the experiment. Water replacement was carried out again using a pump at constant and gentle flow (104 L/h) ensuring the re-establishment of the desired salinity in ca. 50 minutes. After 24 hours (i.e., 48 hours from the freshwater injection), sediments were collected by scraping the sediment from the remaining CTRL and TR mesocosms, previously not sampled to guarantee any dependency effect of consecutive samplings. All sediment samples were stored in Petri dishes at -20 °C until the analyses in the laboratory.

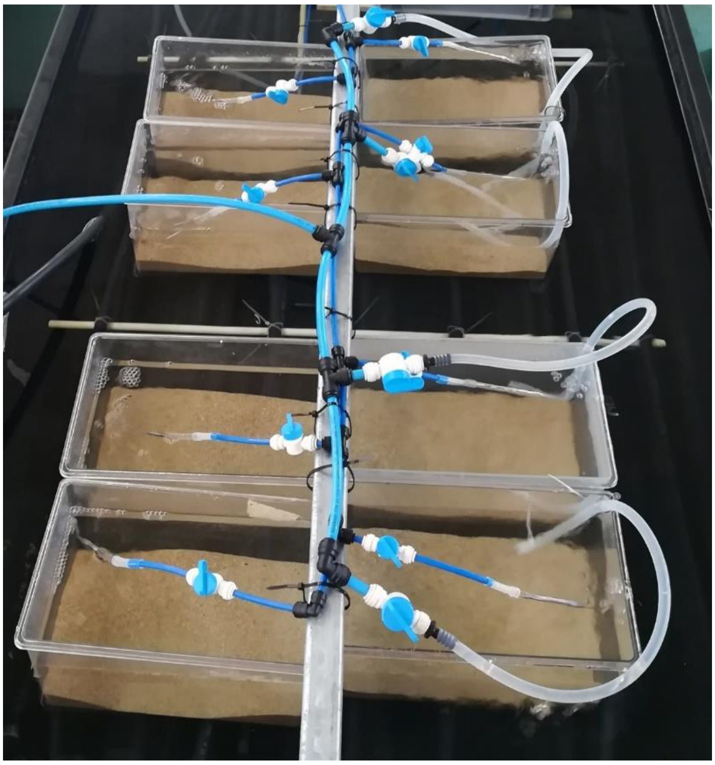

Figure 2. The mesocosms set-up at the aquaculture facility of the Santa Gilla Consortium.

2.2.3 Grain size analysis

Grain size is used to indirectly investigate the dynamic conditions of sediment transport and deposition in different aquatic environments, especially when streams flow into a lagoon and biogeochemical processes are of interest (McLaren and Bowles, 1985; Alves Martins et al., 2015).

Grain size fractions of river and lagoon sediments obtained during the field experiment were determined at the Institute of Marine Sciences (ICM-CSIC) in Barcelona using a Horiba Partica LA950V2 particle size analyzer, with an accuracy of 0.6 % and a precision of 0.1 %. Prior to analysis and after being oven-dried at 60 °C (until reaching a constant weight), 1-5 g of sample were oxidized to remove OM, using 20 % H_2O_2, and sediment particles were disaggregated with 2.5 % $P_2O_7^-$ (Paradis et al., 2019). Grain size fractions were then grouped in the ranges: clay (< 4 µm), silt (4-63 µm), sand (63 µm - 2 mm), and gravel (> 2 mm).

2.2.4 Organic carbon, total nitrogen, and stable C-N isotopes analyses in lagoon sediments

Organic carbon (OC) and total nitrogen (TN) were determined only in lagoon sediments obtained during the field experiment using the procedure described in Nieuwenhuize et al. (1994), coupled with the stable isotopic composition of OC ($\delta^{13}C$) and TN ($\delta^{15}N$). Analyses were carried out with a Thermo NA 2100 elemental analyzer coupled with a continuous-flow isotope-ratio mass spectrometer at the Isotopic Ratio Mass Spectrometry (IRMS - CCiTUB) laboratories of the University of Barcelona.

After being oven-dried at 60 °C (until reaching a constant weight), sediments were grounded to a fine powder and homogenized in an agate mortar mill. An aliquot of 0.5 g was first decarbonated (i.e., to eliminate the inorganic C fraction) by acid-fuming the samples in the presence of 1 M HCl at complete saturation. After 24 h the supernatant was removed, and the samples were placed overnight in a 50 °C pre-heated oven to completely remove HCl residuals. A sub-sample of 25 mg was precisely weighed with an ultra-microbalance inside a tin capsule. Moreover, triplicate samples of IAEA reference standards were prepared. In detail, Acetanilide, Fructose, IAEA 600, IAEA CH_7, IAEA N1, UCGEMA CH, UCGEMA F, UCGEMA K, UCGEMA P, and UCGEMA S were used. Percentage of OC and TN were used to calculate the OC/TN ratio in lagoon sediments. Isotopic data were expressed in parts per thousand (‰) in the conventional $\delta^{13}C$ and $\delta^{15}N$ notations. Their values derived from the formula:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3$$

where R is the ratio of ^{13}C / ^{12}C or ^{15}N / ^{14}N. The $\delta^{13}C$ and $\delta^{15}N$ values are relative to Vienna Pee Dee Belemnite and atmospheric N_2, respectively (Fry, 2006; Cresson et al., 2011; Guerra et al., 2013). The C/N ratio and their stable isotopes were used as proxies of OM origin in different aquatic ecosystems (Sanchez-Vidal et al., 2009; Rumolo et al., 2011 and citations therein; Cazzanelli et al., 2021; Sun et al., 2021).

2.2.5 Biochemical composition and freshness of sedimentary organic matter

Protein, carbohydrate, and lipid contents were determined spectrophotometrically based on the protocols detailed in Danovaro (2010). More specifically, proteins were determined according to Lowry et al. (1951), as modified by Hartree (1972) and Rice (1982), using the Folin-Ciocalteau reagent in a basic environment and expressed as bovine serum albumin equivalents. The procedure proposed by Gerchakov and Hatcher (1972), based on the phenol and concentrated sulfuric acid reaction with saccharides, was used to determine carbohydrates, then expressed as D (+) Glucose equivalents. Lipids, after extraction in chloroform: methanol (1:1, vol:vol) (Bligh and Dyer, 1959), and evaporation in a dry hot bath at 80 to

100°C for 20 min, were determined after the sulfuric acid carbonization procedure (Marsh and Weinstein, 1966) and expressed as tripalmitin equivalents. For each biochemical assay, blanks were obtained using pre-calcinated sediments (450°C for 4 h). Protein, carbohydrate, and lipid concentrations were converted into C equivalents using the conversion factors 0.49, 0.40, and 0.75 mgC mg^{-1}, respectively, obtained from the C contents of the respective standard molecules (albumin, glucose and tripalmitin, respectively), and their sum was reported as the biopolymeric C (BPC) (Fabiano et al., 1995).

Chlorophyll-a (Chl-a) and phaeopigment analyses were carried out according to Danovaro (2010). Pigments were extracted (overnight at 4°C in the dark) from 0.1 g sediment samples using 5 ml of 90% acetone as the extractant. Extracts were analyzed fluorometrically (430 nm excitation and 665 nm emission wavelengths) to estimate Chl-a, and, after acidification with 200 µl 0.1N HCl, phaeopigment concentrations. Total phytopigment was defined as the sum of Chl-a and phaeopigment concentrations and, once converted into C equivalents using 30 µgC µg^{-1} as a conversion factor, I calculate the algal fraction of BPC as the percentage of phytopigment-to-BPC concentrations, used to estimate the fraction of the organic material of autotrophic origin including either the living (Chl-a) and senescent/detrital (phaeopigment) fractions (Danovaro, 2010; Pusceddu et al. 2010, 2014b). I chose the algal fraction of BPC and the protein to carbohydrate ratio as descriptors of the aging, nutritional quality, and enrichment of the sedimentary OM (Dell'Anno et al., 2002; Pusceddu et al. 2009b, 2010).

2.2.6 Extracellular enzymatic activities, C degradation rates and turnover time

OM degradation rates were estimated from aminopeptidase and β-glucosidase activities, determined by the cleavage of artificial fluorogenic substrates (L-leucine-4-methylcoumarinyl-7-amide, for aminopeptidase; 4-methylumbelliferone-D-glucopyranoside, for β-glucosidase) at saturating concentrations (Danovaro, 2010; Bianchelli et al., 2020). Extracellular enzymatic activities were measured after the addition of 100 µl of substrate to 1 ml of a slurry prepared using 1:1 volume of filtered (0.2 µm) and sterile seawater and sediment (substrate final concentration 200 µM) (Danovaro, 2010; Bianchelli et al., 2020). Substrate incubations were performed in the dark at in situ temperature for 1 h. After incubation, the slurries were centrifuged (3000 rpm, 5 min) and supernatants were analyzed fluorometrically (at 380 nm excitation and 440 nm emission for aminopeptidase; at 365 nm excitation and 455 nm emission for β-glucosidase) (Danovaro, 2010; Bianchelli et al., 2020). Data were normalized to sediment dry weight (60 °C, 24 h) and reported as nanomole of hydrolyzed substrate released per g of sediment dry weight h^{-1}. Protease and glucosidase activities were converted into C equivalents using 72 as a conversion factor (estimated from the C content of the fluorescent component released after reaction with the enzymes) and their sum, reported as the potential C degradation rate (µgC g^{-1} h^{-1}). The turnover times (per day) of the whole protein and carbohydrate pools (hereafter C turnover time) were calculated as the inverse number of the ratios of the hourly C degradation rates (once multiplied by 24) and the whole protein and carbohydrate C contents in the sediment. Although these estimates are only potential (maximum) rates of C turnover, they are considered good proxies of ecosystem functioning (Pusceddu et al., 2014b).

2.2.7 Statistical analyses

The experimental design for the field experiment, applied separately for the two habitats (river and lagoon), consisted of two orthogonal factors: 1) Impact (fixed, with 2 levels: Before and After); 2) Station (fixed, with 3 levels for the river habitat: FAN, ISP, SLU, and with 2 levels for the lagoon habitat: INT and EXT). Since the two events differed in terms of intensity and duration, the analyses were conducted separately for the two events. The experimental design for the mesocosm experiment included two orthogonal factors: Phase [2 fixed levels: Flooding (FL) and Recovery (RE)] and Treatment [2 fixed levels: Control (CTRL) and Treatment (TR)]. Permutational analyses of variance (PERMANOVA) (Anderson, 2001) were carried out in either the univariate (i.e., each variable separately) and multi-variate context to test the following null hypotheses:

1) OM quantity, biochemical composition (in terms of protein, carbohydrate, lipid, and phytopigment contents), nutritional quality, C degradation rates, C turnover time (both in river and lagoon habitat), OC and TN percentage, C/N ratio, and $\delta^{13}C$ and $\delta^{15}N$ stable isotopes (only in the lagoon habitat) do not vary after rainfall events.
2) OM quantity, biochemical composition, nutritional quality, extracellular enzymatic activities, C degradation rates and turnover time do not vary after a flooding event nor once the event is ceased.

The analyses were carried out on Euclidean distance-based resemblance matrixes (with previously normalized data only for sedimentary OM), using 999 random permutations of the appropriate units. PERMANOVA tests were followed by a post-hoc tests in case of significant effects of the putative causative factors (rainfall, flooding, or recovery). Canonical analysis of principal coordinates (CAP) was used in the multivariate context to ascertain the allocation of experimental groups to those established a priori. Results from the CAP were then used to visualize, using biplots, differences among experimental groups. PERMANOVA and CAP tests were carried out through the software PRIMER 6+, using the included routine package PERMANOVA (Anderson et al., 2008).

5.3 Results

5.3.1 Magnitude of rainfall events and consequences on salinity

Data on total daily rainfall (**Figure 3**) show that the first rainfall period (November 7-27) was characterized by four rain peaks (max 29.8 mm), whilst the second one (January 10-22) was characterized by a single peak of minor intensity (max 16.4 mm). The cumulative rain input were 124.40 mm and 22.60

mm, in the first and second rainfall periods, respectively. In the river stations salinity remained constant during all samplings. Before-after changes in water salinity were instead observed in both lagoon stations (**Table 1**). In both periods and in both lagoon stations a drop in salinity occurred after the rainfall along with the formation of a salinity vertical gradient. More in details, in November in the internal station salinity dropped from 33.7 to 2.2 and from 33.9 to 20.0 in the surface and bottom waters, respectively, whereas in the external station it dropped from 37.8 to 4.0 and from 37.5 to 25.0 in the surface and bottom waters, respectively. In January in the internal station the salinity dropped from 12.0 to 4.3 and from 18.0 to 4.3 in the surface and bottom waters, respectively, whereas in the external station it dropped from 13.0 to 8.0 and from 35.8 to 18.5.

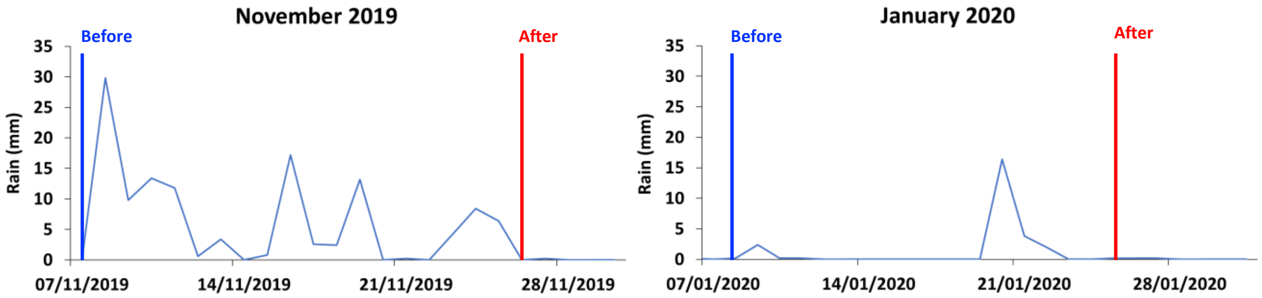

Figure 3. Total daily rainfall in November 2019 and January 2020. Data from Sardinian Regional Agency for the Protection of the Environment (A.R.P.A.S.). The weather station is located at 62 m (m.s.l.), N 4339016.161. E 1494637.434; 39° 12' 01.00" N. lon 8° 56' 15.30" E.

Table 1. Surface and bottom water salinity in the Capoterra lagoon stations before and after the rainfall.

Station	Rainfall	Date	Surface	Bottom
Internal	Before	07/11/2019	33.7	33.9
	After	27/11/2019	2.2	20.0
	Before	10/01/2020	12.0	18.0
	After	22/01/2020	4.3	4.3
External	Before	07/11/2019	37.8	37.5
	After	27/11/2019	4.0	25.0
	Before	10/01/2020	13.0	35.8
	After	22/01/2020	8.0	18.5

5.3.2 Sediment grain size variations after rainfalls

Despite the different magnitude of the rainfall events, the sediment grain size composition varied after both rainfall events in all investigated stations, either in the Santa Lucia River and in the Capoterra lagoon. Variations in grain size composition in river stations after both rainfall events are illustrated in **Figure 4A**. After the rainfall in November 2019, the gravel fraction increased in the two upmost river stations (from 0.5 to 17.8 % and from 9.2 to 30.0 % in Fanebas and Is Pauceris, respectively), whereas in the downstream one (S. Lucia) the gravel fraction decreased (from 10.9 to 7.6 %) and the silt one increased (from 3.1 to 5.5 %). After the minor rainfall in January 2020 the grain size composition varied differently in the three stations. More specifically: the sediments of the top stream station (Fanebas) were characterized by the decrease of the gravel fraction (from 61.3 to 35.0 %) and the increase of the sand and silt ones (from 37.5 to 62.6 % and from 1.3 to 2.4 %, respectively); the sediments of the intermediate station (Is Pauceris) were characterized by the increase of the gravel fraction (from 21.2 to 31.2 %) and the decrease of the sand one (from 76.6 to 66.2 %); the sediments of the downstream station (S. Lucia) were characterized by the increase of the sand fraction (from 21.9 to 49.7 %) and the decrease of the silt and clay fractions (from 28.4 to 3.6 % and from 6.0 to 0.2 %, respectively).

Variations in grain size composition in the two lagoon stations after both rainfall events are illustrated in **Figure 4B**. After the rainfall in November 2019, the sediments of the internal lagoon station were characterized by the decrease of the gravel (from 3.6 to 0.1 %), silt (from 36.0 to 16.3 %) and clay (from 10.9 to 2.9 %) fractions, and the increase of the sand one (from 49.4 to 80.7 %). In the external lagoon sediments were characterized by the increase of the gravel (from 0.6 to 10.1 %), silt (from 10.0 to 19.3 %), and clay (from 2.8 to 3.2%) fractions, and the decrease of the sand one (from 86.6 to 67.5 %). After the January 2020 rainfall, the sediments of the internal lagoon were characterized by the doubling of the sand fraction (from 48.1 to 96.1 %) and the decrease of the silt and clay fractions (from 41.0 to 3.2 % and from 10.9 to 0.5 %, respectively). In the external lagoon sediments were characterized by the increase of the gravel fraction (from 5.6 to 24.2 %) and the decrease of the sand (from 35.9 to 29.5 %), silt (from 46.3 to 36.8 %), and clay ones (from 12.3 to 9.5 %).

Overall, the coarser gravel fraction slipped from the upstream river station to the intermediate one and from the internal to the external lagoon station. Contemporary, the sand fraction, slipped and accumulated downstream in the river and in the internal lagoon station; the finer silt and clay fractions slipped downstream in the river and moved towards the external station of the lagoon in November 2019 and, possibly, left the lagoon in January 2020.

