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PRIMARY RESEARCH PAPER

Effects of rainfall-induced torrential freshwater injections on river and lagoon sediments biogeochemistry

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Abstract We investigated changes in sediment grain size, elemental (total organic C, TOC; total N, TN), isotopic ($\delta^{13}C$, $\delta^{15}N$), biochemical composition (proteins, carbohydrates, lipids, phytopigments), nutritional quality, potential degradation of sedimentary organic matter across a river-to-lagoon continuum before-after high (HRP) and low (LRP) rainfall periods. Both rainfalls relocated the coarser sediment fraction downstream in the river, whilst only the finer fraction reached the lagoon. The high swell associated to the storm preceding the LRP contributed to the seawater overwash over the outer lagoon, which masked the effects of the freshwater discharge. After the HRP, TOC and TN in the outer lagoon increased 5–6 times, whilst after the LRP both decreased 2–8 times in the whole lagoon. Rainfalls caused a $\delta^{13}C$ enrichment (from -27.1 to -22.0 ‰) of the lagoon sediments and changes in the biogeochemistry of both river and lagoon sediments. After both rainfalls, time for the degradation of the biopolymeric C increased downstream the river (5–11 times) and in the outer lagoon (3 times). We conclude that the effects of rainfall-driven river runoff and their spatial extent towards the adjacent lagoon depend on the magnitude and duration of the rainfall, which differently alter sediment biogeochemistry.

Keywords Organic matter · C turnover · C and N stable isotopes · Mediterranean river and coastal lagoon · Climate change · Rainfall

Introduction

Freshwater flash flood events caused by episodic and anomalously short lasting and very intense rainfalls (convective rains) are among the major threats for coastal ecosystems worldwide (Christensen & Christensen, 2003; Jentsch & Beierkuhnlein, 2008; Ummenhofer & Meehl, 2017). In the short term, severe flash floods can cause large physical, chemical and biological consequences on the receiving watersheds (rivers and coastal lagoons). These include, among the others, transport of large volumes of water and sediment, organic matter, nutrients and contaminants from upstream towards the terminal branches of the receiving watersheds (Tesi et al., 2013; Wohl et al., 2015; Raymond et al., 2016). Sediment erosion and transport processes can modify the sediment texture, which, in turn,

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can lead nutrients reallocation, eutrophication of the downstream watersheds, at times accompanied by oxygen depletion (Poff et al., 1997; Leigh et al., 2015). Ultimately, all these physical–chemical changes caused by floodings can alter biogeochemical cycles of the watersheds, impacting microbial communities' composition and ability to store or mobilize organic C (Baldwin & Mitchell, 2000; Aufdenkampe et al., 2011; Caillon et al., 2021). Freshwater flash flood events and extremely intense rainfall are becoming increasingly frequent and their consequences progressively more catastrophic, with large effects widely documented on ecosystems and humans' wellbeing (Jonkman, 2005; Jentsch & Beierkuhnlein, 2008; Wei et al., 2009; Alderman et al., 2012; Brandolin et al., 2012; Petrucci, 2022). Moreover, severity and magnitude of large river floods and their impacts on European coastal ecosystems are expected to further increase by the end of the century, especially in the Mediterranean area (Kundzewicz et al., 2013; Dottori et al., 2020).

The Mediterranean region is a semi-enclosed basin and is an ideal hotspot to investigate episodic climatic events by virtue of its peculiar pluviometric and ecological characteristics (Luterbacher et al., 2006; Giorgi & Lionello, 2008; Hilmi et al., 2022). In this context, Mediterranean transitional aquatic ecosystems, including coastal lagoons, being located between freshwater and the sea, are affected by several naturally ample fluctuations in temperature and salinity and by multiple anthropogenic pressures (Bonotto, 2001; Brehmer et al., 2011; Meredith et al., 2022). Despite this, coastal lagoons provide important ecosystem services that include, for instance, flood lamination, sea shoreline stabilization, sediment and nutrient retention, water quality control, biodiversity and biomass reservoirs, recreation, tourism, and cultural values (Levin et al., 2000; Vanina et al., 2006; Sousa et al., 2013; Newton et al., 2018; El Mahrad et al., 2020). Moreover, coastal lagoons act as hotspots of detrital organic C accumulation and degradation (Pusceddu et al., 2003; Danovaro & Pusceddu, 2007), which makes them important global contributors to the blue carbon storage/release processes (Boadella et al., 2021; Newton et al., 2018; Sousa et al., 2016). Nonetheless, local eutrophication, which increases lagoons' vulnerability to many other multiple anthropogenic pressures (Kennish & Paerl, 2010), can alter their capacity of storing blue carbon.

Normally, in coastal lagoons, the natural mixing of freshwater and seawater depends on the fluctuations in freshwater inputs and seawater intrusion (depending also on the width of the connection with the sea and its conditions, the wind, the waves and the sea level). Riverine inputs, governed by patterns of precipitations inland, influence directly lagoon's physical–chemical and trophic characteristics, such as salinity, nutrient concentrations, sedimentary organic matter (OM) quantity, composition and nutritional quality, microbial ecology, and the biogeochemical cycles (Pusceddu et al., 1996; Bianchelli et al., 2020; Gravina et al., 2020; Magri et al., 2020; Bartoli et al., 2021).

Trophic conditions of the sediments, besides being regulated by hydrodynamics and sedimentological patterns, are influenced also by many concurrent biological mechanisms that include organic C in situ production, heterotrophic consumption and microbe-mediated degradation. Trophic status of sediments is generally more conservative than that of the water column, and this holds particularly true in shallow water ecosystems, like coastal lagoons. Indeed, the faster hydrological and biological dynamics in the water column make descriptors of waters' trophic state far more variable than those in the sediment (Dell'Anno et al., 2002). Most investigations on sediment trophic status in coastal marine and lagoon ecosystems have been carried out considering total organic C (TOC) contents, which, however, do not distinguish between refractory and labile fractions (Pusceddu et al., 2009a). In this regard, the trophic status of coastal ecosystems, including lagoons, has been increasingly assessed in terms of contents, biochemical composition and nutritional quality of their organic deposits (Pusceddu et al., 2011). More in details, the biopolymeric fraction of organic C (as the sum of protein, carbohydrate and lipid C equivalents, hereafter BPC) has been increasingly used for this purpose, as it combines quantitative and qualitative information (Fabiano et al., 1995; Pusceddu et al., 2009b). Moreover, sediment biogeochemical processes are also influenced by the relative contribution of different C stocks, which, in turn, depend also on their origin. In this regard, the analysis of C and N isotopic signature ($\delta^{13}C$ and $\delta^{15}N$, respectively) can be used to assess OM origin, budgets and transformations (Carneiro et al., 2021). In fact, these proxies have been recurrently used to trace nutrient cycling and OM flow within different aquatic food webs (Vizzini & Mazzola, 2006; Rooze & Meile, 2016; Ke et al., 2017; Calizza et al., 2022; Priestley et al., 2022) and, therefore, appear altogether as promising tools for investigating the origin of solid materials transported by rainfall-driven runoffs (Amir et al., 2019).

Other studies have previously investigated the effects of river flash floods on water biogeochemistry in Mediterranean coastal lagoons (e.g. Fouilland et al., 2012). Nonetheless, to the best of our knowledge, no synoptic analyses of the many above-described sedimentary biogeochemical proxies in response to rain-induced torrential runoff have been conducted so far along a river-to-lagoon continuum in the Mediterranean region. To provide insights on such gap of knowledge, we determined sediment grain size (granulometry), organic matter quantity (in terms of TOC, TN and BPC), nutritional quality (in terms of the algal fraction of BPC and protein-to-carbohydrate ratio), origin (C and N stable isotopes), degradation (extracellular enzymatic activities) and turnover time along a river-to-lagoon continuum before and after two rainfall events characterized by different magnitude. More specifically, we expected differential effects of rainfall events characterized by different magnitude on the sediment biogeochemistry along a river-to-lagoon continuum, with shifts in the origin and composition of organic deposits (e.g. Raymond & Bauer, 2001; Dhillon & Inamdar, 2013), and in their trophic role (e.g. Ward et al., 2017). As a corollary, we also expected these effects to vary along the river-lagoon continuum with upstream sediments reflecting stronger terrestrial OM signals, and the lagoon ones variably altered due to the concurrent effects of marine inputs from the adjacent sea (e.g. Bianchi, 2011; Seidel et al., 2015).

