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Short-term recovery of inland and marine environments after the 2023 Emilia-Romagna flood



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Extreme floods can strongly affect connected inland and marine environments, yet their short-term recovery remains poorly resolved. Here we use an integrated multidisciplinary approach to assess the short- and medium-term effects of the 2023 Emilia-Romagna flood in Italy on aquatic environments and their recovery capacity. We combined three monitoring campaigns conducted immediately after the flood, about one month later, and after five months with in-situ measurements of abiotic and biotic variables, environmental DNA community assessment, pharmaceutical residue analyses, and satellite-derived data across rivers, drainage canals and marine waters. Despite the large spatial extent and intensity of the disturbance, most parameters showed partial to near-complete recovery within five months. However, this reflected movement toward post-event trajectories rather than full return to pre-flood conditions. The lack of pre-event baselines limits precise before-after impact assessment and highlights the need for long-term, standardized monitoring frameworks.

Extreme weather events are becoming increasingly frequent and severe, driven by the accelerating effects of climate change. Among these events, flooding is one of the most devastating¹. Over the past three decades, more than 4700 floods were recorded in 168 countries, which affected more than 3.2 billion people, caused over 218,000 fatalities and were responsible for more than 1.3 trillion United States dollars of economic losses². The most recent events include the 2022 mega-flood in Pakistan, caused by extreme monsoon rains following a multi-year drought³; the 2022 floods in Queensland and New South Wales (Australia), resulting from a combination of intense rainfall and near-saturation of initial soil water conditions⁴; the 2024 regional floods in Bangladesh, which were driven by a unique convergence of climatic and hydrological factors and further exacerbated by water releases from the Dambur Dam^{5,6}; and the 2024 Valencia flood in Spain, triggered by a *gota fría* (or “cold drop”) event, in which cold air met warm Mediterranean waters, producing intense rainfall⁷.

Floods, categorized into riverine, coastal, urban and flash types are caused by prolonged rainfall, rapid storm surges or the overflow of rivers and streams beyond their banks^{8,9}. Population growth and consequent urbanization, and land-use changes substantially increase flood exposure and vulnerability by enhancing sediment transport, altering water quality,

and disrupting hydrological cycles¹⁰, thereby further exacerbating the impacts of such events^{11,12}.

Floods not only impact terrestrial and aquatic environments but also mobilize pollutants toward downstream areas, altering biogeochemical dynamics and amplifying stress on coastal ecosystems^{13,14}. Satellite remote sensing has been widely used to map and track river flood plumes in coastal waters, providing estimates of plume extent, persistence and exposure to terrestrially-derived materials, particularly in highly vulnerable systems such as the Great Barrier Reef and other semi-enclosed coastal environments^{15–17}.

While these approaches are effective for characterizing plume-scale dynamics, they are often complemented by in situ and biological observations to assess biogeochemical and ecological responses, which remain less frequently documented following extreme flood events.

Excessive nutrient concentrations and terrestrial organic matter from river inputs often lead to harmful algal blooms¹⁸, hypoxia, and eutrophication, particularly in semi-enclosed seas^{19,20}. These nutrients enhance primary production in surface waters, increasing the deposition of organic matter to sediments, where its decomposition further depletes oxygen levels^{21,22}. Floodwater also acts as vectors for anthropogenic contaminants,

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including pesticides, heavy metals, and contaminants of emerging concern (CECs) which include perfluorinated compounds, plasticizers, plastic additives and pharmaceutical residues. The altered redox conditions during flood-induced deoxygenation events can increase the mobility and toxicity of these pollutants, with profound implications for aquatic ecosystems, water quality, and human health^{23–25}.

Among CECs, environmental pharmaceuticals are of increasing relevance as indicators of untreated urban wastewater discharge and pollutant remobilization during extreme hydrological events. Recent studies suggest that intense rainfall and associated sewer overflows may exacerbate pharmaceutical inputs into sensitive coastal environments, raising concern for the health of aquatic ecosystems under climate change scenarios²⁶.

The monitoring after extreme events generally involves the assessment of the immediate and direct impacts on urban infrastructures, ecosystems and human health. However, understanding the complex interplay between extreme flood events and their visible and unseen cascading effects on coastal and marine ecosystems is crucial but understudied. Short- and long-term monitoring is essential to capture impacts that often remain undetected in routine programs, such as shifts in biological communities, delayed responses to disturbances or the spread of contaminants. Detecting these changes is critical for predicting ecosystem resilience, protecting biodiversity and preventing ecological, economic (e.g., fisheries, tourism) and public health losses.

Between early May (1st–4th) and mid-May (16th–17th) 2023, the Emilia-Romagna region in northern Italy experienced two extreme meteorological events that led to catastrophic flooding, landslides and widespread destruction^{27–29}. The May 2023 events resulted from the convergence of extreme meteorological and hydrological conditions and were considered unprecedented in the region's hydrological history³⁰. The event occurred after a prolonged period of severe drought that had affected the entire Po River Basin since February 2021. Rainfall return periods exceeded 100 years in many areas, reaching over 500 years in the most affected zones. The first event was caused by a polar low-pressure system that stalled over central Italy, resulting from the convergence of moist air masses from the Adriatic Sea and cold bora winds from the north. This interaction, intensified by orographic effects from the Apennine Mountains, led to over 200 mm of rainfall in 48 h in some areas.

Two weeks later, a second low-pressure system exacerbated the crisis, producing even more intense rainfall, up to 240 mm over the same areas already affected in the previous days, exceeding historical records. Together, these events brought an estimated $350 \times 10^6 \text{ m}^3$ of water, equivalent to six months of average rainfall within a remarkably short period³¹. Rivers across Emilia-Romagna experienced record-breaking floods that breached embankments at 23 locations and flooded over 540 km² of lowlands.

During the first event, the high-water levels led to numerous overflows and inundations in unembanked sections of the rivers in the piedmont area and as the floods moved downstream, the persistent high-water stage caused infiltration and riverbank boils. Under this already critical condition, nearly all rainfall of the second mid-May event contributed directly to runoff due to the extreme soil saturation, amplifying floodings, sediment transport and erosion. The precipitation, delivered in multiple pulses, caused rapid rises in river levels and successive flood peaks that merged downstream, producing large flood waves with crest levels exceeding, in many cases, the maximum measurable limits of hydrometric instruments. Despite volume losses from widespread overbank flooding in the foothill sections, small and medium-sized basins, such as Lamone, Montone, Ronco, Savio, experienced further bank overtopping and new breaches, while some previously repaired breaches failed again (Fig. 1).

The city of Ravenna and its surrounding coastal plain were among the most impacted areas, reflecting the high vulnerability of this region to flooding and storm surges driven by low topography (at or below mean sea level) and ongoing land subsidence³². Its landscape and development have been profoundly shaped by centuries of hydraulic engineering, land reclamation, and human intervention, making water management a central and continuous concern. Nowadays, Ravenna depends on a complex artificial

drainage network that allows agricultural activities, protect infrastructures, and accommodate human settlements by controlling vadose zone depth and maintaining constant water table depth in the range of 1.5–2.0 m below the ground during the year³³. Additional details on Ravenna's vulnerability and discharge values of the main rivers and canals are provided in the "Material and Methods" section and in Supplementary Table 1.

On the 16th–17th May, along the Adriatic coastal areas, storm surge also occurred. It was generated by Bora winds (NE wind), with remarkable wave heights reaching up to 3.23 m and sea levels rising up to 0.89 m asl³⁴. The event, lasting approximately 35.5 h, showed a synchronization between astronomical tide and meteorological components, a condition that increases the coastal hazard as it causes a rise in sea level and facilitates coastal flooding. Its return period (T_r) of ~1 year for wave height and <1 year for sea level, indicating a moderate marine anomaly whose impact was amplified by its coincidence with the river floods³⁴. In fact, storm surges worsened the 2023 May flooding by slowing the drainage of floodwaters. The drainage infrastructure, with narrow embankments not designed to handle this excess water, failed to efficiently discharge water to the sea and, in some cases, water stagnated over croplands and urban areas for several days after the event. In the Apennine region, prolonged saturation triggered nearly 66,000 landslides, disrupting critical infrastructure and isolating several communities. The impacts of this extreme event are well documented and include widespread evacuations, with 17 deaths and 36,600 evacuees; extensive flooding of farmlands leading to crop destruction and contamination³⁵, as well as livestock mortality; and severe structural damage in urban areas³⁶. The floods also caused important environmental impacts³⁷ as floodwater transported large quantities of sediments, organic matter, and pollutants into the surface water bodies (rivers and drainage networks), eventually reaching the Adriatic Sea. This led to algal blooms, the death of aquatic organisms³⁸, and severe impacts on the local economy.

Beyond these impacts, the study aims to assess additional direct and indirect effects on inland and marine waters that were not captured by standard routine monitoring conducted under major legislative protocols following the May 2023 flood. Following the event, the Regional Environmental Protection Agency of the Emilia-Romagna (ARPAE), carried out standard assessments, including physico-chemical and microbiological analyses to detect potential contamination of surface and ground water bodies (according to Water Framework Directive 2000/60/EC and the Italian D.Lgs. 152/2006), and the evaluation of bathing water quality (D.Lgs 116/2008). However, these programs are not designed to detect short-lived or subtle impacts such as shifts in biological communities, delayed ecological responses or the movement of CECs, which are the focus of the present study.

Here, we apply a multidisciplinary and integrated protocol to investigate geochemical, environmental, and biological responses to the May 2023 Emilia-Romagna flood.

The approach combines in situ measurements of abiotic and biotic parameters, characterization of protist communities using environmental DNA (eDNA), analyses of contaminants of emerging concern and satellite-derived data to assess water quality across the river-to-sea continuum.