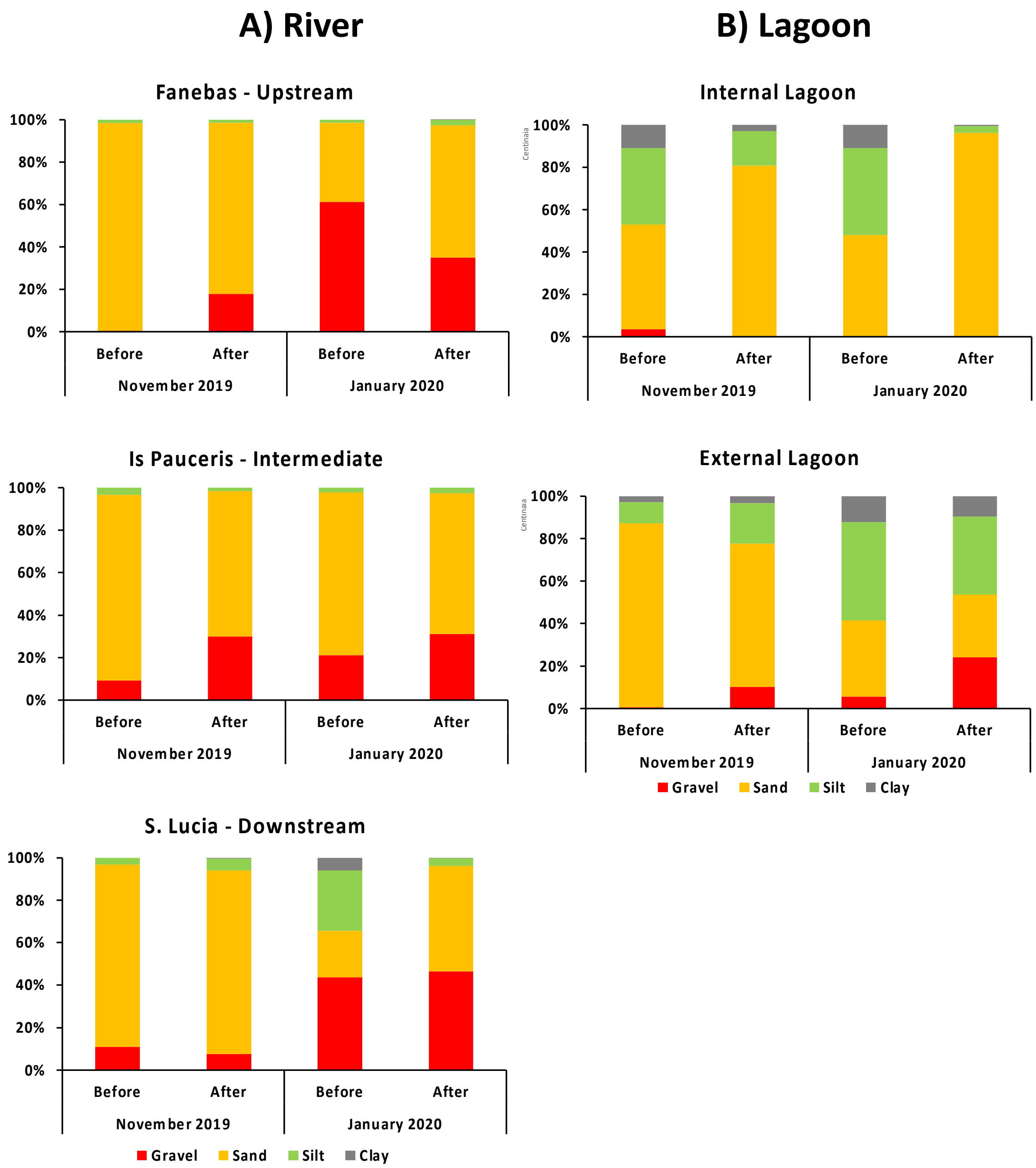

Figure 4. Grain size composition of Santa Lucia River (A) and Capoterra lagoon (B) sediments before and after the rainfall events in November 2019 and January 2020.

5.3.3 Total OC, N, C/N ratio and stable C and N isotope variations in lagoon sediments after rainfalls

Total OC, N, C/N ratio and stable C and N isotope variations in lagoon sediments after rainfalls are illustrated in **Figure 5**. In the Capoterra lagoon sediments both total C and N along with $\delta^{13}C$ were significantly affected by the interaction among the tested factors (Impact × Station), but $\delta^{15}N$ (**Table 2**). The results of post-hoc tests are reported in **Table S1**).

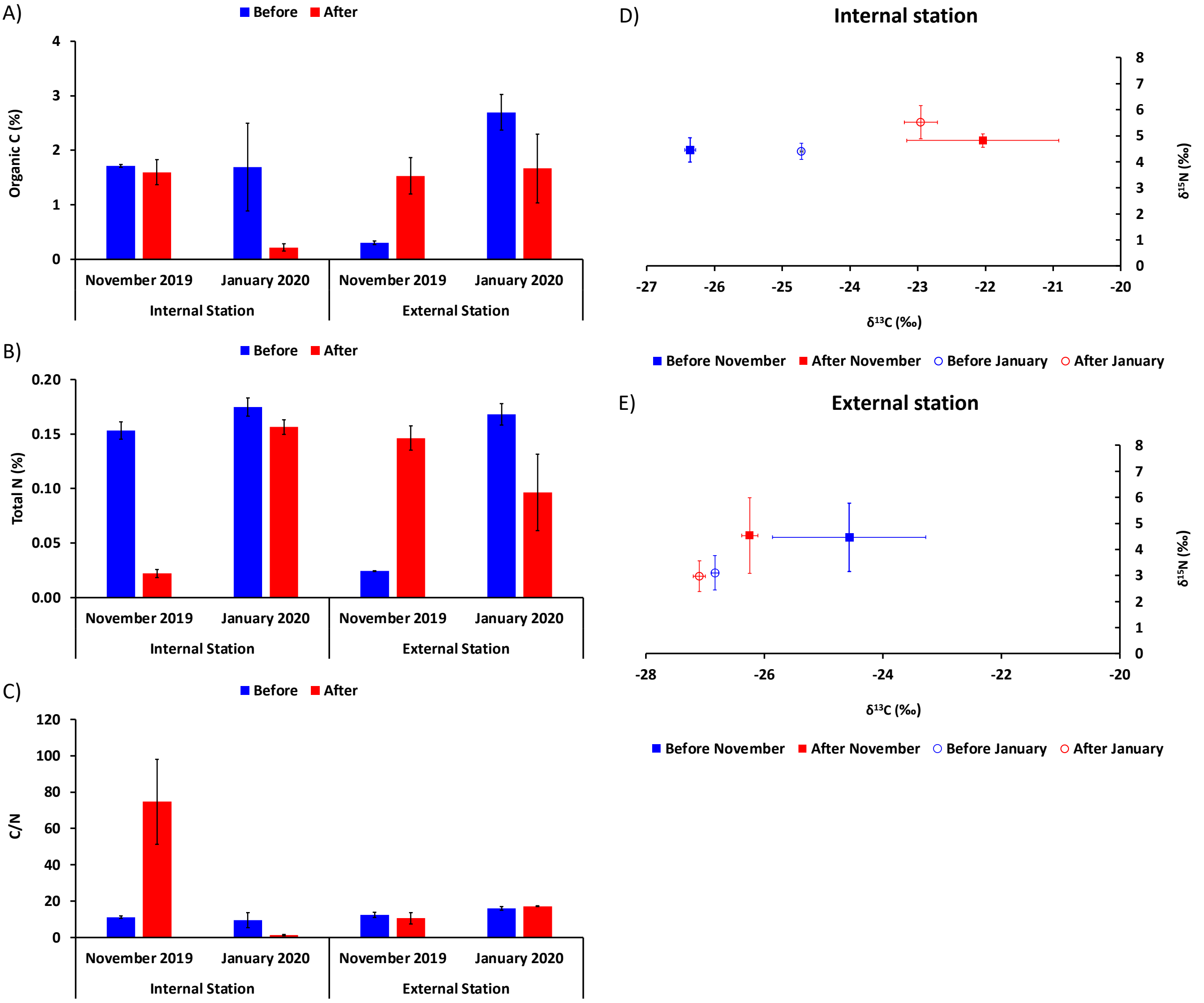

Figure 5. Changes in organic carbon (A) and nitrogen (B) contents, C/N ratio (C), isotopic composition of the internal and external stations in the Capoterra lagoon before and after rainfall in November 2019 (NOV) and January 2020 (JAN). Error bars indicate standard deviation (n = 3).

Table 2. Results of PERMANOVA testing for differences in total C and N contents, C/N ratio, and stable isotopes in lagoon sediments in November 2019 and January 2020 between impact (Im; before and after) and station (St; internal and external). df = degrees of freedom; MS = mean square; Pseudo-F = F statistic; P(MC) = probability after Monte Carlo simulations. * = $p < 0.05$; ** = $p < 0.01$; ns = not significant. Reported is also the percentage of variation explained by each factor, their interaction and residual (i.e., unexplained) variance.

Event	Variable	Source	df	MS	Pseudo-F	P(MC)	% EV
November 2019	**OC**	Impact	1	0.176	22.162	**	17
		Station	1	0.310	39.096	**	30
		Im × St	1	0.257	32.464	**	49
		Residual	8	0.008			5
	TN	Impact	1	0.009	1.213	ns	0
		Station	1	0.002	0.313	ns	0
		Im × St	1	6.780	95.359	**	100
		Residual	8	0.007			0
	C/N	Impact	1	1.763	20.372	**	22
		Station	1	1.832	21.165	**	23
		Im × St	1	1.981	22.887	**	49
		Residual	8	0.087			7
	$\delta^{13}C$	Impact	1	0.481	7.097	*	7
		Station	1	0.402	5.936	*	6
		Im × St	1	2.470	36.464	**	81
		Residual	8	0.068			7
	$\delta^{15}N$	Impact	1	0.098	0.132	ns	0
		Station	1	0.043	0.057	ns	0
		Im × St	1	0.048	0.064	ns	0
		Residual	8	0.746			100
January 2020	**OC**	Impact	1	0.893	16.305	**	32
		Station	1	0.856	15.629	**	31
		Im × St	1	0.028	0.509	*	25
		Residual	8	0.055			13
	TN	Impact	1	0.853	16.686	**	40
		Station	1	0.469	9.170	**	21
		Im × St	1	0.301	5.897	*	25
		Residual	8	0.051			15
	C/N	Impact	1	0.023	7.930	*	6
		Station	1	0.230	80.465	**	67
		Im × St	1	0.041	14.212	**	22
		Residual	8	0.003			5
	$\delta^{13}C$	Impact	1	0.154	90.946	**	4
		Station	1	2.692	15.878	**	79
		Im × St	1	0.281	16.592	**	16
		Residual	8	0.002			0
	$\delta^{15}N$	Impact	1	0.532	2.282	ns	3
		Station	1	8.016	34.392	**	73
		Im × St	1	0.850	3.649	ns	12
		Residual	8	0.233			13

OC sedimentary contents in the internal station did not vary after the November 2019 rainfall and dropped from 1.69 to 0.22 % after the January 2020 one, whereas in the sediments of the external station OC contents raised from 0.30 to 1.53 % after the November 2019 rainfall and did not vary significantly after the January 2020 one (**Figure 5A**).

N sedimentary contents in the internal station decreased significantly both after the November 2019 and January 2020 rainfall (from 0.15 to 0.02 % and from 0.17 to 0.15 %, respectively), whereas in the external station sediments N contents increased from 0.02 to 0.15 % after the November 2019 rainfall and decreased from 0.17 to 0.10 % after the January 2020 one (**Figure 5B**).

The C/N ratio in the sediments of the internal station increased significantly (by ca. 7 times) after the rainfall in November 2019 and decreased (by ca. 7 times) after the January 2020 one, whereas in the sediment of the external station the C/N ratio did not vary after both rainfalls (**Figure 5C**).

Sediments of the internal station after both rainfall events were characterized by a $\delta^{13}C$ enrichment from -26.4 to -22.0 ‰ and from -24.7 to -23.0 ‰, respectively, accompanied by no significant changes in the $\delta^{15}N$ values (**Figure 5D**). Sediments of the external station were characterized by a $\delta^{13}C$ impoverishment from -24.6 to 26.2 ‰ and from -26.8 to -27.1 ‰ in November 2019 and January 2020, respectively, and invariant $\delta^{15}N$ values after both events (**Figure 5E**).

5.3.4 Effects of rainfalls on the biogeochemistry of the Santa Lucia River sediments

Data on the sedimentary OM quantity, nutritional quality, extracellular enzymatic activities, and degradation rates in the Santa Lucia River are reported in **Table S2**. In the Santa Lucia River, all investigated variables and the OM biochemical composition were significantly affected by the interaction between the tested factors (Impact × Station), except for carbohydrate contents, protein to carbohydrate ratio and algal fraction to BPC in November 2019 and except aminopeptidase activity in January 2020 (**Tables 3 and 4**). The results of the pairwise tests are illustrated in **Figure S2**. Variations in OM contents and nutritional quality in the sediments of the Santa Lucia River after the November 2019 and January 2020 rainfalls are illustrated in **Figure 6**, whereas extracellular enzymatic activities, C degradation rates and turnover time are illustrated in **Figure 7**.

After the November 2019 rainfall, protein content significantly increased either upstream or downstream (by 23 and 89%, respectively), whereas it did not vary in the intermediate station. Carbohydrate contents did not vary at all, whereas lipid contents significantly decreased only in the intermediate station (by 64%). BPC contents significantly increased only downstream (by 68%). Total phytopigment content and the algal fraction of BPC significantly decreased (by 55-85%) in all stations. Protein to carbohydrate ratio significantly increased only upstream (by 109%).

Table 3. Results of PERMANOVA testing for differences in the quantity, biochemical composition, nutritional quality, C degradations rates and turnover time in river sediments in November 2019 rainfall event between impacts (Im; before and after), and among stations (St; Fanebas, Is Pauceris, Santa Lucia). df = degrees of freedom; MS = mean square; Pseudo-F = F statistic; P(MC) = probability after Monte Carlo simulations. * = p <0.05; ** = p <0.01; ns = not significant. Reported are also the percentage of variation explained by each factor, their interaction and residual (i.e., unexplained) variance.

Variable	Source	df	MS	Pseudo-F	P(MC)	% EV
Protein	Impact	1	4.106	21.473	**	17
	Station	2	9.479	49.576	**	59
	Im × St	2	1.534	8.021	**	17
	Residuals	12	0.191			7
Carbohydrate	Impact	1	0.001	0.320	ns	0
	Station	2	0.118	38.380	**	78
	Im × St	2	0.010	3.265	ns	9
	Residuals	12	0.003			13
Lipid	Impact	1	0.018	0.837	ns	0
	Station	2	1.288	58.597	**	64
	Im × St	2	0.311	14.155	**	29
	Residuals	12	0.022			7
Biopolymeric C	Impact	1	0.142	11.151	**	8
	Station	2	0.803	63.060	**	70
	Im × St	2	0.100	7.889	**	16
	Residuals	12	0.013			7
Protein to Carbohydrate ratio	Impact	1	3.140	23.362	**	50
	Station	2	0.864	6.432	*	18
	Im × St	2	0.381	2.837	ns	12
	Residuals	12	0.134			20
Chlorophyll-a	Impact	1	18.179	106.870	**	74
	Station	2	1.534	9.019	**	8
	Im × St	2	1.126	6.617	*	12
	Residuals	12	0.170			6
Phytopigments	Impact	1	4.275	133.260	**	74
	Station	2	0.383	11.930	**	9
	Im × St	2	0.259	8.058	**	12
	Residuals	12	0.032			5
Biochemical composition	Impact	1	22.819	58.022	**	43
	Station	2	12.631	32.117	**	35
	Im × St	2	3.019	7.677	**	15
	Residuals	12	0.393			7
Algal fraction of BPC	Impact	1	6.594	166.070	**	85
	Station	2	0.479	12.058	**	9
	IM × ST	2	0.099	2.482	ns	2
	Residuals	12	0.040			5

Aminopeptidase	Impact	1	2.221	14.285	**	8
	Station	2	8.645	55.610	**	48
	IM × ST	2	3.535	22.735	**	38
	Residuals	12	0.155			5
β-glucosidase	Impact	1	1.905	26.841	**	5
	Station	2	15.189	214.010	**	63
	IM × ST	2	3.661	51.577	**	30
	Residuals	12	0.071			2
C degradation rates	Impact	1	2.367	20.695	**	7
	Station	2	10.705	93.606	**	52
	IM × ST	2	3.907	34.162	**	37
	Residuals	12	0.114			3
C turnover time	Impact	1	0.058	20.019	**	6
	Station	2	0.179	62.066	**	28
	IM × ST	2	0.200	69.334	**	63
	Residuals	12	0.003			3

Table 4. Results of PERMANOVA testing for differences in the quantity, biochemical composition, nutritional quality, C degradations rates and turnover time in river sediments in January 2020 rainfall event between impacts (Im; before and after), and among stations (St; Fanebas, Is Pauceris, Santa Lucia). df = degrees of freedom; MS = mean square; Pseudo-F = F statistic; P(MC) = probability after Monte Carlo simulations. * = p <0.05; ** = p <0.01; ns = not significant. Reported are also the percentage of variation explained by each factor, their interaction and residual (i.e., unexplained) variance.

Variable	Source	df	MS	Pseudo-F	P(MC)	% EV
Protein	Impact	1	0.380	26.728	**	25
	Station	2	0.396	27.827	**	40
	Im × St	2	0.140	9.834	**	26
	Residuals	12	0.014			9
Carbohydrate	Impact	1	9.388	54.778	**	18
	Station	2	9.392	54.801	**	27
	Im × St	2	8.926	52.081	**	52
	Residuals	12	0.171			3
Lipid	Impact	1	0.071	2.534	ns	2
	Station	2	0.228	8.146	**	16
	Im × St	2	0.436	15.576	**	67
	Residuals	12	0.028			14
Biopolymeric C	Impact	1	8.316	53.594	**	16
	Station	2	9.656	62.236	**	29
	Im × St	2	8.733	56.284	**	52
	Residuals	12	0.155			3
Protein to Carbohydrate ratio	Impact	1	11.685	39.523	**	34
	Station	2	3.907	13.216	**	16
	Im × St	2	4.914	16.622	**	42
	Residuals	12	0.296			8

Chlorophyll-a	Impact	1	0.006	0.559	ns	0
	Station	2	0.336	29.231	**	34
	Im × St	2	0.289	25.093	**	58
	Residuals	12	0.011			7
Phytopigments	Impact	1	0.010	3.548	ns	1
	Station	2	0.060	20.962	**	16
	Im × St	2	0.140	48.801	**	78
	Residuals	12	0.003			5
Biochemical composition	Impact	1	9.854	43.555	**	17
	Station	2	10.412	46.023	**	27
	Im × St	2	9.855	43.557	**	52
	Residuals	12	0.226			4
Algal fraction of BPC	Impact	1	0.004	0.170	ns	0
	Station	2	0.033	1.452	ns	1
	Im × St	2	0.861	37.673	**	92
	Residuals	12	0.023			8
Aminopeptidase	Impact	1	0.038	12.026	**	14
	Station	2	0.119	37.705	**	69
	Im × St	2	0.008	2.627	ns	6
	Residuals	12	0.003			11
β-glucosidase	Impact	1	0.003	0.560	ns	0
	Station	2	0.097	18.714	**	35
	Im × St	2	0.074	14.251	**	53
	Residuals	12	0.005			12
C degradation rates	Impact	1	0.023	9.719	*	13
	Station	2	0.066	27.849	**	61
	Im × St	2	0.009	3.660	*	12
	Residuals	12	0.002			14
C turnover time	Impact	1	7.374	25.279	**	15
	Station	2	9.736	33.379	**	31
	Im × St	2	7.731	26.503	**	48
	Residuals	12	0.292			6

Aminopeptidase and β-glucosidase activity along with C degradation rates significantly increased upstream (by 282-1688%) and decreased downstream (by 56-64%), whereas C turnover time significantly decreased upstream (by 78%) and increased downstream (by 357%). The biochemical composition of sedimentary OM varied significantly in all stations (**Figure S2**). Most variations after the November 2019 rainfall depended by the decreasing patterns in chlorophyll-a and phaeopigment contents, with before-after changes more pronounced in the downstream station (**Figure 8A**).

After the January 2020 event, protein content significantly decreased (by 60%) only in the intermediate station. Carbohydrate content did not vary upstream, whereas it significantly increased in the intermediate station and downstream (by 312 and 5149%, respectively). Lipid contents significantly decreased upstream (by 62%) and decreased downstream (by 70%).