Materials and methods

Study area

The Capoterra lagoon (Southern Sardinia, Italy), with an average depth of approximately 1 m, corresponds to the southwestern pond of the larger Santa Gilla lagoon (Fig. 1), one of the most important wetlands of the Central Mediterranean Sea, protected under the Ramsar Convention and the Natura 2000 Network. On its northern limit, the Capoterra lagoon receives the freshwater inflow from the torrential Santa Lucia River (Fig. 1). Several meteorological stations located near the lagoon have pointed out a large variability in the precipitation patterns over the last decade (Istat, 2024; Scanu et al., 2024), when the annual precipitation values have ranged between a minimum of ca. 300 mm and a maximum of > 600 mm per year, with

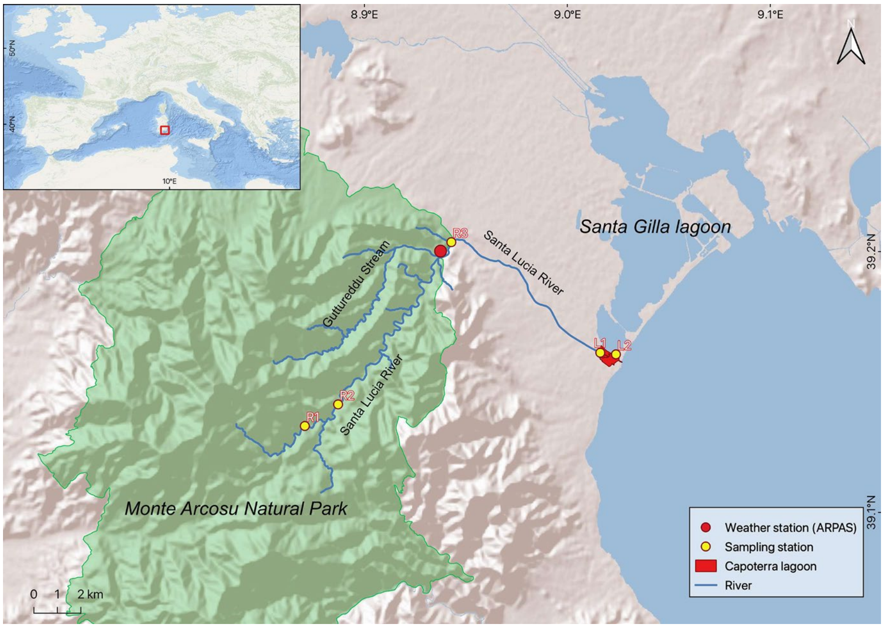

Fig. 1 Study area and sampling station locations. R1 = upstream, R2 = intermediate, R3 = downstream, L1 = internal lagoon, L2 = external lagoon. Reported is also the location of the Sardinian Regional Agency for the Protection of the Environment (ARPAS) weather station

an average of ca. 544 mm. The heaviest rainfalls predominantly occur in winter, typically between November and December. During the last decade, this area has been affected by several severe rainfall events. For instance, in October 2018, it was hit by about ca. 356 mm of rain in less than 20 h (corresponding to the amount of rain that typically would fall in 4–5 months). Such event caused the flood of the Santa Lucia River, with harmful consequences for reared mussel farms in the lagoon, because of the sudden drop in salinity and the extreme raise in water turbidity that impaired their physiology and nutrition, accompanied by a mass mortality event (Addis et al., 2021). Furthermore, the study area is characterized by a high risk of soil erosion and intense anthropic disturbance (e.g. industrial, agricultural, and fishing activities) with significant increase in nitrogen, and phosphorous loads and algal blooms causing local eutrophication (Scanu et al., 2024).

Sampling strategy and environmental parameters

Sediment sampling was carried out at five stations before and after a period of 4 consecutive heavy rain events in November 2019 (7–11 November 2019; high rainfall period, hereafter HRP) and before and after another period with only 1 severe rain event in January 2020 (10–22 January 2020; low rainfall period, hereafter LRP). Three stations were located along the Santa Lucia River: R1 (upstream), R2 (intermediate), R3 (downstream), and two additional stations were located along a putative salinity gradient within the Capoterra lagoon: L1 (internal lagoon) nearest to the Santa Lucia River mouth, and L2 (external lagoon) nearest to the sea water inlet (Fig. 1). In each riverine station, replicated sediment samples (n = 3) were collected by gently scraping the top first centimetre of the sediment with Falcon-type tubes. In the lagoon stations, replicates (n = 3) were collected manually using plexiglass cores (4.7 cm internal diameter) and the top first cm of each replicate stored in Petri dishes at – 20 °C until the analysis.

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 at all stations. Daily rainfall data (mm) were made available by the Geological Department of the Sardinian Regional Agency for the Protection of the Environment (ARPAS). Wind speed, wind direction, and significant wave height data from the adjacent marine coastal area were obtained from the satellite-based Copernicus Climate Change Service (2023).

Grain size analysis

Grain size fractions of river and lagoon sediments obtained during the field experiment were determined using a Horiba Partica LA950V2 particle size analyser, 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 sediment sample was oxidized to remove OM, using 20% H_2O_2, and sediment particles were disaggregated with 2.5% $P_2O_7^{4-}$ (Paradis et al., 2019). Grain size fractions were grouped in the ranges: clay (< 4 µm), silt (4—63 µm), sand (63 µm—2 mm), and gravel (> 2 mm).

Total organic carbon, total nitrogen, and stable C-N isotopes analyses in lagoon sediments

Due to the coarse grain size of river sediment samples, TOC and TN were determined only in lagoon sediments (i.e. L1-L2 stations) using the procedure described in Nieuwenhuize et al. (1994), along with the stable isotopic composition of $\delta^{13}C$ and $\delta^{15}N$. Analyses were carried out with a Thermo NA 2100 elemental analyser coupled with a continuous-flow isotope-ratio mass spectrometer.

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. 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-micro-balance 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. The percentages of TOC and TN were used to calculate the TOC/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 are derived from the formula:

$$\delta^{13}\mathrm{C} \text{ or } \delta^{15}\mathrm{N} = \left[\left(\mathrm{R}_{\mathrm{sample}}/\,\mathrm{R}_{\mathrm{standard}}\right) - 1\right] \times 10^{3}$$

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., 2012; Guerra et al., 2013). The TOC/TN ratio, $\delta^{13}C$, and $\delta^{15}N$ 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).

Biochemical composition and bioavailability of sedimentary OM

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 & Hatcher (1972), based on the phenol and concentrated sulfuric acid reaction with saccharides, was used to determine carbohydrates, and then expressed as D (+) Glucose equivalents. Lipids, after extraction in chloroform: methanol (1:1, vol: vol) (Bligh & Dyer, 1959), and evaporation in a dry hot bath at 100 °C for 20 min, were determined after the sulfuric acid carbonization procedure (Marsh & 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 (i.e. cPRT, cCHO, cLIP) 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 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 analysed fluorometrically (430 nm excitation and 665 nm emission wavelengths) to estimate chlorophyll-a, and, after acidification with 200 µl 0.1N HCl, phaeopigment concentrations. Total phytopigment was defined as the sum of chlorophyll-a and phaeopigment concentrations. After conversion into C equivalents using a factor of 30 µgC μg^{-1}, we calculate the algal fraction of BPC as the percentage of phytopigment-to-BPC concentrations. This was used to estimate the fraction of the organic material of autotrophic origin, including both the living (chlorophyll-a) and the senescent/detrital (phaeopigment) components (Danovaro, 2010; Pusceddu et al., 2010, 2014b). The algal fraction of BPC and the protein-to-carbohydrate ratio were chosen as descriptors of the ageing, nutritional quality, and enrichment of the sedimentary OM (Dell'Anno et al., 2002; Pusceddu et al., 2009b; Pusceddu et al., 2010).