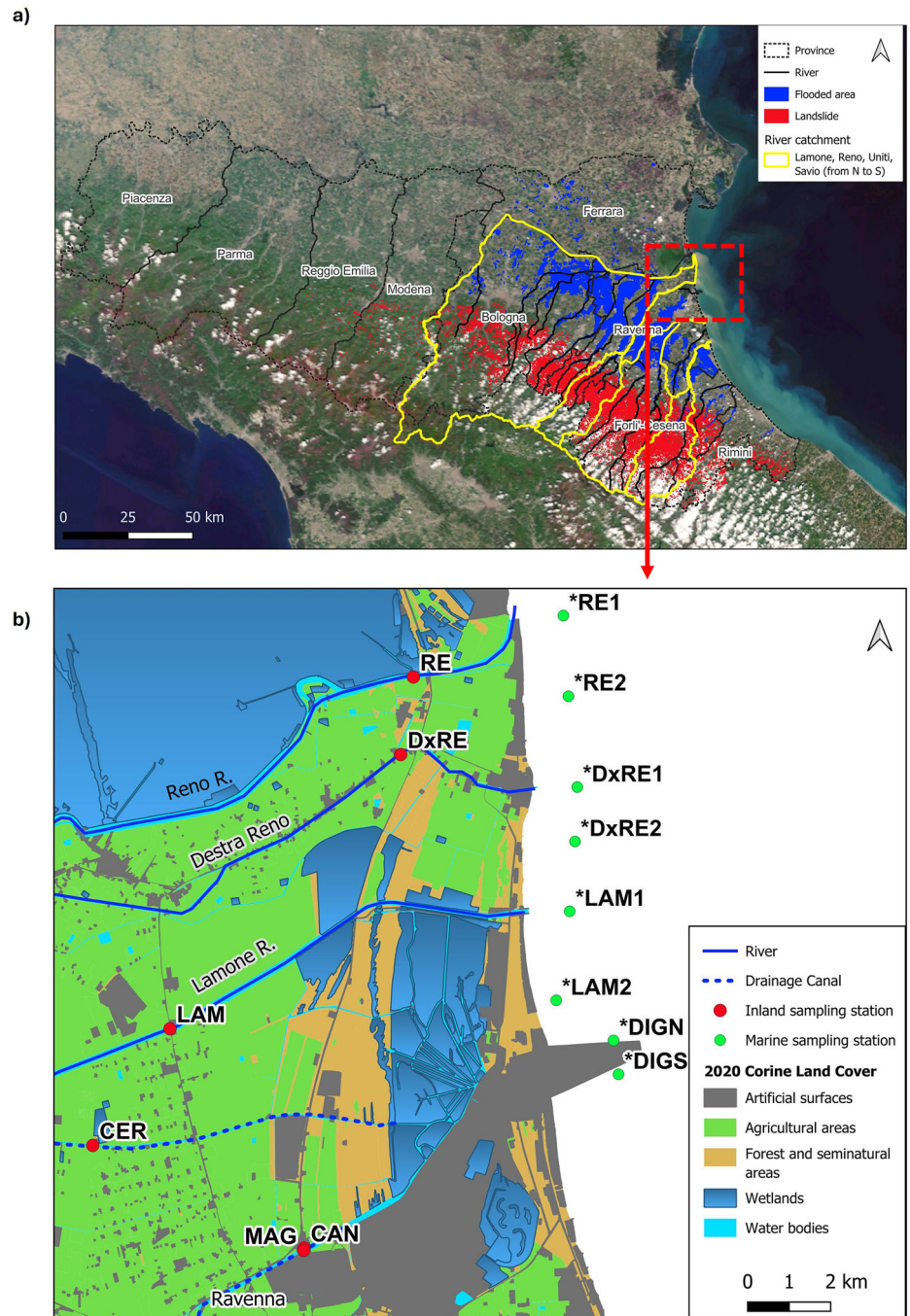
By integrating these complementary observations, this study addresses a critical gap in understanding how multiple components of aquatic ecosystems respond to extreme hydrological disturbances. Given the increasing frequency and intensity of extreme precipitation events and the scarcity of observations collected immediately after such events, our work provides a timely framework for assessing short- and medium-term environmental impacts and system recovery following large flood pulses.

Results

Environmental responses to the flood

Three coordinated surveys were carried out on 30 May (T1), 16 June (T2), and 10 October 2023 (T3) across a river-to-sea transect encompassing inland rivers and drainage canals, as well as coastal stations located in front of the main river mouths (Fig. 1). This sampling design captured the spatial gradient of flood influence and its short- to medium-term evolution, with

Fig. 1 | Areas affected by floods and landslides in May 2023 and location of the study area. a River catchments (yellow), rivers (black) and areas of the Emilia Romagna region affected by landslides (red) and flood (blue) in May 2023. (Data source: Emilia-Romagna data geoportal; base map: European Union, Copernicus Sentinel-2 imagery, date 05/05/2023); **b** Inland (red) and marine (green) sampling stations selected along the main rivers and drainage channels involved in the flood.



unaffected inland (Reno River, Cerba canal) and coastal (south of the Ravenna jetties) stations providing reference conditions. Across all stations, key physical, chemical, optical and biological parameters were measured in situ and from surface water samples to assess flood-related changes (see Materials and Methods section).

Following the flood event, the hydrological and ecological dynamics across the river-to-sea continuum underwent short-term but intense alterations, followed by a progressive attenuation of these anomalies over subsequent months (Fig. 2; full list of environmental parameters in Supplementary Table 2). To contextualize these changes, measurements from 2023 were compared with long-term ARPAE datasets: decade-long riverine records (2010–2022) for the Reno, Destra Reno and Lamone rivers (Fig. 3) and seasonal climatologies (2010–2022) from coastal station 6 for marine waters (Fig. 4). Although the two baselines differ in temporal structure,

together they show that the 2023 anomalies extended far beyond the typical variability of the system.

Figure 2 illustrates the spatial-temporal evolution of all measured parameters across the river-to-sea transects, whereas Figs. 3, 4 compare 2023 values with long-term inland and coastal baselines, respectively. Inland waters responded immediately to the exceptional freshwater discharge. Temperature (T), electrical conductivity (EC) and dissolved inorganic nitrogen concentrations $N(NO_3^- + NO_2^-)$ remained broadly within historical ranges, whereas dissolved oxygen (DO), pH and dissolved inorganic phosphate $P(PO_4^{3-})$ departed markedly from baseline conditions (Fig. 3). These deviations are quantitatively supported by the baseline comparison (Supplementary Table 3): T1–T2 DO and pH values consistently fell within the lowest percentiles (often <5%) of the 2010–2022 distribution across all stations, while phosphate peaks at DxRE and RE ranked above the 95th

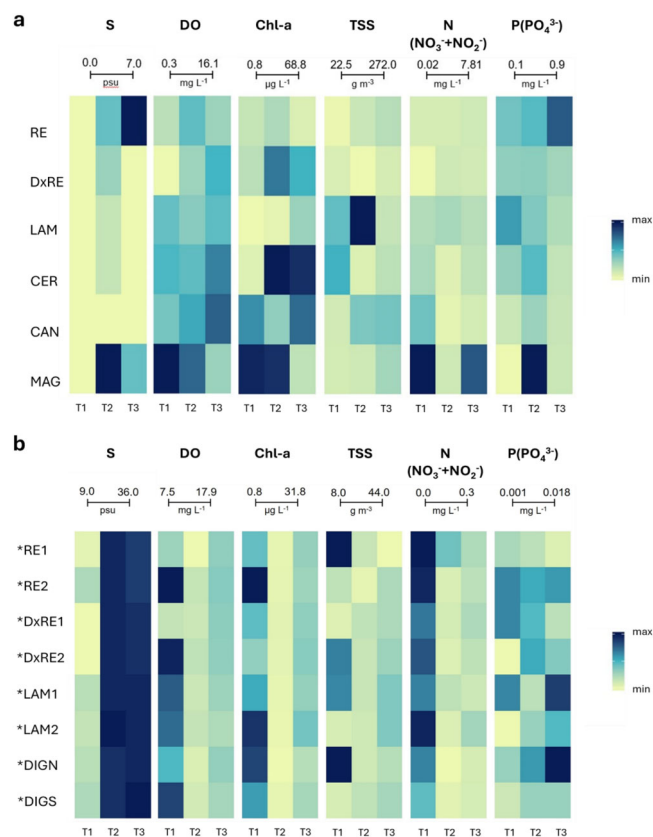


Fig. 2 | Heatmap of environmental parameters measured at inland and marine sampling stations following the May 2023 flood. a River and drainage-canal stations; **b** Marine stations. Panels represent, from left to right: salinity (S), dissolved oxygen (DO), chlorophyll-a (Chl-a), total suspended solids (TSS), dissolved organic nitrogen $N(NO_3^- + NO_2^-)$ and dissolved inorganic phosphorus $P(PO_4^{3-})$. For each parameter, values were normalized using min-max scaling to facilitate comparison of relative spatial and temporal variations across stations and sampling times. Absolute minimum and maximum values for each parameter are reported above the corresponding panels to provide a quantitative reference. Color intensity, therefore, reflects relative, not absolute, differences within each parameter and should not be compared across different variables.

percentile. Conversely, T, EC and $N(NO_3^- + NO_2^-)$ remained within the central portion of the historical range.

DO minima occurred during T1–T2, reaching near-anoxic levels at DxRE (0.3 mg L^{-1}), before gradually increasing by T3 (Fig. 2). Total suspended solids (TSS) increased strongly from T1 to T2, peaking at 272 g m^{-3} at CAN, consistent with intense sediment resuspension and transport within the drainage network. CDOM also rose sharply in T1 due to terrestrial inputs, remained elevated in T2 and declined by T3 (Fig. 2).

These inland responses were directly mirrored in the adjacent coastal zone. The massive freshwater pulse caused an exceptional drop in sea-surface salinity during T1, down to 9.0 psu at *RE and *DxRE1, values far outside the historical spring distribution (Fig. 4; $z = -5.9$, ≈ 0 th percentile; numerical details reported in Supplementary Table 4). Salinity recovered rapidly, returning to typical marine levels ($\sim 35 \text{ psu}$) by T2. Marine DO also reached hypoxic conditions in T1–T2 (5 mg L^{-1} at *RE1), improving by autumn. Suspended sediments displayed a more heterogeneous pattern across coastal stations, with maxima occurring either in T1 (*RE1, *DIGN) or in T2 (*DxRE1, *LAM2), reflecting spatial variability in plume retention and resuspension dynamics.

Nutrient patterns further illustrated the propagation of the flood signal from rivers to the sea. Inland dissolved inorganic nitrogen showed a classic flood-pulse response, with a sharp increase in T1 along a north–south gradient, a decline in T2 and a partial rebound in T3, while phosphate

exhibited a more variable inland pattern. In marine waters, nitrate+nitrite concentrations during spring and summer (T1–T2) fell within the historical range ($|z| < 1$), but autumn values were exceptionally low ($z = -2.3$; ~ 1 st percentile), indicating unusually nitrogen-poor post-recovery conditions. Conversely, $P(PO_4^{3-})$ remained within or slightly above typical values in spring and autumn ($|z| \approx 0.3$) but reached extremely high concentrations in summer ($z = 3.0$; ~ 99.9 th percentile), revealing a pronounced N–P decoupling linked to the flood and subsequent stratification.

Phytoplankton biomass responded strongly but transiently to these physical and chemical perturbations. In marine waters, chlorophyll-a (Chl-a) peaked immediately after the event (up to $31.8 \mu\text{g L}^{-1}$ at *RE2), corresponding to nearly four standard deviations above the historical spring mean ($z = 3.7$; >99 th percentile; Fig. 4). This bloom collapsed in T2, coinciding with elevated turbidity and partially recovered in T3, when Chl-a approached or exceeded the upper historical autumn range. Inland waters showed a temporal lag relative to the coast, with Chl-a and phaeopigments peaking in T2 and decreasing by T3 (Fig. 2), consistent with delayed primary production under confined freshwater conditions.

Overall, the combined inland and marine analyses indicate that the 2023 Emilia-Romagna flood produced environmental conditions far outside the typical variability of the river-to-sea system, especially for salinity, suspended sediments, oxygen dynamics, nutrient stoichiometry and Chl-a, before progressively returning toward baseline conditions by autumn.