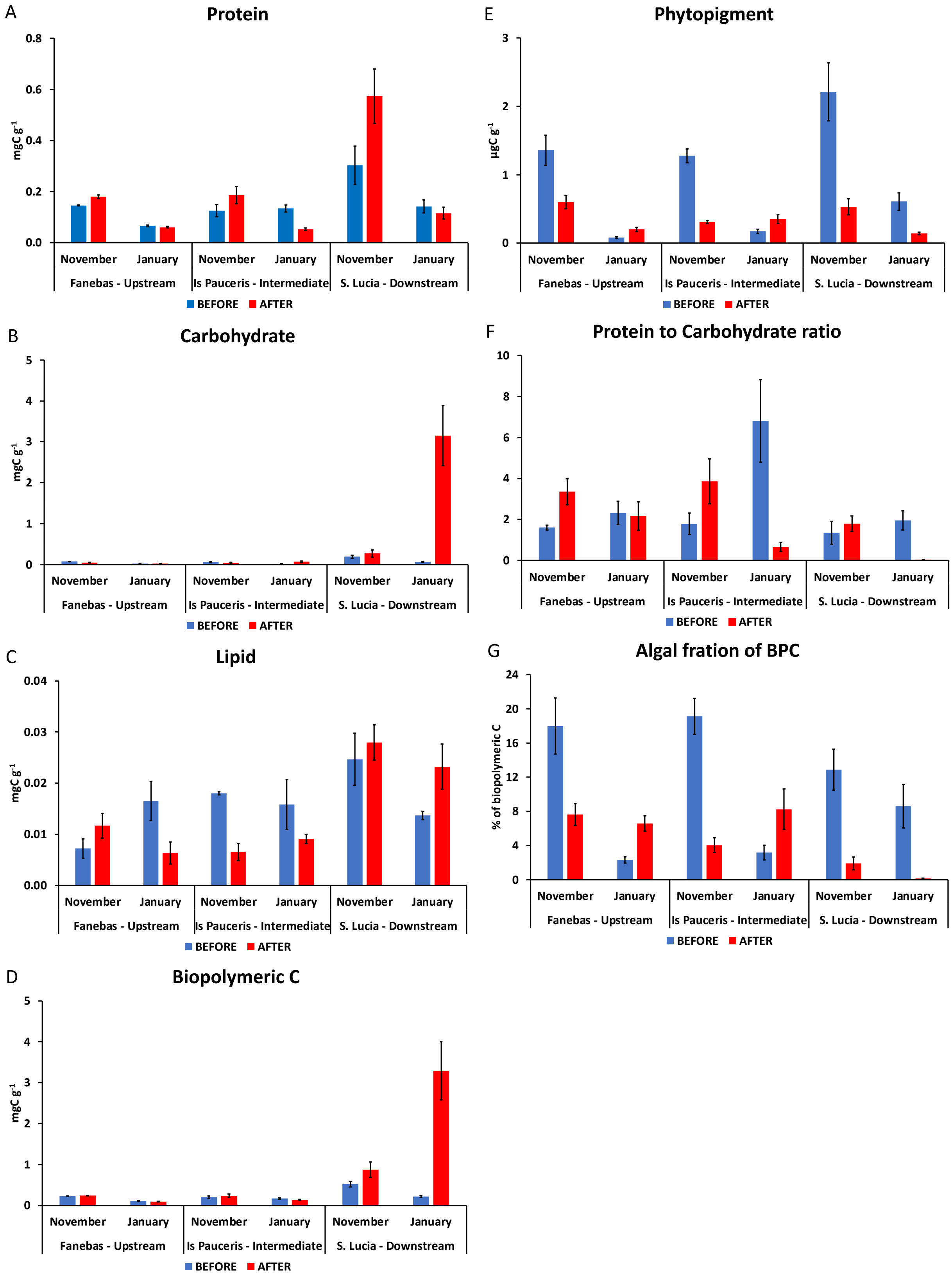

Figure 6. Protein (A), carbohydrate (B), lipid (C), biopolymeric C (D) and phytopigment contents (E), protein to carbohydrate ratio (F) and algal fraction of biopolymeric C (G) in the sediments of the upstream, intermediate, and downstream stations in the Santa Lucia River before and after the rainfall in November 2019 and January 2020. Error bars indicate standard deviation (n = 3).

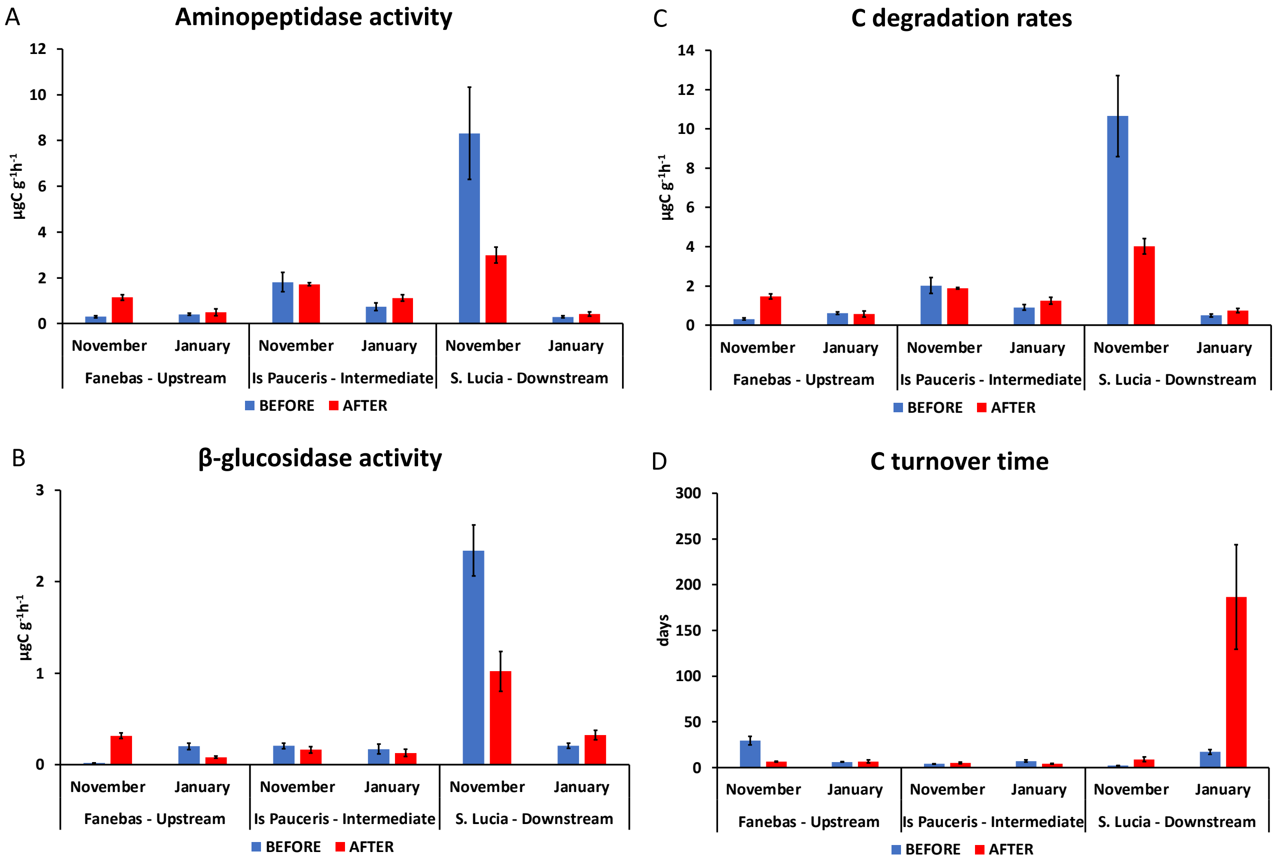

Figure 7. Aminopeptidase (A), β-glucosidase (B), C degradation rates and turnover time in the sediments of the upstream, intermediate, and downstream stations in the Santa Lucia River before and after the rainfalls in November 2019 and January 2020. Error bars indicate standard deviation (n = 3).

BPC contents increased significantly only downstream (by 1425 %). Total phytopigment content, and algal fraction significantly increased in the two uppermost river stations (by 52-436%) and decreased downstream (by 76-98%). Protein to carbohydrate ratio significantly decreased only in the intermediate station and downstream (by 6 and 90%, respectively). Aminopeptidase activity significantly increased only in the intermediate station (by 52%), whereas β-glucosidase activity significantly decreased upstream (by 60%) and increased downstream (by 57%). C degradation rates significantly increased in the intermediate station and downstream (by 38-50%). C turnover time significantly decreased in the intermediate station (by 42%) and increased downstream (by 994%). After the January 2020 rainfall event, differences in the biochemical composition were mostly explained by an increase in chlorophyll-a and phaeopigment contents upstream, by an increase in carbohydrate content in the intermediate station, and by a marked decrease in chlorophyll-a and phaeopigment contents downstream (**Figure 8B**).

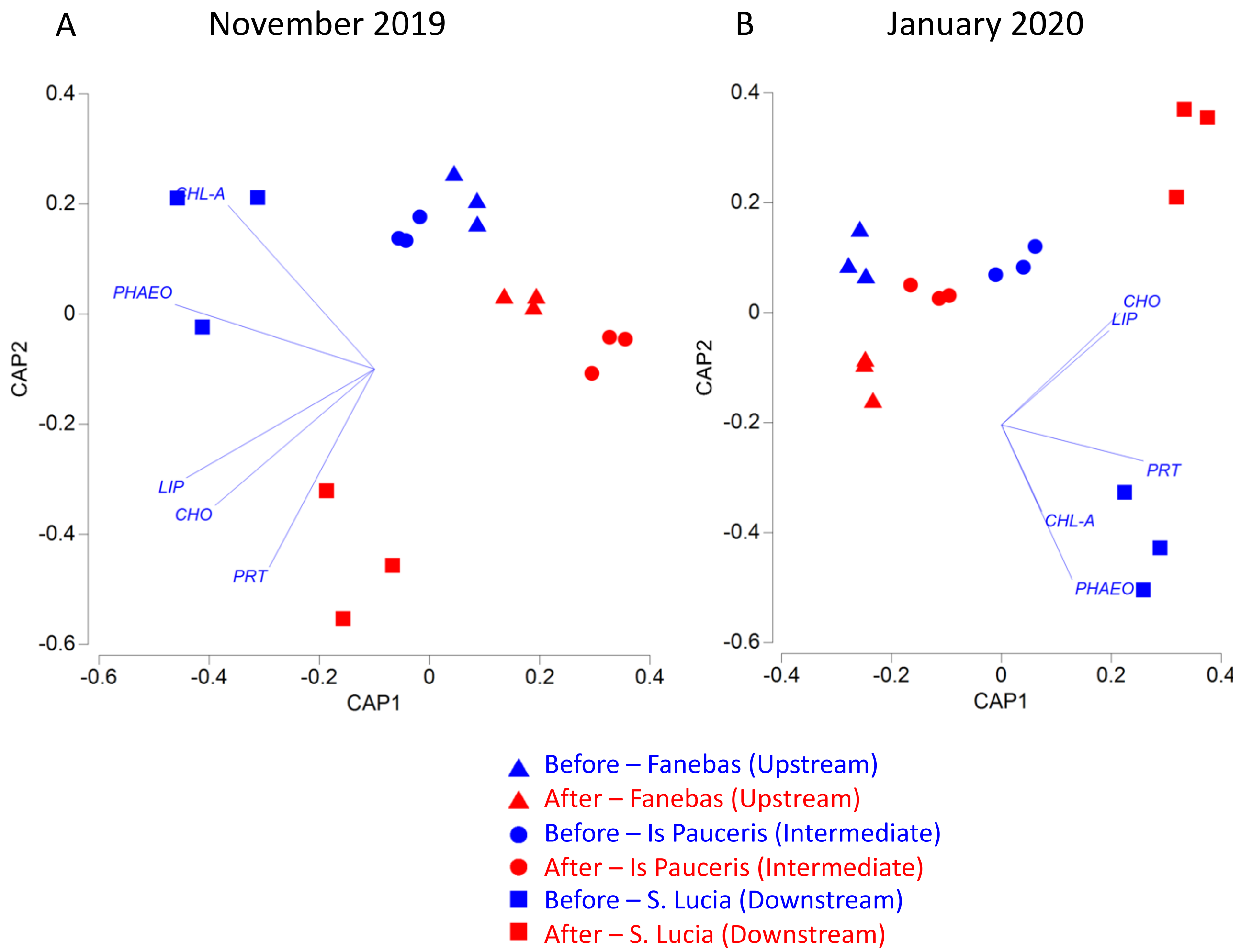

Figure 8. Biplot obtained after the CAP showing differences in the biochemical composition of sedimentary OM before and after the November 2019 (A) and January 2020 (B) rainfalls in the Santa Lucia River (Fanebas - Upstream; Is Pauceris - Intermediate; Santa Lucia - Downstream). PRT = protein; CHO = carbohydrate; LIP = lipid; Chl-a = chlorophyll-a; Phaeo = phaeopigment.

5.3.5 Effects of rainfalls on the biogeochemistry of the Capoterra lagoon sediments

Data on the sedimentary OM quantity, nutritional quality, extracellular enzymatic activities, and degradation rates in the Capoterra lagoon are reported in **Table S3**. Here, all investigated variables were significantly affected by the interaction between the tested factors (Impact × Station), except for protein to carbohydrate ratio and the algal fraction of BPC in November 2019, and except for protein and phaeopigment contents in January 2020 (**Tables 5 and 6**). The results of the pairwise tests are illustrated in **Figure S3**. Variations in OM contents and nutritional quality in the sediments of the Capoterra lagoon after the November 2019 and January 2020 rainfalls are illustrated in **Figure 9**, whereas extracellular enzymatic activities, C degradation rates and turnover time are illustrated in **Figure 10**.

Table 5. Results of PERMANOVA testing for differences in the quantity, biochemical composition, nutritional quality, C degradations rates and turnover time in lagoon sediments in November 2019 between impacts (Im; before and after), and stations (St; Internal vs. External). df = degrees of freedom; MS = mean square; Pseudo-F = F statistic; P(MC) = probability after Monte Carlo simulations. * = p <0.05; ** = p <0.01; ns = not significant. Reported are also the percentage of variation explained by each factor, their interaction and residual (i.e., unexplained) variance.

Variable	Source	df	MS	Pseudo-F	P(MC)	% EV
Protein	Impact	1	0.663	21.926	**	38
	Station	1	0.237	7.840	*	13
	Im × St	1	0.346	11.437	*	38
	Residuals	8	0.030			11
Carbohydrate	Impact	1	0.097	130.870	**	36
	Station	1	0.145	196.180	**	53
	Im × St	1	0.013	18.176	**	9
	Residuals	8	0.001			2
Lipid	Impact	1	0.001	13.114	**	9
	Station	1	0.008	104.550	**	76
	Im × St	1	0.001	8.428	*	11
	Residuals	8	0.000			4
Biopolymeric C	Impact	1	0.056	65.878	**	44
	Station	1	0.065	75.866	**	51
	Im × St	1	0.000	0.248	*	1
	Residuals	8	0.001			4
Protein to Carbohydrate ratio	Impact	1	1.329	3.035	ns	9
	Station	1	7.322	16.719	**	66
	Im × St	1	0.140	0.319	ns	0
	Residuals	8	0.438			25
Chlorophyll-a	Impact	1	0.012	0.624	ns	0
	Station	1	0.002	0.092	ns	0
	Im × St	1	0.604	30.743	**	91
	Residuals	8	0.020			9
Phytopigments	Impact	1	0.004	7.586	*	3
	Station	1	0.008	15.145	**	7
	Im × St	1	0.050	91.926	**	87
	Residuals	8	0.001			3
Biochemical composition	Impact	1	0.776	15.234	**	22
	Station	1	0.401	7.881	**	11
	Im × St	1	0.984	19.323	**	57
	Residuals	8	0.051			9
Algal fraction of BPC	Impact	1	0.353	14.103	**	22
	Station	1	1.044	41.690	**	68
	Im × St	1	0.002	0.067	ns	0
	Residuals	8	0.025			10

Aminopeptidase	Impact	1	0.765	32.853	**	47
	Station	1	0.446	19.163	**	27
	Im × St	1	0.162	6.953	*	18
	Residuals	8	0.023			9
β-glucosidase	Impact	1	0.011	1.467	ns	0
	Station	1	0.951	127.310	**	61
	Im × St	1	0.284	38.057	**	36
	Residuals	8	0.007			3
C degradation rates	Impact	1	0.681	32.794	**	58
	Station	1	0.234	11.283	*	12
	Im × St	1	0.089	4.308	*	19
	Residuals	8	0.021			11
C turnover time	Impact	1	0.002	0.234	ns	0
	Station	1	0.726	92.130	**	63
	Im × St	1	0.192	24.343	**	32
	Residuals	8	0.008			4

Table 6. Results of PERMANOVA testing for differences in the quantity, biochemical composition, nutritional quality, C degradations rates and turnover time in lagoon sediments in January 2020 between impacts (Im; before and after), and stations (St; Internal and External). df = degrees of freedom; MS = mean square; Pseudo-F = F statistic; P(MC) = probability after Monte Carlo simulations. * = $p < 0.05$; ** = $p < 0.01$; ns = not significant. Reported are also the percentage of variation explained by each factor, their interaction and residual (i.e., unexplained) variance.

Variable	Source	df	MS	Pseudo-F	P(MC)	% EV
Protein	Impact	1	0.527	8.358	*	13
	Station	1	2.553	40.530	**	68
	Im × St	1	0.241	3.819	ns	10
	Residuals	8	0.063			10
Carbohydrate	Impact	1	0.188	8.021	*	9
	Station	1	0.010	0.432	ns	0
	Im × St	1	0.824	35.056	**	84
	Residuals	8	0.023			7
Lipid	Impact	1	0.152	468.840	**	67
	Station	1	0.017	51.540	**	7
	Im × St	1	0.029	88.087	**	25
	Residuals	8	0.000			1
Biopolymeric C	Impact	1	0.216	60.176	**	28
	Station	1	0.028	7.801	*	3
	Im × St	1	0.250	69.528	**	66
	Residuals	8	0.004			3
Protein to Carbohydrate ratio	Impact	1	0.000	0.000	ns	0
	Station	1	0.486	8.184	*	16
	Im × St	1	0.987	16.611	**	70
	Residuals	8	0.059			14

Chlorophyll-a	Impact	1	0.770	32.965	**	10
	Station	1	3.324	142.310	**	42
	Im × St	1	1.825	78.144	**	46
	Residuals	8	0.023			2
Phytopigments	Impact	1	0.092	90.689	**	51
	Station	1	0.024	23.375	**	13
	Im × St	1	0.030	29.805	**	33
	Residuals	8	0.001			3
Biochemical composition	Impact	1	1.681	15.158	**	12
	Station	1	5.907	53.265	**	42
	Im × St	1	2.919	26.322	**	41
	Residuals	8	0.111			5
Algal fraction of BPC	Impact	1	0.194	51.824	**	8
	Station	1	0.250	66.888	**	10
	Im × St	1	0.995	266.400	**	81
	Residuals	8	0.004			1
Aminopeptidase	Impact	1	15.505	131.980	**	88
	Station	1	0.446	3.795	ns	2
	Im × St	1	0.609	5.185	*	6
	Residuals	8	0.117			4
β-glucosidase	Impact	1	11.092	67.981	**	75
	Station	1	0.180	1.106	ns	0
	Im × St	1	1.457	8.928	*	18
	Residuals	8	0.163			7
C degradation rates	Impact	1	16.899	144.190	**	88
	Station	1	0.314	2.676	ns	1
	Im × St	1	0.793	6.767	*	7
	Residuals	8	0.117			4
C turnover time	Impact	1	0.051	47.565	**	7
	Station	1	0.200	186.560	**	29
	Im × St	1	0.212	197.490	**	62
	Residuals	8	0.001			1

After the November 2019 rainfall event, protein content did not vary in the internal station, whereas it significantly increased (by 233%) in the external one. Carbohydrate, lipid, and BPC significantly increased in both stations (by 33-286%). Total phytopigment content significantly decreased in the internal station (by 42 %) and increased in the external one (by 118%). The algal fraction of BPC significantly decreased in the internal station (by 64 %), whereas it did not vary in the external one. Protein to carbohydrate ratio did not vary at all, whereas the algal fraction of BPC significantly decreased only in the internal station (by 64%). Aminopeptidase activity significantly increased in the internal station (by 183%), and β-glucosidase activity increased in the external one (by 522%). C degradation rates significantly increased only in the internal station (by 129%).