C degradation and turnover time

Degradation rates of the protein and carbohydrate pools (representing the most abundant fractions of BPC; Pusceddu et al., 2014a, b) were estimated from aminopeptidase and β-glucosidase activities using the artificial fluorogenic substrates (L-leucine-4-methyl-coumarinyl-7-amide, for aminopeptidase; 4-methyl-umbelliferone-D-glucopyranoside, for β-glucosidase) as described in Danovaro (2010) and adapted for lagoon sediments (Bianchelli et al., 2020). Data were reported as nanomole of hydrolysed substrate released per g of sediment dry weight h^{-1}. Aminopeptidase and β-glucosidase activities (hereafter LEU and BETA) were converted into C equivalents using 72 as a conversion factor and their sum, reported as the potential C degradation rate (µgC g^{-1} h^{-1}(1)). The daily C turnover time of the whole protein and carbohydrate pools were calculated using (2) and (3):

$$\text{C degradation rates } \left(\mu\text{gC g}^{-1}\text{h}^{-1}\right) = \text{LEU} + \text{BETA} \tag{1}$$

$$\text{C turnover (d - 1)} = \frac{\text{(C degradation rates)} \bullet 24}{\text{cPRT} + \text{cCHO}} \tag{2}$$

$$\text{C turnover time (d)} = \frac{1}{\text{C turnover}}, \tag{3}$$

where

LEU and BETA = aminopeptidase and β-glucosidase activities (μgC g^{-1} h^{-1}).
cPRT and cCHO = protein and carbohydrate contents converted into C equivalents using 0.49 and 0.40 as a conversion factor, respectively (μgC g^{-1}).

Although these estimates are only potential (maximum) rates of C turnover time, they are considered good proxies of ecosystem functioning (Pusceddu et al., 2014a; Soru et al., 2022).

Effect size estimate and statistical analyses

Forest plots have been employed to ensure a clear and standardized representation of the reported effects on OM quantity (i.e. BPC and phytopigment contents), nutritional quality (i.e. algal fraction of BPC and protein-to-carbohydrate ratio), C degradation rates and C turnover time on both riverine and lagoon stations (Soru et al., 2022). The effect size metric was used to quantify the magnitude of these effects as the log-proportional change between the mean (X) of the after (A) and before (B) groups, calculated as follows:

$$R_i = \ln\left(XA_i / XB_i\right),$$

where Ri represents the log–response ratio for the variable i, and XA_i and XB_i are the mean values of the metric for study i in after (A) and before (B) conditions, respectively.

The experimental design, applied separately for the river and lagoon, consisted of two orthogonal factors: 1) Impact (2 fixed levels: Before and After); 2) Station (3 fixed levels for the river: R1, R2, R3, and 2 fixed levels for the lagoon: L1 and L2). Since the two rainfall periods differed in terms of intensity and duration, the analyses were conducted separately for HRP and LRP. To test the effect of the two factors and their interactions, permutational analyses of variance (PERMANOVA) (Anderson, 2001) were carried out in either the univariate (i.e. each variable separately) or multivariate context.

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 & Millar, 2004), thus avoiding the assumption of normality (Anderson, 2014). Only data of sedimentary OM were previously normalized.

The analyses were carried out on Euclidean distance-based resemblance matrixes obtained from previously normalized data, using 999 random permutations of the appropriate units. PERMANOVA tests were followed by a post hoc tests in case of significant effects of the rainfall. 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).

Results

Rainfall events and hydrographic changes

During the HRP, the total daily rainfall peaked up during 4 distinct events (max 29.8 mm; mean 7.71 ± 5.93), whilst during LRP, a single rainfall peak event was observed (16.4 mm; mean 4.55 ± 1.74) (Fig. 2A). The cumulative rain inputs were 124.40 mm and 22.60 mm, in HRP and LRP, respectively (Fig. 2B).

In all riverine and lagoon stations, the temperature showed a minimal but detectable decrease (0.2–0.4 °C) after both HRP and LRP (Table 1). Salinity remained consistently low (~ 0.2–0.3) at the three riverine stations after both rainfall periods. Conversely, it decreased drastically after both HRP and LRP in lagoon stations (Table 1). In detail, after HRP salinity dropped significantly at both lagoon stations from ~ 34–37 to ~ 2–4 in the surface waters and to ~ 20–25 in the bottom ones. After LRP the salinity at L1 decreased from 12.0–18.0 to 4.3 in both surface and bottom waters, whilst at L2 it dropped slightly from 13.0 to 8.0 and from 35.8 to 18.5 in surface and bottom waters, respectively.

Wind direction and speed and significant wave heights showed some differences between the two

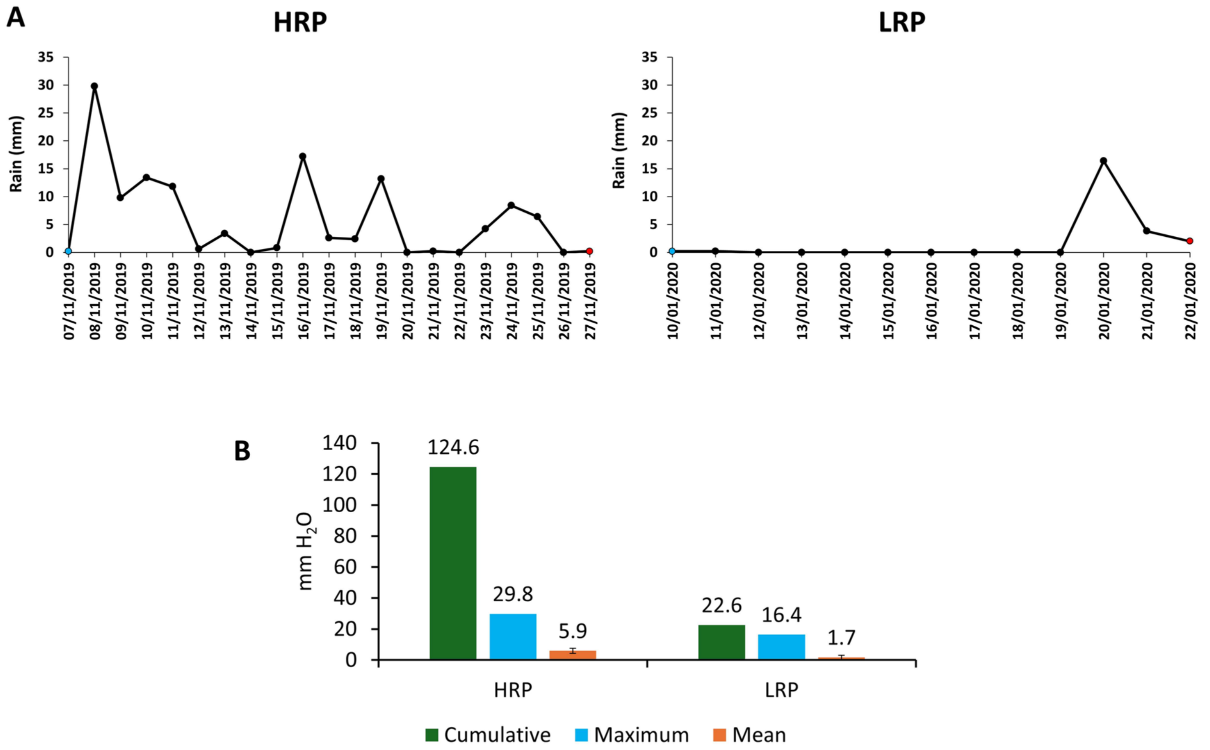

Fig. 2 Total daily rainfall (**A**) and cumulative, maximum and mean rain inputs (**B**) during HRP (November 2019) and LRP (January 2020). Data obtained from the ARPAS weather station

rainfall periods (Supplementary Fig. S1). During HRP, the wind came predominantly from W-NW and from S-SW, with speeds never exceeding 30 km/h (Supplementary Fig. S1 A-B). The significant wave height at sea during HRP never exceeded 1.5 m (Supplementary Fig. S1C). During LRP, the wind came predominantly from S-SE (Supplementary Fig. S1D), reaching peak speeds > 40 km/h. The significant wave height exceeded 3 m causing a large swell (Supplementary Fig. S1 E–F).

