



Effects of acidification on the biogeochemistry of unvegetated and seagrass marine sediments

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ABSTRACT

Many studies addressed ocean acidification (OA) effects on marine life, whereas its effects on sedimentary organic matter (OM) have received less attention. We investigated differences in OM features in sediments from unvegetated and seagrass (*Posidonia oceanica*) beds in a shallow hydrothermal area (Aeolian Archipelago, Mediterranean Sea), under natural (8.1–8.0) and acidified (7.8–7.9) conditions. We show that a pH difference of -0.3 units have minor effects on OM features in unvegetated sediments, but relevant consequences within acidified seagrass meadows, where OM quantity and nutritional quality are lower than those under natural pH conditions. Effects of acidified conditions on OM biogeochemistry vary between unvegetated and seagrass sediments, with lower C degradation rates and longer C turnover time in the former than in the latter. We conclude that OA, although with effects not consistent between unvegetated and vegetated sediments, can affect OM quantity, composition, and degradation, thus having possible far-reaching consequences for benthic trophic webs.

1. Introduction

The increase of atmospheric carbon dioxide (CO₂) levels since pre-industrial values has concurred not only to global warming but also to ocean acidification (OA) (the so-called other CO₂ problem; Doney et al., 2009), with ocean pH decreasing by a rate of 0.016–0.020 per decade in the subtropics and 0.002–0.026 per decade in the subpolar and polar zones since the 1980s (Cooley et al., 2022).

OA can cause changes at all hierarchical levels of ecological organization, with effects detectable at physiological, biological, and ecological scales (Hall-Spencer and Harvey, 2019; Doney et al., 2020). OA can cause changes in the biochemical composition of marine organisms (especially primary producers; Rossoll et al., 2012; Jin et al., 2020) and in the composition and diversity of marine communities (Molari et al., 2018; Foo and Byrne, 2021; Zunino et al., 2021; Nagelkerken and Connell, 2022). OA can also alter the relationships between

consumers and their food sources by modifying their nutritional quality (Duarte et al., 2016), as well as nutrient cycling (Molari et al., 2018; Simone et al., 2022). These changes, in turn, may also alter C burial in the ocean bottom, ultimately affecting benthic microbial degradation of organic detritus (Ravaglioli et al., 2019) and thus impacting benthic food webs (Weinbauer et al., 2011).

Sedimentary organic matter (OM) plays a crucial role for the nutrition of benthic communities, especially for deposit-feeders who mostly rely on detrital (non-living) OM and the attached microbial fraction for their food requirements (Lopez and Levinton, 1987, 2011). Indeed, the quantity and the nutritional quality (in terms of biopolymeric C; Fabiano et al., 1995) of sedimentary OM can be used as proxies of the trophic status (i.e., food availability) of coastal marine sediments (Dell'Anno et al., 2002; Pusceddu et al., 2009; Bianchelli et al., 2016). OM nutritional quality (ultimately determined by the variable contribution of labile, semi-labile, and refractory fractions of organic matter; Van

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Oevelen et al., 2011) can also influence benthos growth rates, community respiration, and benthic food webs (Vos et al., 2004; Middelburg and Levin, 2009; Pusceddu et al., 2009; Campyà-Llovet et al., 2017). Therefore, any change in OM nutritional quality and biochemical composition can have far-reaching consequences on the whole ecosystem functioning (Liu et al., 2010).

Seagrasses are among the most productive ecosystems on Earth (Larkum et al., 2006) and are primary sources of pre-eminently refractory OM (Lawrence et al., 1989; Pusceddu et al., 1999). They play a crucial role in sedimentary biogeochemistry, C storage, and nutrient cycling in marine coastal environments (Fourqurean et al., 2012; Duarte et al., 2013; Macreadie et al., 2017). Seagrass meadows are unfortunately in global decline because of many anthropogenic environmental disturbances (Orth et al., 2006; Williams, 2007; Waycott et al., 2009; Giakoumi et al., 2015; Unsworth et al., 2015; Ceccherelli et al., 2018), including OA (Guilini et al., 2017; Seymour et al., 2018; Zunino et al., 2019; Mecca et al., 2020; Berlinghof et al., 2022). Restoration is one of the most examined methods to counteract the decline of seagrass habitats (Fraschetti et al., 2021), but our knowledge of the environmental factors possibly affecting restoration plans are still far from being achieved (Pazzaglia et al., 2021), and this holds true for pH as well.

Volcanic submarine CO₂ vents, characterized by naturally reduced pH of the surrounding waters are ideal model systems to explore the effects of OA at the ecosystem level, because they exhibit a complex set of environmental conditions that cannot be faithfully replicated at the laboratory scale (Foo et al., 2018; Gonzalez-Delgado and Hernandez, 2018; Aiuppa et al., 2021; Rizzo et al., 2022). The island of Panarea (southern Tyrrhenian Sea, Italy), together with its numerous nearby small islets, is the largest shallow hydrothermal system of the Mediterranean Sea and represents an ideal natural laboratory to investigate the effects of OA on benthic habitats (Rogelja et al., 2016; Esposito et al., 2022, and citations therein).

Most of the studies carried out in naturally acidified environments have been to date focused on the effects of acidification on benthic communities and reported progressive simplification of communities' structure and trophic complexity with decreasing pH (Linares et al., 2015; Vizzini et al., 2017; Foo et al., 2018; Esposito et al., 2022; Fanelli et al., 2022). However, only few field studies addressed the effects of OA on benthic biogeochemistry or organic carbon cycling in different types of sediments (Molari et al., 2018; Ravaglioli et al., 2020; Tangherlini et al., 2021). Moreover, to the best of our knowledge, no studies have simultaneously investigated the effects of acidification on the quantity, quality, and degradation rates of organic detritus in unvegetated and vegetated (seagrass) sediments in natural acidified environments.

To shed light on the effects of OA on sedimentary OM quantity, biochemical composition, and degradation rates and the potential role of seagrasses in mitigating or exacerbating these effects (Hendriks et al., 2014; Seymour et al., 2018), with a perspective eye to the potential effects of acidification on the edaphic factors (OM contents, composition and degradation rates) possibly affecting seagrass restoration success, we investigated two coastal areas (Panarea and Bottaro Islands, Mediterranean Sea) both characterized by natural and acidified pH values and the co-occurrence of bottoms unvegetated and vegetated by the seagrass *Posidonia oceanica* L. Delile. At both sites, we investigated differences in quantity, biochemical composition, and degradation rates of OM in sediments characterized by natural (ca. 8.1–8.0; current value) and "acidified" pH (7.8–7.9; consistent with the IPCC high greenhouse gas emissions scenario for 2100) (Gattuso et al., 2015; Abd-Elgawad et al., 2019; Cooley et al., 2022). Specifically, we tested the (null) hypothesis that the amount, biochemical composition, C degradation rates, and C turnover times of sedimentary OM do not differ between current and acidified pH in both vegetated and unvegetated habitats.