Protist communities

The eDNA metabarcoding analysis of protist communities revealed strong compositional shifts across inland and marine stations (Fig. 5). A total of 74 taxa were detected, distributed across 29 different classes, whose relative abundances are provided in Supplementary Fig. 1. Inland stations were predominantly characterized by high abundances of Heterokontophyta (mainly diatoms), Cryptista, Ciliophora, and Chlorophyta. Conversely, marine stations exhibited higher taxa diversity, dominated mainly by Heterokontophyta and Dinoflagellata. Immediately following the flood (T1), marine stations experienced an influx of freshwater-associated taxa, particularly Bigyra, Chlorophyta, and Ciliophora, reflecting freshwater and terrestrial microorganisms input into coastal waters. By T2 and T3, these groups had declined in their abundance, while typical marine taxa re-established dominance, marking the system's ecological recovery to the typical seasonal phytoplankton assemblages described in ARPAE's annual monitoring reports³⁹, which serve as a reference for expected marine community conditions during this period.

Redundancy analysis (RDA; Fig. 6) was used to relate protist community structure to the main environmental gradients. In the ordination space, samples positioned closer together share a more similar community structure, whereas greater distances indicate increasing dissimilarity. Arrows represent environmental variables, with their direction and length indicating the strength and direction of their influence on community structure. The analysis confirmed that S, T, DO, TSS, and nutrients were the main environmental drivers structuring these communities, with the first two RDA axes explaining 31.7% of variance (RDA1: 21.4 %, RDA2: 10.3%) in the community structure. ANOVAs (Table 1) confirmed that all environmental predictors significantly influenced community structure (S $p < 0.001$; T and DO $p < 0.01$; TSS , $N(NO_3^- + NO_2^-)$, $P(PO_4^{3-})$ $p < 0.05$). Inland samples were more dispersed in ordination space immediately after the flood but became progressively clustered by T3, indicating gradual ecological stabilization. These temporal patterns reveal a strong but transient disturbance followed by a clear trend of recovery and resilience within 5 months.

Occurrence of pharmaceuticals

Flood-induced hydrological perturbations also mobilized contaminants of emerging concern, notably pharmaceutical residues (Fig. 7 and Supplementary Table 5 for the full list). Pharmaceuticals analyzed in this work include four anti-inflammatories (i.e., Ketoprofen, Diclofenac, Ibuprofen, Naproxen), two diuretics (Furosemide and Hydrochlorothiazide) and the

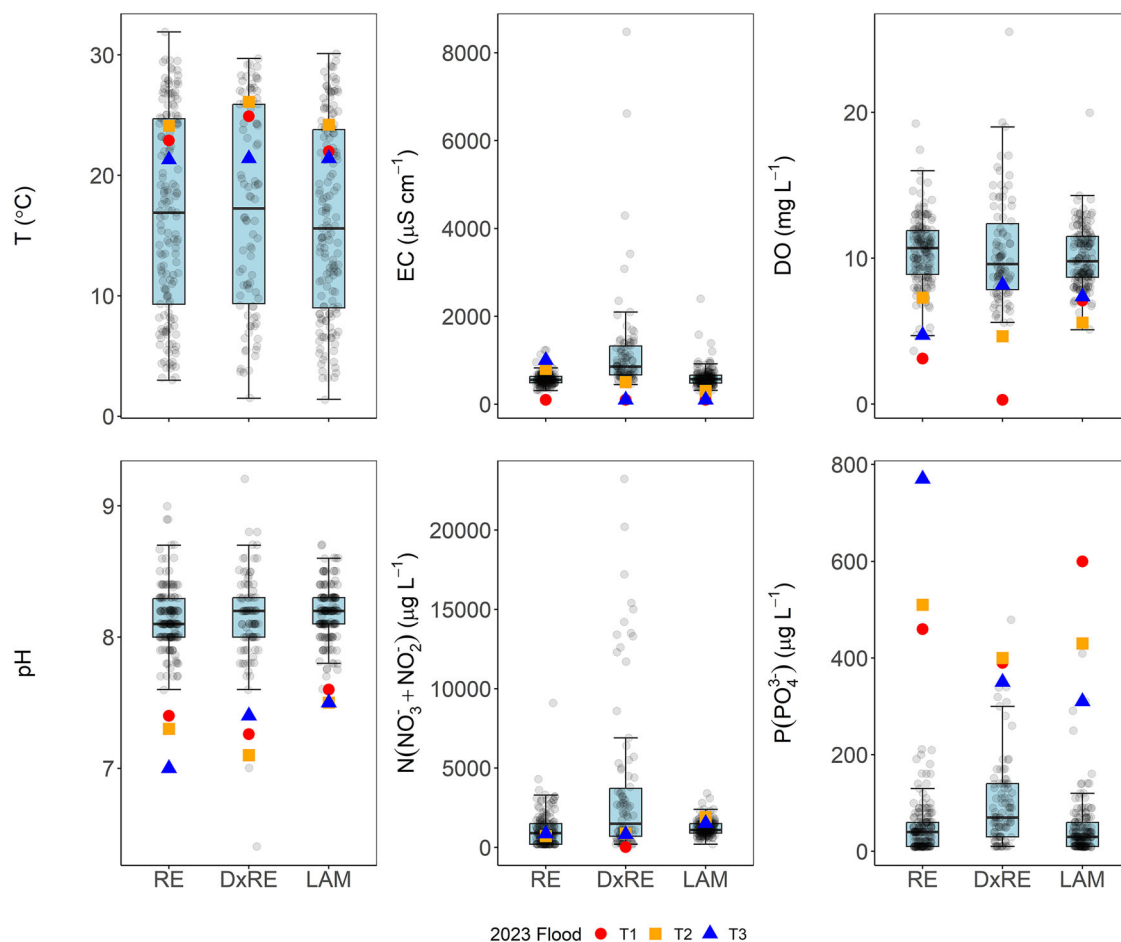


Fig. 3 | Comparison of environmental parameters measured during the 2023 flood event with historical baseline data (2010–2022; boxplots) from ARPAE inland sampling stations RE, DxRE, and LAM. Boxplots represent historical seasonal distributions, while colored symbols indicate measurements from the 2023

post-flood sampling periods (T1–T3). Parameters shown include salinity (S), chlorophyll-a (Chl-a), dissolved inorganic nitrogen $\text{N}(\text{NO}_3 + \text{NO}_2)$ and dissolved inorganic phosphorus $\text{P}(\text{PO}_4^{3-})$. Note the marked deviations observed during the flood event, highlighting substantial short-term environmental alterations.

antipyretic Paracetamol. LC-MS/MS analyses indicated the widespread occurrence of these compounds; in particular Diclofenac, Ketoprofen and Ibuprofen have been detected in almost all samples analyzed, both in surface and marine waters and in the different sampling campaigns (Supplementary Table 5). Fig. 7 reports the sum of pharmaceutical concentrations occurring in each sampling campaign. The inland stations RE e DxRE exhibited elevated values in May (T1) RE: 72.24 ng L^{-1} , DxRE: 27.33 ng L^{-1} followed by a gradual decrease in June (T2) and October (T3).

The same was observed for all the marine stations: pharmaceutical concentrations were higher in T1 than in T3, mirroring the physical and biological recovery trends. Pharmaceutical occurrence in some inland stations did not display a temporal gradient: total pharmaceutical concentrations at the inland MAG station reached 395.98 ng L^{-1} in T1, dropped to 42.83 ng L^{-1} in T2, and rebounded to 395.77 ng L^{-1} by T3. Differently, the LAM station showed a peak-concentration at T2 and lower levels at T1 and T3. A common feature in pharmaceuticals occurrence is represented by the spatial gradient: marine stations exhibited much lower pharmaceutical levels with respect to inland stations, decreasing steadily from T1 to T3 in each monitoring campaign.

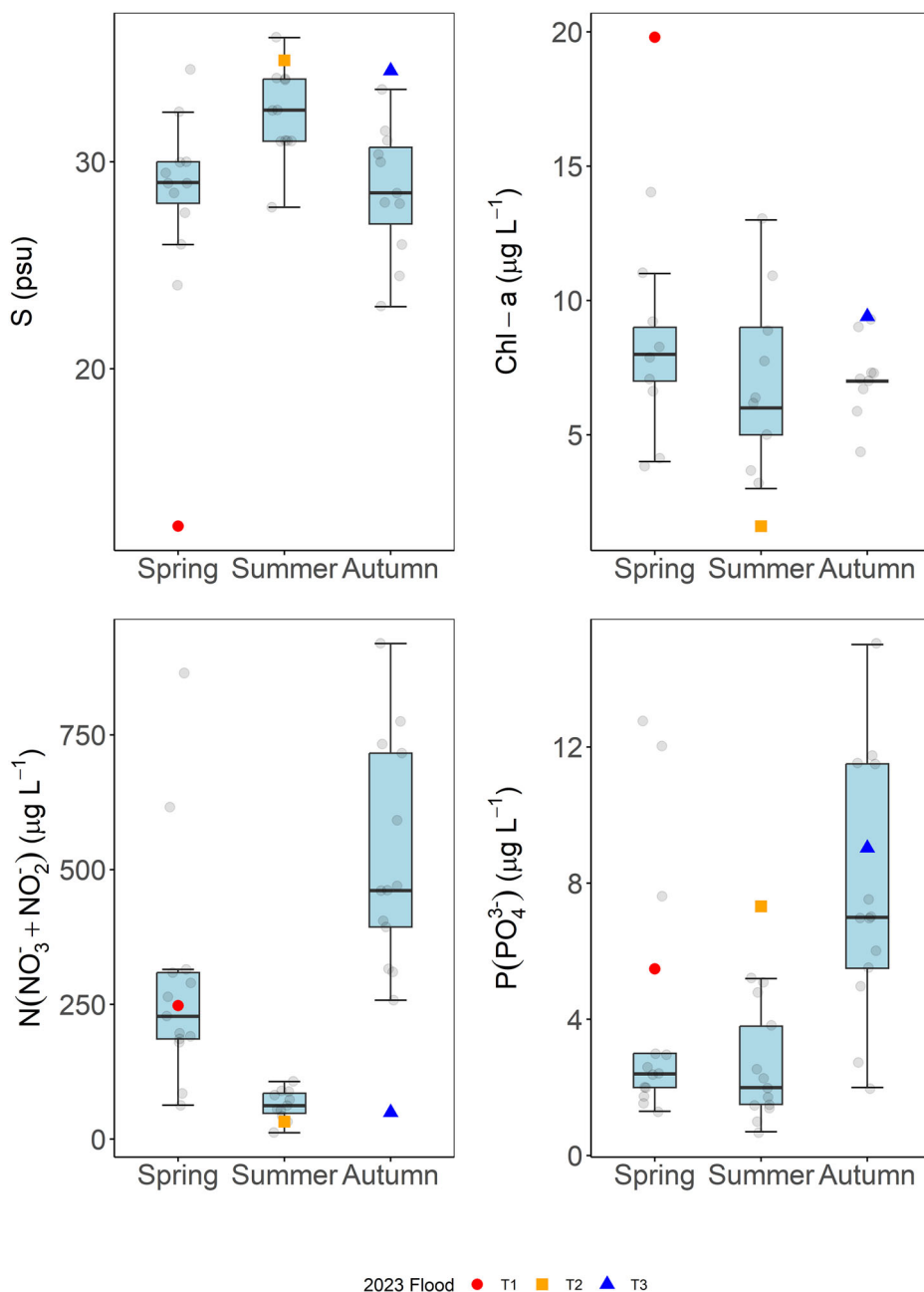
Remote sensing and image-based water quality

To further assess how exceptional the May 2023 conditions were, beyond what could be inferred from in situ ARPAE observations, we also examined long-term satellite-derived Chl-a climatologies. Remote sensing provides the only continuous multi-decadal record for the region, allowing us to place the 2023 anomaly in the context of historical May variability.