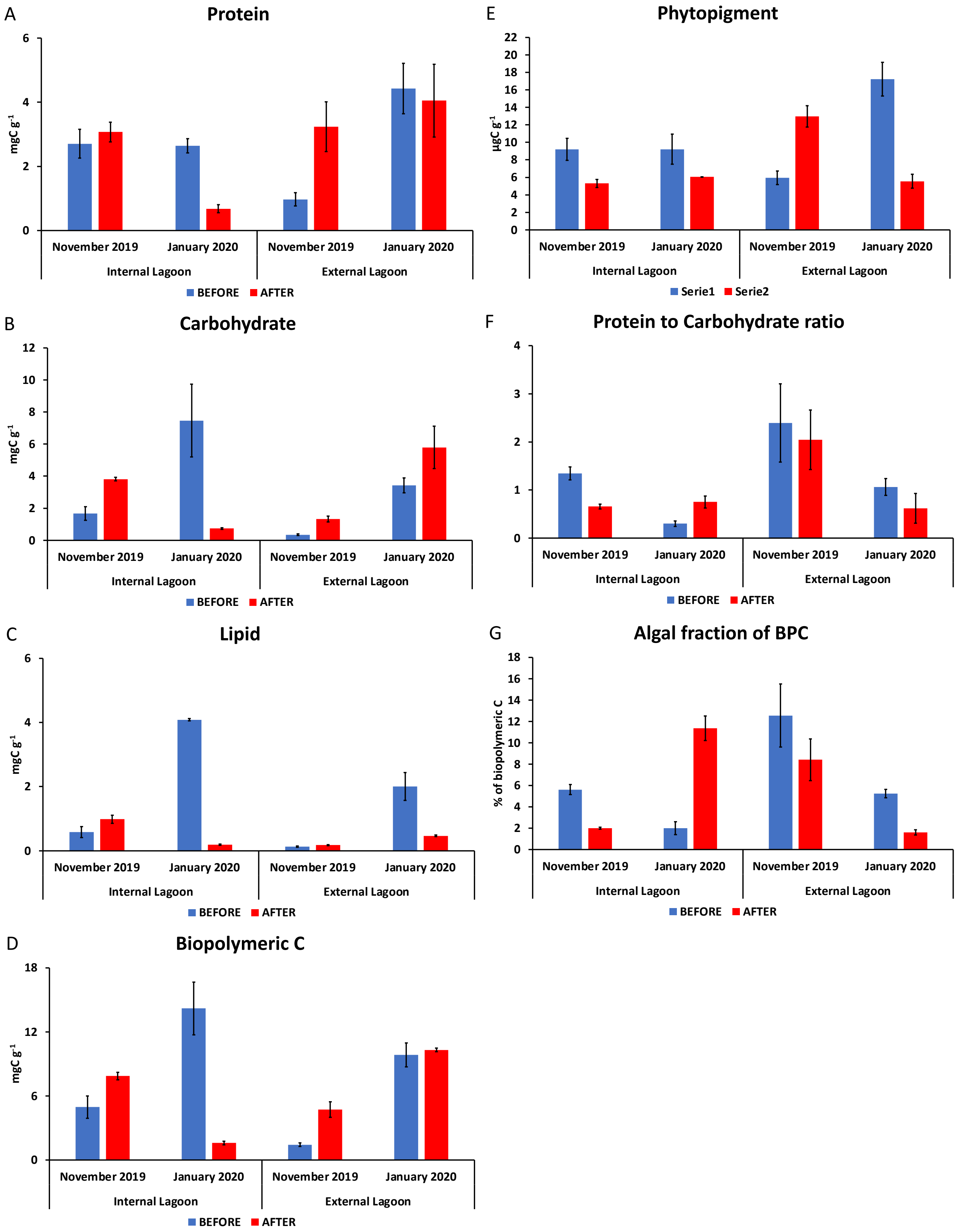

Figure 9. Protein (A), carbohydrate (B), lipid (C), biopolymeric C (D) and phytopigment contents (E), protein to carbohydrate ratio (F) and algal fraction of biopolymeric C (G) in the sediments of the Capoterra lagoon and after the rainfalls in November 2019 and January 2020. Error bars indicate standard deviation (n = 3).

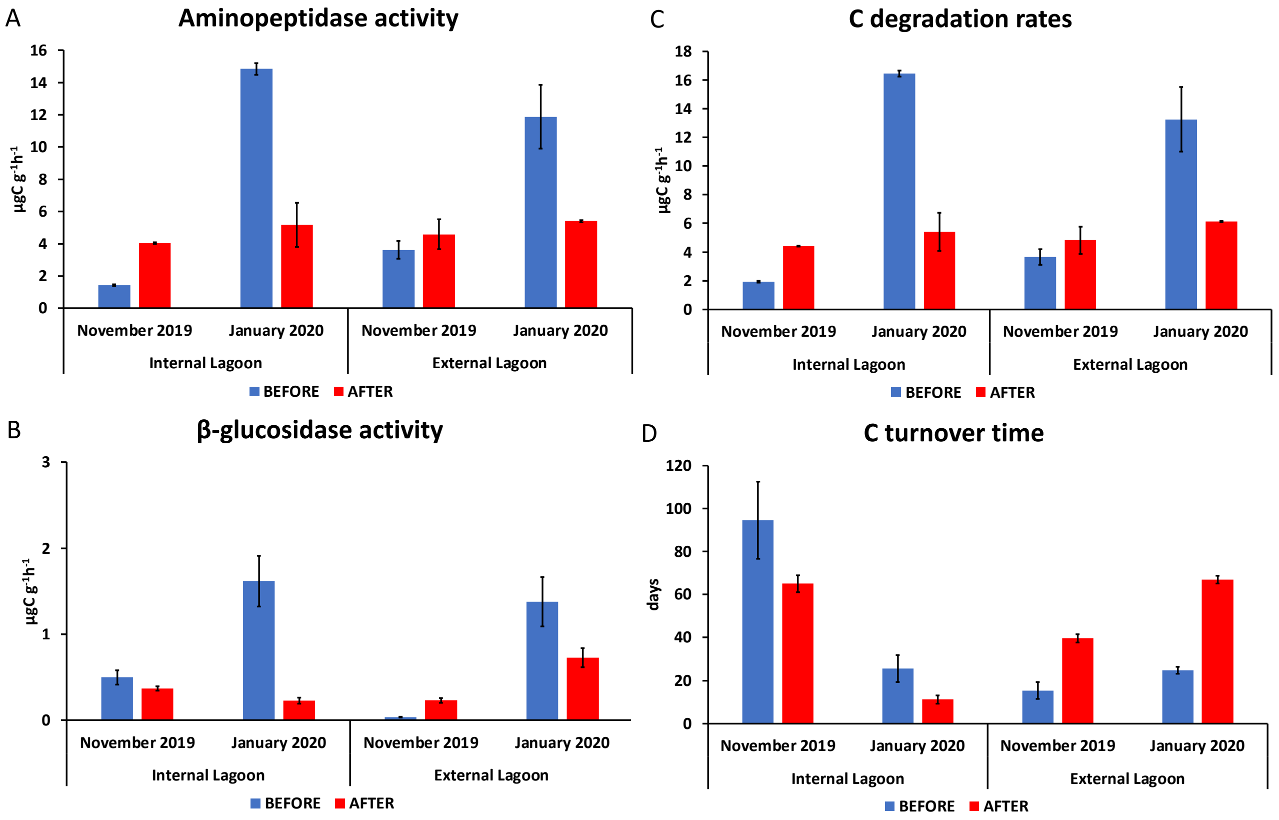

Figure 10. Aminopeptidase (A), β-glucosidase (B), C degradation rates and turnover time in the sediments of the Capoterra lagoon before and after the rainfalls in November 2019 and January 2020. Error bars indicate standard deviation (n = 3).

C turnover time significantly decreased in the internal station and increased in the external one (by 31 and 158%, respectively). After the November 2019 event, differences in the biochemical composition were mostly explained by an increase in carbohydrate and lipid contents in the internal station, and by an increase in chlorophyll-a and phaeopigment contents in the external one (**Figure 11A**).

After the January 2020 rainfall event, protein, carbohydrate, lipid, and BPC contents decreased in the internal station (by 74-95%), whereas carbohydrate content increased, and lipid content decreased in the external one (by 69 and 77%, respectively). Total phytopigment content significantly decreased in both lagoon stations (by 34-68%). The algal fraction of BPC increased (by 466%) in the internal station, whereas it decreased in the external one (by 69%). Protein to carbohydrate ratio significantly increased only in the internal station (by 149%). Aminopeptidase, β-glucosidase activity, and C degradation rates significantly decreased in both lagoon stations (by 47-86%). C turnover time significantly decreased in the internal station (by 56%) and increased in the external one (by 170%). Differences in the biochemical composition were mostly explained by a decrease in carbohydrate and lipid contents in the internal station, and by a decrease in chlorophyll-a content in the external station (**Figure 11B**).

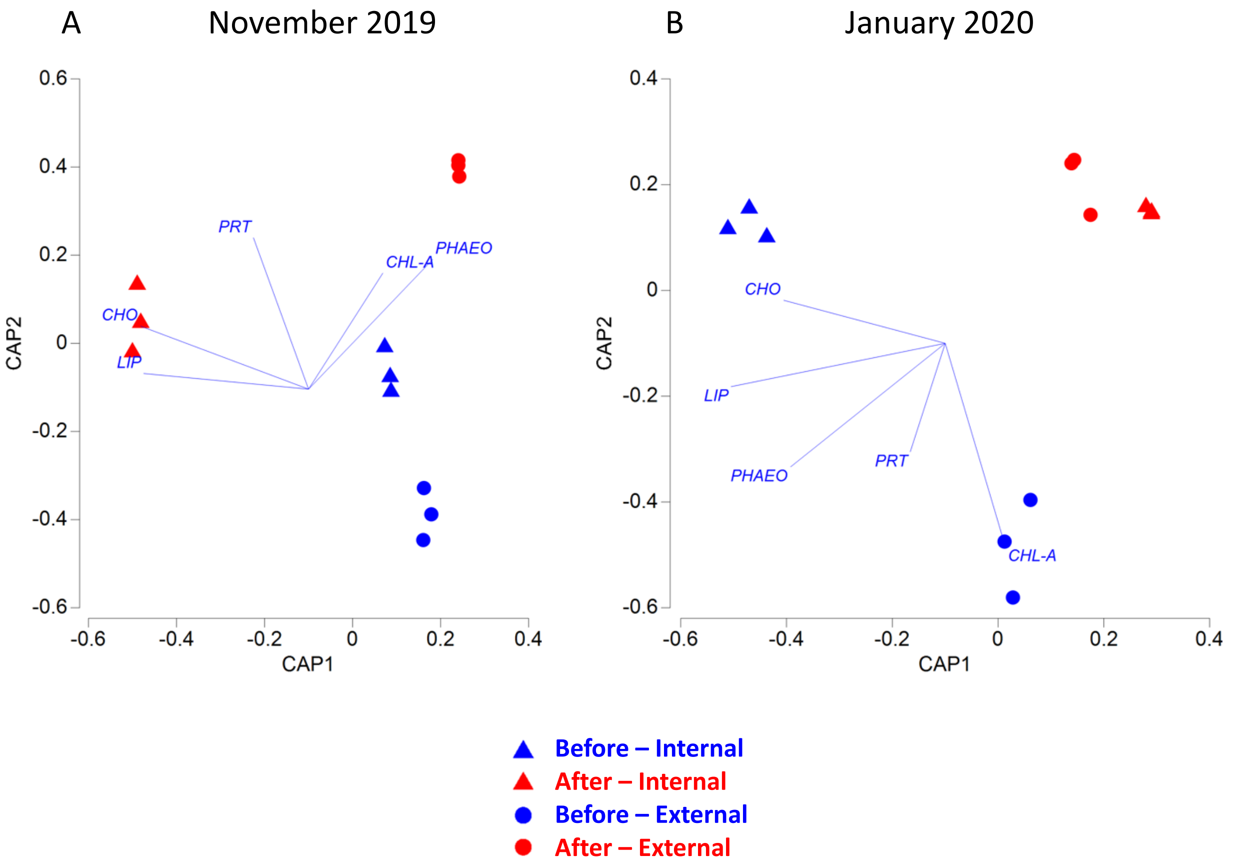

Figure 11. Biplot obtained after the CAP showing differences in the biochemical composition of sedimentary organic matter before and after the November 2019 (A) and January 2020 (B) rainfalls in the internal and external stations of the Capoterra lagoon. PRT = protein; CHO = carbohydrate; LIP = lipid; Chl-a = chlorophyll-a; Phaeo = phaeopigment.

5.3.6 Effects of a simulated flooding and recovery on the biogeochemistry sediments

Data on the sedimentary OM contents, nutritional quality, and C degradation rates and turnover time during the mesocosm experiment simulating a freshwater flooding on lagoon sediments are reported in **Table S4**. Differences in OM contents and nutritional quality in the lagoon sediments exposed to the freshwater flooding and the subsequent recovery are illustrated in **Figure 12**, whereas extracellular enzymatic activities, C degradation rates and turnover time are illustrated in **Figure 13**.

The PERMANOVA tests revealed significant interactions between the two tested factors (Phase × Treatment) for protein, chlorophyll-a and phytopigment contents, OM biochemical composition, protein to carbohydrate ratio, and β-glucosidase activity (**Table 7**). Other variables (discussed below) showed also significant differences within the Treatment factor (control vs. treatment) only. Post hoc tests contrasting control vs. treatment were carried out separately for the two phases (flooding and recovery) (**Table S5**).

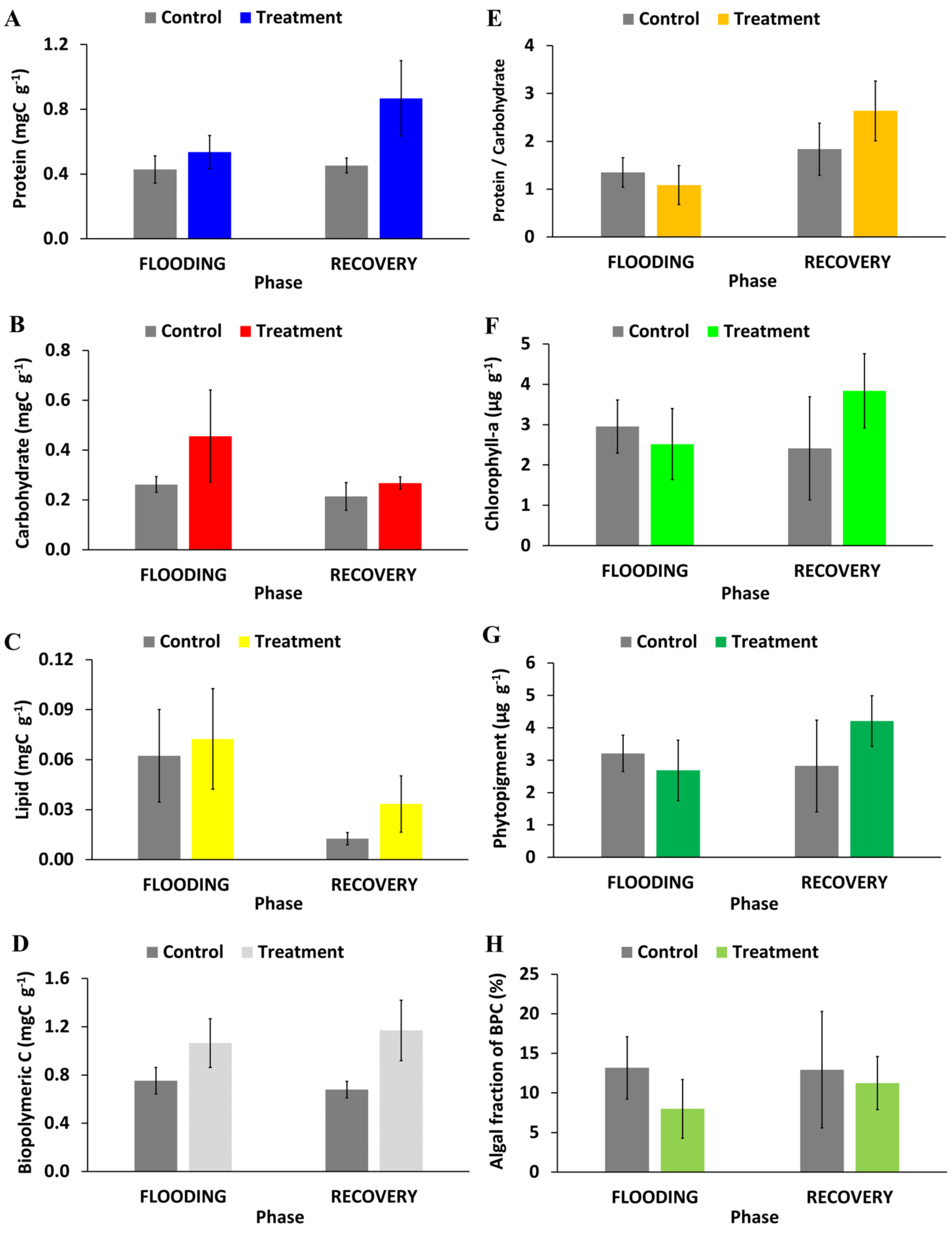

Figure 12. Changes in protein (A), carbohydrate (B), lipid (C), biopolymeric C (D), protein to carbohydrate ratio (E), chlorophyll-a (F), phytopigment (G), algal fraction of biopolymeric C (H) of lagoon sediments after the freshwater flooding and recovery in control and treatment tanks. Error bars indicate standard deviation (n=3).