Sediment grain size variations during the study periods

Sediment grain size composition in the river-lagoon continuum was predominantly characterized by sandy sediments after both rainfall periods, although the percentage of gravel, silt, and clay varied among stations and between HRP and LRP.

After the HRP (Fig. 3A), the gravel fraction increased by about 35-fold and threefold at R1 and R2, respectively, whilst the silt fraction doubled at R3. In contrast, at L1, both silt and clay fractions decreased by about twofold and fourfold, respectively, whereas the sand fraction doubled. Moreover, at L2, both gravel and silt fractions increased by about 16-fold and twofold, respectively.

After the LRP (Fig. 3B), the gravel fraction halved at R1 and increased by 1.5 times at R2. Notably, at R3-L1, the sand fraction doubled, whereas the finer fractions dropped: the silt fraction decreased eightfold and 13-fold, and the clay fraction decreased 13-fold and 22-fold at R3 and L1, respectively. Similarly, at station L2, the finer (silt and clay) fractions decreased by 1.3 times, whilst the gravel fraction increased fourfold.

TOC, TN, and stable C-N isotope signatures in lagoon sediments

TOC and TN sedimentary contents, δ^{13}C values, but not δ^{15}N values, were significantly affected by the interaction Impact × Station (Supplementary Table S1).

Table 1 Water temperature (°C), surface and bottom salinity (PSU) in the Santa Lucia River (R1, R2, R3) and in the Capoterra lagoon (L1, L2) before and after the HRP (07–27/11/2019) and LRP (10–22/01/2020)

Station	Rainfall	Impact	Date	Temperature	Salinity (surface)	Salinity (Bottom)
R1	HRP	Before	07/11/2019	15.0	0.2	0.2
		After	27/11/2019	14.8	0.2	0.2
	LRP	Before	10/01/2020	10.0	0.2	0.2
		After	22/01/2020	9.8	0.2	0.2
R2	HRP	Before	07/11/2019	14.2	0.2	0.2
		After	27/11/2019	13.9	0.2	0.2
	LRP	Before	10/01/2020	9.9	0.2	0.2
		After	22/01/2020	9.7	0.2	0.2
R3	HRP	Before	07/11/2019	14.2	0.3	0.3
		After	27/11/2019	14.0	0.3	0.3
	LRP	Before	10/01/2020	13.3	0.3	0.3
		After	22/01/2020	12.9	0.3	0.3
L1	HRP	Before	07/11/2019	16.1	33.7	33.9
		After	27/11/2019	15.9	2.2	20.0
	LRP	Before	10/01/2020	14.1	12.0	18.0
		After	22/01/2020	13.9	4.3	4.3
L2	HRP	Before	07/11/2019	16.0	37.8	37.5
		After	27/11/2019	15.7	4.0	25.0
	LRP	Before	10/01/2020	14.4	13.0	35.8
		After	22/01/2020	14.1	8.0	18.5

After the HRP, L1 was characterized by a significant sevenfold decrease in TN contents, a sevenfold increase of the TOC/TN ratio values, a concurrent δ^{13}C enrichment (from − 26.4 to -22.0‰), and invariant sedimentary TOC content (Fig. 4 A-D). L2 showed a 5–6 time increase in TOC and TN contents (Fig. 4 A-B) and no significant variation in both TOC/TN ratio and δ^{13}C values (Fig. 4 C-D).

After the LRP, L1 was characterized by a significant decrease in TOC and TN sedimentary contents and TOC/TN ratio values (by 8, 1.1, and 7 times, respectively) (Fig. 4 A-C), and a δ^{13}C enrichment (from − 24.7 to − 23.0‰) (Fig. 4E). L2 showed no significant variations in TOC contents and TOC/TN ratio values but a TN halving (Fig. 4 A-C), and a δ^{13}C impoverishment (from − 26.8 to − 27.1‰) (Fig. 4E).

Biochemical composition and bioavailability of riverine and lagoon sediments

Data on the sedimentary OM contents and extracellular enzymatic activities are reported in Supplementary Table S2. All investigated variables were significantly affected by the interaction Impact × Station in both riverine and lagoon stations (Supplementary Tables S3-S6). Exceptions to this were observed only after the HRP for the protein-to-carbohydrate ratio and the algal fraction of BPC in both riverine and lagoon stations.

The largest effect size of HRP on BPC contents occurred in L2 (positive effect), whereas the largest effect of LRP occurred in R3 (positive) and L1 (negative) (Fig. 5A). In more detail, after both rainfall periods, no significant changes were observed for BPC content in R1-R2 (Supplementary Fig. S2A). Along the R3-L1-L2 continuum, BPC contents increased significantly (by 2–3 times) only after the HRP, whereas after the LRP, BPC contents increased (up to 15 times) in R3 and decreased (by—89%) in L1 (Supplementary Fig. S2A).

The effect size of HRP on total phytopigment content resulted generally negative in all stations, with exception in L2, whereas the effect size of LRP resulted positive in R1-R2 and negative in the R3-L1-L2 continuum (Fig. 5B). More in detail, after HRP, total phytopigment content decreased (by ca. 2–4 times) in all stations except for L2 where values doubled, whereas after LRP they increased 2-folds

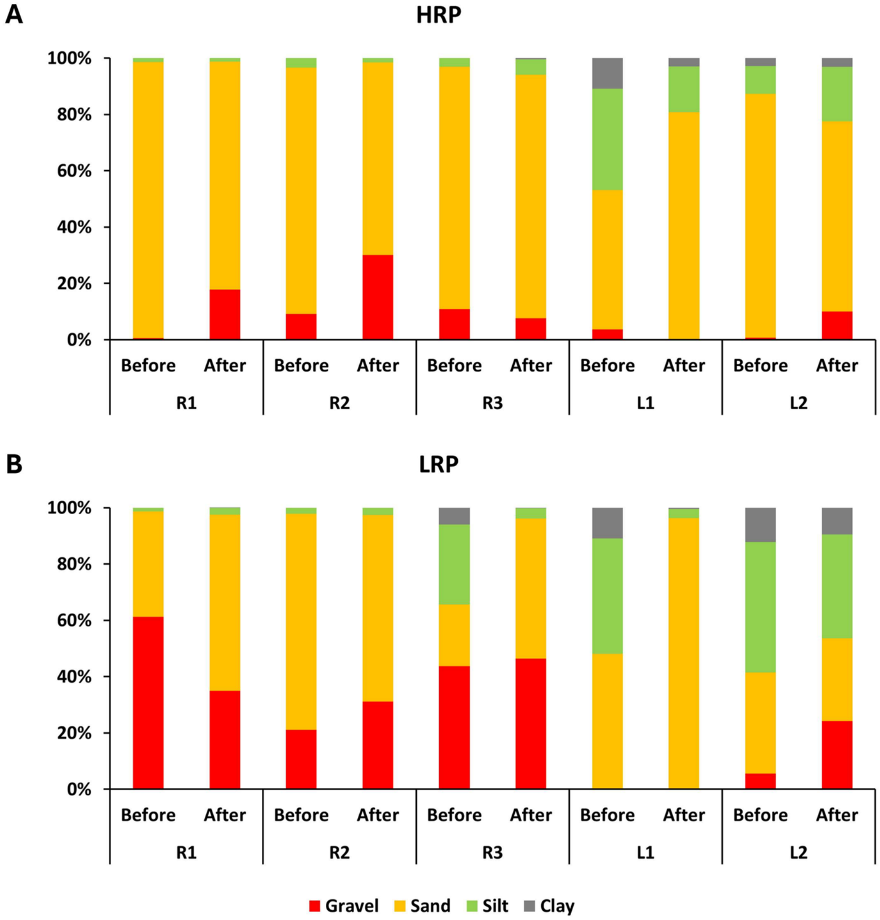

Fig. 3 Grain size composition of sediments in the Santa Lucia River (R1, R2, R3) and the Capoterra lagoon (L1, L2) before and after HRP (**A**) and LRP (**B**)

in R1-R2 and decreased (by ca. 2–4 times) in the R3-L1-L2 continuum (Supplementary Fig. S2B).