Across all coastal stations, Chl-a concentrations during May 2023 were markedly higher than the 2013–2022 baseline (Table 2). Mean anomalies ranged from $+7$ to $+16 \mu\text{g L}^{-1}$, corresponding to standardized anomalies between 0.7 and 2.0 σ . The strongest deviations occurred at the southernmost stations (*LAM2, *DIGN, *DIGS), where Chl-a levels exceeded both the long-term mean and the upper percentile range of the recent climatology. The most pronounced anomaly was observed at *DIGS ($Z = 2.02$; $\approx 98\text{th}$ percentile), indicating an exceptional bloom intensity relative to the variability of the last decade. *LAM2 ($Z = 1.56$; $\approx 94\text{th}$ percentile) and *DIGN ($Z = 1.40$; $\approx 92\text{nd}$ percentile) also showed strong positive anomalies, while northern stations (*RE1, *DxRE2) exhibited moderate but consistent increases (75th–88th percentile).

This spatial pattern indicates a southward intensification of coastal productivity during the May 2023 event, with a coherent regional signal superimposed on local variability. Overall, the 2023 bloom stands out clearly from the background variability of the OLCI-VIIRS era, representing a high-intensity event located within the statistical upper tail of the satellite-derived distribution. The daily evolution of Chl-a further highlights the anomalous nature of the event (Fig. 8). While baseline conditions typically remained below $20 \mu\text{g L}^{-1}$, 2023 values showed a distinct late-May enhancement, with peaks between days 25–30. Southern stations recorded the largest departures, with daily concentrations temporarily exceeding the May-specific 95th percentile and, at *DIGS, reaching values nearly an order of magnitude above the climatological median. Northern stations displayed a similar timing but lower magnitude, indicating a spatially coherent but intensity-graded response along the coastline.

Fig. 4 | Seasonal baseline distributions (2010–2022) for salinity (S), chlorophyll-a (Chl-a), dissolved inorganic nitrogen $N(NO_3^- + NO_2^-)$ and dissolved inorganic phosphorus $P(PO_4^{3-})$ from ARPAE monitoring station 6 (Casalborsetti), overlaid with 2023 post-flood observations. For each sampling time (T1, T2, T3), values represent the mean of eight stations sampled in this study, all located within ~5 km of Casalborsetti. Because ARPAE provides seasonally aggregated long-term data, each 2023 sampling time was compared with the corresponding ARPAE season (e.g., T1 with Spring, T2 with Summer, T3 with Autumn). Box-plots represent historical seasonal distributions, while colored points show 2023 values relative to this baseline.



The synchrony of bloom peaks across stations suggests a common regional-scale driver, likely linked to the post-flood nutrient enrichment that following the extreme rainfall and river discharge event. Together, the historical comparison and daily-scale analysis confirm that May 2023 represents an extraordinary Chl-a anomaly in both magnitude and temporal dynamics.

Having established that the 2023 flood triggered an exceptional Chl-a anomaly, we then evaluated how accurately current satellite sensors and algorithms can capture water-quality variability under such extreme optical conditions.

Satellite-based monitoring effectively captured the spatial footprint and temporal evolution of the flood-driven changes. Sentinel-2 and Sentinel-3 retrievals of Chl-a and TSS aligned with in situ observations (see Supplementary Tables 6, 7 for full results), although their performance varied depending on turbidity and dissolved organic matter levels.

During T1 (May 2023, peak turbidity), Sentinel-2 algorithms (ACOLITE chl_oc3 and C2Nets C2XCOMPLEX) achieved the best performance (MAE $\approx$ 32–34%), accurately reproducing the strong Chl-a variability

associated with the flood pulse. TSS retrievals showed higher errors (MAE $\approx$ 54–58%), reflecting the challenging optical conditions created by suspended matter and CDOM.

By T2, Sentinel-3 OLCI chl_nn and C2Nets C2X showed improved accuracy (MAE $\approx$ 18–24%), whereas Sentinel-2 Chl-a retrievals displayed substantial overestimation, likely due to the low concentration range and reduced spectral contrast of summer waters. TSS retrievals improved markedly (MAE $\approx$ 18–26%).

Under T3, Chl-a retrieval errors fell below 20% for both Sentinel-2 and Sentinel-3, and TSS models (particularly C2X) performed with high accuracy (MAE 49%, bias -8.1). Spatial distribution maps derived from C2X (Fig. 9) clearly captured the extensive sediment plumes and elevated Chl-a during T1, their progressive attenuation during T2 and the re-establishment of typical regional patterns by T3, consistent with in situ observations.

Finally, we compared satellite-derived CDOM absorption (acdom 443 nm) with laboratory measurements, where available. Since only the C2Nets framework and Sentinel-3 OLCI WFR provide CDOM products, this validation was restricted to a subset of dates and algorithms. Results

Fig. 5 | Spatiotemporal changes in protist community composition across inland and marine environments. Relative abundance (%) of protist communities at the phylum level across sampling stations and time points (T1–T3), based on eDNA metabarcoding. Colors indicate protist phyla as shown in the legend.

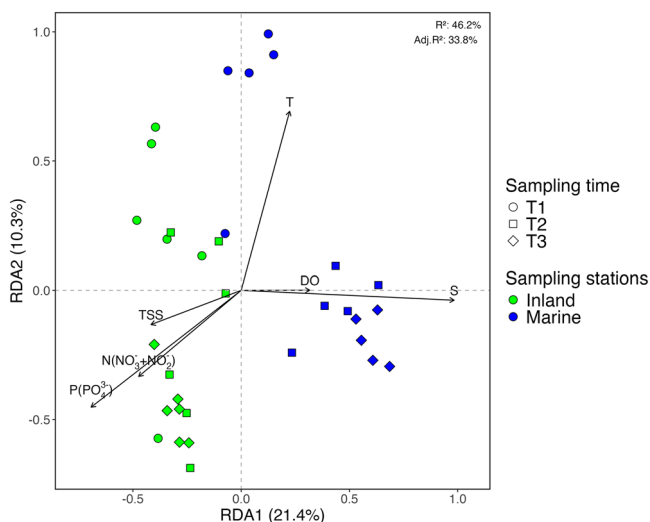
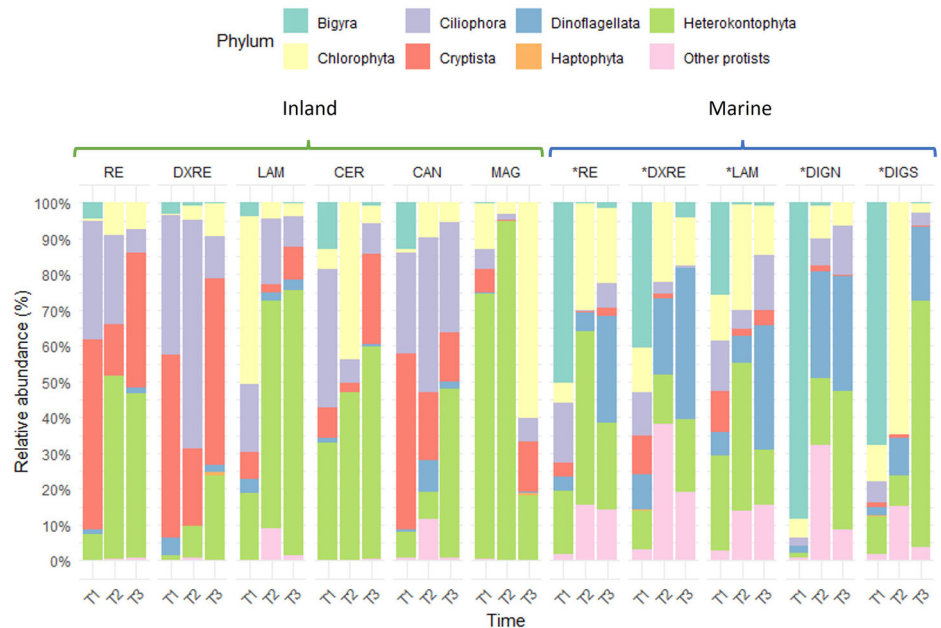


Fig. 6 | Redundancy analysis (RDA) showing the relationship between protist community composition and environmental variables across inland (green) and marine (blue) stations. Points represent individual samples, shaped by sampling time (T1–T3). Arrows indicate environmental gradients, with length proportional to their influence on community structure. Samples closer in ordination space exhibit more similar community composition.

showed substantial overestimation and high uncertainty, indicating limited reliability over the study period; therefore, CDOM retrieval metrics are reported exclusively in Supplementary Table 8.

Overall, these results confirm that remote sensing offers an effective complementary tool for detecting flood-driven disturbances and subsequent ecosystem recovery, despite the known limitations of bio-optical algorithms under extreme turbidity and CDOM conditions.

Discussion

This multidisciplinary study provides a comprehensive assessment of the short- and medium-term impacts of the May 2023 Emilia-Romagna flood, documenting for the first time the environmental, biological, and chemical alterations triggered by an extreme hydrological disturbance along this

Table 1 | Results of ANOVAs testing the influence of each environmental variable on protist community structure based on redundancy analysis (RDA)

Terms	Df	Variance	F	Pr (>F)
S (psu)	1	0.0811	6.3741	0.0001***
T (°C)	1	0.0352	2.7704	0.004**
DO (mg L ⁻¹)	1	0.0302	2.3735	0.0097**
TSS (g m ⁻³)	1	0.0272	2.1362	0.0117*
N(NO ₃ ⁻ + NO ₂ ⁻) (μg L ⁻¹)	1	0.0261	2.0504	0.0202*
P(PO ₄ ³⁻) (μg L ⁻¹)	1	0.0313	2.4605	0.0032**
Residual	26	0.3308		

Notes: Degrees of freedom are reported as Df. Significance codes: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

All predictors significantly influenced community structure, with salinity (S), temperature (T), dissolved oxygen (DO), total suspended solids (TSS), dissolved inorganic nitrogen N(NO₃⁻ + NO₂⁻) and phosphorus P(PO₄³⁻) showing varying degrees of explanatory power.

coastal region. By integrating inland and marine observations, we reveal a coherent response of disturbance and recovery across the river–sea continuum, captured through coordinated inland observations and near-surface marine sampling. The May 2023 flood transported massive freshwater, sediments, nutrients, and organic matter into the river–drainage–sea system, driving pronounced departures from standard environmental conditions. These inputs triggered a cascade of short-term physical, chemical, and biological responses, followed by progressive but spatially uneven recovery.