2. Materials and methods

2.1. Study area

The island of Panarea lies about 19 km south of the active volcano Stromboli (Espa et al., 2010). The Panarea Volcanic Complex represents the emergent part of a submarine composite volcano, belonging to the Aeolian Arc in the southern Tyrrhenian Sea (Italy), largely dismantled by erosion and mainly submerged (Espa et al., 2010; Esposito et al., 2018). On the eastern side of Panarea, a group of islets (Basiluzzo, Dattilo, Panarelli, Lisca Bianca, Bottaro, Lisca Nera and Formiche), that represent the remnants of a crater rim, form a small archipelago emerging from the submarine platform at the top of the volcano (Italiano and Nuccio, 1991; Esposito et al., 2018). The eastern coast of Panarea is characterized by shallow submarine hydrothermal exhalations that have been active since historical times (Italiano and Nuccio, 1991; Esposito et al., 2006; Maugeri et al., 2010; Vizzini et al., 2010), releasing both gases and thermal waters with temperatures between 40 and 95 °C and pH values between 4.7 and 5.4 (Italiano and Nuccio, 1991; Gugliandolo et al., 2006).

The gas of the fumarolic field is predominantly CO₂ (98 vol%) with traces of H₂S and other gases (Italiano and Nuccio, 1991; Esposito et al., 2006). The CO₂ released as a gas (with traces of H₂S and other gases) from the stable hydrothermal vents creates a pH gradient that encompasses the predicted decrease in sea surface pH of about 0.06 to 0.32 units for the year 2100 (Abd-Elgawad et al., 2019). Such peculiarities make Panarea Island and its adjacent area ideal field laboratories for investigating the effects of OA on sediment biogeochemistry (Rogelja et al., 2016).

Our study was carried out at two distinct sites, about 2.7 Km apart (Fig. 1): one characterized by the presence of a dense meadow of the seagrass *Posidonia oceanica* (hereafter vegetated site; Esposito et al., 2006; Vizzini et al., 2010; Rogelja et al., 2016; Di Bella et al., 2022) within the so-called Bottaro crater, near the Bottaro Islet, and one characterized by incoherent sediments (hereafter unvegetated site), northeast of the Panarea Island.

The vegetated site (38° 38'13.58" N; 15° 6'33.95" E, 8–10 m depth) is characterized by the presence of a crater (Bottaro crater) from which CO₂ constantly escapes giving rise to a pH gradient from the centre of the crater up to 30–35 m distance. The rim of the crater is characterized by large boulders and stones and is colonized by several macroalgae, including *Cystoseira brachycarpa* var. *balearica*, *Codium bursa*, *Flabellia petiolata* and *Lobophora variegata*; a few meters from the rim, patches of the seagrass *Posidonia oceanica* are interspersed with rocky and coarse sand substrate (Gaglioti and Gambi, 2018). The Bottaro crater is a depression (approximately 14 m wide and 20 m long with a mean depth of 11 m) that was created by a massive gas eruption that took place in November 2002 (Caracausi et al., 2004; Esposito et al., 2006). At the Bottaro site, sediments were collected at ca. 7 m from the crater's edge, where the pH was 7.9, and the temperature was ca. 22.6 °C (acidified conditions). Sediments were also collected at approximately 30 m out from the crater's edge, where the pH was about 8.02 and the temperature was 22.7 °C (natural pH conditions).

The unvegetated site (38° 38.536 N; 15° 04.714 E, 10–12 m depth) is characterized a patchy meadow of *Posidonia oceanica* interspersed with clearings of coarse sand substrate (Gaglioti et al., 2019). There, the gasses come from spots of hydrothermal emission zones, recognizable by the presence of yellowish patches, hotspot of thermal water flooding directly from the substrate. The unvegetated site is characterized by highly variable pH (range 5.6–7) and temperature (range 26–60 °C, depending on the proximity to the vent) (Rogelja et al., 2016). There, we collected sediments from patches of incoherent sediments in either acidified (pH 7.85, temperature 23.7 °C) or natural (pH 8.07, temperature 24.6 °C) pH conditions. Information about temporal variability of temperature and pH in the different sites are reported in Appendix A (Table A1).

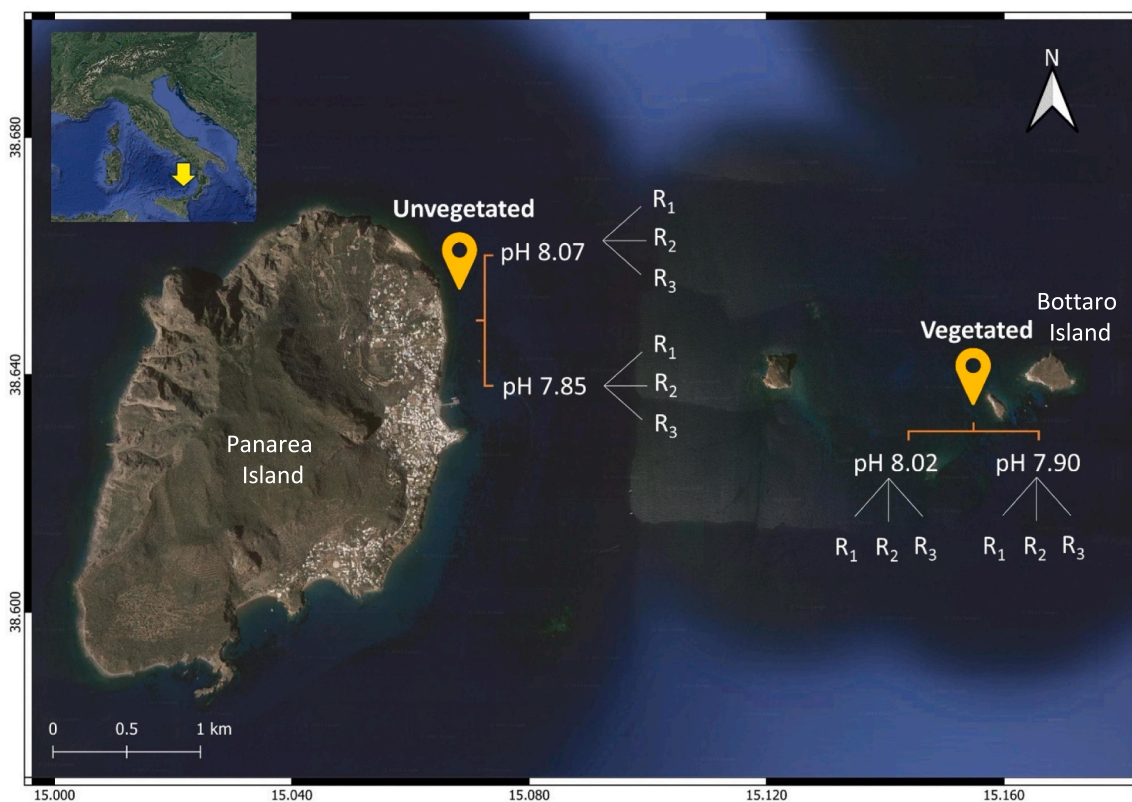


Fig. 1. Study area off the Panarea and Bottaro islands (Aeolian archipelago, Mediterranean Sea) and the sampling design (modified from Google Earth 2021, accessed on 10/29/2021).

The predominant components of the gas emissions in both sampling sites are CO_2 , with an average of 98 vol%, and H_2S , which ranges from 0.4 to 4.0 vol%, and volumes of other gases (i.e., N_2 , H_2 , He, and CH_4) typically very small (0.4 vol%, 1100 vol. ppm, 11 vol. ppm, and 10 vol. ppm, respectively) (Voltattorni et al., 2009; Rogelja et al., 2016).

At both sites, the sediments were generally composed mainly by coarse and medium-coarse sand with negligible contribution of the mud (silt + clay) fraction. A white mat surrounded the areas of thermal waters discharge (Gugliandolo et al., 2006; Rogelja et al., 2016; Di Bella et al., 2022).