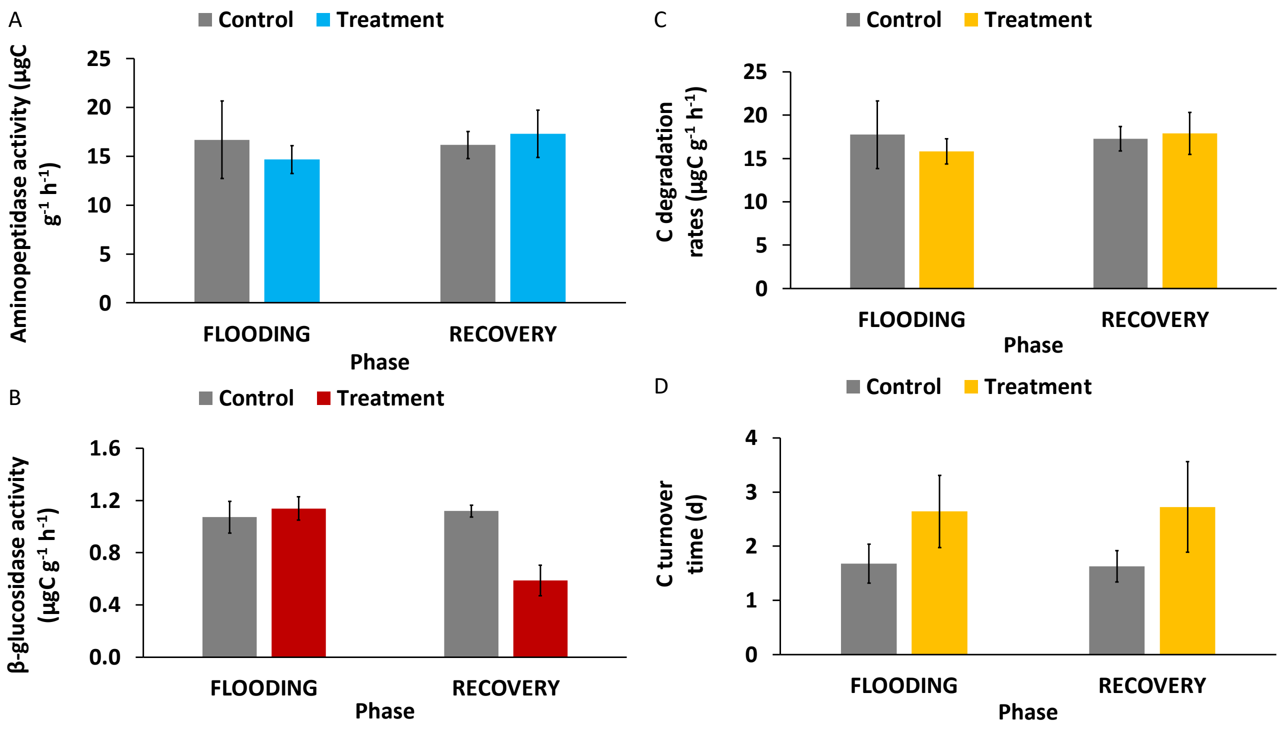

Figure 13. Changes in extracellular aminopeptidase (A) and β-glucosidase (B) activities, C degradation rates (C) and turnover time (D) in lagoon sediments after the freshwater flooding and recovery in control and treatment tanks. Error bars indicate the standard deviation (n=3).

Table 7. Results of PERMANOVA testing for differences in sedimentary organic matter contents, biochemical composition, nutritional quality, extracellular enzymatic activities, C degradation rates and turnover time between phase (Ph; Flooding vs. Recovery) and treatment (Tr; Control vs. Treatment). df = degrees of freedom; MS = mean square; Pseudo-F = F statistic; P(MC) = probability after Monte Carlo simulations. * = p <0.05; ** = p <0.01; *** = p <0.001; ns = not significant. Reported are also the percentage of variation explained by each factor, their interaction and residual (unexplained) variance.

Variable	Source	df	MS	Pseudo-F	P(MC)	EV %
Protein	Phase	1	3.936	10.350	**	17
	Treatment	1	8.501	22.352	***	38
	Ph × Tr	1	2.956	7.773	*	24
	Residual	20	0.380			21
Carbohydrate	Phase	1	4.793	8.561	*	24
	Treatment	1	5.304	9.475	*	26
	Ph × Tr	1	1.706	3.047	ns	13
	Residual	20	0.560			37
Lipid	Phase	1	11.655	23.872	***	62
	Treatment	1	1.412	2.893	ns	5
	Ph × Tr	1	0.168	0.345	ns	0
	Residual	20	0.488			33

Biopolymeric C	Phase	1	0.022	0.050	ns	0
	Treatment	1	13.701	31.876	***	70
	Ph × Tr	1	0.681	1.584	ns	3
	Residual	20	0.430			27
Chlorophyll-a	Phase	1	0.823	1.011	ns	0
	Treatment	1	1.298	1.594	ns	3
	Ph × Tr	1	4.594	5.642	*	42
	Residual	20	0.814			55
Phytopigments	Phase	1	1.616	2.032	ns	5
	Treatment	1	0.926	1.165	ns	1
	Ph × Tr	1	4.555	5.729	*	42
	Residual	20	0.795			53
Biochemical composition	Phase	1	25.031	7.892	***	25
	Treatment	1	17.050	5.376	***	16
	Ph × Tr	1	9.488	2.992	*	15
	Residual	20	3.172			44
Algal fraction of BPC	Phase	1	0.543	0.575	ns	0
	Treatment	1	2.828	2.993	ns	14
	Ph × Tr	1	0.733	0.776	ns	0
	Residual	20	0.945			86
Protein to Carbohydrate ratio	Phase	1	10.890	26.066	***	50
	Treatment	1	0.751	1.796	ns	2
	Ph × Tr	1	3.004	7.190	*	25
	Residual	20	0.418			24
Aminopeptidase activity	Phase	1	1.019	1.044	ns	0
	Treatment	1	0.165	0.169	ns	0
	Ph × Tr	1	2.300	2.356	ns	18
	Residual	20	0.976			81
β-glucosidase activity	Phase	1	6.097	39.456	***	20
	Treatment	1	5.194	33.613	***	17
	Ph × Tr	1	8.618	55.763	***	57
	Residual	20	0.155			6
C degradation rates	Phase	1	0.622	0.610	ns	0
	Treatment	1	0.417	0.410	ns	0
	Ph × Tr	1	1.593	1.564	ns	9
	Residual	20	1.018			91
C turnover time	Phase	1	0.003	0.005	ns	0
	Treatment	1	11.120	18.793	***	60
	Ph × Tr	1	0.043	0.073	ns	0
	Residual	20	0.592			40

Protein and chlorophyll-a contents, and protein to carbohydrate ratio were significantly higher than in the control only during the recovery phase (by 92, 59 and 44%, respectively), whereas carbohydrate contents were higher than in the control only during the flooding one (by 74%). BPC was significantly higher than in the control during both flooding and recovery phases (by 41-72%, respectively). During both phases, lipid, and total phytopigment contents, the algal fraction of BPC, the aminopeptidase activity and the C degradation rates did not vary between the treatment and control tanks. β-glucosidase activity was significantly lower than in the control only during the recovery treatment (by 48%). C turnover time was significantly higher than in the control during both flooding and recovery phases (by 58 and 67%, respectively).

The OM biochemical composition differed between control and treated tanks during both phases (**Table S5**, **Figure 14**). More in details, during the flooding phase differences between treatment and control tanks were mostly explained by an increase in carbohydrate contents, whereas during the recovery one by an increase in protein and chlorophyll-a contents.

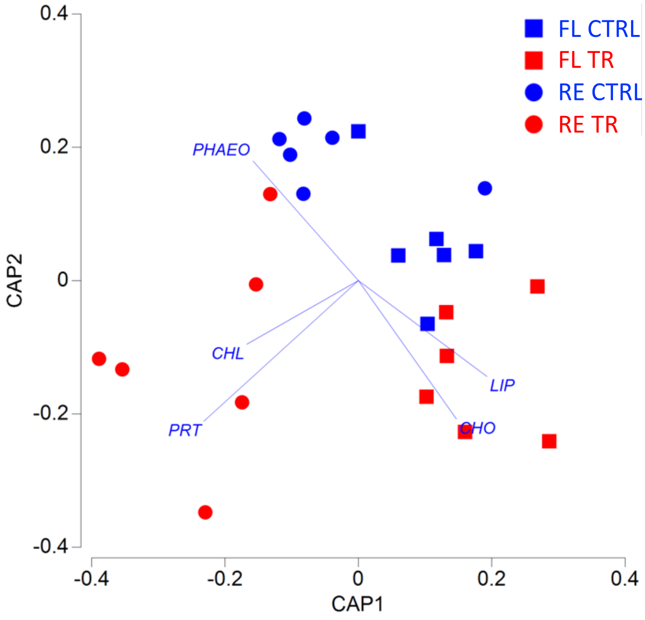

Figure 14. A) Biplot obtained after CAP showing differences in the biochemical composition of sedimentary organic matter between treatment (TR) and control (CTRL) tanks during flooding (FL) and recovery (RE) phases. PRT = protein; CHO = carbohydrate; LIP = lipid; Chl-a = chlorophyll-a; Phaeo = phaeopigment.

5.4 Discussion

Riverine inputs directly influence lagoon's physical-chemical characteristics (i.e., salinity, nutrients, OM) (Badosa et al., 2007; Laut et al., 2016; Meredith et al., 2022) mainly following anomalous rainfall and flooding events. These phenomena will become increasingly frequent, with several consequences on ecosystem functioning (Jentsch and Beierkuhnlein, 2008; Dottori et al., 2020), especially in rivers and coastal lagoons. Both systems are nowadays commonly affected by eutrophication, a natural phenomenon which however has been exacerbated by climate change and anthropic activities (Cloern, 2001; de Jonge and Elliott, 2001; Dodds, 2006). Nonetheless, the effects of intense rainfall-induced freshwater injections on river and lagoon sediment biogeochemistry are still poorly investigated.

5.4.1 Effects of rainfalls on C, N, C/N and isotopic signature in the Capoterra lagoon sediments

Generally large quantities of C and N accumulate in lagoon sediments due to both natural river runoffs and human activities, thus stimulating in the short-term growth of phytoplankton and phytobenthos (Karydis and Kitsiou, 2012; Franzo et al., 2015). Nonetheless my results do not fit with such expectation. Indeed, with exception of the external lagoon station after the heavier rainfall period, C and N contents decreased after rainfall. The general decrease in C and N contents in the internal lagoon could be the result of a major transportation of finer sediments towards the outer lagoon, which tend to deposit in the external station, but only after the heavier rainfall. This result suggests that the "eutrophicating" effect of rainfall on the outer lagoon sediments depends largely on the extent of rainfall.

The C/N ratio, an indicator of sedimentary OM quality (Danovaro et al., 2001), did not vary in the external lagoon station after both rainfall periods, whereas in the internal one increased (up to 7-folds) after the heavier rainfall and decreased after the lighter one. The lack of effects of both rainfall periods on the C/N ratio in the external lagoon suggests that, possibly, the effects of rainfall-driven run-off in the outer lagoon could have been partly masked by inputs from the sea. On the other hand, the 7-fold increase in C/N ratio after the heavier rainfall in November 2019 in the internal station could be associated with larger inputs of terrestrial plant material transported by the river (Meyers, 1997; Rumolo et al., 2011; Nasi et al., 2020). This hypothesis is corroborated by the patterns of $\delta^{13}C$ values which, since terrestrial C4 plant material such as weed and cane species and other C4 (common in the riparian area / watershed of the study area) which have enriched (up to -10 ‰) $\delta^{13}C$ values (Hedges et al., 1997), decreased after both rainfall periods, more pronouncedly (from -26.4 to -22.0‰) after the heavier precipitation period in November 2019 than after the lighter one in January 2020 (from -24.7 to -23.0‰). Since higher $\delta^{15}N$ values could be linked to the incorporation of anthropogenic N inputs in the river (e.g., fertilizer, wastewater, etc.) by aquatic primary producers (Cazzanelli et al., 2021 and citations therein) and since $\delta^{15}N$ values did not change in this study after both rainfall periods, I conclude that the two rainfall periods did not transport major quantities of anthropogenic material in the lagoon.

5.4.2 *Effects of rainfalls on sediment biogeochemistry in river and lagoon sediments*

The total organic OC sedimentary contents are only a rough descriptor of the food available for the benthos, as they represent the bulk of OM, without any possibility to discriminate between the labile and refractory fractions (Pusceddu et al., 2009). On the other hand, the BPC, as the sum of protein, carbohydrate, and lipid contents (as defined by Fabiano et al., 1995), representing 50-70% of total organic C (Tselepides et al., 2001; Pusceddu et al., 2009), are currently considered the labile or semi-labile fraction of sedimentary organic C (van Oevelen et al., 2011).

In the Santa Lucia River, overall, both rainfall periods were followed by a significant increase in the BPC content only in the downstream station, where, unexpectedly, the positive effect of the lighter rainfall was higher than that after the heavier one. Indeed, after the January 2020 rainfall period the downstream meso-eutrophic Santa Lucia station, according to the classification proposed by Pusceddu et al. (2009b, 2011), has turned into eutrophic (BPC > 3 mgC g^{-1} and algal fraction of BPC < 12 %). Moreover, I notice here that the enrichment in BPC observed after the lighter rainfall period in January 2020 was not observed in the adjacent internal Capoterra lagoon station, where BPC contents even decreased ca. 9-folds, nor in the external one, where BPC contents did not vary. On the other hand, after the heavier rainfall period in November 2019, BPC contents increased in both lagoon stations, more pronouncedly in the internal one. These results are difficult to explain but suggest that the heavier rainfall period in November 2019 was able to move BPC particles from the river to the lagoon, whereas the lighter one in January 2020 exerted an "eutrophicating" effect only within the river. This result highlights that the expected eutrophication of sediments exposed to rainfall-driven river run-off and its spatial extent is tightly dependent on the magnitude of the rainfall.

The response of benthic consumers to increased OM supply is influenced more by OM quality (e.g., bioavailability) rather than by bulk concentration (Cebrián et al., 1998, Huxel, 1999; Pusceddu et al., 2009). Accordingly, the trophic status of sediments depends also on the biochemical composition and, in turn, in the nutritional quality of sedimentary OM, rather than simply on its quantity (Grall and Chauvaud, 2002). Accordingly, in the last two decades, the nutritional quality of sedimentary OM has been assessed in terms of the algal fraction of BPC, being linearly related to the digestible fraction of OM (Pusceddu et al., 2003), whereas its ageing and heterotrophic nature has been determined using the protein to carbohydrate ratio, with values > 1 typically associated with "younger" material of heterotrophic origin (Pusceddu et al., 2000).

I show here that in the Santa Lucia River sediments the biochemical composition of sedimentary OM varied significantly after both rainfall periods. Nonetheless, the effects were ample and consistent in all river stations only after the heavier rainfall period in November 2019, where differences before and after the rainfall were markedly driven by the decrease in the phytopigment contents and the decrease of the algal fraction of BPC. Instead, after the January 2020 rainfall period, differences were clearly seen only in the downstream river station, because of a major increase in the carbohydrate fraction and a decrease of the algal fraction of BPC only in the downstream station. Notably, the protein to carbohydrate ratio values increased in almost all river stations only after the heavier rainfall period in November 2019. Overall, these results indicate that the organic material found after the heavier rainfall period was enriched in the heterotrophic fraction but depleted in its overall nutritional quality (i.e., algal fraction of BPC). This apparent discrepancy is explained by the fact that, as outlined above, during the November 2019 rainfall period larger inputs of terrestrial plant material (enriched in organic N) entered the river. Since terrestrial plants have a much lower nutritional quality for the benthic consumers (as dominated by structural and refractory carbohydrates; Pusceddu et al., 1999; Manini et al., 2003), these results indicate that in the Santa Lucia River, rainfalls exerted an overall negative effect on the nutritional quality of sedimentary OM only after the heavier precipitation period in November 2019 or, after that in January 2020, only in the downstream station.

Both rainfall periods determined significant effects also on the biochemical composition of sedimentary OM in the Capoterra lagoon. After the heavier rainfall in November 2019, these effects were associated with an increase in the carbohydrate and lipid fractions in the internal lagoon station and an increase in the phytopigment content, as well as with a decrease in the algal fraction of BPC and the protein to carbohydrate values in both stations. After the lighter rainfall in January 2020 differences in the OM biochemical composition in the lagoon were associated with a general decrease in carbohydrate and lipid contents in the internal station and by a decrease in protein and phytopigment contents in the external one. Altogether, these results, corroborated by the biplots produced after the CAP, pinpoint that rainfall events can have important effects on the biochemical composition of lagoon sedimentary OM and, more specifically, on its nutritional quality. I highlight here that, generally, these effects, as observed for OM loads, are related to the magnitude of the rainfall. After the heavier rainfall period the nutritional quality of the sediment dropped down in both lagoon stations because of the documented larger inputs of OM of terrestrial origin, associated with plant material of prevalent refractory nature. On the other hand, after the lighter rainfall period the nutritional quality of sediments increased in the internal station and decreased in the external one. The increase in the OM nutritional quality of the internal lagoon after the lighter rainfall period is difficult to explain. However, I recall here that in the internal lagoon after the lighter rainfall period, contrarily to what observed in the external station after both events and in the internal one after the heavier rainfall, OM contents decreased along with an increase in the sandy fraction of the sediment. This pattern suggests that the lighter rainfall period brought to the internal station lower amounts of refractory OM of plant origin (as outlined by the d^{13}C values, see above). Altogether these results confirm that the weak injection of freshwater inputs below certain threshold levels can exert an even positive effect on the trophic conditions of the sediments of the recipient lagoon, enhancing the nutritional quality of sedimentary OM. Moreover, these results suggest that heavy rainfall events (i.e., characterized by greater freshwater injections) could favor the downstream transport of nutrients out of the river and even towards the external lagoon, causing a long-distance effect associated with a consistent reduction in the nutritional quality of sedimentary OM, because of the larger inputs of refractory material of terrestrial origin. Nonetheless, lighter rainfall events in riverine systems can be as impactful as heavier rainfall periods, whereas the consequences on lagoon sediments can be highly variable, most likely depending on factors uncontrolled in this study.

Previous studies showed that river-transported OM inputs (within certain threshold levels) can stimulate extracellular enzymatic activities in recipient coastal lagoons and adjacent marine sediments (Keith and Arnosti, 2001; Dell'Anno et al., 2008). I show here that in the Santa Lucia River, after the heavier rainfall period in November 2019, both enzymatic activities and, consequently, C degradation rates increased in the upstream station, did not vary in the intermediate one, and decreased in the downstream one. These patterns in C degradation rates could be related to the differential bioavailability of organic substrates across the river auction, whose quantity was significantly affected by the increased river runoff only in the downstream station, because of inputs of more refractory compounds. This hypothesis is indeed corroborated by the increase in C turnover time occurred, after both rainfall events, in the downstream river station. Nonetheless changes in enzymatic activities and C degradation rates after the lighter rainfall period in January 2020 in the Santa Lucia River were much less evident, whereas, again, C turnover time in the downstream station increased by > 10 times. As outlined above for the other investigated variables, I pinpoint here that, overall, C degradation rates and turnover time in river sediments are related with the magnitude of the rainfall, with larger changes occurring after heavier rainfall events.

Changes in extracellular enzymatic activities and C degradation and turnover time in the Capoterra lagoon sediments after rainfalls were rather different from those observed in the affluent Santa Lucia River. After the heavier rainfall period, aminopeptidase activity increased in the internal station and remained almost constant in the external one, whereas β-glucosidase activity decreased in the internal station and increased in the external one. After the lighter rainfall period, both activities decreased in both lagoon stations. As aminopeptidase activity is the most important one, C degradation rates followed the same pattern, with an increase after the heavier precipitation period and a decrease after the lighter one. Notably, after both rainfall C turnover time decreased in the internal station and increased in the external one. These results pinpoint that, overall, the effects of rainfalls on sedimentary C degradation vary with the magnitude of the rainfall exerting a negative effect after heavier precipitations and a positive one after lighter events. This result is consistent with the hypothesis of enzymatic activity inhibition following large OM inputs on sediments, firstly postulated by Meyer-Reil and Köster (2000) and, more recently, reported by Dell'Anno et al. (2008) after a major run-off of the Po River on the coastal sediments of the Adriatic Sea. In fact, although it is known that increasing substrate availability can stimulate extracellular enzymatic activities (Keith and Arnosti, 2001), when concentrations of organic substrates exceed certain threshold levels, enzymatic activities may be inhibited (Meyer-Reil and Köster, 2000). This event might impair the balance between degradation and utilization processes of organic compounds, thus promoting their accumulation in the sediment and letting the system shift towards eutrophication.