In line with the observed physical-chemical changes, marine protist communities responded rapidly to the flood-induced stress. In fact, salinity, dissolved oxygen, and nutrient loads result as the primary drivers shaping protist community structures, consistent with global findings in flood-impacted systems^{40,41}. High river discharge into the sea during and immediately after the May 2023 flood event created conditions characterized by elevated nutrients, reduced salinity and light availability that favored the proliferation of freshwater and opportunistic taxa^{42,43}, mainly Bigyra, Chlorophyta and Cryptista, into near-surface coastal waters, while high TSS and CDOM limited light penetration, constraining in situ primary production. The temporary increase in Cryptista and other nutrient-tolerant

groups, as well as heterotrophic taxa such as Bigyra and Ciliophora, is consistent with post-flood dynamics reported in other estuarine systems^{43–45}. Their short-lived dominance reflected the high availability of organic matter in the turbid near-surface waters influenced by the freshwater plume.

The simultaneous presence of high Chl-a and turbidity recorded both in situ data and satellite imagery during the first post-flood sampling (T1, May), suggests advected upstream phytoplankton biomass and short-lived local blooms driven by nutrient pulses due to freshwater inflows. The temporary increase in photosynthetic activity, despite sub-optimal optical conditions, is a phenomenon also reported in other coastal systems following major hydrological events^{44,46}. It should be noted that T1 (30 May 2023) represents the earliest feasible post-event survey under emergency conditions, rather than the instantaneous peak following the flood pulses earlier in May. Therefore, some short-lived maxima (e.g., TSS and CDOM) may have been higher immediately after the event and could be underestimated in our T1 measurements, although sustained drainage from flooded areas likely maintained markedly perturbed conditions at the end of May. As the hydrological signal weakened from T1 to T3, seawater salinity increased rapidly (~35 psu), suspended solids diminished and oxygen concentrations increased, collectively enabling the re-establishment of

typical marine protist assemblages dominated by Dinoflagellata and Heterokontophyta by autumn. Our marine sampling targeted the upper 0–50 cm of the water column, i.e., the layer most directly affected by riverine plumes. Therefore, the marine patterns discussed here primarily describe near-surface responses, while vertical gradients may have occurred under stratified conditions. This near-surface focus is also consistent with satellite ocean-color observations, which are inherently limited to the upper optical layer of the water column and therefore rely on surface in situ measurements for calibration and validation.

While highly energetic hydrological conditions during the immediate post-flood phase (T1) likely promoted strong mixing in rivers, drainage channels and near-surface coastal waters, such conditions cannot be assumed for the later sampling periods. During T2 and T3, hydrodynamic forcing progressively weakened, and the development of vertical or lateral gradients, particularly in the marine environment and in low-flow inland channels, cannot be excluded. Accordingly, interpretations for T2 and T3 reflect conditions at the sampled depth rather than the entire water column. The lack of depth-resolved measurements represents a limitation of the present study and constrains our ability to fully assess stratification and vertical heterogeneity during the recovery phase. Future studies would benefit from vertical profiling (e.g., CTD casts, depth-resolved nutrient and oxygen measurements) and higher-frequency sampling to better resolve mixing processes and their temporal evolution following extreme flood events.

Inland waters exhibited more heterogeneous and slower trajectories of recovery but shared a similar successional pattern: opportunistic taxa declined over time and were progressively replaced by Heterokontophyta, consistent with transitional freshwater systems undergoing post-disturbance stabilization^{47,48}. This community change through time appeared broadly consistent across inland stations, indicating that ecological succession following extreme events may follow common pathways even in different freshwater contexts. However, the pace and magnitude of these changes differed among stations. The marked spatial variability observed across inland stations likely reflects the complex interplay between hydrology, land use and emergency water-management conditions during and after the flood. In the Ravenna lowlands, drainage channels received water volumes far beyond their design capacity, and pumping operations created prolonged zones of stagnation. Consequently, the waters entering the network varied greatly in composition, from agricultural and urban runoff carrying mobilized contaminants to stagnant floodwaters enriched through prolonged contact with inundated areas. These contrasts likely

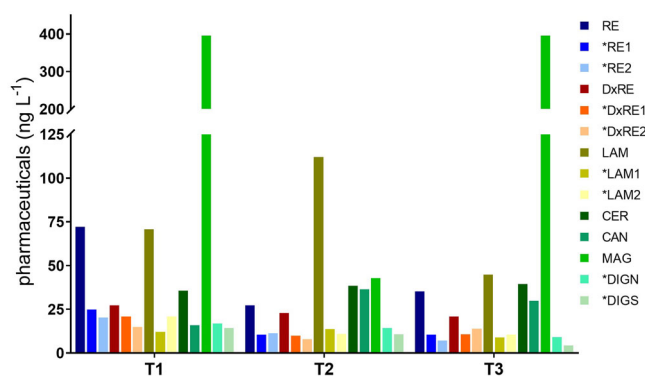


Fig. 7 | Pharmaceuticals were measured in water samples collected during three campaigns in 2023. Bars show the sum of the seven pharmaceuticals analyzed. Limit of quantification (ng L^{-1}) and relative standard deviation (RSD %) ($n = 3$) for each compound analyzed are indicated in “Materials and Methods”. T1, May; T2, June; T3, October.

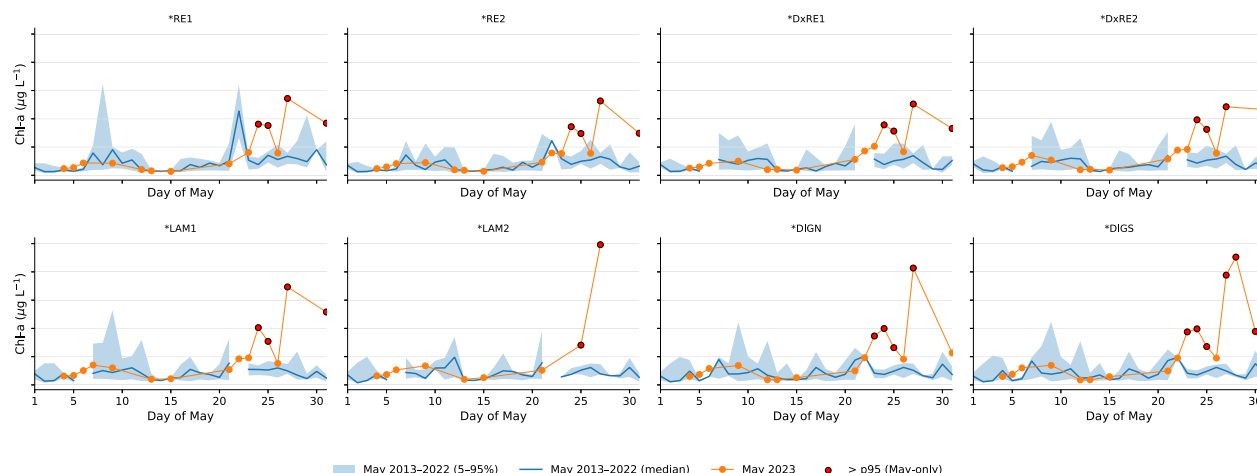


Fig. 8 | Daily chlorophyll-a (Chl-a) concentrations ($\mu\text{g L}^{-1}$) at the eight coastal stations during May 2023 (orange line and dots), compared with the May climatology derived from the 2013–2022 baseline (blue line = median; shaded area = 5th–95th percentile range). Red markers indicate days when May 2023

concentrations exceeded the station-specific 95th percentile (p95) computed from the 2013–2022 May baseline. Data originates from the CMEMS OCEANCOLOUR_MED_BGC_L3_MY_009_143 Level-3 daily multisensor product (1 km resolution).

Table 2 | Baseline-event comparison of satellite-derived chlorophyll-a concentrations for the marine stations during May 2023 relative to the 2013–2022 climatology

Station	Baseline mean	Baseline SD	Baseline 95th percentile	Event mean	Z-score (σ)	Percentile rank (%)	N (baseline)	N (May 2023)
*RE1	9.9	10.9	24.7	17.1	0.7	75	116	14
*RE2	8.3	6.4	22.4	16.0	1.2	88	116	15
*DxRE1	9.3	7.4	23.9	17.1	1.1	86	113	15
*DxRE2	9.5	7.5	23.2	18.0	1.1	87	109	16
*LAM1	9.9	8.7	24.4	20.9	1.3	89	110	15
*LAM2	9.1	7.3	21.3	20.5	1.6	94	73	9
*DIGN	9.6	7.7	23.7	20.3	1.4	92	122	15
*DIGS	9.6	7.8	24.9	25.5	2.0	98	125	17

Baseline statistics (mean, standard deviation, 95th percentile) were computed from all available May observations in the 10-year historical record. Event mean represents the average chlorophyll-a concentration retrieved for May 2023. Z-scores (σ) quantify deviations from the baseline mean in standard deviation units, and percentile ranks indicate the empirical position of 2023 values within the climatological distribution (0% and 100% indicate values outside the historical range). Units for all chlorophyll-a concentrations are $\mu\text{g L}^{-1}$.

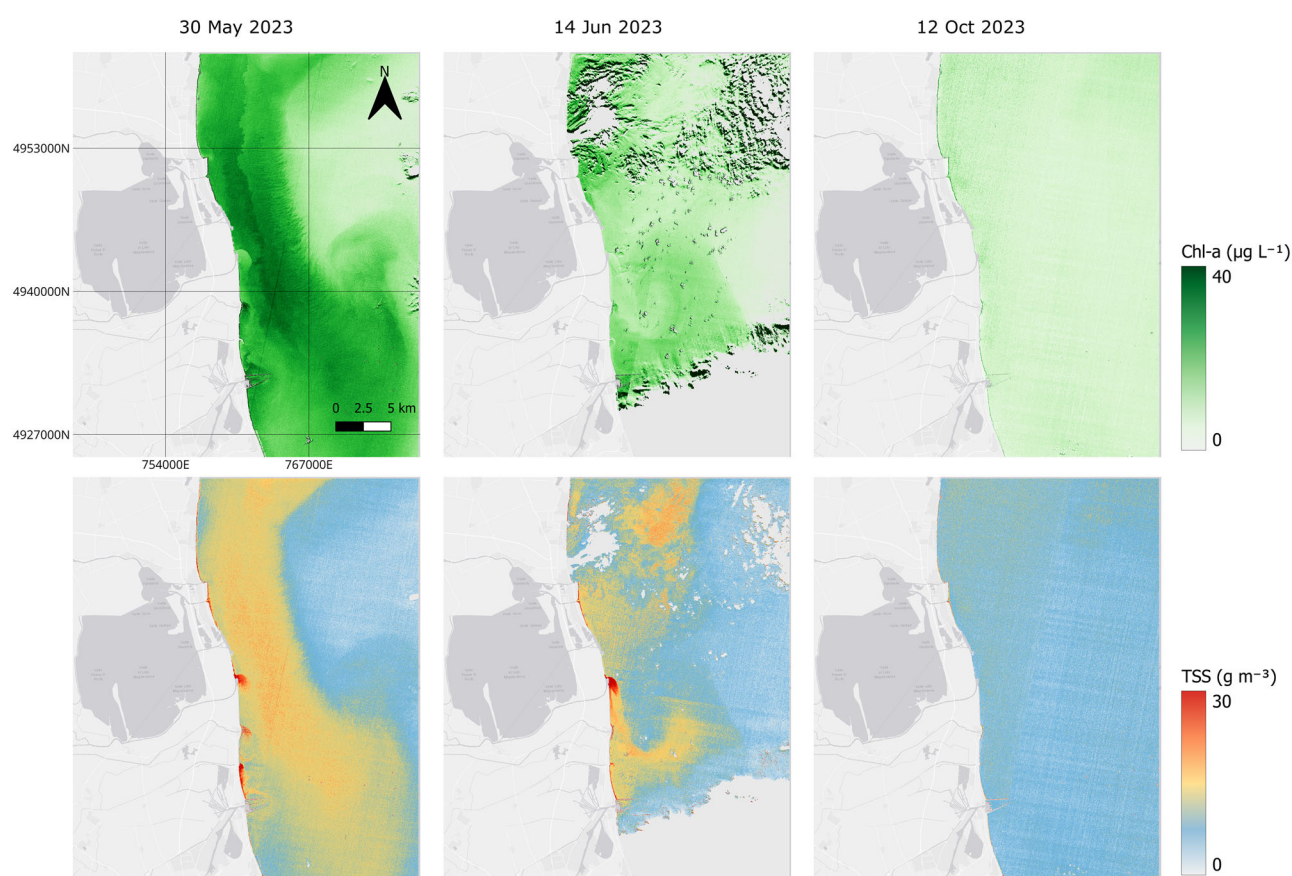


Fig. 9 | Sentinel-2 maps of chlorophyll-a and total suspended solids along the Ravenna coastal zone derived with the C2X processor (C2RCC plugin in SNAP). Upper row: chlorophyll-a (Chl-a , $\mu\text{g L}^{-1}$). Lower row: total suspended solids (TSS, g m^{-3}). Acquisition dates are shown above each column. Prior to processing, 20-m products were masked using the Sen2Cor land/sea mask, retaining only pixels