The HRP effect size on the algal fraction of BPC was mostly negative, whereas the LRP one was positive in R1-R2-L1 and negative in R3-L2 (Fig. 5C). In detail, HRP caused an up to sevenfold decrease of the algal fraction of BPC in all stations, whereas the LRP reduced the algal fraction of BPC in R3 and L2 (by ca. 63- and threefold, respectively) and enhanced it in R1-R2 and L1 by ca. 3–sixfold (Supplementary Fig. S2C).

The HRP effect size on the protein-to-carbohydrate ratio resulted positive in R1 and negative in L1, whereas the LRP one was negative in R2-R3 and slightly positive in L1 (Fig. 5D). In detail, the HRP did not cause any change in the protein-to-carbohydrate ratio at most riverine stations, except for a significant twofold increase in R1, and caused a twofold decrease of the ratio in L1, whereas the LRP reduced the values ratio in R2-R3 and increased it in L1 (by ca. 10–60 and 2 times, respectively) (Supplementary Fig. S2D).

The biochemical composition of sedimentary OM varied significantly after both rainfall periods and in all investigated stations (Table 2). After the HRP, changes in the biochemical composition of riverine sediments (especially in R3) depended upon the decrease of phytopigment contents (Fig. 6A), whereas changes in lagoon sediments were mostly explained by increases in carbohydrate and lipid contents in L1, and by an increase in the phytopigment content in L2 (Fig. 6B). Changes in the biochemical composition of riverine sediments after the LRP were mostly explained by an increase in the phytopigment content in R1, a decrease in carbohydrate and lipid contents in R2, and by a remarkable decrease in the phytopigment content

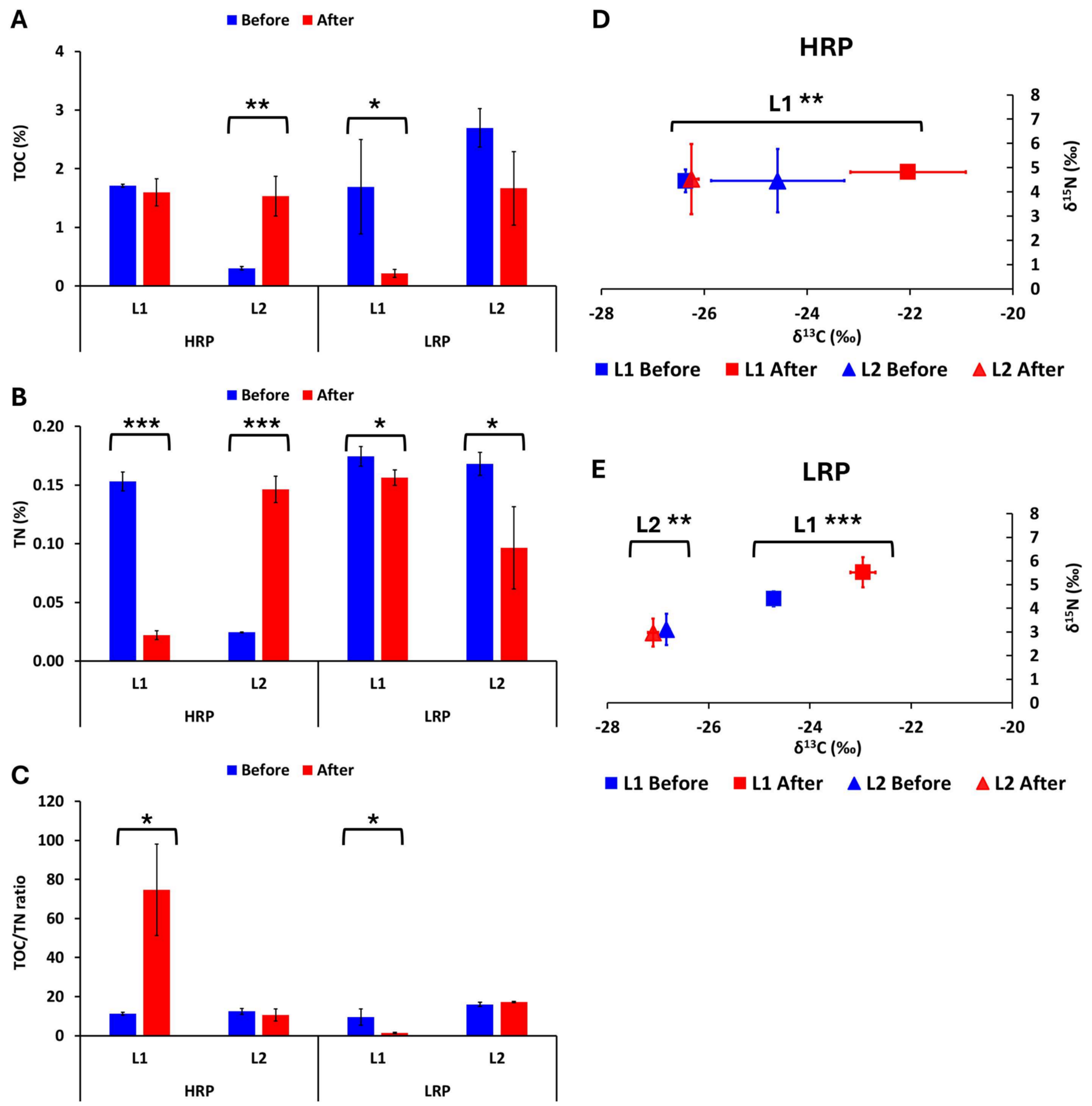

Fig. 4 Changes in total organic C (**A**) and nitrogen (**B**) sedimentary contents, TOC/TN ratio values (**C**), and isotopic composition (**D**-**E**) of the Capoterra lagoon sediments (stations L1 and L2) before and after the HRP and the LRP. Error bars indicate standard deviation (n = 3). Asterisks indicate significant differences: $* = P < 0.05$, $** = P < 0.01$, $*** = P < 0.001$. In D-E, asterisks refer only to $\delta^{13}C$ (before vs. after)

in R3 (Fig. 6C). Lagoon sediments biochemical composition variations were mostly explained by a decrease in carbohydrate and lipid contents in L1, and by a decrease in the phytopigment content in L2 (Fig. 6D).