During sampling, water samples were collected at the two sampling sites using Niskin bottles manually operated by divers. Each sample was then analyzed at the OGS Panarea ECCSEL NatLab, Italy, to determine the parameters of the carbonate system. Water samples were analyzed for pH (spectrophotometric), total alkalinity (AT, by potentiometric titration in an open cell), inorganic nutrients concentration, and other carbonate system parameters (partial pressure of CO_2 , dissolved inorganic carbon, and saturation state of calcite and aragonite) (Table A2). Temperature was measured during sampling at each site using a data logger (HOBO® Pendant Temperature Logger MX2201).

2.2. Sediment sampling

Samples were collected in October 2020 under different pH conditions: 8.07 vs. 7.85 at the unvegetated site (Panarea) and 8.02 vs. 7.90 at the vegetated one (Bottaro). The decision to use such a restricted sampling design, which does not account for the variability at the scale of each single site and pH conditions, was chosen giving priority to the comparison of sediment biogeochemistry features at similar contrasting values of pH (with similar temperatures) in the two habitats (unvegetated sediments and sediments vegetated by the seagrass *P. oceanica*), which limited the possibility of finding more spatial replicates of the same combination of substrate and pH conditions.

The top 1st centimetre of the sediment was sampled by means of manual plexiglass corers (4.7 cm internal diameter) operated by SCUBA divers at the unvegetated site and, because of the presence of seagrass roots, by gently scraping the surface sediment with a spatula at the site vegetated by *P. oceanica* (Fig. 1).

In all sites, sediment samples were collected in three independent replicates. Once collected, sediments were stored in Petri dishes and 50 mL plastic tubes, respectively, and preserved at $-20\text{ }^\circ\text{C}$ until analysis.

2.3. Biochemical composition of sedimentary organic matter

The contents of chlorophyll-a and phaeopigment (after acidification with 200 μL 0.1 N HCl) in the sediment were determined using fluorometric assays (Danovaro, 2010), after extraction (overnight at $4\text{ }^\circ\text{C}$ in the dark) with 90 % acetone. Total phytopigment content was defined as the sum of chlorophyll-a and phaeopigment concentrations and, once converted into carbon (C) equivalents using $40\text{ }\mu\text{gC }\mu\text{g phytopigment}^{-1}$ as a conversion factor, utilized to estimate the fraction of organic material of autotrophic origin (Pusceddu et al., 2010). Although the C:Chla can vary from 10 to 100 (Cloern et al., 1995), we used the conversion factor proposed in Pusceddu et al. (2009) to allow comparison with other studies carried out in a variety of shallow coastal aquatic environments.

Protein, carbohydrate, and lipid contents were determined spectrophotometrically (Varian Cary 50 UV-VIS spectrophotometer) according to the protocols described 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-Ciocalteu 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-calculated sediments (500 °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). Since phytopigments and proteins are tightly related to the bioavailability of organic matter for heterotrophic nutrition (Pusceddu et al., 2003), both phytopigment and protein contributions to biopolymeric C have been here used as descriptors of the nutritional quality of sedimentary OM.

2.4. Extracellular enzymatic activities, C degradation rates and turnover time of sedimentary organic matter

Extracellular enzymatic activities (aminopeptidase, β-glucosidase) were determined by cleavage of analogue fluorogenic substrates (L-Leucine-4-methylcoumarinyl-7-amide, Leu-MCA; 4-MUF-β-D-glucopyranoside, Glu-MUF, respectively) at saturating concentrations (Danovaro et al., 2005). Measurements were carried out in triplicate by adding to the sediment 100 μL of Glu-MUF or Leu-MCA (final concentration 200 μM) in a final volume of 5 mL filtered (GF/F glass microfiber filter, Whatman™) sterile seawater. Substrate incubations were performed in the dark at in situ temperature for 1 h. After incubation, the sediment slurries were centrifuged (Eppendorf 5804, Eppendorf AG, Germany) at 2500 rpm for 5 min and the supernatants were analyzed fluorometrically (at 380 nm excitation, 440 nm emission for Leu-MCA and 365 nm

excitation, 455 nm emission for Glu-MUF) using a fluorometer (Shimadzu RF-6000). Fluorescence of samples was measured immediately after addition of the substrate and after incubation. The increase of fluorescence units during incubation was converted into activity by comparison with standard curves of 7-amino-4-methylcoumarin for Leu-MCA and of 4-methylumbelliferone for both Glu-MUF. Enzymatic activities were expressed as nmol of substrate hydrolysed g⁻¹ h⁻¹ (Danovaro et al., 2005). Data were normalized to sediment dry weight (60 °C, 24 h) and reported as nmol 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).

2.5. Statistical analyses

To test the null hypothesis by which there are no differences in organic matter quantity, biochemical composition (in terms of protein, carbohydrate, lipid, and phytopigment contents), degradation rates and turnover time between the different pH values in both habitats (unvegetated vs vegetated), permutational analyses of variance (PERMANOVA) (Anderson, 2001) were carried out in either the uni- or multivariate context using 2 fixed and orthogonal factors: habitat ($n = 2$,

Table 1

Results of PERMANOVA testing for differences in sedimentary OM contents, biochemical composition, and nutritional quality between habitats and pH conditions. df = degrees of freedom; MS = mean square; F = statistical F; P(MC) = probability level after Monte Carlo simulations; * = $P < 0.05$; ** = $P < 0.01$; ns = not significant. Reported is also the % of explained variance.

Variable	Source	df	MS	F	P(MC)	% explained variance
Organic matter composition	Habitat (H)	1	1.219	121.0	**	61.9
	pH	1	0.237	23.5	**	11.6
	H x pH	1	0.238	23.7	**	23.4
	Residuals	8	0.010			3.1
	Habitat (H)	1	0.615	237.2	**	47.5
Protein C	pH	1	0.221	85.1	**	16.9
	H x pH	1	0.224	86.4	**	34.3
	Residuals	8	0.003			1.2
	Habitat (H)	1	0.594	120.8	**	64.5
	pH	1	0.016	3.2	ns	1.2
Carbohydrate C	H x pH	1	0.014	2.9	ns	2.1
	Residuals	8	0.005			32.3
	Habitat (H)	1	0.009	3.9	ns	47.8
	pH	1	0.000	0.0	ns	0
	H x pH	1	0.000	0.1	ns	0
Lipid C	Residuals	8	0.002			53.3
	Habitat (H)	1	2.714	270.6	**	87.4
	pH	1	0.125	12.4	**	3.7
	H x pH	1	0.117	11.6	*	6.9
	Residuals	8	0.010			1.9
Biopolymeric C	Habitat (H)	1	0.002	6.5	*	44.9
	pH	1	0.001	1.8	ns	6.2
	H x pH	1	0.000	0.1	ns	0.0
	Residuals	8	0.000			48.9
	Habitat (H)	1	0.013	12.7	*	42.7
Total phytopigment	pH	1	0.009	8.7	*	28.1
	H x pH	1	0.002	2.0	ns	7.3
	Residuals	8	0.001			21.9
	Habitat (H)	1	0.020	1.8	ns	3.1
	pH	1	0.061	5.5	ns	17.6
Algal fraction of biopolymeric C	H x pH	1	0.089	8.1	*	55.7
	Residuals	8	0.011			23.6