classified as marine water (land and inland-water pixels were removed), consistent with the C2RCC/C2X optimization for coastal and marine waters. Sampling station locations are shown only in the upper-left panel for reference and are identical for all panels; they are not repeated to avoid map overcrowding.

contributed to the heterogeneous recovery trajectories observed across inland stations.

Pharmaceuticals exhibited an inland-to-coast gradient. Moreover, pronounced T1 peaks of pharmaceuticals occurred only at selected inland stations (RE, MAG and DxRE), indicating localized mobilization of anthropogenic sources within portions of the inundated catchment. Concentrations generally declined in subsequent samplings, especially in marine stations and toward the coastal zone, consistent with dilution, degradation

and downstream transport^{49,50}. Their decrease over time mirrored the recovery of physical and biological parameters. The concentrations detected in drainage canals (particularly elevated in MAG), which drain urbanized areas next to the city, underscore their role as key conduits of pollutants, and the need for dedicated monitoring given their current lack of systematic surveillance.

Beyond situ observations, remote sensing offered an additional, independent perspective on the magnitude and spatio-temporal footprint of the

flood. Satellite data not only allowed us to place the 2023 event within a multi-decadal context but also provided a means to assess how effectively current algorithms capture water-quality dynamics under the extreme optical conditions generated by the flood.

Remote-sensing analyses provided two complementary lines of evidence that help contextualize the May 2023 event. First, the long-term satellite climatology (2013–2022) allowed us to assess whether the flood-driven response was detectable against multi-decadal background variability. The comparison showed that Chl-*a* concentrations during May 2023 exceeded the historical envelope at all stations, with anomalies particularly pronounced in the southern sector, where values reached the 95th–98th percentiles of the multisensor record. Daily trajectories highlighted a coherent late-May peak across the coastline, confirming that the post-flood enhancement was not only evident *in situ*, but also clearly detectable from space when viewed against the full OLCI–VIIRS era. This demonstrates that the flood produced a bloom of sufficient magnitude, duration and spatial coherence to be captured by operational ocean-color products, even in a region characterized by high optical complexity.

A second set of analyses examined how accurately current satellite algorithms can retrieve water-quality parameters under such extreme optical conditions, but their performance was strongly modulated by the hydrodynamic and optical complexity produced by the flood. During T1, when suspended sediments and CDOM dominated the reflectance signal, algorithm accuracy deteriorated for both Sentinel-2 and Sentinel-3 products, reflecting the inherent challenges of Case-2 waters under hydrological disturbance. Even algorithms designed for turbid or optically complex waters, including C2RCC variants, ACOLITE empirical and semi-analytical approaches, and red/NIR band-based models^{51–54}, showed variable performance, reflecting their sensitivity to region-specific optical conditions⁵⁵. As conditions stabilized in T2 and T3, suspended solids declined and water clarity increased, leading to a marked improvement in retrieval accuracy across most algorithms with Chl-*a* and TSS errors falling to levels comparable to or better than those reported for other European coastal systems^{56,57}. This highlights both the limitations of current bio-optical approaches during highly dynamic events and their robustness once the system returns to more typical optical regimes.

Together, these two remote-sensing perspectives, historical context and algorithm evaluation demonstrate that satellite products can both detect the exceptional nature of extreme events and track their recovery, while also revealing the conditions under which their accuracy is compromised. This underscores the value of integrating long-term multisensor archives with targeted matchups during extreme events and points to the need for region-specific calibration strategies capable of capturing the rapid optical shifts characteristic of flood-impacted coastal waters.

Taken together, the *in situ* measurements, historical ARPAE comparisons, biological responses, contaminant patterns and remote-sensing analyses converge toward a consistent picture: the May 2023 flood produced environmental conditions that were both extreme and spatially coherent across the entire river–sea continuum. This integrated evidence provides a strong basis for placing the event in a broader temporal context. To evaluate whether such a disturbance has any precedent in the region, we place our observations in the context of documented extreme floods of the northern Adriatic basin. The 2023 flood stands out both in magnitude and in the spatial coherence of its impact. Earlier Po River floods in 2000⁵⁸ and 2014⁴⁶ also altered the marine system but produced smaller quantitative changes. During those events, salinity minima ranged from 6–7 psu in 2000 to values remaining above 29 psu in 2014, whereas in 2023 salinity dropped to 9–10 psu simultaneously across all marine stations, indicating a more intense and widespread freshwater influence. CDOM absorption at 443 nm (a_{443} up to 1.9 m^{-1}) and suspended-solids concentrations (up to 44 mg m^{-3}) also exceeded previously reported maxima for high-discharge conditions in the northern Adriatic^{46,59}, suggesting enhanced transport of sediment and humic-rich material⁶⁰. In contrast, nutrient concentrations were lower than those documented during the 2000 and 2014 floods, likely reflecting rapid dilution, intense flushing and/or swift biological uptake during the early

post-flood phase, although persistently elevated values in several drainage channels point to chronic anthropogenic inputs.

Overall, despite the severity of the disturbance, the coupled inland–marine system demonstrated a remarkable capacity for short-term recovery, as evidenced by the temporal and spatial gradients observed across the measured variables (see also conceptual summary in Fig. S2). Within five months, most physical, chemical, biological and contaminant-related parameters had returned close to baseline conditions. Yet, the limited availability of high-resolution pre-event data, particularly for contaminants and protist communities, remains a major constraint for fully quantifying the magnitude and ecological footprint of extreme floods. Our findings highlight the need for integrated, long-term monitoring frameworks combining conventional analyses, molecular tools and multi-sensor remote sensing to capture the cascading effects of future hydrological extremes in this increasingly vulnerable region.

Conclusions

Despite the absence of complete site-specific pre-event baselines, which represents a critical limitation, this study offers a crucial reference point, a post-event snapshot against which future monitoring efforts and environmental assessments can be compared. The results underscore the value of a sampling design that integrates rivers, canals and marine stations, which allowed us to trace the pathways and characteristic times required for nutrients and pollutants to travel from the coastal plain to the sea, particularly within the surface plume layer. We showed that the flood mobilized contaminants from agricultural and urban areas, delivering a concentrated pulse of pollutants to the coast, further exacerbated by drainage network failures.

Regardless of the severity of the initial impacts, including pronounced shifts in water chemistry, biological communities and concentrations of contaminant of emerging concern, ecosystems showed a notable capacity for short-term resilience, with substantial chemical and biological recovery within a few months, particularly by October 2023. However, important questions remain regarding the long-term resilience of these coastal systems under future scenarios of more frequent and intense hydrological extremes driven by climate change. Our findings point to a critical need for continuous, long-term, multi-parameter monitoring that bridge traditional assessment protocols with innovative high resolution remote sensing and molecular tools. Such integrated strategies are essential to capture environmental dynamics and assess ecosystem responses under extreme event scenarios, ultimately improving preparedness and management capacity in the face of climate-driven hydrological disturbances.

Materials and methods

Study area

Originally part of the Po River delta and its extensive alluvial plain, the study area was historically characterized by meandering rivers that transported water and sediments from both the Alps to the north and the Apennines to the south. The plain is filled with Plio-Quaternary sediments, reaching up from 4 to 8 km in thickness in the study area⁶¹.

Since Roman times, human activities have progressively modified the natural hydrographic network. In the alluvial plain, the rivers were straightened all the way to the sea, causing sediment to accumulate within the artificial embankments. Today, these rivers flow as perched channels above the surrounding plain, confined by embankments and hydraulic structures that keep them several meters above ground level. Large-scale reclamation works, mainly implemented between 1920 and 1960, transformed marshlands into agricultural land through the construction of hundreds of kilometers of drainage canals, sluices, and water pumping stations, which regulate groundwater levels, prevent flooding, and maintain optimal vadose zone depths (1.5–2.0 m below ground) for farming throughout the year. Initially conceived for land reclamation and agricultural expansion, this infrastructure today also plays a vital role in flood defense, infrastructure protection, and urban safety³³. Over the last five decades, drainage volumes have shown a persistent upward trend, primarily

driven by factors such as subsidence-induced vertical gradients. Climatic drivers, such as altered precipitation patterns, have contributed secondarily to these long-term trends⁶². The Ravenna coastal zone is low-lying, with large portions at or even below mean sea level, and in the last century has been experiencing notable land subsidence, both natural and anthropogenic, with maximum rates during the 1970s due to intense groundwater and natural gas extraction^{63,64}. Although mitigation measures and regulations on groundwater withdrawal have since reduced subsidence rates, vertical ground movements remain an active process, particularly along the coast⁶⁵.

These characteristics make the system particularly vulnerable, as water management relies on a very delicate balance between the primary hydrographic network (rivers) and the secondary network (drainage system). Under normal conditions, this balance allows for the regulation of water flows and helps prevent flooding or water stagnation. However, during extreme weather events, such as the intense and prolonged rainfall of the 2023 event, or in case of overflows and levee breaches, the sudden increase in water volume can exceed the system drainage capacity and lead to flooding and damage to infrastructure.

Field measurements

The study focused on the monitoring of rivers and freshwater canals (hereafter indicated as inland sampling stations), as well as in several station of the portion of the Adriatic Sea in front of the Ravenna coast, which were impacted and affected by the May 2023 flood event (hereafter indicated as marine sampling stations). The sampling strategy was designed under post-emergency conditions to capture the main gradients of flood impact both inland and at sea, while ensuring safe and temporally consistent operations. Sampling campaigns were carried out in a coordinated manner by a large multidisciplinary team. Fieldwork was organized into specialized teams operating simultaneously across inland and marine environments to minimize the duration of sampling. In May and June, inland and marine samples were collected on the same day (morning and afternoon, respectively), while in October the two environments were sampled on consecutive days. This coordinated design ensured temporal coherence between compartments and minimized short-term variability.