C degradation rates and turnover time in river and lagoon sediments

The HRP effect size on C degradation rates resulted positive in R1-L1 and negative in R3, whereas the LRP one was null or slightly positive in

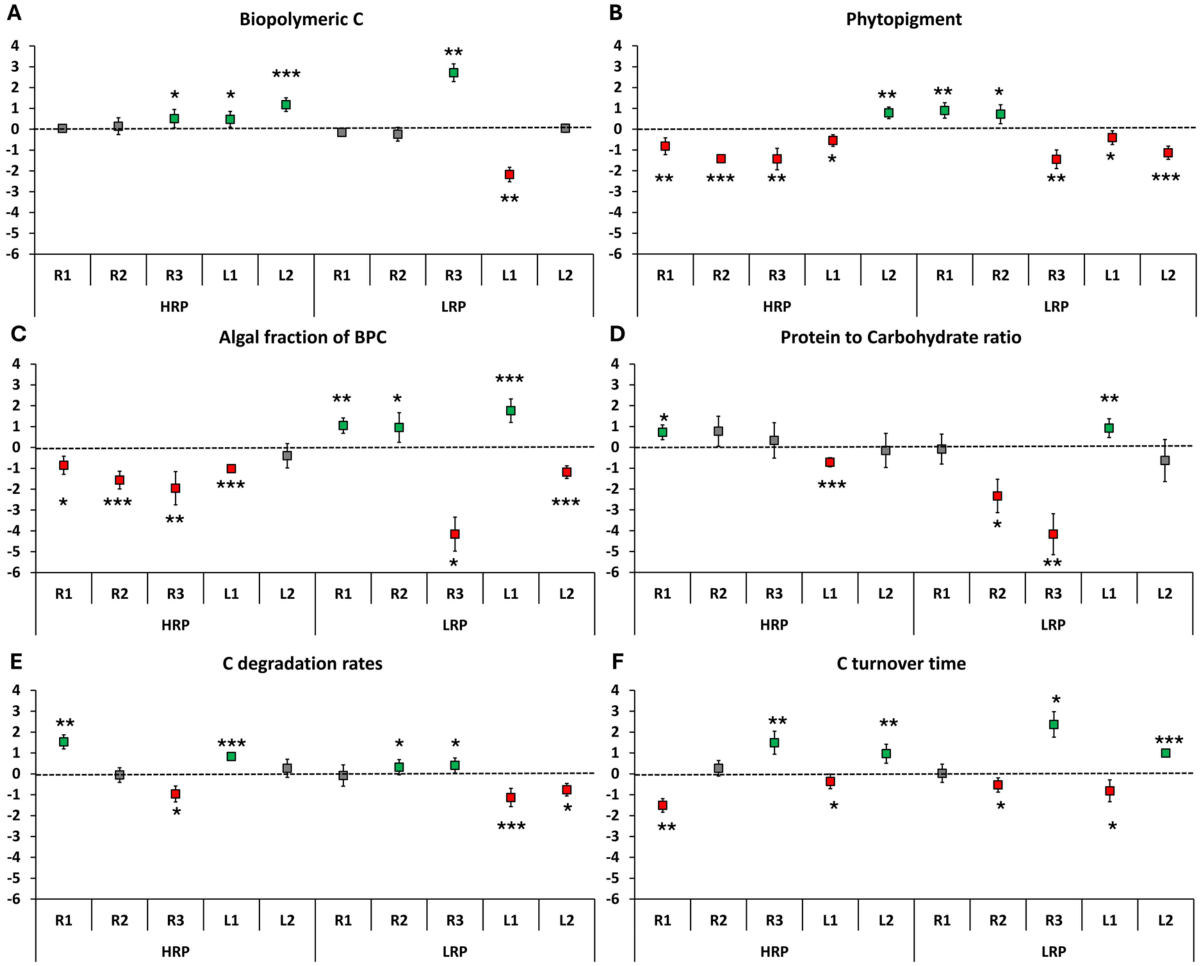

Fig. 5 Effect size of rainfalls on biopolymeric C (**A**) and total phytopigment sedimentary contents (**B**), algal fraction of BPC (**C**), protein-to-carbohydrate ratio values (**D**), C degradation rate (**E**) and C turnover time (**F**) after the HRP and the LRP in the Santa Lucia River (stations R1, R2, R3) and Capoterra lagoon (stations L1, L2). Green dots = positive effect, red dots = negative effect, grey dots = no effect. Error bars indicate standard deviation (n = 3). Asterisks indicate significant deviation from the null effect: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

riverine stations and negative in both lagoon stations (Fig. 5E). HRP significantly enhanced C degradation rates in R1-L1 (by ca. 5 and 2 times, respectively) and reduced them (by ca. 3 times) in R3, whereas LRP slightly stimulated (by ca. 1.5 times) C degradation in R2-R3 but decreased it in L1-L2 (by 3 and 2 times, respectively) (Supplementary Fig. S2E).

The HRP effect size on C turnover time was negative in R1-L1 and positive in R3-L2, whereas the LRP one was slightly negative in R2-L1 and more pronouncedly positive in R3-L2 (Fig. 5F). HRP caused a significant decrease (up to 5 times in R1) in R1-L1 and an increase in R3-L2 (by ca. 5 and 3 times, respectively). LRP decreased C turnover time in R2-L1 (by approximately 50%) and increased it in R3-L2 (by ca. 11 and 3 times, respectively) (Supplementary Fig. S2F).

Discussion

Effects of rainfalls on salinity and sediment grain size

Salinity of the river did not change during the entire study period (Table 1). Before the HRP, the high salinity values observed in both the surface and

Table 2 Pairwise tests identifying significant changes in biochemical composition of sedimentary organic matter (in the multivariate context) after the HRP and the LRP in the Santa Lucia River (R1, R2, R3) and Capoterra lagoon (L1, L2) stations

	HRP		LRP	
Station	t	P(MC)	t	P(MC)
R1	5.1374	**	4.0134	**
R2	8.5514	**	4.2084	**
R3	4.5886	**	6.8671	**
L1	2.5605	*	6.8658	**
L2	5.3577	**	3.8451	**

Asterisks indicate significant differences: $* = P < 0.05$, $** = P < 0.01$

bottom waters of the lagoon suggest that before this flood, the river had only a minor influence on the lagoon, which was instead mainly affected by the intrusion of marine waters. This was not the case for the salinity values before the LRP, when both surface and bottom waters of the lagoon showed brackish salinities, particularly in the internal station L1 and near the bottom at the outer station L2. The expected decrease in the lagoon salinity following the two rainfall events was highest in surface waters than in the bottom ones. Moreover, the salinity drop after the HRP was expectedly larger than that after the LRP, presumably due to the higher water discharge caused by the cumulative rain (Fig. 2B). This result clearly demonstrates that the increase of river freshwater discharge following both rainfall periods was able to retreat the marine seawater intrusion in the lagoon, particularly at the internal station.

The above delineated scenario is consistent also with the observed changes in sediment grain size after the two rainfall periods (Fig. 3). The increase of the silt sediment fraction in the outer lagoon station L2 after HRP is indicative of the transport of finer sediments seaward, promoted by the intense rainfall (Fig. 2). The milder river discharge following the LRP showed a different response of the outer lagoon sediments at station L2, characterized by an increase of the gravel fraction. This grain size change in the outer lagoon sediments after the LRP was likely the consequence of the large swell that occurred in the days before the rainfall. The strong (> 30 km/h) southeast winds along with the high waves at sea (> 3 m; Supplementary Fig. S1) could have induced the overwash of coarse costal sediments into the outer lagoon and favoured a larger intrusion of seawater that could not be fully retreated after the LRP. This could have presumably promoted a stronger vertical mixing of the outer lagoon, as outlined by the minor differences in salinity between surface and bottom waters at L2 (Table 1).

Effects of rainfalls on TOC, TN, 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 (promoted by rainfall) and human activities, thus stimulating, in the short term, growth of phytoplankton and phytobenthos (Karydis & Kitsiou, 2012; Franzo et al., 2015). For example, studies carried out in other Mediterranean lagoons reported increased N sedimentary contents after large rainfall-driven influx of rivers (Magri et al., 2020; Ngadi et al., 2023). Our results do not entirely fit with such expectation. In fact, TOC and TN sedimentary contents (Fig. 4) increased after rainfall only in the outer lagoon station L2 and only after the HRP, concomitantly with the observed large transportation of finer river sediments, characterized by larger C and N contents. The lack of a positive response of TOC and TN contents in the outer lagoon after the LRP, possibly masked by the intrusion of seawater, the resuspension promoted by the coastal swell overwash and the lower river water discharge, suggests, therefore, that the eventual "eutrophicating" effect of rainfall on the outer lagoon sediments depends upon the magnitude of the resulting freshwater discharge, and the forcing conditions in the neighbouring marine area.