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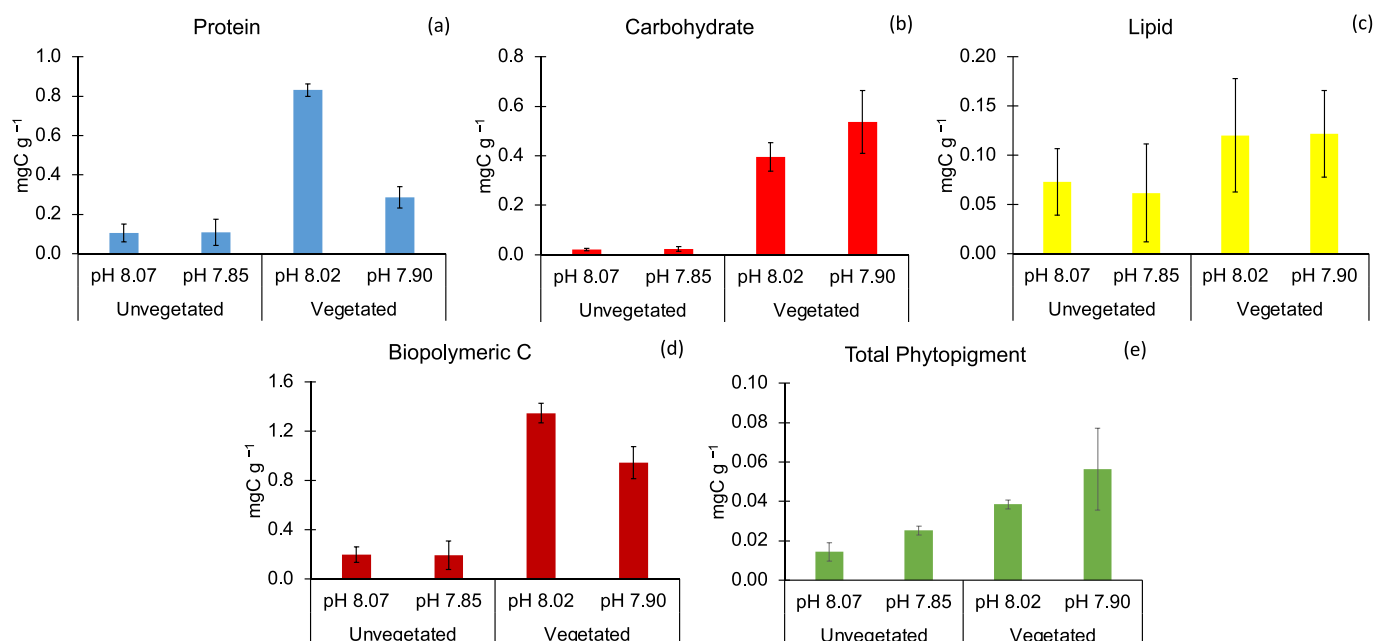


Fig. 2. (a) Protein, (b) carbohydrate, (c) lipid, (d) biopolymeric C, and (e) total phytopigment contents in the top first cm layer of unvegetated and vegetated sediments at the two pH values. Error bars are standard deviations ($n = 3$).

“unvegetated” and “vegetated”), and pH ($n = 2$, “normal” pH 8.02–8.07 for the vegetated and unvegetated sediment respectively, and “acidified” pH 7.85–7.90 for the unvegetated and vegetated sediment respectively). 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, post-hoc pairwise tests were also carried out to ascertain patterns of differences among pH values. Multivariate differences in organic matter biochemical composition (in terms of protein, carbohydrate, and lipid contents) were visualized with a biplot after a canonical analysis of the principal coordinates (CAP), which 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 ascertain the homogeneity of multivariate and univariate dispersion among the data,

PERMDISP tests were also carried out. PERMDISP test is based on the method proposed by Anderson (2006) that uses the ANOVA F statistic to compare (among different groups) the distances from observations to their group centroid and the permutation of appropriate residuals to obtain P -values.

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

3. Results

3.1. Quantity, biochemical composition, and nutritional quality of sedimentary organic matter

Differences in OM sedimentary contents, biochemical composition, and nutritional quality (estimated in terms of algal and protein fractions of biopolymeric C) between habitats and pH conditions are reported in

Table 2

Results of the pairwise test assessing differences in sedimentary organic matter quantity, biochemical composition, and nutritional quality between pH conditions separately for each habitat. t = statistic t ; $P(\text{MC})$ = probability level after Monte Carlo simulations; * = $p < 0.05$; ** = $p < 0.01$; ns = not significant.

Variable	Term	Contrast (pH)	t	$P(\text{MC})$
Organic matter composition	Unvegetated	8.07 vs. 7.85	0.275	ns
	Vegetated	8.02 vs. 7.90	5.621	**
Protein	Unvegetated	8.07 vs. 7.85	0.043	ns
	Vegetated	8.02 vs. 7.90	15.113	**
Carbohydrate	Unvegetated	8.07 vs. 7.85	0.487	ns
	Vegetated	8.02 vs. 7.90	1.754	ns
Lipid	Unvegetated	8.07 vs. 7.85	0.327	ns
	Vegetated	8.02 vs. 7.90	0.040	ns
Biopolymeric C	Unvegetated	8.07 vs. 7.85	0.087	ns
	Vegetated	8.02 vs. 7.90	4.542	*
Total phytopigment	Unvegetated	8.07 vs. 7.85	0.212	ns
	Vegetated	8.02 vs. 7.90	0.577	ns
Algal fraction of biopolymeric C	Unvegetated	8.07 vs. 7.85	2.471	ns
	Vegetated	8.02 vs. 7.90	1.636	ns
Protein fraction of biopolymeric C	Unvegetated	8.07 vs. 7.85	0.258	ns
	Vegetated	8.02 vs. 7.90	11.220	**

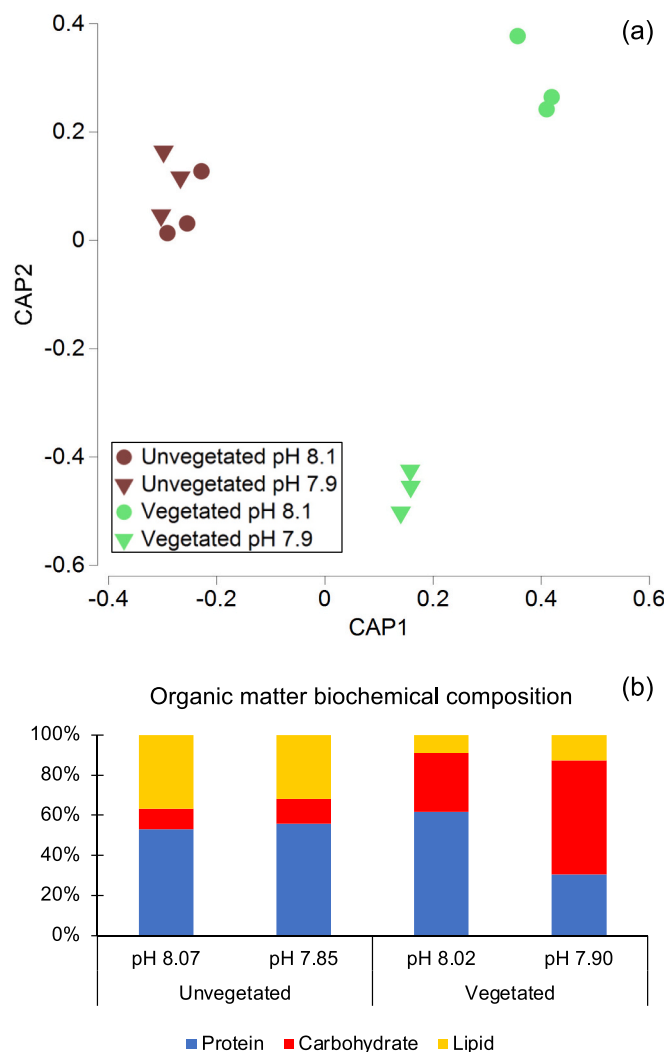


Fig. 3. (a) Biplot obtained after the canonical analysis of the principal coordinates (CAP) illustrating differences in the biochemical composition of organic matter between sediments at different pH in the two habitats and (b) the relative contributions to biopolymeric C of proteins, carbohydrates and lipids in both habitats and pH values.