Inland stations were selected along channels and rivers that experienced the highest flood-induced discharge and sediment transport and they included the terminal reaches of the Reno, Destra Reno, and Lamone rivers to the north of the city, as well as the Cerba, Canala, and Magni drainage canals, which drain the western part of the city and discharge into the Baiona lagoon before reaching the sea (Fig. 1). Reno River and Cerba drainage canal were designed as control points as they remained unaffected by the flood. Although the Reno River catchment experienced elevated water levels during the rainfall period, no flooding was recorded within its watershed. Similarly, the Cerba canal drains a small coastal basin in the north of Ravenna that did not experience inundation. As such, these stations served as reference points for water quality and biological analyses, providing representative conditions for non-impacted areas. The characteristics of rivers and canals included in the monitoring are reported in Supplementary Table 1.

Marine sampling stations were identified based on contemporaneous satellite imagery showing sediment plumes and turbid water masses extending from the main river mouth. They were selected about 1 km offshore and located in front of each river mouth and at the midpoints between them. Two additional marine stations were situated ~1.5 km offshore, south and north of the Ravenna port's jetties. Water depth at the marine stations was generally 5–8 m (Supplementary Table 2). Due to the presence of the long jetty infrastructure (2.4 km) acting as a physical barrier, the southern station was used as control point because it was protected from the direct influence of northern fluvial and canal plumes originating from the flood. It was considered a suitable reference point for assessing baseline marine conditions unaffected by the flood-induced inputs.

Three monitoring campaigns were carried out on the 30th of May 16th June, and 10th October 2023. This timeline enabled a comprehensive

evaluation of both the short- and medium-term impacts of the flooding on inland and marine ecosystems of the study area, as well as the processes of environmental recovery. May 30th was selected as the earliest feasible sampling date, coinciding with the lifting of emergency restrictions related to hydrogeological risk. Because fieldwork was constrained by emergency conditions, the first sampling (T1, 30 May 2023) represents the earliest feasible post-event survey and may not capture the instantaneous maxima of short-lived parameters immediately after the flood. June was chosen to capture early post-event conditions, while October 10th represented a later time point, allowing evaluation of the system's potential recovery and the re-establishment of pre-event conditions.

At each sampling station, physical and chemical parameters such as temperature (T), electrical conductivity (EC), pH, redox potential (Eh), and dissolved oxygen (DO) were measured by a HQ4300 Portable Multi-Meter (HACH®, Loveland, CO, USA), while surface-water samplings were collected to perform chemical and biological community analysis. Water samples, from both inland and marine stations, were collected within the upper 50 cm of the water column. In the marine environment, this sampling depth was selected to characterize near-surface waters most directly influenced by riverine plumes; therefore, marine results primarily represent near-surface conditions rather than the full water column. For each sampling station were collected:

- 0.5 l for eDNA metabarcoding
- 1 l for contaminant of emerging concern (CECs)
- 2 l for biological analyses (chlorophyll-a (Chl-a), phaeopigments, inorganic dissolved nitrogen $\text{N}(\text{NO}_3^- + \text{NO}_2^-)$ and inorganic dissolved phosphorus $\text{P}(\text{PO}_4^{3-})$, Total Suspended Solids (TSS) and Chromophoric Dissolved Organic Matter (CDOM)

Samples were stored in pre-washed rigid 1-l plastic bottles (10% HCl or NaClO). Salinity (S) was measured in the field using a refractometer. All samples were then transported to the laboratory immediately after collection and processed according to the different standardized analytical procedures. At each station and for each sampling period, replicated samples were collected and processed independently to ensure the representativeness and reproducibility of the measurements. This approach allowed us to account for small-scale spatial variability and to obtain robust average values for each site.

Water quality assessment

Water-quality samples were collected at inland (rivers and drainage canals) and marine stations (Fig. 1). Several analytical procedures were identical across water types, while others differed according to standard protocols for fresh and marine systems.

For all stations, Chl-a and phaeopigments were measured following the same procedure. Water volumes of 200–600 mL were filtered onto pre-combusted Whatman GF/F filters (0.7 μm , 47 mm). Pigments were extracted in 90% acetone, and Chl-a concentrations were determined spectrophotometrically at 665 and 750 nm and phaeopigments were quantified after acidification (final concentration: 0.66 M HCl)⁶⁶.

Total Suspended Solids (TSS) were also analyzed consistently at all stations, filtering water onto pre-weighed 0.45 μm membrane filters and following the gravimetric protocol⁶⁷. Filters from marine samples were rinsed with 0.5 M ammonium formate (2× the filtered volume) to remove salts before drying at 105 °C.

Inland-water samples were filtered through 0.45 μm membrane filters, stored in pre-cleaned 100 mL HDPE bottles at 4 °C, and transported immediately to the laboratory. Major anions, including nitrate and nitrite, and alkalinity were analyzed within 24 h by Ion Chromatography (883 Basic IC Plus, Metrohm). Major cations and potentially toxic elements were quantified by ICP-OES (Agilent 5800) after acidification with 1% HNO_3 . Calibration followed certified standards and national APAT-IRSA (2003) guidelines; ionic balances were within $\pm 5\%$, and accuracy and precision were better than 10%.

Regarding nutrient fluxes, it was not possible to derive reliable estimates for the event because several rivers and drainage channels overflowed their banks before reaching the sea, and many pumping stations operated at full capacity, and in some case additional temporary external pumps were used to accelerate the removal of the exceptional flooded water volumes. Consequently, accurate discharge data for both rivers and water pumping stations during and immediately after the flood were either unavailable or not representative of the actual transport pathways and flux calculations would have introduced substantial uncertainty. Nutrient concentrations were thus retained as the most robust and comparable metric of enrichment across stations and time periods.

Marine-water samples underwent separate analyses for inorganic nutrients and CDOM. Filtered samples (GF/F) were stored in polyethylene bottles pre-washed with 10% HCl, and dissolved inorganic nitrogen as $\text{N}(\text{NO}_3^{2-} + \text{NO}_2^-)$ and dissolved inorganic phosphorous as $\text{P}(\text{PO}_4^{3-})$ were quantified spectrophotometrically according to Strickland & Parsons (1972). CDOM absorption was measured on 0.2 μm -filtered samples following Mannino et al., collecting UV-Vis spectra from 250 to 800 nm and deriving absorption coefficients at 443 nm. During the October campaign (T3), CDOM values were frequently near or below detection limits; these were excluded from comparative analyses to ensure reliability of the performance assessment.

To contextualize 2023 measurements from both inland and marine stations, long-term environmental data were retrieved from the Regional Environmental Protection Agency of Emilia-Romagna (ARPAE) (<https://dati.arpae.it/dataset/rete-regionale-per-la-qualita-ambientale-acque-superficiali-fluviali-dati-2010-2023>). Inland samples were compared with river monitoring data (2013–2023) for Reno, Destra Reno, and Lamone rivers available in the ARPAE open database. For comparison with long-term datasets, nitrate concentrations were used as the reference measure, consistent with ARPAE historical records in which nitrite was generally below detection limits; dissolved inorganic nitrogen is therefore reported as $\text{NO}_3^- + \text{NO}_2^-$.

For marine waters, historical values (2010–2022) were extracted from ARPAE's Annual Marine Water Quality Reports³⁹ (available on <https://www.arpae.it/it/temi-ambientali/mare/report-e-bollettini/qualita-ambientale-acque-marine>). Among the ARPAE stations, Casalborsetti (Site 6) was selected as the most representative long-term marine site due to its central position relative to our study area and its consistent surface-water sampling (0–1 m). Seasonal climatologies were compiled for salinity, Chl-a, $\text{N}(\text{NO}_3^- + \text{NO}_2^-)$, and $\text{P}(\text{PO}_4^{3-})$. Each 2023 sampling period (T1–T3) was matched to the corresponding historical season (Spring, Summer, Autumn), and the mean values across our eight marine stations were compared with the 2010–2022 baseline.

The deviation of 2023 values from the historical climatology was quantified through z-scores ($z = (x_{2023} - \mu_{\text{baseline}}) / \sigma_{\text{baseline}}$). Percentile ranks derived from these z-scores were used to express the relative extremeness of 2023 measurements, acknowledging that the limited length of the baseline series provides relative rather than absolute estimates of anomaly.

Protist communities' assessment

In order to analyze the protist communities, eDNA metabarcoding analysis was performed on all inland and marine water samples. They were pre-filtered on 3 μm nitrocellulose filters and re-filtered on a 0.22 μm nitrocellulose filter. After filtration, filters were stored at -20°C . Total DNA from the filters was extracted using the BioFast Soil Genomic DNA Extraction Kit (BioFlux, Hangzhou, Zhejiang, China), following the manufacturer's instructions, with two negative controls included per extraction batch to check for contaminations. PCR reactions for the V4–V5 region of the 18S rRNA were carried out in a total volume of 20 μL and included 17 μL of AmpliTaq Gold™ 360 Master Mix (Applied Biosystems), 0.5 μL of each primer (F-566, R-1200; 10 μM)⁶⁸ that already included 12-bp tags for multiplexing, and 2 μL of DNA template, with the following thermal profile: 95 $^\circ\text{C}$ for 15 min, followed by 35 cycles of 95 $^\circ\text{C}$ (45 s), 60 $^\circ\text{C}$ (45 s), 72 $^\circ\text{C}$ (1 min), and a final extension at 72 $^\circ\text{C}$ for 10 min. PCR products were

evaluated by agarose gel electrophoresis (1.5% gel, 0.5X TBE). For the 18S library, PCR products were pooled and purified with the Macherey-Nagel™ NucleoSpin Gel and PCR Clean-up Kit to remove DNA fragments <70 bp. DNA concentration was measured with a Qubit fluorometer (Broad Range kit, Invitrogen). The libraries were sequenced in-house with a MinION Mk-1C (Oxford Nanopore Technologies). The 18S library was prepared with the SQK-LSK110 kit and sequenced on a FLO-MIN111 flow cell with a 22-h run. Basecalling was conducted using Guppy (ONT) with a quality threshold of 8. Demultiplexing and length filtering were performed with OBITOOLS⁶⁹. The taxonomic assignment was conducted using the RDP classifier with the SILVA 138 reference database at genus level^{70,71}. Data were cleaned keeping only protist taxa that had a total read count of at least 10 and a similarity threshold of 97%. Since some samples did not amplify and ensure consistency, only samples present in both biological and environmental datasets were considered for the Redundancy Analysis (RDA). Environmental parameters considered for the RDA were N, P, TSM, DO, temperature, and salinity.

Protist abundance data were square-root transformed, and environmental variables were standardized with *decostand()* to allow comparison among different scales. RDA analysis allows the samples and environmental factors to be represented on the same two-dimensional ordination plot to visualize their relationships. RDA was performed with *rda()* using scaling 2, to highlight the effects of explanatory variables (environmental parameters). The significance of each environmental variable in explaining community structure was assessed with *anova.cca()*. All analyses were performed in R version 4.4.1 (R Core Team, 2024), using the vegan package⁷² and ggplot2 for data visualization.