Overall, these results suggest that the rainfall events, especially the heavier ones (at least when not destructive as in this study), could lead to accentuate eutrophication of recipient sediments, not only influencing OM loads and nutritional quality but also C degradation processes, whose extent and consequences could severely affect rivers and coastal lagoons trophic status.

5.4.3 Effects of a simulated flooding and recovery on the biogeochemistry sediments

I observed higher sedimentary BPC contents in treated sediments than those in the control, either during the flooding or the recovery phase. More specifically, sediments exposed to the flooding were characterized by a shift from oligotrophic (BPC < 1.0 mgC g^{-1}) to meso-eutrophic (BPC in the range of 1-3 mgC g^{-1}) status (according to the classification proposed by Pusceddu et al., 2009b, 2011). Such increase in the BPC contents in tanks exposed to flooding was associated with a remarkable increase of the carbohydrate fraction, the more refractory class of organic compounds (Pusceddu et al., 2003), but, at the same time, with invariant values of the protein to carbohydrate ratio values and of the algal fraction of BPC. This result suggests that a sudden change in salinity of lagoon waters can concur to increase the amount of OM in the sediment, without altering the overall nature (heterotrophic vs. autotrophic, in terms of the protein to carbohydrate ratio) and nutritional quality (in terms of the algal fraction of BPC) of organic substrates. Interestingly, these changes do not fit with those observed after natural and, overall, weaker events of salinity drop in the field. Such a discrepancy between the results obtained from the field survey and the mesocosm simulation could be caused by the different duration and amplitude of exposure of the lagoon sediments to freshwater injection, but, overall, indicates that the major consequence of a severe flooding (like the one simulated in the mesocosm) on lagoon sediments is the increase of organic loads. Reasons for such a positive response of OM loads to sudden exposure to freshwater are difficult to explain, but, among the others, there could be the case that the freshwater injected in the mesocosm could have added discrete amount of OM and other nutrients in the treated tanks. As I did not determine the organic load of injected freshwater, more detailed studies are required to try to demonstrate this hypothesis. Another possible explanation is a positive response of benthic biomass to the input of new macro- and micro-nutrients brought by the freshwater injection. On the one hand, I could hypothesize that the freshwater injection could have brought some inorganic nutrients which stimulated the microphytobenthos. In fact, thanks to their proximity to land, lagoons normally receive considerable amounts of terrestrial nutrients, which stimulate *in situ* primary production (Bianchi, 2007). However, this hypothesis cannot stand as phytopigment sedimentary contents (a proxy for microphytobenthos biomass) remained unchanged during the flooding phase. On the other hand, the increase in OM matter could have been the result of heterotrophic biomass enhancement, which, however and again, should have not been the case, as, overall, the protein to carbohydrate ratio (a proxy for the relative importance of heterotrophic vs. autotrophic nature of OM) did not vary during the flooding phase. Ultimately, the accumulation of OM in sediments exposed to the flooding could have been caused by a concurrent impairment of the balance between production and degradation processes: indeed, the C degradation rates during the flooding were slightly (though not significantly) lower than those in the control tank, suggesting a slowing of C degradation, also corroborated by a clear increase of the C turnover time. Such an effect on C degradation rates could have been, then, associated with important changes in the composition and function of prokaryotic communities (Moghadam et al., 2022).

The difference in OM sedimentary loads between control and treatment tanks observed during the flooding phase remained unchanged during the recovery one, indicating that, whatever the cause of the OM enrichment of lagoon sediments during the flooding, its effects persist in the short term also after the recovery of natural values of salinity. On the other hand, during the recovery phase changes in the biochemical composition of sedimentary OM were clearly explained by increasing contents of proteins and chlorophyll-a, as well as by increasing values of the protein to carbohydrate ratio. Such finding leads to infer that, while a short-lasting freshwater flashflood could impair lagoon sedimentary OM loads but not their nutritional quality, the sudden recovery of natural salinity values could lead lagoon sediments to be characterized by a pre-eminently heterotrophic nature of the sedimentary OM loads.

5.5 Conclusions

The current knowledge about the consequences of rainfalls and floodings on river and coastal lagoon benthic ecosystems are still far from being fully understood. Although this study cannot exhaustively identify clear and unbiased cause-effect relationships between the occurrence of rainfalls and floodings on the trophic status of river and coastal lagoon sediments, the results of my thesis pinpoint that these events can have differential consequences on quantity, biochemical composition, nutritional quality, and degradation processes of sedimentary OM in either rivers or coastal lagoons. The observed consequences, their direction and amplitude vary, at times inexplicably, according to the magnitude of the rainfall and, possibly, with the exposure time of the impacted sediments to flooding events, which consequences, when observed, that can last also during the entire recovery of natural salinity values. The ongoing climate change is causing the occurrence of increasingly more intense and more frequent rainfall and river flashflood events, especially in the Mediterranean region (Jentsch and Beierkuhnlein, 2008; Kundzewicz et al., 2013; Wei et al., 2009; Dottori et al., 2020; Petrucci, 2022). The results of my thesis opened additional questions (to initial ones) to be answered, so that further correlative and manipulation experiments are needed to achieve a clearer picture of the effects of rainfall and flooding events on benthic aquatic ecosystems.

5.6 References

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5.7 Supplementary tables

Table S1. Pairwise tests on total C, N, their ratio, and C stable isotope between impacts (before and after) and lagoon stations after November 2019 and January 2020 rainfalls.

		November 2019		**January 2020**	
Variable	**Station**	**t**	**P(MC)**	**t**	**P(MC)**
OC	Internal	0.880	ns	3.164	*
	External	6.293	**	2.516	ns
TN	Internal	25.695	***	2.953	*
	External	19.022	***	3.393	*
C/N	Internal	4.699	*	3.397	*
	External	0.930	ns	1.943	ns
δ^{13}C	Internal	6.662	**	12.460	***
	External	2.228	ns	3.829	*

Table S2. Sedimentary contents of proteins (PRT), carbohydrates (CHO), lipids (LIP), biopolymeric C (BPC) and phytopigments (PHY), aminopeptidase (LEU) and β-glucosidase (BETA) activities, and C degradation rate (Cdr) in the sediments of the Santa Lucia River before and after the November 2019 and January 2020 rainfalls (FAN = Fanebas, upstream; ISP = Is Pauceris, intermediate; SLU = Santa Lucia, downstream). Reported are standard deviations (n=3).

Rainfall	**Station**	**Event**	**PRT** mgC g^{-1}	**CHO** mgC g^{-1}	**LIP** mgC g^{-1}	**BPC** mg g^{-1}	**PHY** µg g^{-1}	**LEU** µgC g^{-1}h^{-1}	**BETA** µgC g^{-1}h^{-1}	**Cdr** µgC g^{-1}h^{-1}
November 2019	**FAN**	**Before**	0.15±0.00	0.07±0.00	0.01±0.00	0.23±0.00	1.36±0.22	0.30±0.05	0.02±0.00	0.32±0.05
		After	0.18±0.01	0.04±0.01	0.01±0.00	0.24±0.00	0.60±0.10	1.15±0.12	0.32±0.03	1.46±0.14
	ISP	**Before**	0.14±0.05	0.06±0.01	0.02±0.00	0.22±0.04	1.28±0.10	1.81±0.42	0.21±0.03	2.02±0.40
		After	0.19±0.03	0.04±0.01	0.01±0.00	0.24±0.04	0.31±0.02	1.72±0.06	0.16±0.03	1.88±0.04
	SLU	**Before**	0.30±0.08	0.19±0.04	0.02±0.01	0.52±0.07	2.21±0.42	8.31±2.01	2.34±0.28	10.65±2.06
		After	0.57±0.11	0.27±0.09	0.03±0.00	0.87±0.19	0.53±0.12	2.99±0.34	1.02±0.22	4.01±0.40
January 2020	**FAN**	**Before**	0.07±0.00	0.02±0.01	0.02±0.00	0.11±0.01	0.08±0.01	0.41±0.05	0.20±0.03	0.61±0.06
		After	0.06±0.00	0.02±0.01	0.01±0.00	0.09±0.00	0.20±0.03	0.49±0.16	0.08±0.01	0.57±0.15
	ISP	**Before**	0.13±0.01	0.02±0.00	0.02±0.00	0.17±0.02	0.17±0.03	0.74±0.17	0.17±0.05	0.91±0.14
		After	0.05±0.00	0.07±0.02	0.01±0.00	0.13±0.02	0.35±0.07	1.12±0.15	0.13±0.04	1.25±0.18
	SLU	**Before**	0.14±0.03	0.06±0.01	0.01±0.00	0.22±0.02	0.61±0.13	0.29±0.05	0.21±0.03	0.50±0.08
		After	0.12±0.02	3.15±0.74	0.02±0.00	3.29±0.71	0.14±0.02	0.42±0.10	0.32±0.05	0.75±0.11

Table S3. Sedimentary contents of proteins (PRT), carbohydrates (CHO), lipids (LIP), biopolymeric C (BPC) and phytopigments (PHY), aminopeptidase (LEU) and β-glucosidase (BETA) activities, and C degradation rate (Cdr) in the sediments of the Capoterra lagoon before and after the November 2019 and January 2020 rainfalls. Reported are standard deviations (n=3).

Rainfall	Station	Event	PRT	CHO	LIP	BPC	PHY	LEU	BETA	Cdr
			mgC g^{-1}	**mgC g^{-1}**	**mgC g^{-1}**	**mg g^{-1}**	**µg g^{-1}**	**µgC $g^{-1}h^{-1}$**	**µgC $g^{-1}h^{-1}$**	**µgC $g^{-1}h^{-1}$**
November 2019	**Internal**	**Before**	2.71±0.45	1.67±0.42	0.58±0.17	4.96±1.04	9.20±1.24	1.43±0.05	0.50±0.08	1.93±0.05
		After	3.07±0.31	3.81±0.11	0.98±0.13	7.87±0.35	5.31±0.46	4.04±0.05	0.37±0.03	4.41±0.04
	External	**Before**	0.97±0.20	0.34±0.05	0.13±0.02	1.45±0.16	5.96±0.77	3.62±0.54	0.04±0.01	3.66±0.54
		After	3.24±0.78	1.32±0.18	0.18±0.02	4.73±0.72	12.97±1.23	4.59±0.93	0.23±0.03	4.82±0.96
January 2020	**Internal**	**Before**	2.64±0.22	7.46±2.27	4.09±0.04	14.19±2.45	9.20±1.72	14.84±0.36	1.62±0.30	16.46±0.21
		After	0.68±0.13	0.74±0.05	0.19±0.02	1.60±0.16	6.04±0.01	5.17±1.37	0.23±0.04	5.40±1.33
	External	**Before**	4.43±0.79	3.42±0.47	2.01±0.43	9.85±1.12	17.21±1.92	11.87±1.98	1.38±0.29	13.25±2.25
		After	4.05±1.14	5.79±1.31	0.47±0.02	10.31±0.17	5.56±0.79	5.41±0.06	0.73±0.11	6.13±0.05

Table S4. Sedimentary contents of proteins, carbohydrates, lipids, biopolymeric C, and phytopigments, aminopeptidase and β-glucosidase activities, C degradation rate and turnover time in the lagoon sediments exposed to flooding and recovery. CTRL = control; TR = treatment. Reported are standard deviations (n=3).

Variable	**Flooding**		**Recovery**	
	CTRL	**TR**	**CTRL**	**TR**
Protein (mgC g^{-1})	0.43±0.08	0.54±0.10	0.45±0.05	0.87±0.23
Carbohydrate (mgC g^{-1})	0.26±0.03	0.46±0.19	0.21±0.06	0.27±0.02
Lipid (mgC g^{-1})	0.06±0.03	0.07±0.03	0.01±0.00	0.03±0.02
Biopolymeric C (mgC g^{-1})	0.75±0.11	1.06±0.20	0.68±0.07	1.17±0.25
Chlorophyll-a (μg g^{-1})	2.95±0.66	2.51±0.88	2.41±1.28	3.84±0.92
Phytopigments (μg g^{-1})	3.21±0.56	2.68±0.93	2.82±1.42	4.21±0.79
Aminopeptidase (μgC $g^{-1}h^{-1}$)	16.68±3.96	14.67±1.43	16.15±1.39	17.31±2.42
β-glucosidase (μgC $g^{-1}h^{-1}$)	1.07±0.12	1.14±0.09	1.12±0.5	0.59±0.12
C degradation rate (μgC $g^{-1}h^{-1}$)	17.75±3.91	15.81±1.45	17.27±1.41	17.89±2.42
C turnover time (d)	1.68±0.36	2.64±0.67	1.63±0.29	2.72±0.84

Table S5. Results of post-hoc test assessing differences in sedimentary organic matter contents, biochemical composition, nutritional quality, extracellular enzymatic activities, C turnover time between control and treatment during the flooding and recovery phases. t = statistic t; P(MC) = probability after Monte Carlo simulations. * = p <0.05; ** = p <0.01; *** = p <0.001; ns = not significant.

Variable	Phase	t	P(MC)
Protein	Flooding	1.9932	ns
	Recovery	4.3015	**
Carbohydrate	Flooding	2.5330	*
	Recovery	2.1799	ns
Lipid	Flooding	0.6043	ns
	Recovery	2.9405	*
Biopolymeric C	Flooding	3.3091	**
	Recovery	4.6109	**
Chlorophyll-a	Flooding	0.9716	ns
	Recovery	2.2187	*
Phytopigments	Flooding	1.1775	ns
	Recovery	2.0926	ns
Protein to Carbohydrate ratio	Flooding	1.2862	ns
	Recovery	2.3565	*
β-glucosidase activity	Flooding	1.0803	ns
	Recovery	10.4520	***
C turnover time	Flooding	3.1239	*
	Recovery	3.0322	*
Biochemical composition	Flooding	1.6238	*
	Recovery	2.4063	**

5.8 Supplementary figures

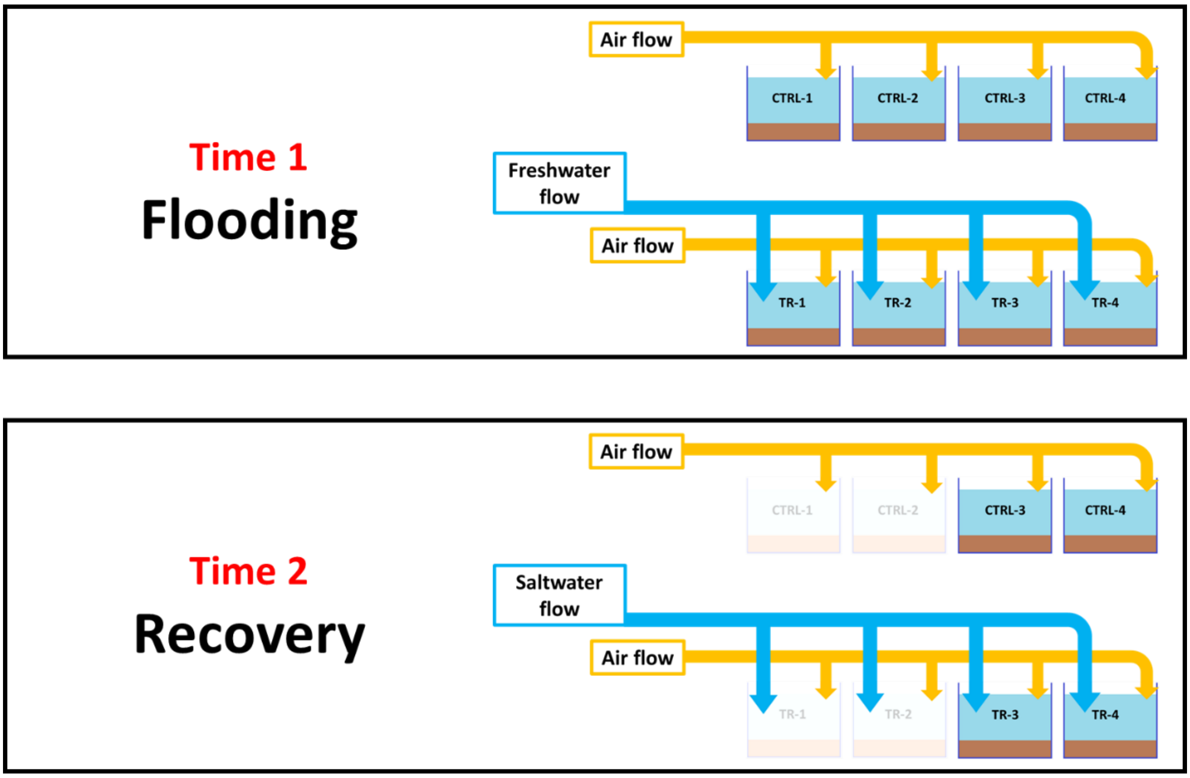
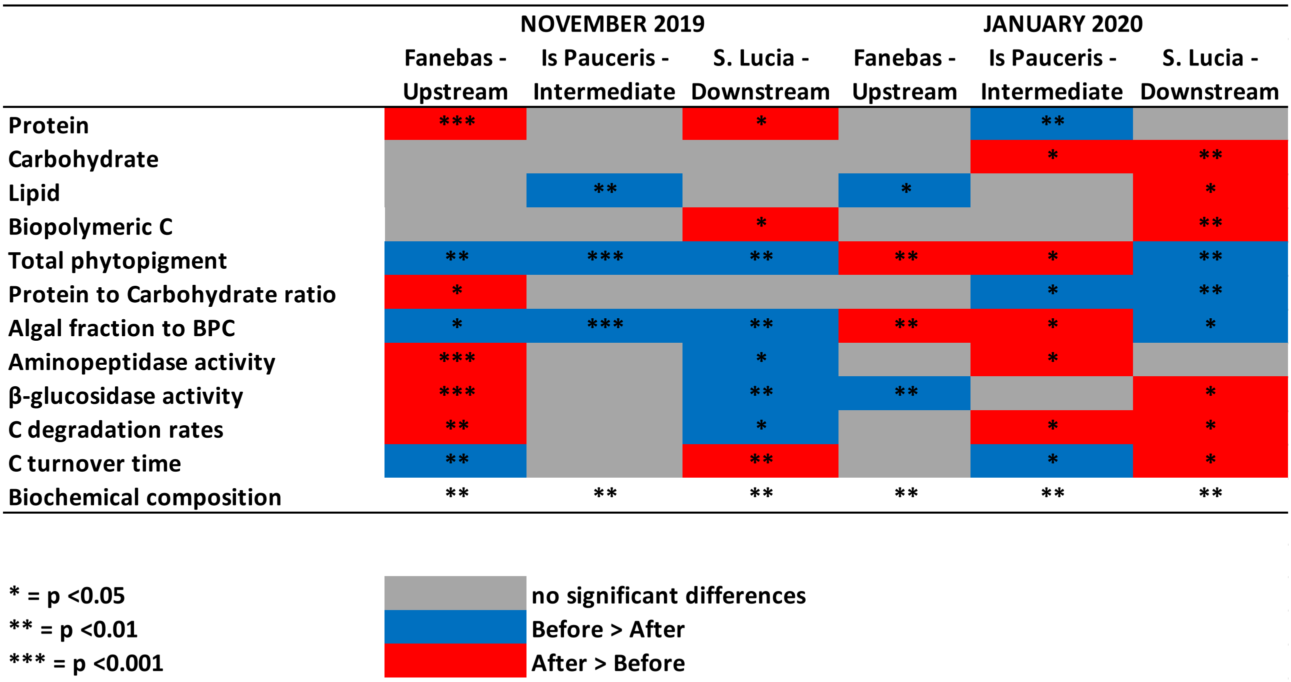


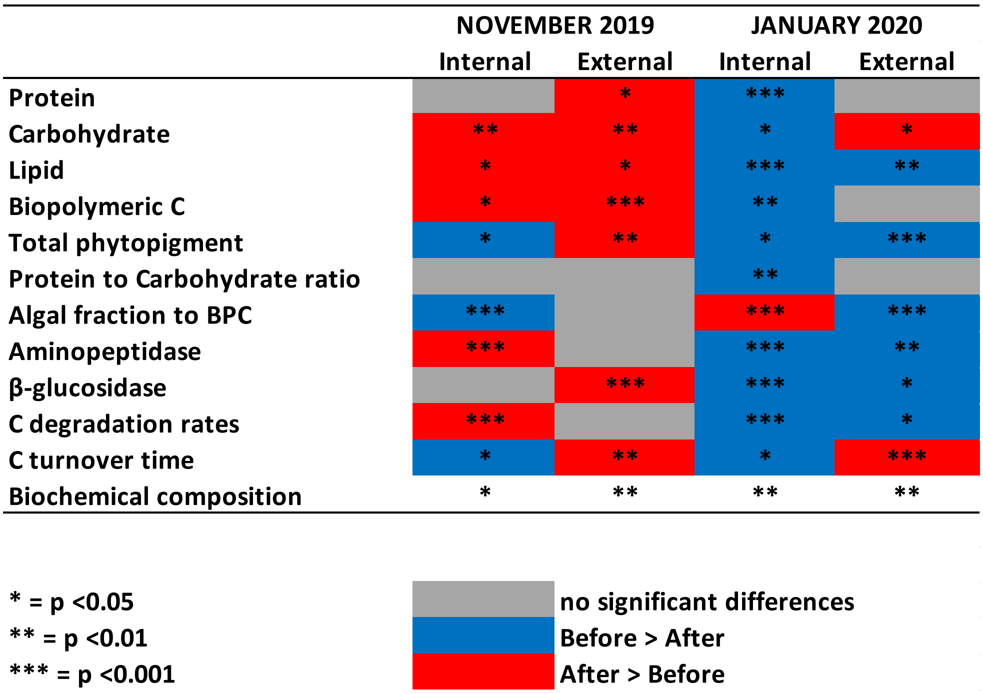
Figure S1. Scheme of the mesocosm experiment.