Table 1.

Overall, total phytopigments, protein, carbohydrate, lipid, and biopolymeric C contents were consistently lower in the unvegetated than in the vegetated sediments, with values 2 to 25 times higher in the latter than in the former (Fig. 2). The differences in protein, carbohydrate, lipid and biopolymeric C sedimentary contents between pH levels were significant only in the vegetated sediments, albeit with some differences for the different organic compounds (Table 2).

More specifically, protein and biopolymeric C contents were 2.9 and 1.4 times higher, respectively, in vegetated sediments at pH 8.07 than at pH 7.9, while carbohydrate and lipid contents did not differ between the two pHs (Fig. 2).

Total phytopigment sedimentary contents were generally higher in vegetated bottoms, whereas in both habitats no significant differences were observed between pH conditions (Fig. 2e, Table 2).

The biochemical composition of sedimentary OM (in terms of proteins, carbohydrates, lipids) differed significantly between the two habitats, and only in the vegetated sediments between the pH conditions (Table 2; Fig. 3a). More specifically, the unvegetated sediments were characterized by higher protein and lipid contents than those observed in seagrass sediments, where the carbohydrate fraction dominated over the protein and lipid ones (Fig. 3b). The results of the PERMDISP routine

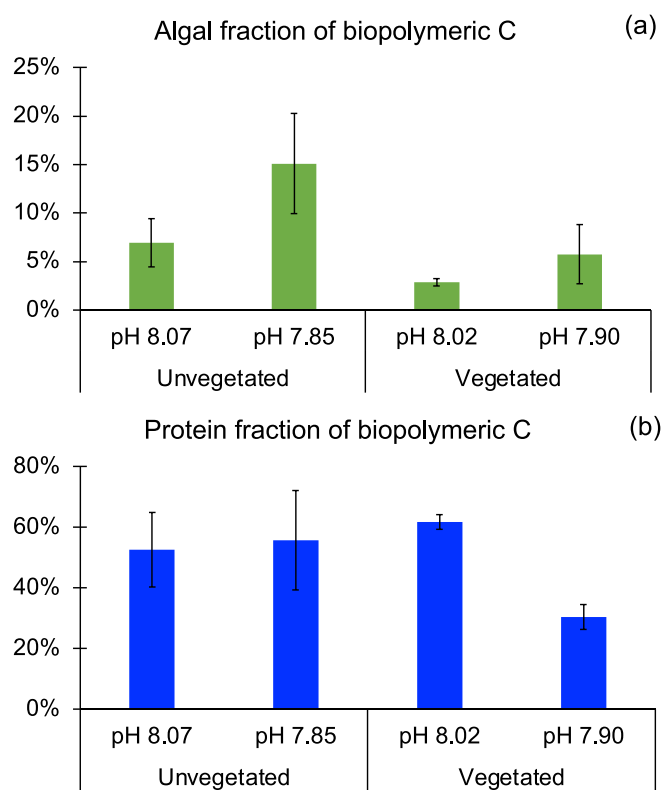


Fig. 4. (a) Algal and (b) protein fractions of biopolymeric C in unvegetated and vegetated sediments at the different pH values. Error bars are standard deviations ($n = 3$). Results of the pairwise tests between pH conditions are not reported as consistently not significant (Table 2).

pairwise tests revealed no significant differences in the OM composition data dispersion between different pH conditions.

In both habitats, the algal fraction of biopolymeric C did not show significant differences between pH values (Fig. 4a). The protein fraction of biopolymeric C did not differ between pH conditions in unvegetated sediments, whereas, in vegetated sediments the values at pH 7.90 were about half those at pH 8.02 (Table 2; Fig. 4b, c).

3.2. Extracellular enzymatic activities, C degradation rate and C turnover time

Differences in extracellular enzymatic activities, C degradation rates and C turnover time between habitats and pH conditions were overall characterized by a significant interaction of both tested factors (Table 3).

Aminopeptidase and β -glucosidase activities were consistently lower in the unvegetated sediments than in the vegetated ones (Fig. 5). Post-hoc tests (Table 4) revealed that in the unvegetated sediments only aminopeptidase activity differed between pH conditions. On the other hand, aminopeptidase activity in the vegetated sediments was about 1.1 times higher at pH 7.90 than at pH 8.02 (Fig. 5a), and β -glucosidase activity at pH 7.90 was about 1.7 times lower than at pH 8.02 (Fig. 5b).

The turnover times of proteins and carbohydrates differed between pH conditions only in the vegetated sediments, where the turnover time of proteins at pH 7.90 was about three times lower than at pH 8.02 (Fig. 6a) and the turnover time of carbohydrates at pH 7.90 was about two times higher than at pH 8.02 (Fig. 6b; Table 4).

Differences between pH conditions in cumulative rates of carbon (C) degradation (expressed as the sum of protein and carbohydrate degradation rates) and cumulative carbon (C) turnover time were significant in both sediments (Table 4). In unvegetated sediments, the C degradation rate at pH 7.85 was about half that at pH 8.07, while the C turnover time at pH 7.85 was about twice that at pH 8.07 (Fig. 6c,d). In vegetated

Table 3

Results of PERMANOVA testing for differences in extracellular enzymatic activities, C degradation rates and turnover time between habitats and pH conditions and their interactions. df = degrees of freedom; MS = mean square; F = statistical F; P(MC) = probability level after Monte Carlo simulations; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; ns = not significant. Reported is also the % of explained variance.

Variable	Source	df	MS	F	P(MC)	% of explained variance
Aminopeptidase	Habitat (H)	1	220.87	145.9	**	49.3
	pH	1	26.78	17.7	**	5.7
	H x pH	1	97.29	64.3	**	43.0
	Residuals	8	1.15			2.0
β -glucosidase	Habitat (H)	1	4.63	65.5	**	65.6
	pH	1	0.70	9.9	*	9.1
	H x pH	1	0.74	10.4	**	19.2
	Residuals	8	0.07			6.1
Protein turnover time	Habitat (H)	1	336.73	91.1	***	29.2
	pH	1	135.73	36.7	***	11.6
	H x pH	1	329.75	89.2	***	57.2
	Residuals	8	3.70			1.9
Carbohydrate turnover time	Habitat (H)	1	53382	419.2	**	64.4
	pH	1	10166	79.8	**	12.1
	H x pH	1	9432	74.1	**	22.5
	Residuals	8	127			0.9
C degradation rate	Habitat (H)	1	1.50	182.1	**	58.6
	pH	1	0.19	22.8	**	7.1
	H x pH	1	0.42	51.1	**	32.4
	Residuals	8	0.01			1.9
C turnover time	Habitat (H)	1	1209.20	245.2	**	71.3
	pH	1	47.50	9.6	*	2.5
	H x pH	1	211.80	42.9	**	24.5
	Residuals	8	4.90			1.8