Pharmaceuticals analyses

To analyze pharmaceuticals, water samples were spiked with a labeled internal standard (Ibuprofen-d3) at a concentration of 30 ng L^{-1} . Samples were filtered using glass microfiber filters (1.60 μm), then with cellulose acetate filters (0.45 μm), and extracted using Solid Phase Extraction (SPE) Oasis HLB cartridges as reported in ref. 73. Cartridges were eluted with 6 mL of methanol, evaporated under a nitrogen gentle stream up to a volume of 25 μL and reconstituted in 250 μL of water/methanol (90:10) mixture for LC-MS-MS analysis. Samples were transferred into 2 mL vials, after filtration (0.22 μm CA membrane filters) for analysis. Sample extracts concentrated 2000 times were measured along with calibration standards, which corresponded to a range of 1–300 $\mu\text{g L}^{-1}$. Chemical analysis of pharmaceuticals was performed at the Mario Negri Institute for Pharmacological Research, IRCCS (Department of Environmental Health Sciences, Milan, Italy), following established procedures⁷⁴.

Remote sensing and image processing

To evaluate whether the May 2023 flood-induced anomaly was detectable against the natural variability of the region, we reconstructed a decade-long satellite-based climatology of surface Chl-a concentration for the Emilia-Romagna coastal zone. Daily multisensor ocean-color data were obtained from the Copernicus Marine Environment Monitoring Service (CMEMS) product OCEANCOLOUR_MED_BGC_L3_MY_009_143, which provides Level-3 (L3) daily composites at 1-km spatial resolution for the Mediterranean Sea. This dataset integrates observations from multiple satellite missions (SeaWiFS, MODIS-Aqua, MERIS, VIIRS, and Sentinel-3 OLCI), ensuring long-term continuity from 1997 onward and radiometric consistency across sensors. Because the most recent decade of the record is dominated by OLCI and VIIRS, the climatology is representative of the modern observational regime and suitable for comparison with 2023 conditions.

Eight fixed coastal stations corresponding to our in situ marine sampling locations were selected using GPS coordinates. For each station, daily Chl-a values were extracted as the mean of all valid pixels within a 1.5-km geodesic buffer. This radius ensured sufficient pixel availability across the full historical record, particularly during early-sensor years, while minimizing land contamination. Pixel selection followed the CMEMS quality-

flagging scheme: only observations flagged as valid (mask = 0) were retained, and non-physical or missing values were excluded.

Analyses were restricted to the month of May for each year between 2013 and 2022, corresponding to the pre-stratification period and matching the timing of the 2023 flood event (T1). Two complementary analyses were conducted:

1. Statistical Baseline Characterization

For each station, all valid daily May Chl-a measurements from 2013–2022 were aggregated to compute baseline statistics, including mean, standard deviation, and the 95th percentile. The May 2023 station means were then compared with this baseline to quantify the magnitude of the anomaly. Metrics included the absolute anomaly (ΔMean), standardized anomaly ($Z\text{-score} = \Delta\text{Mean} / \sigma$), and percentile rank relative to the historical distribution.

2. Daily Climatology Comparison

A daily climatology (1–31 May) was constructed by computing the median and 5th–95th percentile envelope for each day across 2013–2022. Observations from May 2023 were then overlaid onto this baseline to visualize departures from expected seasonal variability, identify days exceeding the May-specific 95th percentile, and characterize the timing and intensity of the bloom relative to the historical window.

All data preprocessing and analysis were performed in Python using xarray, pandas, and matplotlib. This integrated approach enabled consistent evaluation of the May 2023 anomaly within a multi-decadal framework while leveraging the spatial and temporal continuity of the CMEMS multisensor archive.

The satellite climatology provided the long-term context needed to assess whether the May 2023 bloom exceeded the natural variability of the region. To complement this historical evaluation and to understand how well satellite products can resolve flood-driven changes at finer spatial and temporal scales, we also tested the performance of current retrieval algorithms applied to Sentinel-2 and Sentinel-3 imagery. This second component of the analysis focused on determining whether operational and semi-operational processors can accurately estimate water-quality parameters under the extreme optical conditions generated by the flood.

The aim of the remote sensing use in this study was to evaluate whether ready-to-use (e.g., Sentinel-3 OLCI) and semi-operational products (based on Sentinel-2 data processing) could reliably estimate water quality parameters, especially under extreme hydrological conditions, such as those that followed the May 2023 flood.

We applied and compared multiple algorithms across satellite products to identify those most accurate and operationally useful for post-flood environmental monitoring. Each satellite of the Sentinel-2 constellation is equipped with the Multispectral Instrument (MSI), which captures data across 13 spectral bands, ranging from visible (VIS) and near-infrared (VNIR) to the shortwave infrared (SWIR) spectrum. The MSI provides spatial resolutions of 10 m (VIS and VNIR), 20 m (red-edge and vegetation-related bands), and 60 m (bands for atmospheric correction). For this study, Sentinel-2 Level-1C products were downloaded from the Copernicus Browser, accessible at: <https://browser.dataspace.copernicus.eu>.

The images selected correspond to dates closest to in situ sampling. In cases where satellite overpass or high cloud cover prevented acquisition on the exact sampling date, the nearest available image was used. Images were pre-processed using ESA's SNAP software (v.1.10.0) (software available at: <https://step.esa.int/main/toolboxes/snap>), including subsetting to the area of interest and resampling to a spatial resolution of 20 m. This processing step follows previous findings showing that 20 m resolution provides more stable and accurate water quality estimates from Sentinel-2 imagery⁷⁵.

Image processing included atmospheric correction followed by the retrieval of Chl-a, suspended particulate matter, and CDOM absorption at 443 nm. Although satellite algorithms formally retrieve suspended particulate matter (SPM or TSM), this quantity is widely considered equivalent to gravimetrically measured total suspended solids (TSS) in coastal and transitional waters. Accordingly, satellite-derived SPM/TSM estimates were directly compared with in situ TSS measurements, and hereafter the term

TSS is used for consistency. These parameters were retrieved using two distinct processing frameworks, each based on different algorithms and coefficients (see Supplementary Table 9):

- The Case 2 Regional Coast Colour processor (C2RCC), developed by ref. 76 and integrated within SNAP, applies a two-step neural network inversion approach. First, atmospheric correction is performed by estimating top-of-atmosphere (TOA) radiance contributions (e.g., water vapor, aerosols), followed by the inversion of surface reflectance to derive inherent optical properties (IOPs) of the water column. Neural networks are trained using an extensive dataset (millions of simulations) based on radiative transfer model that covers a wide range of IOPs^{76,77}, including pigment absorption (a_{pig}), detrital absorption (a_{det}), CDOM absorption (a_{gelb}), and also scattering by both sediment (b_{part}) and calcareous particles (b_{wit}). Three variants of the model were applied, allowing the processor to adapt to a broad variety of water conditions. Specifically, the suite of C2RCC neural networks (collectively referred to here as C2Nets) includes: i) C2RCC, optimized for mesotrophic and moderately turbid marine waters; ii) C2X, designed for highly turbid or extreme optical conditions; and iii) C2XCOMPLEX, targeting optically complex waters with intermediate turbidity levels. All three variants were tested to evaluate their performance under the extreme conditions caused by the flood event.
- ACOLITE (software available at: <https://github.com/acolite/acolite>, version 20231023.0) was also employed for atmospheric correction, applying the Dark Spectrum Fitting (DSF) algorithm, which estimates aerosol optical thickness and path reflectance through dynamic dark pixel selection^{78,79}. ACOLITE is particularly suited for turbid and optically complex waters, providing robust correction under highly dynamic aquatic conditions. In addition to atmospheric correction, ACOLITE offers a suite of empirical and semi-analytical algorithms (see the full list in Supplementary Table 9) to estimate Chl-a, turbidity (expressed in Formazin Nephelometric Units, FNU), and suspended particulate matter (SPM, in g/m^3) from remote sensing reflectance (Rrs). While turbidity is an optical parameter related to water clarity, SPM TSM represents the concentration of suspended particulate material in the water column and was compared against in situ TSS measurements, following the terminology defined above.

To further assess the potential of ready-to-use satellite products, we also employed data from Sentinel-3, which carries the Ocean and Land Colour Instrument (OLCI), specifically designed for inland and marine monitoring, among other applications. OLCI is equipped with 21 spectral bands spanning the visible to near-infrared (VIS–NIR) regions, enabling the detection of key water quality parameters at large spatial scales. The OLCI Water Full Resolution (WFR) products are Level-2 outputs providing remote sensing reflectance (Rrs) and derived biogeophysical parameters such as Chl-a and TSS and they are available at: <https://browser.dataspace.copernicus.eu>. These products are generated through ESA's operational processing chains using standard algorithms (see Supplementary Table 8 for a full list of algorithms). OLCI WFR products offer a native spatial resolution of 300 meters, which, while coarser than Sentinel-2, provides the advantage of high temporal coverage and consistent processing standards, making them valuable for large-scale or regional monitoring. Once the Level-2 products were obtained, a match-up analysis was performed between satellite-derived estimates from each algorithm and the corresponding in situ measurements. This was conducted separately for the products derived from Sentinel2 (C2Nets-derived and ACOLITE-derived products) and Sentinel-3 OLCI. For each product and acquisition date, a 3×3 pixel window centered on the GPS coordinates of each sampling station was extracted. Within each window, quality flags were analyzed to exclude unreliable pixels, thereby ensuring data quality. Subsequently, the remaining pixel values were averaged to derive a representative satellite-based estimate for comparison with laboratory measurements. To quantitatively evaluate the satellite-derived estimates against in situ reference measurements, three

statistical metrics commonly used for the validation of remote sensing products were employed: Mean Absolute Error (MAE), Root Mean Square Error (RMSE), and Bias.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Environmental datasets and derived data products supporting the findings of this study are available in the Zenodo repository at <https://doi.org/10.5281/zenodo.18376796>⁷⁹. Raw sequencing data have been deposited in the European Nucleotide Archive under study accession PRJEB107152 and will be publicly released upon publication of this article.

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Author contributions

Riccardo Bentivogli contributed to formal analysis, data curation, visualization, and writing of the original draft. Carlotta Carlini contributed to data curation, investigation, and writing of the original draft. Federica Costantini contributed to conceptualization, methodology, supervision, and manuscript review and editing. Elena Fabbri contributed to conceptualization, methodology, supervision, and manuscript review and editing. Beatrice Maria Sole Giambastiani contributed to conceptualization, methodology, formal analysis, supervision, and manuscript review and editing. Nicolas Greggio contributed to conceptualization, methodology, formal analysis, supervision, and manuscript review and editing. Martina La Torre contributed to formal analysis and investigation. Cristina Luccarini contributed to formal analysis and investigation. Laura Pezzolesi contributed to conceptualization, methodology, formal analysis, supervision, and manuscript review and editing. Alessandro Piazza contributed to formal analysis, investigation, and manuscript review and editing. Paola Valbonesi contributed to conceptualization, methodology, formal analysis, supervision, and manuscript review and editing. Sonia Silvestri contributed to conceptualization, methodology, project administration, funding acquisition, supervision, and manuscript review and editing. All authors approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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