	NOVEMBER 2019			JANUARY 2020		
	Fanebas - Upstream	Is Pauceris - Intermediate	S. Lucia - Downstream	Fanebas - Upstream	Is Pauceris - Intermediate	S. Lucia - Downstream
Protein	🟥 ***	⬜	🟥 *	⬜	🟦 **	⬜
Carbohydrate	⬜	⬜	⬜	⬜	🟥 *	🟥 **
Lipid	⬜	🟦 **	⬜	🟦 *	⬜	🟥 *
Biopolymeric C	⬜	⬜	🟥 *	⬜	⬜	🟥 **
Total phytopigment	🟦 **	🟦 ***	🟦 **	🟥 **	🟥 *	🟦 **
Protein to Carbohydrate ratio	🟥 *	⬜	⬜	⬜	🟦 *	🟦 **
Algal fraction to BPC	🟦 *	🟦 ***	🟦 **	🟥 **	🟥 *	🟦 *
Aminopeptidase activity	🟥 ***	⬜	🟦 *	⬜	🟥 *	⬜
β-glucosidase activity	🟥 ***	⬜	🟦 **	🟦 **	⬜	🟥 *
C degradation rates	🟥 **	⬜	🟦 *	⬜	🟥 *	🟥 *
C turnover time	🟦 **	⬜	🟥 **	⬜	🟦 *	🟥 *
Biochemical composition	**	**	**	**	**	**

*** = p <0.05**	⬜	**no significant differences**
**** = p <0.01**	🟦	**Before > After**
***** = p <0.001**	🟥	**After > Before**

Figure S2. Pairwise tests identifying significant changes in sedimentary organic matter content, nutritional quality, C degradation rate, C turnover time, and biochemical composition after November 2019 and January 2020 rainfalls in the Santa Lucia River.



	NOVEMBER 2019		JANUARY 2020	
	Internal	External	Internal	External
Protein	▨	🟥 *	🟦 ***	▨
Carbohydrate	🟥 **	🟥 **	🟦 *	🟥 *
Lipid	🟥 *	🟥 *	🟦 ***	🟦 **
Biopolymeric C	🟥 *	🟥 ***	🟦 **	▨
Total phytopigment	🟦 *	🟥 **	🟦 *	🟦 ***
Protein to Carbohydrate ratio	▨	▨	🟦 **	▨
Algal fraction to BPC	🟦 ***	▨	🟥 ***	🟦 ***
Aminopeptidase	🟥 ***	▨	🟦 ***	🟦 **
β-glucosidase	▨	🟥 ***	🟦 ***	🟦 *
C degradation rates	🟥 ***	▨	🟦 ***	🟦 *
C turnover time	🟦 *	🟥 **	🟦 *	🟥 ***
Biochemical composition	*	**	**	**

* = p <0.05	▨	no significant differences
** = p <0.01	🟦	Before > After
*** = p <0.001	🟥	After > Before

Figure S3. Pairwise tests identifying changes in sedimentary organic matter contents, nutritional quality, C degradation rate, C turnover time, and biochemical composition after the November 2019 and January 2020 rainfalls in the Capoterra lagoon.

6. General conclusions and perspectives: a metanalytical approach

6.1 Premise

Sedimentary compartments of aquatic ecosystems can be exposed to an array of single and combined natural and anthropogenic disturbances and perturbations (Humphries and Baldwin, 2003; Murphy and Romanuk, 2012; Harris, 2014; Monk et al., 2019). While the effects of a variety of disturbance sources on benthic biodiversity and, partially, on ecosystem functioning have been repeatedly investigated, the effects on sedimentary organic matter (OM) attributes have been only limitedly addressed. In most cases, information on the effects of disturbance on sedimentary OM are oriented to specific disturbance sources and are also often spatially and temporally fragmented.

While I did not consider in my thesis the effects of spatial or temporal variability of those effects nor their possible interactions, I had the opportunity to investigate the effects of several different sources of disturbance including marine bottom trawling, heat waves, sea warming, rainfalls and floodings. For each of these sources I assessed the effects of each disturbance across levels of perturbation or across a gradient of temperature (mimicking the current climate change). Though being aware of the many potential biases in comparing the outcomes of disturbance on sediments from different aquatic habitats and ecosystems, I applied a metanalytical approach to carry out a comparative analysis of: 1) the effect magnitude of each separate selected disturbance on quantity and nutritional value of sedimentary OM as well as on C degradation rate and turnover time, and 2) the effect magnitude of all selected disturbances on each of the above-mentioned variables.

The general aim of these metanalyses was identifying the strongest effect of each selected disturbance on the tested variables and the most threatening disturbance (among those investigated in my thesis) on sedimentary organic stocks (using biopolymeric C as a proxy for bioavailable organic substrates; Pusceddu et al., 2009), nutritional value (in terms of the algal fraction of biopolymeric C; Pusceddu et al., 2009), C degradation and turnover time (as proxies of benthic ecosystem functioning; Pusceddu et al., 2014).

6.2 Method

Meta-analysis is defined as "*the quantitative synthesis and analysis of a collection of experimental studies*" (Osenberg et al., 1999) or "*the formal application of quantitative methods to summarize evidence across studies*" (Hedges et al., 1999). Considering the numerosity and complexity affecting any set of environmental data, it must be noted that any meta-analysis can be, in turn, affected by several potential pitfalls (e.g., inadequate presentation of data summaries). Nonetheless, I decided to adopt this approach as all the data I collected during my thesis are quantitative, adequately replicated and rigorously based on the presence of either affected or control conditions for each of the investigated disturbance, according to before-after or control-impact sampling designs. Here, I used the effects magnitude to model the differences between disturbed and control conditions (Hedges et al., 1999; Claudet and Fraschetti, 2010). I calculated the effect magnitude with log–response ratios (Hedges et al., 1999) according to the following algorithm:

$$R_i = \ln (XT_i / XC_i)$$

where R_i represents the log–response ratio for the variable *i* (namely: biopolymeric C content, algal fraction of biopolymeric C, C degradation rate and C turnover time), and XTi and XCi are the mean values of the metric for study *i* in treated (T) and control (C) conditions, respectively. XT and XC for each typology of disturbance passed through the metanalysis are described in **Table 1**. The metanalysis has been carried out in two separate sessions to assess:

1) differences among the effect magnitude of each single selected disturbance on biopolymeric C contents, algal fraction of biopolymeric C (not available for the sea warming disturbance), protein to carbohydrate ratio (only for the effects of sea warming), C degradation rates and C turnover time (not available for the sea warming disturbance)
2) differences among the effect magnitude of the selected disturbances (in all experimental conditions) separately for each of the above-mentioned variables (with exception of the algal fraction of biopolymeric C, C degradation rate and turnover time in sediments exposed to sea warming).

In both cases, differences have been assessed by means of permutational paired post-hoc tests, using the PERMANOVA routine included in the PRIMER 6+ package (Anderson et al., 2008). For each of the above-mentioned variables, differences in the effect magnitude among the selected disturbances have been illustrated using forest plots.

6.3 Results and Conclusions

6.3.1 Differential effects of disturbances on different attributes of sedimentary organic matter

The results of the pairwise tests carried out to ascertain paired differences in the effect magnitude of each level of the selected disturbance on the selected variables are reported in **Table S2**. The overall disturbance effect magnitudes of each level of the selected disturbance on each investigated sedimentary OM feature (biopolymeric C content, algal fraction of biopolymeric C, C degradation rates and C turnover time) are illustrated in **Figure 1**.

Table 1. Treatment and Control data for each typology of disturbance included in the metanalysis and source of the data. C-I = control-impact design; B-A = Before-After design.

Disturbance	**Habitat**	**Data from Chapter**	**Typology of the study**	**Treatment (T)**	**Control (C)**
Bottom trawling	Marine	2	C-I in the field	Low or Medium	Null
Heatwaves	Marine	3	Manipulative C-I in the field	Medium or High	Natural temperature
Warming*	Marine oligo-mesotrophic	4	Manipulative C-I in mesocosm	26 (Medium) or 29°C (High)	Natural temperature (23°C)
Warming*	Marine meso-eutrophic	4	Manipulative C-I in mesocosm	26 (Medium) or 29°C (High)	Natural temperature (23°C)
Rainfall	River	5	B-A in the field	Low or High	Before
Rainfall	Lagoon	5	B-A in the field	Low or High	Before
Flooding	Lagoon	5	C-I in mesocosm	Flooding	Null

* The effect magnitude of heat snaps (Experiment 2, Chapter 4) was estimated only for sediments occupied by *H. tubulosa* but not for their feces (calculated separately).

The results of the metanalysis show that, overall, bottom trawling (**Figure 1A**), whatever its intensity, can exert negative effects on OM quantity (in terms of biopolymeric C contents) and nutritional quality (in terms of algal fraction of biopolymeric C) as well as on C degradation and turnover. The analysis shows also that the largest effects are observed on OM quantity and on C degradation rates. This result is aligned with several previous studies who identified bottom trawling as a major threat for quantity and nutritional quality of food available for benthic consumers as well as for benthic ecosystem functioning either in coastal or deep-sea environment (Bhagirathan et al., 2010; Pusceddu et al., 2014). This result, though limited to a very local basin, seriously pose the question of whether this kind of fishery needs further restrictive rules and limitations or should be even prohibited.

Marine heatwaves can exert positive effects on OM quantity, nutritional quality, C degradation (with exception of minor temperature anomalies) and turnover time, with magnitudes of the effect that increase with the increase of the temperature anomaly caused by the heatwave (**Figure 1B**). Marine heatwaves are increasingly being more frequent, intense, and long-lasting across the world oceans (Oliver et al., 2019; Benedetti Cecchi, 2021). Since the effects of marine heatwaves appear to promote a general eutrophication of marine coastal sediments, the results of my analysis anticipate that, net of the effects of other multiple stressors, marine heatwaves will cause not only direct effects on benthic biomass and biodiversity (Garrabou et al., 2022), but will most likely alter sediment biogeochemistry and, ultimately, the food availability for benthic consumers.

The results of the metanalysis show that sea warming can have variable effects on either OM stocks and biochemical composition, the direction of which varies with the background trophic conditions: meso-eutrophic sediments can indeed be characterized by a positive effect of sea warming on biopolymeric C contents and a concurrent decrease in the protein to carbohydrate ratio, whereas oligo-mesotrophic sediments could experience a decrease of organic sedimentary stocks and no variations in its biochemical composition (**Figure 1C**). Sea warming, along with ocean acidification (the so-called "other CO_2 problem"; Doney et al., 2009) is the most evident manifestation of current global change on oceans worldwide (Rogelj et al., 2012; Cheng et al., 2019). Overall, the results of my metanalysis suggest that benthic marine coastal environments characterized by different trophic status could experience opposite changes in response to oceans' warming with oligotrophic ones being most likely exposed to further impoverishment of (more bioavailable) food for the benthos and the eutrophic ones intended to experience a decrease in sedimentary food availability. This generalization, if confirmed by additional study cases, would imply that any action aimed at adapting fisheries or rearing activities of benthic deposit-feeders in the incoming century will need to be modulated differently according to the current benthic trophic status of the interested habitats.

Though the data used in this metanalysis refer to minor rainfall events in a localized hydrographic basin located in Sardinia (central Mediterranean Sea; see Chapter 5 for further details) and the effect magnitudes have been calculated as mean values among river or between lagoon stations (thus smoothing spatial differences of the effects in both habitats), the metanalysis reveals that the effect magnitude of rainfalls on sedimentary OM attributes in river can be far different from that in the adjacent lagoons. The effect magnitude of both rainfalls on OM stocks and degradation rates in the river was positive, with values after the lighter rainfall event higher than that following the heavier one. On the contrary, rainfalls exerted negative effects on the nutritional quality of the organic substrates and a clear acceleration of C turnover (**Figure 1D**). The effect magnitude of rainfalls on the sedimentary OM attributes of the investigated coastal lagoon (**Figure 1E**) appeared quite variable, depending on the intensity of the rainfall: after the lighter precipitation period OM quantity and C degradation rates decreased, whereas after the heavier one they increased. The nutritional quality of organic matter in lagoon sediments was, instead, lowered after the lighter precipitation period but enhanced after the heavier one. Although these results suggest that rainfalls, whatever their intensity, can modify, in the very short-term, either OM quantity or nutritional quality of river and lagoon sediments, the relative exiguity of the study cases investigated to address this topic in my thesis does not allow me to draw any robust conclusion, generalizable at larger spatial or temporal scales.

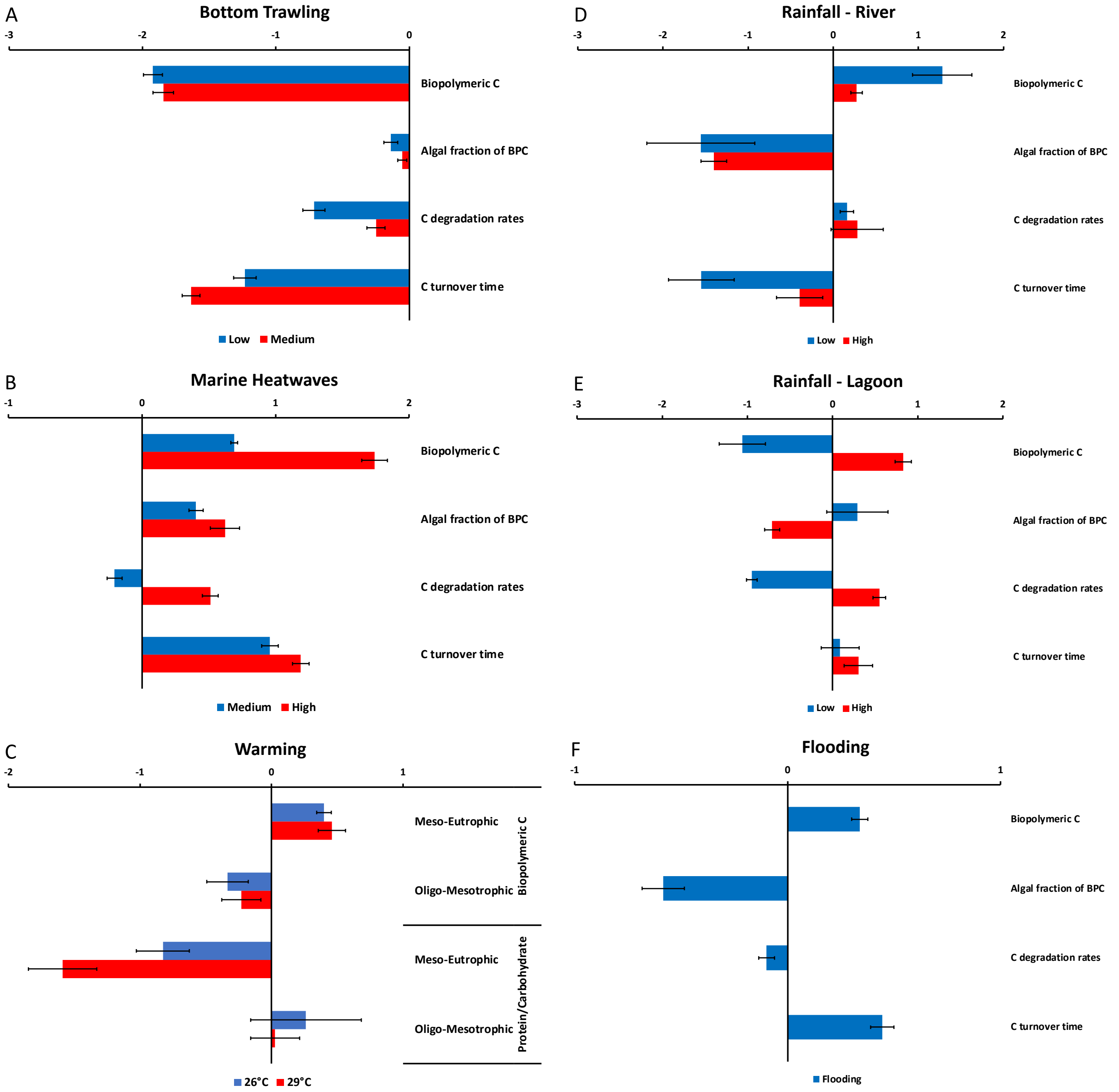


Figure 1. Effect magnitude of different bottom trawling intensities (low vs. medium) (A), marine heatwaves (medium vs. high) (B), warming (26°C vs. 29°C) (C), rainfall (low vs. high) (D), flooding (E) on sedimentary organic matter biopolymeric C, protein to carbohydrate ratio, algal fraction of biopolymeric C, C degradation rates and turnover time. Error bars indicate standard error (n=3).

The metanalysis of the simulated flooding effects on coastal lagoon sediments revealed positive effects on either biopolymeric C contents or C turnover time and negative effects on the OM nutritional quality and C degradation rates (**Figure 1F**). As for marine heatwaves, the intensity of episodic events including flooding, is increasingly exacerbated by current climate change (Trenberth, 2011). Therefore, the results of my study, though based on a simple and temporally limited manipulative experiment in mesocosm, support the anticipation that future, more frequent, and more intense flooding events would not only affect severely the availability of food for benthic lagoon organisms, but could also impair the ecosystem functions of coastal lagoons, thus altering their capacity of buffering the impacts of climatic perturbations before they reach the adjacent coastal marine environments.