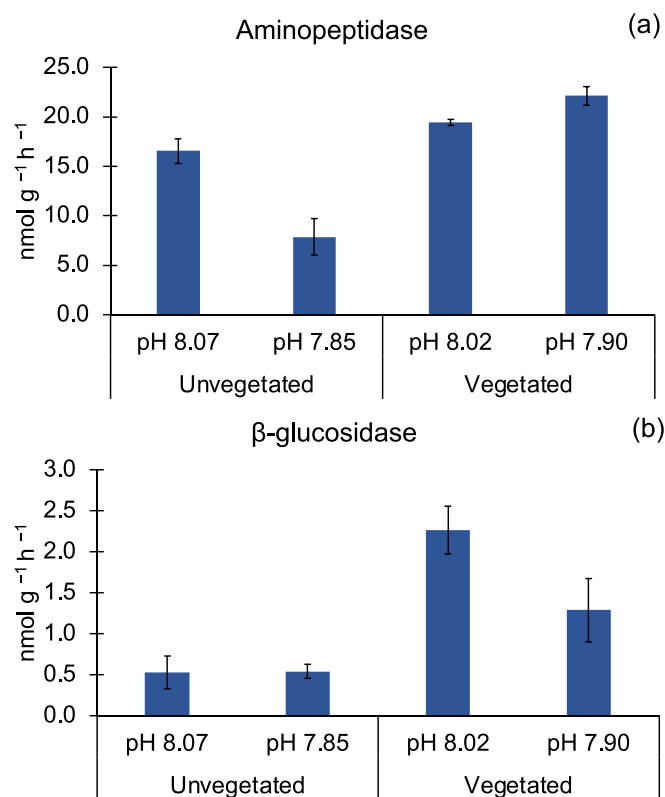


Fig. 5. (a) Aminopeptidase, and (b) β -glucosidase (expressed as $\text{nmol C g}^{-1} \text{h}^{-1}$) activity in the top first cm of unvegetated and vegetated sediments at the two pH values. Error bars are standard deviations ($n = 3$).

sediments, the C degradation rate at pH 7.90 was about 1.1 times higher than at pH 8.02 (Fig. 6c), and the C turnover time at pH 7.90 was about 1.6 times lower than at pH 8.02 (Fig. 6d).

4. Discussion

4.1. Effects of acidification on OM contents

Our results reveal that pH conditions lower than those currently encountered in non-vent systems could significantly affect contents, nutritional quality, and degradation of sedimentary organic matter especially in marine seagrass-vegetated habitats where *P. oceanica* is the dominant species.

Although, as expected, the sediments colonized by the seagrass exhibited biopolymeric C contents almost one order of magnitude higher than the unvegetated bottoms (Pusceddu et al., 2007), both habitats, regardless of pH conditions, were characterized by meso-oligotrophic

Table 4

Results of the pairwise PERMANOVA tests assessing differences in extracellular enzymatic activities, protein, carbohydrate and C degradation rates and turnover times between pHs separately for each habitat. t = statistic t ; $p(\text{MC})$ = probability level after Monte Carlo simulations; * = $p < 0.05$; ** = $p < 0.01$; ns = not significant.

Variable	Term	Contrast (pH)	t	P(MC)
Aminopeptidase	Unvegetated	8.07 vs. 7.85	6.667	**
	Vegetated	8.02 vs. 7.90	4.722	**
β -Glucosidase	Unvegetated	8.07 vs. 7.85	0.097	ns
	Vegetated	8.02 vs. 7.90	3.509	*
Protein turnover time	Unvegetated	8.07 vs. 7.85	1.161	ns
	Vegetated	8.02 vs. 7.90	16.331	**
Carbohydrate turnover time	Unvegetated	8.07 vs. 7.85	0.239	ns
	Vegetated	8.02 vs. 7.90	12.050	**
C degradation rate	Unvegetated	8.07 vs. 7.85	6.491	**
	Vegetated	8.02 vs. 7.90	2.992	*
C turnover time	Unvegetated	8.07 vs. 7.85	2.616	*
	Vegetated	8.02 vs. 7.90	6.406	**

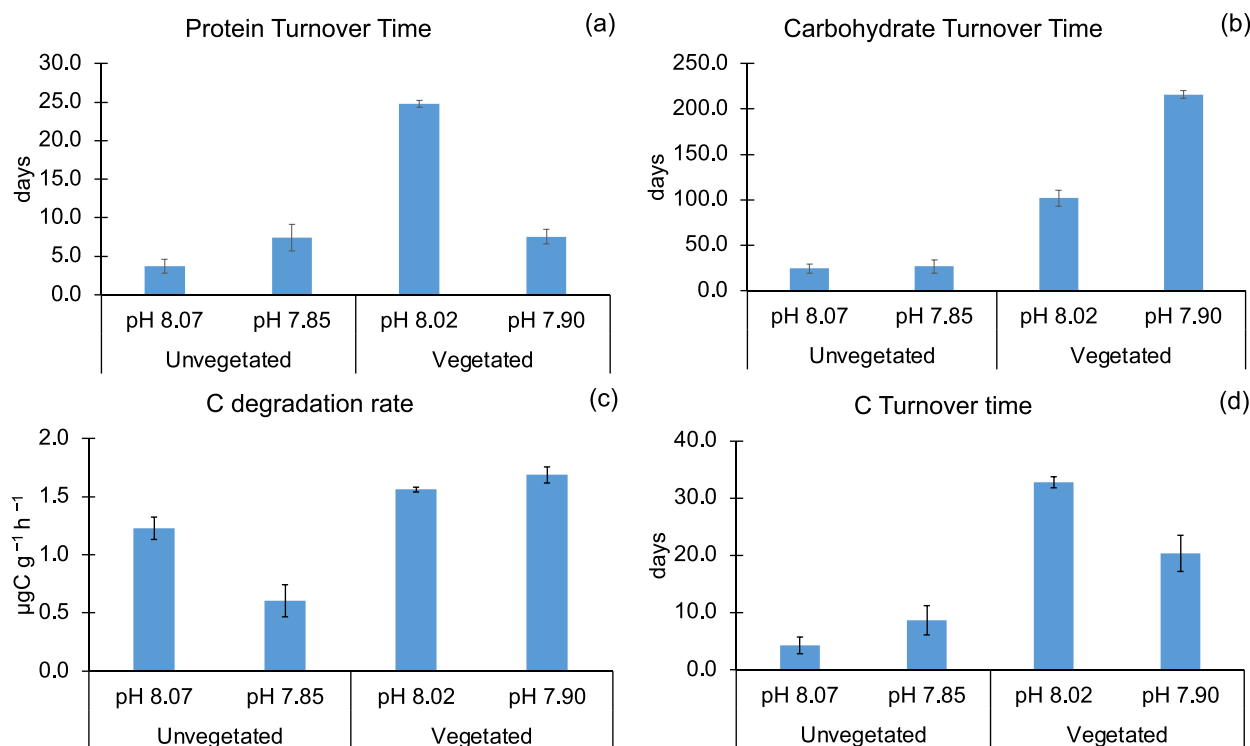


Fig. 6. (a) Protein and (b) carbohydrate turnover time (expressed in days), and (c) C degradation rates (expressed in $\mu\text{gC g}^{-1}\text{h}^{-1}$) and (d) C turnover time (days) in the top first cm of unvegetated and vegetated sediments at the two pH values. Error bars are standard deviations ($n = 3$).

conditions (according to the classification proposed by Dell'Anno et al., 2002 and Pusceddu et al., 2011) with biopolymeric C contents ranging 0.1–0.3 and 0.9–1.4 mgC g^{-1} in unvegetated and vegetated sediments, respectively.