Since N is more limiting than C for heterotrophic nutrition, the TOC/TN ratio has been often used as a proxy of sedimentary OM nutritional quality and ageing (Danovaro et al., 2001). During our study, the values of this ratio did not change in the outer lagoon sediments after both rainfall periods. On the one hand, this result corroborates the hypothesis that after the LRP the effects of rainfall-driven runoff in the outer lagoon could have been partly masked by the inputs of seawater promoted by high swell conditions and sediment resuspension. On the other hand, the values of the TOC/TN ratio increased in the internal lagoon only after the HRP. This result suggests that the nutritional quality of sedimentary OM decreased

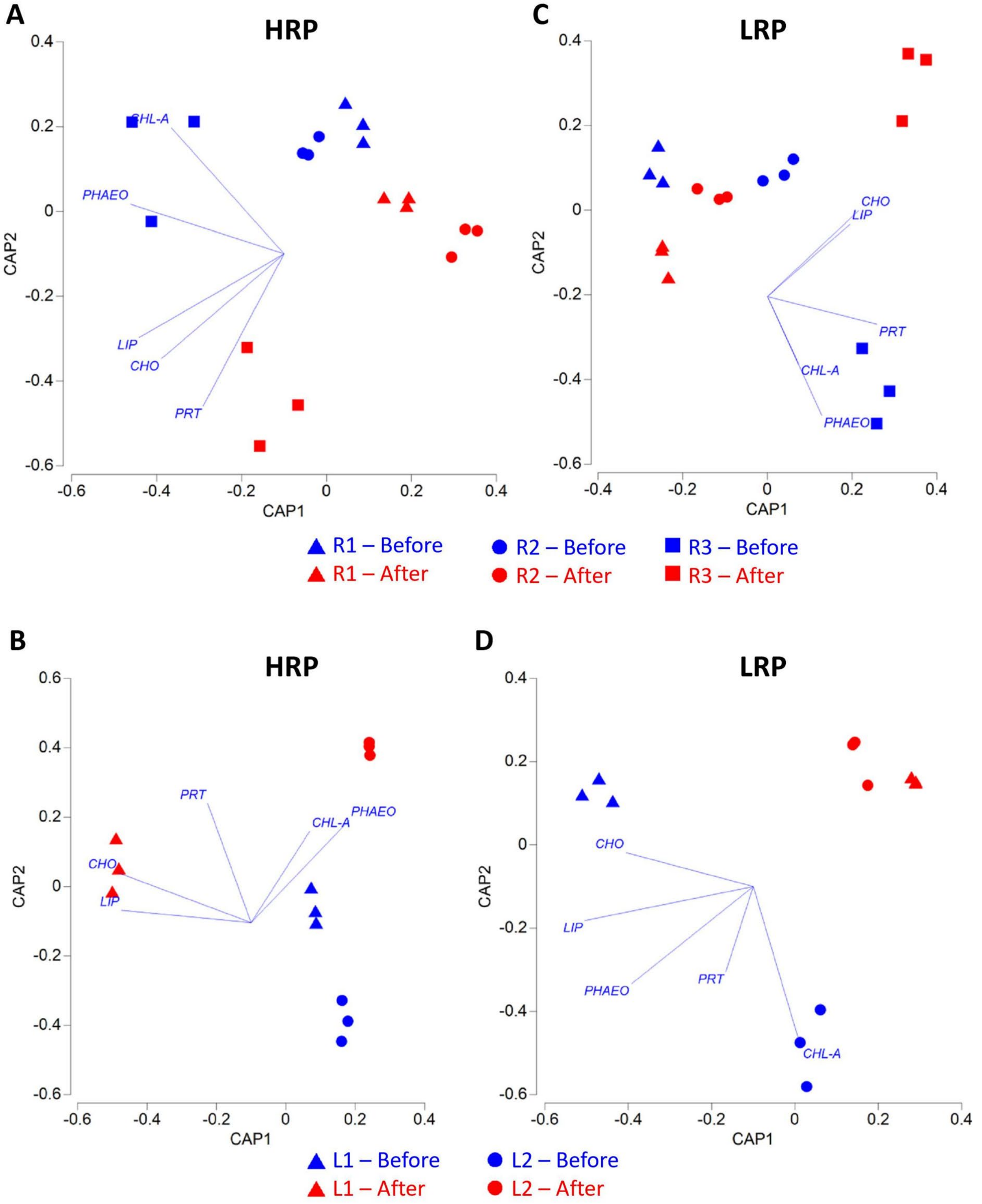

Fig. 6 Biplot obtained after the CAP showing differences in the biochemical composition of sedimentary organic matter before and after the HRP and the LRP in the Santa Lucia River (**A**-**C**) and Capoterra lagoon (**B**-**D**). Vectors are proportional to the proportion of change associated with each of the variables included in the analysis. *PRT* Protein, *CHO* Carbohydrate, *LIP* Lipid, *CHL-A* Chlorophyll-a, *PHAEO* Phaeopigment

in the internal lagoon, hence reducing the sedimentary trophic status (sensu Cloern, 2001; Pusceddu et al., 2009b). The lack of the same effect after the LRP suggests, furthermore, that the changes in the trophic status of lagoon sediments after rainfall events are dependent upon their magnitude and duration. The thresholds of the rainfall characteristics altering the sediments trophic status cannot be derived from the literature, nor can plausibly remain stable over time. Such thresholds, indeed, could be affected by the morphology of the lagoon, sediment characteristics, hydrological, and climatological regimes, but also characteristics of nutrient drainage basins surrounding the lagoon. The increase in the TOC/TN ratio values, associated with a decrease in the TN content in the internal lagoon sediments after the HRP, could be also due to increased inputs of terrestrial plant material (Gordon & Goñi, 2003) transported by the river discharge (Meyers, 1997; Rumolo et al., 2011; Nasi et al., 2020). The occurrence of plant-originated material after the HRP is corroborated by the isotopic signature of organic C (δ^{13}C). In the internal lagoon sediments, δ^{13}C values increased after both rainfall periods, more pronouncedly after the HRP (from − 26.4 to − 22.0‰) than after the LRP (from − 24.7 to − 23.0‰). Terrestrial plants have typically heavier δ^{13}C values (i.e. are more enriched in ^{13}C), are nitrogen-poor and richer in recalcitrant molecules (e.g. lignin, suberin, and hemicelluloses) (Hedges et al., 1997; Priestley et al., 2022). This applies, particularly, to weed and cane species and other C4 species (δ^{13}C from -10 to -14‰), that are common in the riparian area/watershed of the study area (Mossa et al., 1996; Mossa & Bacchetta, 1998). Thus, the enriched δ^{13}C values and the TN sedimentary content decrease after the HRP could have been caused by increased inputs of terrestrial plant debris, transported after the rainfall. Another possible explanation for the enriched δ^{13}C in L1 after HRP and LRP could be also the hydrodynamic washing of the surficial finer sediments after the rainfall. Although we cannot estimate the relative importance of this mechanism on δ^{13}C values changes, we hypothesize that it had likely a role minor than the input of OM of terrestrial origin. Indeed, the sediments across the riverbed were generally characterized by coarse sediments (dominated by gravel and sand; Fig. 3) less prone to be washed away.

Differently from δ^{13}C values, the comparison of δ^{15}N values before–after HRP and LRP did not reveal any significant change (Fig. 4). Values of δ^{15}N during the study (4.3 ± 0.8‰) were on average lower than the expected ranges (+ 5–15‰) associated with anthropogenic organic fertilizers inputs (Inácio et al., 2015; Savage, 2005). Our results, whilst suggesting that the lagoon under scrutiny exhibited a minor environmental alteration due to anthropogenic nitrogen, do not allow us to discriminate effects of rainfall on N sources in the lagoon sediments.

Effects of rainfalls on biopolymeric C in river and lagoon sediments

The TOC sedimentary contents are only a rough descriptor of the food available for the benthos, as they represent the bulk of organic matter, but do not allow to discriminate between the labile and refractory fractions (Pusceddu & Danovaro, 2009a). On the other hand, the biopolymeric C, as the sum of protein, carbohydrate, and lipid C contents (BPC; Fabiano et al., 1995), representing 50–70% of TOC (Tselepides et al., 2000; Pusceddu & Danovaro, 2009a), includes the labile or semi-labile fractions of sedimentary organic C (van Oevelen et al., 2011).