6.3.2 Ranking the effect magnitude of different disturbance sources on quantity and nutritional quality of sedimentary organic matter and benthic ecosystem functioning

Aquatic ecosystems worldwide and, more specifically, the world oceans are progressively subjected to a plethora of increasing and most often unregulated sources of natural and anthropogenic disturbances (Jackson et al., 2001; Lotze et al., 2006; Walling, 2006; Boero and Bonsdorff, 2007; Hoegh-Guldberg et al., 2007; Halpern et al., 2008; Pandolfi et al., 2011). All these disturbances have deep consequences on the global biodiversity, that, in turn, determines a global loss of ecosystems ability to function properly (Cardinale et al., 2012), ultimately leading to a severe loss of the goods and services derived from those ecosystems by humans (Worm et al., 2006). The management of marine ecosystems, their ability to produce goods and services for human uses, and any action aimed at protecting integer habitats or restore the altered ones would in principle require spatial information on current impacts and their ecological consequences (Micheli et al., 2013). Nonetheless, current knowledge of the magnitude of the different impacts on sediment biogeochemistry is still far from being fully achieved, which limits a lot the possibility to generalize trajectories of ecosystems change, adaptation or management over broad scales.

The results of the metanalysis carried out here to ascertain differences among the effect magnitude of a (restricted) selection of natural and anthropogenic disturbances on quantity and nutritional quality of sedimentary OM and benthic ecosystem functioning (in terms of C degradation and turnover time; Pusceddu et al., 2014) across different aquatic ecosystems allowed me to outline a first, albeit limited, rank of disturbance criticality.

The results of the pairwise tests carried out to ascertain paired differences in the effect magnitude of different levels of disturbance caused by bottom trawling, marine heatwaves, sea warming, rainfalls and floodings estimated from the data produced in my thesis on sedimentary biopolymeric C contents, nutritional quality, C degradation and C turnover time are reported in **Table S2**. The results of the metanalysis reveal that bottom trawling, whatever the intensity, is the most concerning source of disturbance affecting OM sedimentary contents (**Figure 2A**), showing an absolute effect magnitude higher than that of all other sources of disturbance and, moreover, being able to remove organic substrates. With exception of sea warming in oligo-mesotrophic conditions and low rainfall events in the lagoon, all other sources of disturbance have positive effects on OM quantity, with the largest magnitude associated with severe (high) marine heatwaves and low rainfall events in the river. As anticipated in Chapter 2, these results indicate that the mechanical disturbance of bottom trawling on marine sediments represents a critical source of anthropogenic disturbance which could be able to severely affect benthic trophic webs, ultimately impoverishing coastal sediments and leading them to a forced, unnatural, oligotrophication. All other sources of disturbance investigated here, though with lower effects, cause the increase of sedimentary OM loads, thus potentially contributing to eutrophication processes associated with enhanced nutrient release in the environment, ultimately representing further critical stressors for aquatic ecosystems. On the other hand, the nutritional quality of sedimentary OM (in terms of the algal fraction of biopolymeric C; Pusceddu et al., 2003, 2009) is mostly negatively affected by rainfall events by rainfalls in river, whatever their intensity and mostly positively by marine heatwaves (**Figure 2B**). At a first glance, these results would suggest that the nutritional quality of sedimentary OM can be differently affected by the investigated sources of disturbance in freshwater and marine ecosystems, which can potentially experience a decrease and an increase in food availability for benthic consumers, respectively.

The results of the metanalysis show also that the most threatening sources of disturbance on sedimentary C degradation rates (**Figure 2C**) are represented by low rainfalls in the lagoon and low trawling activities, which both cause a decrease of C degradation process. On the hand, sedimentary C turnover time appears to be mostly affected negatively by bottom trawling, whatever the intensity, and low rainfalls in river, and positively by marine heatwaves (**Figure 2D**).

Though aware that the study cases included in my thesis are just a few tiles of the complex mosaic of the array of natural and anthropogenic disturbances affecting globally the sedimentary biogeochemistry of aquatic ecosystems, and though my metanalyses are based on rigorous Control-Impact and Before-After sampling designs across variable intensities of each investigated disturbance, these results allow me to draw the following conclusions:

1) bottom trawling represents, overall, the most threatening source of disturbance on the sedimentary biogeochemistry of aquatic ecosystems, negatively affecting both OM loads and benthic ecosystem functioning
2) rainfalls in river sediments may, even if in the very short-term, represent a major threat on the nutritional quality of organic substrates for the benthos
3) marine heatwaves can have large effects on the benthic trophic status of coastal marine sediments, representing an additional source of eutrophication and, thus, a major factor altering benthic trophic webs.

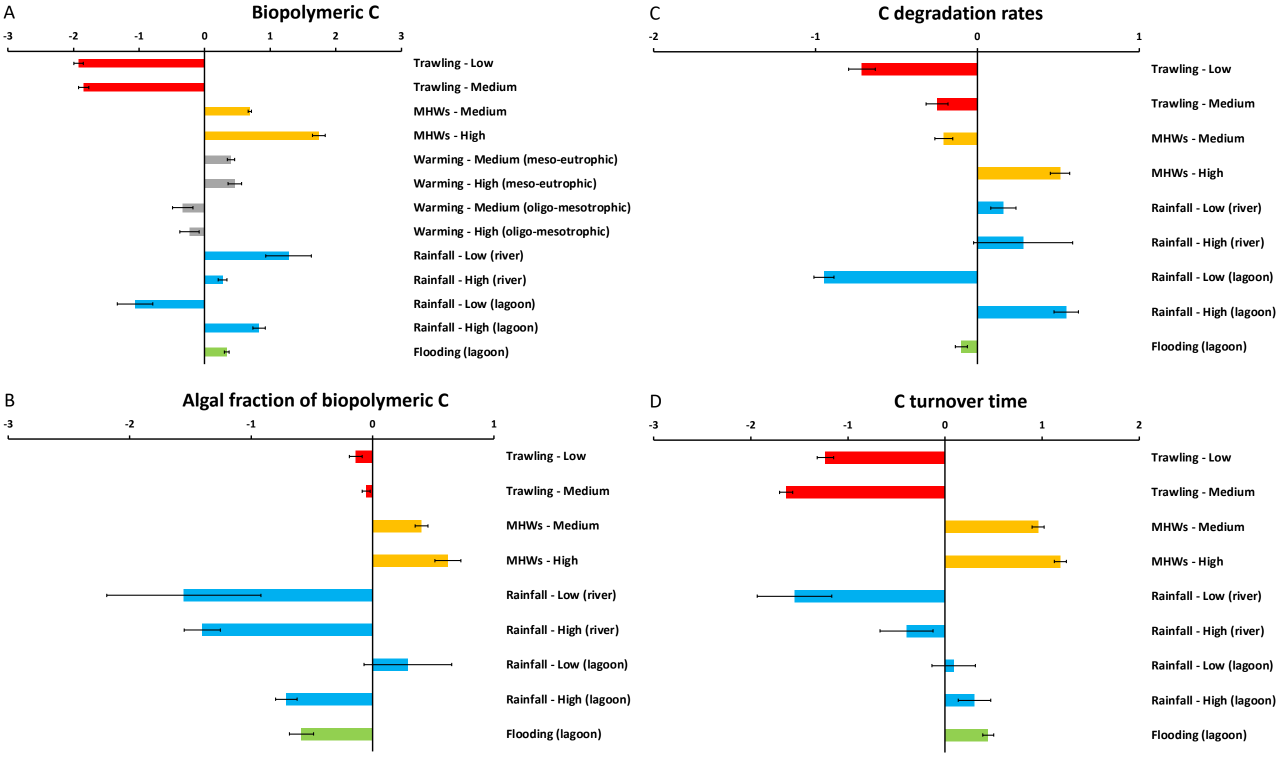


Figure 2. Effect magnitude of bottom trawling (low and medium intensity), marine heatwaves (MHWs; medium and high intensity), sea warming (medium and high thermal shock intensity on meso-eutrophic and oligo-mesotrophic sediments), rainfall (low and high intensity on river and lagoon sediments), and flooding (on lagoon mesocosm sediments) on biopolymeric C (A), algal fraction of biopolymeric C (B), C degradation rates (C), and C turnover time (D). Error bars indicate standard error (n=3). Note that for the sea warming, only effects on biopolymeric C are reported.

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6.5 Supplementary tables

Table S1. Post-hoc test identifying differences in the effect magnitude of the different levels of each typology of selected disturbance on biopolymeric C, protein to carbohydrate ratio (only for the effects of sea warming), algal fraction of biopolymeric C, C degradation rates and C turnover time in the sediments. t = statistic t; P(MC) = probability level after Monte Carlo simulation; * = p <0.05; ** = p <0.01; *** = p <0.001; ns = not significant.

Disturbance	Contrast	Variable	t	P(MC)
Bottom trawling	Low vs. Medium	Biopolymeric C	1.282	ns
		Algal fraction of Biopolymeric C	2.385	ns
		C degradation rates	7.571	**
		C turnover time	6.441	**
Marine Heatwaves	Medium vs. High	Biopolymeric C	18.277	***
		Algal fraction of Biopolymeric C	3.114	*
		C degradation rates	15.194	***
		C turnover time	4.522	*
Warming (sediments)	Medium vs. High (Meso-eutrophic)	Biopolymeric C	0.883	ns
		Protein to Carbohydrate ratio	4.028	*
	Medium vs. High (Oligo-mesotrophic)	Biopolymeric C	0.845	ns
		Protein to Carbohydrate ratio	0.881	ns
Warming (feces)	Medium vs. High (Meso-eutrophic)	Biopolymeric C	6.395	**
		Protein to Carbohydrate ratio	0.410	ns
	Medium vs. High (Oligo-mesotrophic)	Biopolymeric C	8.372	**
		Protein to Carbohydrate ratio	6.097	*
Rainfall (river)	High vs. Low	Biopolymeric C	4.900	**
		Algal fraction of Biopolymeric C	0.406	ns
		C degradation rates	0.668	ns
		C turnover time	4.238	*
Rainfall (lagoon)	High vs. Low	Biopolymeric C	11.392	***
		Algal fraction of Biopolymeric C	4.655	*
		C degradation rates	26.442	***
		C turnover time	1.329	ns
Flooding	Flooding vs. Recovery	Biopolymeric C	6.077	**
		Algal fraction of Biopolymeric C	6.031	**
		C degradation rates	5.173	*
		C turnover time	0.914	ns

Table S2. Post-hoc tests identifying differences in the effect magnitude of pairs of each of the selected disturbances on biopolymeric C content, protein to carbohydrate ratio (only for the sea warming), algal fraction of biopolymeric C, C degradation rates and C turnover time. t = statistic t; P(MC) = probability level after Monte Carlo simulation; * = p <0.05; ** = p <0.01; *** = p <0.001; ns = not significant; ME = meso-eutrophic; OL = oligo-mesotrophic.

	Biopolymeric C		**Protein to Carbohydrate ratio**		**Algal fraction of biopolymeric C**		**C degradation rates**		**C turnover time**	
Groups	**t**	**P(MC)**	**t**	**P(MC)**	**t**	**P(MC)**	**t**	**P(MC)**	**t**	**P(MC)**
TRAWLING (low) vs. TRAWLING (medium)	1.282	ns			2.385	ns	7.571	***	6.441	**
TRAWLING (low) vs. HEATWAVE (medium)	60.140	***			12.575	***	8.801	**	35.903	***
TRAWLING (low) vs. HEATWAVE (high)	53.003	***			10.896	***	20.891	***	39.714	***
TRAWLING (low) vs. WARMING (medium, ME)	44.399	***	9.467	***						
TRAWLING (low) vs. WARMING (medium, OL)	15.920	***	0.254	ns						
TRAWLING (low) vs. WARMING (high, ME)	32.833	***	12.485	***						
TRAWLING (low) vs. WARMING (high, OL)	17.897	***	2.625	ns						
TRAWLING (low) vs. RAINFALL (low, river)	15.575	***			3.853	*	13.393	***	1.389	ns
TRAWLING (low) vs. RAINFALL (high, river)	39.042	***			13.848	***	5.455	**	5.086	**
TRAWLING (low) vs. RAINFALL (low, lagoon)	5.310	**			2.031	ns	3.921	*	9.565	***
TRAWLING (low) vs. RAINFALL (high, lagoon)	40.232	***			9.525	**	19.569	***	14.094	***
TRAWLING (low) vs. FLOODING	48.645	***			6.878	**	11.829	**	28.692	***
TRAWLING (medium) vs. HEATWAVE (medium)	53.626	***			12.715	***	0.807	ns	49.048	***
TRAWLING (medium) vs. HEATWAVE (high)	50.089	***			10.285	***	14.600	**	53.468	***
TRAWLING (medium) vs. WARMING (medium, ME)	40.420	***	13.697	***						
TRAWLING (medium) vs. WARMING (medium, OL)	14.880	***	2.402	ns						
TRAWLING (medium) vs. WARMING (high, ME)	30.760	***	15.845	***						
TRAWLING (medium) vs. WARMING (high, OL)	16.751	***	7.225	**						
TRAWLING (medium) vs. RAINFALL (low, river)	15.134	***			4.096	*	6.888	**	0.385	ns
TRAWLING (medium) vs. RAINFALL (high, river)	35.749	***			15.327	***	2.939	*	7.665	**
TRAWLING (medium) vs. RAINFALL (low, lagoon)	4.797	***			1.634	ns	13.167	***	12.798	**
TRAWLING (medium) vs. RAINFALL (high, lagoon)	37.717	***			11.943	**	13.611	***	18.531	***
TRAWLING (medium) vs. FLOODING	43.596	***			8.820	**	3.363	*	41.677	***
HEATWAVE (medium) vs. HEATWAVE (high)	18.277	***			3.114	*	15.194	***	4.522	*
HEATWAVE (medium) vs. WARMING (medium, ME)	8.192	***	6.991	**						
HEATWAVE (medium) vs. WARMING (medium, OL)	11.146	**	0.651	ns						

HEATWAVE (medium) vs. WARMING (high, ME)	3.754	*	10.424	**						
HEATWAVE (medium) vs. WARMING (high, OL)	10.649	***	0.569	ns						
HEATWAVE (medium) vs. RAINFALL (low, river)	2.919	*			5.327	**	6.656	***	11.128	**
HEATWAVE (medium) vs. RAINFALL (high, river)	10.086	**			19.807	***	2.732	*	8.387	***
HEATWAVE (medium) vs. RAINFALL (low, lagoon)	11.141	**			0.538	ns	15.267	***	6.485	**
HEATWAVE (medium) vs. RAINFALL (high, lagoon)	2.433	ns			18.589	***	13.897	***	6.311	**
HEATWAVE (medium) vs. FLOODING	13.411	***			15.245	***	2.795	*	10.702	**
HEATWAVE (high) vs. WARMING (medium, ME)	20.828	***	8.372	**						
HEATWAVE (high) vs. WARMING (medium, OL)	19.488	***	0.261	ns						
HEATWAVE (high) vs. WARMING (high, ME)	15.689	***	11.613	***						
HEATWAVE (high) vs. WARMING (high, OL)	19.370	***	1.494	ns						
HEATWAVE (high) vs. RAINFALL (low, river)	2.208	ns			5.851	**	6.186	**	12.151	***
HEATWAVE (high) vs. RAINFALL (high, river)	21.655	***			19.025	***	1.268	ns	9.816	**
HEATWAVE (high) vs. RAINFALL (low, lagoon)	16.866	***			1.513	ns	29.315	***	8.206	**
HEATWAVE (high) vs. RAINFALL (high, lagoon)	11.703	**			16.406	***	0.689	ns	8.534	**
HEATWAVE (high) vs. FLOODING	23.454	***			14.211	**	15.143	***	15.530	***
RAINFALL (low, river) vs. RAINFALL (high, river)	4.900	*			0.406	ns	0.668	ns	4.238	*
RAINFALL (low, river) vs. RAINFALL (low, lagoon)	9.178	**			4.376	*	19.279	**	6.369	**
RAINFALL (low, river) vs. RAINFALL (high, lagoon)	2.165	ns			2.281	ns	6.193	***	7.632	**
RAINFALL (low, river) vs. FLOODING	4.648	*			2.614	ns	5.256	**	8.878	**
RAINFALL (high, river) vs. RAINFALL (low, lagoon)	8.288	**			7.498	***	6.833	**	2.377	ns
RAINFALL (high, river) vs. RAINFALL (high, lagoon)	8.263	***			6.891	**	1.465	ns	3.778	*
RAINFALL (high, river) vs. FLOODING	1.427	ns			7.900	***	2.150	ns	5.237	**
RAINFALL (low, lagoon) vs. RAINFALL (high, lagoon)	11.392	**			4.655	**	26.442	***	1.329	ns
RAINFALL (low, lagoon) vs. FLOODING	8.854	**			4.046	*	20.344	***	2.681	*
RAINFALL (high, lagoon) vs. FLOODING	8.310	**			1.621	ns	13.369	***	1.384	ns
WARMING (medium, ME) vs. WARMING (medium, OL)	7.609	**	4.030	*						
WARMING (medium, ME) vs. WARMING (high, ME)	0.883	ns	4.028	*						
WARMING (medium, ME) vs. WARMING (high, OL)	6.899	**	5.368	**						
WARMING (medium, ME) vs. RAINFALL (low, river)	4.317	*	4.129	**						
WARMING (medium, ME) vs. RAINFALL (high, river)	2.464	ns	10.695	***						
WARMING (medium, ME) vs. RAINFALL (low, lagoon)	9.135	***	5.864	**						

WARMING (medium, ME) vs. RAINFALL (high, lagoon)	6.741	**	3.019	*
WARMING (medium, ME) vs. FLOODING	1.555	ns	4.541	*
WARMING (medium, OL) vs. WARMING (high, ME)	7.301	***	6.482	**
WARMING (medium, OL) vs. WARMING (high, OL)	0.845	ns	0.881	ns
WARMING (medium, OL) vs. RAINFALL (low, river)	7.308	**	6.282	**
WARMING (medium, OL) vs. RAINFALL (high, river)	6.180	**	1.062	ns
WARMING (medium, OL) vs. RAINFALL (low, lagoon)	4.015	*	0.416	ns
WARMING (medium, OL) vs. RAINFALL (high, lagoon)	10.966	***	2.782	ns
WARMING (medium, OL) vs. FLOODING	7.204	**	2.121	ns
WARMING (high, ME) vs. WARMING (high, OL)	6.619	**	8.769	***
WARMING (high, ME) vs. RAINFALL (low, river)	3.906	*	1.613	ns
WARMING (high, ME) vs. RAINFALL (high, river)	2.589	ns	13.457	***
WARMING (high, ME) vs. RAINFALL (low, lagoon)	9.073	***	9.115	**
WARMING (high, ME) vs. RAINFALL (high, lagoon)	4.548	**	7.223	**
WARMING (high, ME) vs. FLOODING	1.899	ns	8.542	***
WARMING (high, OL) vs. RAINFALL (low, river)	6.903	**	6.930	***
WARMING (high, OL) vs. RAINFALL (high, river)	5.397	**	4.208	*
WARMING (high, OL) vs. RAINFALL (low, lagoon)	4.667	*	0.760	ns
WARMING (high, OL) vs. RAINFALL (high, lagoon)	10.448	***	3.774	*
WARMING (high, OL) vs. FLOODING	6.457	**	2.497	*

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