We show here that in unvegetated sediments the potential influence of lower pH values on organic matter content, whatever the class of organic compounds, was almost nihil. On the other hand, those exposed to acidified pH values showed lower biopolymer C concentrations compared to vegetated sediments under natural pH conditions. This was mainly due to a noticeably lower protein content, while the amounts of carbohydrates, lipids, and phytopigments remained unaltered. Accordingly, the overall biochemical composition of the vegetated sediments under acidified conditions differed remarkably from that under natural pH conditions. At a first glance, these results would suggest that decreasing pH (as expected under OA) could have no significant effects on organic loads and biochemical composition in unvegetated sediments, but variable effects on the different classes of organic compounds in sediments colonized by the seagrass *P. oceanica*. Causes for such a different pattern between vegetated and unvegetated sediments are difficult to be ascertained and could also be related to other environmental covariates characterizing the two habitats, but unaccounted in this study. One hypothesis could be that different patterns of organic loads under different pH conditions in the two habitats are related to the different pH gap between ambient and acidified conditions in each of the two habitats. This, however, was much lower in vegetated ($\Delta\text{pH} = 0.12$) than in unvegetated ($\Delta\text{pH} = 0.22$) sediments, suggesting that organic loads in the more acidified bare sediments would have not been affected at all, whereas the decrease in the protein sedimentary content appears to be peculiar of less acidified seagrass sediments only. Nevertheless, in this regard, other short-term (20-weeks) mesocosm experiments conducted in unvegetated sediments reported higher protein and biopolymeric C contents with the increase in CO_2 concentration and the duration of exposure (Rastelli et al., 2015, 2016), suggesting that lower pH conditions would not always deplete, but even enhance organic loads. Such a pattern has also been reported in field studies conducted in

other natural acidified and unvegetated sediments (Tangherlini et al., 2021). Determining the reasons of such discrepancy between our results and those available in the literature is challenging and would require more investigation. Indeed, CO_2 emissions, and consequently the environmental pH values in marine sediments, vary over space and time (Queiros et al., 2014; Molari et al., 2018; Tangherlini et al., 2021). Such pH fluctuations could interfere with fluctuations of other environmental covariates (e.g., sediment granulometry, gas composition of the hydrothermal emissions, temperature and so on), making more difficult the disentanglement of pH effects from those of other contingent covariates. For these reasons, we cannot exclude that the observed differences in BPC contents among sediments exposed to different pH values could be ascribed to other covariates, not considered here, that may have acted as confounding factors and contributed to shaping the observed patterns.

The differences in BPC contents in vegetated sediments exposed to different pH values reported here are consistent with previous observations showing lower levels of organic C in naturally acidified superficial sediments populated by the seagrass *Cymodocea nodosa* (Vizzini et al., 2019). These results could be also ascribed to the direct negative effects of low pH on the biology of the seagrass. This, in turn, could hamper the ability of these plants to efficiently trapping organic particles and, consequently, limit C accumulation in sediments (Vizzini et al., 2019). Whatever the mechanism behind the decrease in organic C content in seagrass sediments, these results would suggest that a further decrease in oceans' pH will specifically impact seagrass habitats, unless adaptive mechanisms occur that are to date unpredictable. In this regard, it is noticeable that, in the same study area of our investigation, *P. oceanica* has, most likely, adaptively shifted to a dwarf "bonsai" size (Gambi et al., 2023). This, on the one hand, could explain the similar effects of acidification that we and other authors (Vizzini et al., 2019) observed in the sediment of both species, and, also, provide support to the decreased capacity of undersized seagrass shoots to trap and retain organic particles.

4.2. Effects of acidification on biochemical composition and nutritional quality of sedimentary OM

Our results, which confirm previous findings obtained either under relatively pristine conditions (Danovaro et al., 1994; Pusceddu et al., 1999) or under other anthropogenic impacts (e.g., Pusceddu et al., 2007), also revealed that the biochemical composition of seagrass sediments differs significantly from that in unvegetated sediments. Such differences are mostly related to the greater input of structural carbohydrates released by the seagrasses (Danovaro, 1996; Cresson et al., 2019) compared to unvegetated sediments.

As for the sedimentary contents of OM, the biochemical composition of unvegetated sediments did not differ between natural and acidified conditions, indicating that the food availability for a model benthic detritus-feeder would be not affected by a pH decrease of about 0.3 units. This, instead, is not the case for seagrass sediments, where lower pH values are associated with a sedimentary OM biochemical composition and, consequently, nutritional quality far different from those observed under natural pH conditions. We report here, indeed, that acidified seagrass sediments are characterized by a lower protein contribution to BPC, mostly compensated by an increased contribution of carbohydrates. This is also evident from the values of the protein to carbohydrate ratio, which in acidified seagrass sediments was about one third of that in sediments at natural pH (0.45 ± 0.14 and 1.74 ± 0.28 , respectively). Seagrass sediments are characterized by organic detritus that mostly originates from the plant itself (leaves, rhizomes, sheaths), and hence it reflects plant's biochemical composition (Kaal et al., 2016). Therefore, we can hypothesize that the differences in the biochemical composition between acidified seagrass sediments and seagrass sediments exposed to natural pH could be the result of compositional modifications of the seagrass, directly caused by acidification.

This hypothesis is corroborated by previous studies suggesting that a further decrease in pH could drive the biochemical composition and the nutritional characteristics of marine primary producers (Duarte et al., 2016; Hernán et al., 2016; Scartazza et al., 2017; Cresson et al., 2019; Jin et al., 2020). In this regard, it must also be noticed that altered C:N ratios, lipid metabolism and fatty acid composition, characterizing seagrasses exposed to OA (Jiang et al., 2010; Rossoll et al., 2012; Campbell and Fourqurean, 2013; Scartazza et al., 2017; Cresson et al., 2019), causing changes in their palatability, may even influence herbivores' feeding behavior (Hernán et al., 2016) and grazing pressure (Apostolaki et al., 2014; Donnarumma et al., 2014). Nevertheless, we also notice that the lower protein contribution to BPC and the lower values of the protein to carbohydrate ratios in acidified seagrass sediments are likely not attributable to effects on microphytobenthos production, as total phytopigment contents did not differ between acidified seagrass sediments and seagrass sediments at natural pH. Since nitrogen is the most limiting element for heterotrophic nutrition and proteins are the richest in N among the classes of organic compounds, the contribution of proteins to BPC and the values of the protein to carbohydrate ratio are often used as proxies for the nutritional quality of organic detritus (Pusceddu et al., 2009, 2010). Accordingly, our results suggest that seagrass sediments exposed to OA, differently from the unvegetated ones, could experience a decreased food quality. As acidified seagrass sediments appear to be poorer in OM contents of a nutritionally poorer quality, we anticipate that, under future scenarios of oceans pH, a model benthic deposit-feeder, according to the optimal foraging theory (Stephens and Krebs, 1986), would be forced to ingest larger amounts of sedimentary organic matter to fulfil its energetic demand. Nevertheless, we are not, at the present stage, able to predict which will be the effects on energy budget allocation and life histories of organisms due to changes of organic matter content and nutritional quality of more acidified sediments. We acknowledge, however, that such an aspect deserves further investigations. Also, although we cannot project the consequences of these responses at larger spatial scales, we can hypothesize that seagrass habitats, particularly vulnerable to the

detrimental consequences of decreasing pH, would not only be exposed to the direct effects on the production, metabolism, and physiology of the plants, but also by the potentially severe indirect effects on the trophodynamics of the benthos inhabiting seagrass sediments.