In the Santa Lucia River, both rainfall periods were followed by a significant increase in the BPC sedimentary content only in the downstream station R3 (Fig. 5A). Here, unexpectedly, the positive effect of the lighter and shorter LRP was higher than that after the HRP. According to the thresholds proposed by Pusceddu et al. (2009b) and Pusceddu et al. (2011), the sediments of the downstream station R3 before the rainfall could be ranked as mesotrophic (with BPC content of 0.22 mgC g^{-1} and the algal fraction of BPC 8.6%). According to the same thresholds, after the LRP, those sediments became eutrophic (with BPC content of 3.29 mgC g^{-1} and algal fraction of BPC 0.14%). Such increase in the trophic status of sediments after the LRP was not observed in the lagoon sediments, where BPC contents even decreased (by ca. ninefold) in L1 and did not change in L2. On the other hand, after the HRP, BPC contents slightly increased in both lagoon stations, more pronouncedly in L2. Overall, these results would suggest that only the HRP was able to move BPC particles from the river to the lagoon. This hypothesis fits well with the drop in surface salinity in both lagoon stations, which was strongest after HRP, and is consistent with both manipulative and field studies. For instance, positive and long-term effects of abrupt and abundant arrival of freshwater simulating an extreme rainfall event in mesocosm were observed on BPC sedimentary contents in a lagoon, even during the salinity recovery (Ennas et al., 2024). In other Mediterranean lagoons, higher sedimentary OM contents were observed a week after a rainfall-caused flood (Specchiulli et al., 2018) and comparing sedimentary OM contents between a rainy winter and the following drought summer (Orro & Cabana, 2021).

Once more, these results confirm that the expected eutrophication of sediments exposed to rainfall-driven river runoff and its spatial extent along the river-lagoon continuum can be tightly dependent on the magnitude and duration of the rainfall events.

Effects of rainfalls on biochemical composition and bioavailability of river and lagoon sediments

The response of benthic consumers to increased OM supply is influenced more by its bioavailability rather than by bulk concentrations (Cebrián & Duarte, 1998; Huxel, 1999; Pusceddu & Danovaro, 2009a). Accordingly, the trophic status of sediments depends also on the OM biochemical composition and bioavailability, rather than simply on its quantity (Grall & Chauvaud, 2002). In the last two decades, the OM bioavailability has been assessed in terms of the algal fraction of BPC, which is linearly related to the OM digestible fraction (Pusceddu et al., 2003). OM ageing and origin, instead, can be estimated using protein-to-carbohydrate ratio as a proxy, with values > 1 associated with “younger” material mostly heterotrophic in nature (Pusceddu et al., 2000, 2003).

In the Santa Lucia River sediments, the nature and bioavailability of sedimentary OM varied significantly after rainfall periods, though with different patterns after HRP and LRP (Fig. 5 C-D). After the HRP, almost all river stations experienced a decrease in bioavailability and an increase of the heterotrophic fraction, as outlined by the significant drop in the algal fraction of BPC and an increase in the protein-to-carbohydrate value, respectively. After the LRP, river stations, but R3, experienced an increase in bioavailability and a decrease in the heterotrophic fraction. Our results pinpoint that rainfall events of different magnitude can exert opposite effects on the bioavailability of river sedimentary OM. Since terrestrial plants debris (as dominated by structural and refractory carbohydrates; Pusceddu et al., 1999; Manini et al., 2003) have a much lower bioavailability for benthic consumers, our results highlight that high runoff events might make sedimentary OM less bioavailable for benthic consumers, due to higher fractions of terrestrial plant debris, whereas milder runoffs could make available more bioavailable resources. The effects of runoff periods with different magnitude on the bioavailability of sedimentary OM (i.e. algal fraction of BPC) and its nature (in terms of protein-to-carbohydrate ratio) apparently propagated also in the lagoon, but limitedly to the internal station and with opposite patterns. Here, the effects of HRP on the algal fraction of BPC and the protein-to-carbohydrate ratio were negative, whilst those of LRP were positive. These results, again, suggest that stronger rainfalls, transporting a larger fraction of refractory OM (originating from terrestrial plant debris), can decrease sedimentary OM bioavailability and alter biochemical composition across the entire river-lagoon continuum, whilst milder runoffs can exert the same effects only within the downstream river and the adjacent internal lagoon. Similar effects on the sedimentary OM bioavailability and biochemical composition were observed after a stormy winter, injecting older terrestrial OM characterized by a higher aromaticity (proxy for refractory matter) in another coastal lagoon (Boadella et al., 2021).

Effects of rainfalls on C degradation rates and turnover time in river and lagoon sediments

In the Santa Lucia River, after the HRP, C degradation rates (Fig. 5E) increased in the upstream station (R1), did not vary in the intermediate one (R2), and decreased in the downstream one (R3). Such pattern could be related to the differential bioavailability of organic substrates entering the river, that decreased because of larger inputs of more refractory compounds, associated with terrestrial plant debris. This hypothesis is corroborated by the increase in C turnover time (Fig. 5F) that occurred in the downstream river station (R3). Nonetheless, changes in C degradation rates after the milder LRP in the Santa Lucia River were much less evident, though C turnover time increased also in the downstream station (by > 10 times). We conclude that patterns in C degradation rates and turnover time in river sediments, especially after large rainfalls, could decrease the bioavailability of organic substrates for benthic consumers, mostly because of larger inputs of more refractory compounds.

Changes in C degradation rates and C turnover time in the Capoterra lagoon sediments after the two rainfall periods were rather different from those observed in the Santa Lucia River (Fig. 5E-F). After the HRP, C degradation rates increased only in the internal lagoon station, whereas after the LRP decreased in both lagoon stations. On the other hand, after both rainfall periods, C turnover time decreased in the internal station and increased in the external one. The inconsistency of the responses of C degradation rates and turnover time between the two lagoon stations after the two rainfalls is difficult to explain. The general acceleration of C degradation (accompanied by a decreasing C turnover time) in the internal lagoon station after HRP leads to hypothesize that, despite the decrease in the nutritional quality of sedimentary organic C, the enhanced C input reaching the innermost part of the lagoon stimulated bacterial activities to some extent. However, despite a similar increase in the organic C input after the HRP in the outmost lagoon, the response of enzyme-mediated organic C degradation was not significant, even if the nutritional quality of the C inputs remained unvaried. After the LRP, the responses were even more confused, presumably masked by the overwash of seawater, not allowing to hypothesize any substantial mechanism behind the observed variability in the responses of C degradation (and turnover) in the two lagoon stations. Overall, we cannot exclude that, though limited, the observed decrease in water temperature could have affected C degradation rates and, consequently, C turnover time. Nonetheless, we notice that the temperature decreased with almost the same interval at all stations and after both rainfalls, whereas C degradation rates and turnover time varied across habitats, stations and rainfall periods. We, thus, retain that the observed changes in sedimentary C metabolism were likely more affected by characteristics of the transported material (not directly investigated here) rather than by the temperature variations.

Conclusions

The current knowledge about the consequences of rainfalls (and eventual following floods) on river and connected coastal lagoons' sediments are still far from being fully understood. We showed that relatively milder rainfalls in riverine systems can be as impactful as heavier ones, whereas the consequences of rainfalls with different magnitude on lagoon sediments can be highly variable in extent and direction. Indeed, we can anticipate that rainfall events with different magnitude can have differential consequences on quantity, biochemical composition, bioavailability, degradation and turnover time of sedimentary organic C in either rivers or coastal lagoons, but did not find consistent patterns, causes and consequences of those changes. Since extreme rainfall events are expected to become more frequent and intense because of climate change, we foster the need of further investigations related with the occurrence of a larger spectrum of rainfall events magnitude to identify gradients of river runoff impact on sedimentary OM characteristics.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors have no conflicts of interest or financial involvement to disclose.

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