4.3. Effects of acidified conditions on enzymatic activities, C degradation rates and turnover time

Marine sediments, particularly the nearshore ones, are a large and important global C sink and the main site of OM degradation (Atwood et al., 2020; Lønborg et al., 2020). Degradation of organic detritus mediated by extracellular enzymatic activities produced by prokaryotes is a key-step of C degradation processes (Arnosti, 2011). Enzymatic activities, including either proteases or glucosidases, have been repeatedly used as proxies of ecosystem functioning in relation with biodiversity (Danovaro et al., 2008) or in response to anthropogenic impacts (Pusceddu et al., 2014).

Enzymatic activities exhibit a strong pH dependency because changes in hydrogen ion concentration can modify either the three-dimensional structure of their active sites (Tipton et al., 2009; Yamada and Suzumura, 2010; Endres et al., 2014) or the redox state of cofactors, which can influence any enzymatic reaction at large (Celussi et al., 2017). On this basis, we hypothesized that sediments exposed to lower pH conditions might show different rates of enzymatic activities than those under current pH and that, ultimately, sedimentary C degradation rates could be modified by the projected acidification of the oceans (Weinbauer et al., 2011).

Overall, our results showed that the effects of lower pH values on enzymatic activities in unvegetated and seagrass vegetated sediments differed either between the two enzymes or between the two habitats.

We report here that in unvegetated sediments while β -glucosidase activity was apparently not affected by acidification, the aminopeptidase activity was significantly lower under acidified conditions than in sediments at natural pH. This result was consistent with the results of previous studies carried out in mesocosm experiments (Rastelli et al., 2015, 2016; Celussi et al., 2017), which suggested that OA could impair N-rich organic compounds degradation. Moreover, since the protein turnover time in acidified unvegetated sediments resulted higher than that in sediments exposed to natural pH, our results let us infer also that OA could enhance the accumulation in the sediment of N-rich organic compounds, which, triggering a positive feedback mechanism, could further inhibit aminopeptidase activities (Dell'Anno et al., 2008).

Patterns of enzymatic activities in seagrass sediments were rather different from those observed in unvegetated sediments. Indeed, acidified seagrass sediments were characterized by lower β -glucosidase and higher aminopeptidase activities than those in sediments exposed to natural pH conditions. The apparent stimulation of aminopeptidase activity under acidification in seagrass sediments, contrasting with the observed negative effects in unvegetated sediments, is consistent with other studies (Ravaglioli et al., 2020). Such a stimulation, which does not hold true for β -glucosidase, could be likely ascribable to the seagrass metabolism, which could create patches of microenvironments where acidification is partly buffered by the effects of photosynthesis on pH (Hendriks et al., 2014; Guilini et al., 2017; Ricart et al., 2021; Di Bella et al., 2022). On the other hand, the null (in unvegetated sediments) or negative (in seagrass sediments) effects of acidification on β -glucosidase activity do not get consistent feedback in other studies, in which either low or no response as well as either increasing or decreasing patterns have been observed (Grossart et al., 2006; Yamada and Suzumura, 2010; Sala et al., 2016; Celussi et al., 2017; Ravaglioli et al., 2020; Saïdi et al., 2023). The apparently idiosyncratic response of β -glucosidase activity to OA could be the result of other environmental covariates, more effective than acidification.

As a cumulative consequence of the different effects of OA on the extracellular enzymatic activities in the two habitats, we report here also variable effects of acidification on the C degradation rates and turnover

time between habitats. In acidified unvegetated sediments, C degradation rates and C turnover time resulted lower and higher, respectively, than those in unvegetated sediments exposed to natural pH. The opposite pattern was instead observed in seagrass sediments, where C degradation rates and C turnover time resulted higher and lower, respectively, than those in seagrass sediments exposed to natural pH. These results pinpoint that OA, even under a small pH reduction of 0.3 units, could have major impacts on the C degradation rates in either unvegetated bottoms or seagrass sediments, impairing ecosystem functioning in unvegetated bottoms, but boosting it in seagrass sediments.

5. Conclusions and perspectives

Anthropogenic ocean acidification, resulting from the global increase in atmospheric CO₂ concentrations, is one of the greatest threats to marine habitats (Cooley et al., 2022). Here we observed that changes in organic matter features of sediments exposed to lower pH conditions could be of relevance for benthic trophic webs and, overall, ecosystem functioning.

We showed that the effects of pH on organic matter contents, composition, nutritional quality, degradation rates and turnover time can vary between marine benthic habitats (unvegetated sediments vs. seagrass sediments). Overall, unvegetated sediments exposed to acidified pH conditions are invariant respect to OM contents, biochemical composition, and nutritional value for secondary consumers, but also by decreased C degradation rates and increased C turnover time. Acidified seagrass sediments, instead, are characterized by decreased contents of nutritionally poorer organic matter, increased C degradation rates and decreased C turnover time.

Therefore, whatever the benthic habitat, our results, though limited to a very local situation and despite we cannot exclude that our results could be also partially influenced by other environmental covariates (Apostolaki et al., 2014; Ravaglioli et al., 2020), suggest that a further decrease of pH (as that expected under future climate scenarios) will not only directly and indirectly alter marine ecosystems productivity and biodiversity (Linares et al., 2015; Molari et al., 2018, 2019; Vizzini et al., 2017, 2019), but would also modify the food availability for benthic deposit-feeders as well as patterns of C cycling, thus ultimately altering the structure and functioning of benthic trophic webs. The effects of decreased pH on organic matter features documented here appear also larger in seagrass than unvegetated sediments.

The quantity and quality of organic detritus depends on the development of a microbial community that can modulate the subsurface nutrient cycling and chemical processes of plants (Elgersma et al., 2012; Kourtev et al., 2003; Wolfe and Kironomos, 2005), including seagrasses (Fuggie et al., 2023). As a corollary, we could infer that altered sediment biogeochemistry caused by lower pH values could, indirectly, modify the responses and adaptation of transplanted seagrasses during restoration actions. Restoration experiences of seagrass meadows are widely spread in oceans worldwide (Fraschetti et al., 2021), but, to date, the actual effects of climate change on the efficacy of restoration actions have been mostly concentrated on the effects of oceans warming on plants and unvegetated sediments (Stipich et al., 2022; Soru et al., 2022), while those assessing the effects of decreasing pH on seagrass sediments and ecosystem functioning are still far from being fully achieved. Although in our study we focused only on the effects of acidified conditions on sediment biogeochemistry, we stress here the need of investigating oceans' acidification influence on the effectiveness and success of restoration practices.

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CRedit authorship contribution statement

Santina Soru: Formal analysis, Writing – original draft, Writing – review & editing. **Manuel Berlino:** Investigation, Writing – review & editing. **Gianluca Sarà:** Conceptualization, Funding acquisition, Resources, Writing – review & editing. **Maria Cristina Mangano:** Investigation, Writing – review & editing. **Cinzia De Vittor:** Investigation, Writing – review & editing. **Antonio Pusceddu:** Conceptualization, Funding acquisition, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2023.115983>.

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