

Natural and anthropogenic tipping points in a Mediterranean karstic lake: evidence from Lake Estanya (NE Spain)

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Abstract

Karstic lakes in Mediterranean regions are aquatic ecosystems that are highly sensitive to the strong hydroclimate stress characteristic of Mediterranean climates and the long history and intensity of human activity. Rapid changes in the last decades have been documented in many of them. Understanding the ecological thresholds and tipping points of these systems is essential for the management of water resources, conservation of biodiversity, and preservation of cultural and societal resources. In this study, we examined the hydrodynamic and biogeochemical evolution of Lake Estanya in northeast Spain over the last 300 years by combining continuous, high-resolution limnological monitoring (2018–2023) with multiproxy geochemical and isotopic analyses of short sediment cores. The limnological monitoring revealed a swift transition in real time from a warm monomictic regime to chemical meromixis in 2018. This chemical stratification was triggered by severe summer evaporation during a prolonged drought, which increased salinity and concentrated carbonate and sulphate throughout the water column. This was followed by freshwater inflows from subsequent autumn rainfall, which diluted the surface mixolimnion and created a dense, saline monimolimnion. On a decadal to centennial scale, our paleolimnological record reveals two distinct tipping points. The oldest one occurred after the onset of the Little Ice Age (LIA) (ca. 1850 CE), and it was characterised by a marked shift towards an evaporation-dominated brackish system with a large increase in the $\delta^{34}\text{S}_{\text{V-CDT}}$ of total sulphur and a reduction in detrital inputs. The youngest shift occurred after 2000 CE and it is evidenced by increased $\delta^{15}\text{N}_{\text{AIR}}$ (up to + 6.82‰) and decrease of $\delta^{13}\text{C}_{\text{V-PDB}}$ (down to -31.24‰), indicating eutrophication, increased primary productivity and more frequent anoxic bottom water. While the first transition was caused by hydroclimatic changes after the LIA, the second one reflects an unprecedented anthropogenic tipping point driven by the intensive use of agricultural fertilisers in the watershed. Our findings highlight that the combined effects of climate change and intensive agriculture are pushing Mediterranean karstic lakes into unprecedented states. Urgent watershed management is required to limit nutrient loading and adaptation to new climate conditions to preserve ecological resilience.

Introduction

At a global scale, lakes are facing changes in limnological parameters such as thermal regime, lake heat waves (Woolway et al., 2021), anoxia (Jenny et al., 2016; Nkwalale et al., 2026), increased bioproductivity and eutrophication (Ho et al., 2019), salinisation, lower lake levels and invasive species (Hessen et al., 2024).

Ongoing climate change affects lake ecosystems worldwide through warmer temperatures, prolonged droughts, altered hydrological balance, enhanced salinisation, increased thermal stratification, and declining water levels and changes in biological communities (Woolway et al., 2020).

In Mediterranean-climate regions worldwide, these impacts are amplified by drought-prone conditions and intensive resource exploitation, leading to severe hydrological degradation, altered mixing regimes, and even complete desiccation (Fuentealba et al., 2020; Hallett et al., 2018; Lakane et al., 2025; Petrone

et al., 2010; Ravinandrasana and Franzke, 2025; Riddin et al., 2024; Smith et al., 2023; Soulé and Knapp, 2024; Williams et al., 2022). Recent observations from the Mediterranean Basin further illustrate these ongoing environmental changes, including drastic water level drops, enhanced eutrophication, and shrinking surface areas (Chrif El Idrissi et al., 2025; El- Bouhali et al., 2024; Trajkovski and Apostolova, 2024).

In particular, karstic lakes in Mediterranean climates are unique aquatic ecosystems that are extremely vulnerable to ongoing climate change and anthropogenic pressure (Jeppesen et al., 2015). The inherent dynamics of the Mediterranean climate, characterised by annual cycles of summer drought and winter precipitation and multiannual alternation of prolonged droughts and intense precipitation (Lionello et al., 2006), are the main drivers of the chemical and biological processes in these ecosystems. From a limnological perspective, most of these Mediterranean lakes exhibit seasonal alternation, which is typical of warm monomictic systems. Under this regime, surface and deep waters stratify thermally during the warmer months, differentiating into an epilimnion and hypolimnion before undergoing complete vertical mixing in winter (Boehrer and Schultze, 2008).

Karstic lakes in the Iberian Peninsula are numerous and develop in abundant limestone and evaporite formations (Gutiérrez et al., 2008). Several of these systems have provided high-resolution palaeoenvironmental records and have been monitored during recent decades, revealing substantial limnological and environmental changes. In Lake Arreo, a transition from meromictic to holomictic conditions has been documented together with recent changes in catchment runoff dynamics (Corella et al., 2021, 2013, 2011). Lake Montcortès has recorded increased Hg and Pb deposition, enhanced soil erosion within the watershed, and recent changes in mixing dynamics and hypolimnetic oxygen conditions (Corella et al., 2019, 2017; Vegas-Vilarrúbia et al., 2026, 2018). In southern Iberia, Lake Zoñar, recent climatic variability and anthropogenic pressures have responded to impact both water quality and ecosystem functioning (Martín-Puertas et al., 2011). Likewise, Lake Banyoles has recorded the development of local meromictic conditions since the late nineteenth century, and enhanced organic productivity associated with eutrophication and increasing human impact during the twentieth century (Morellón et al., 2015). Karstic lakes in the Iberian Range have undergone intense human pressure since Medieval times greatly affecting sediment delivery (Barreiro-Lostres et al., 2017).

Together, these studies highlight the sensitivity of Iberian karst lakes to ongoing climatic and anthropogenic forcing. All the lakes are included in areas with a variable degree of government management and protection. Identification of tipping points and the response of these ecosystems to anthropogenic and climate changes is of paramount importance for human and environmental health, resources availability (clean water, food production, fisheries) and biodiversity conservation. The main objective of this study is to characterize the recent (last 300 years) hydrodynamic and biogeochemical evolution of Lake Estanya to identify recent trends and both natural and anthropogenic tipping points. By combining continuous, high-resolution limnological monitoring (2018–2023) with multiproxy geochemical analysis of short sediment cores, this study aims to provide a comprehensive

understanding of the lake's ecological thresholds and its response to multiple environmental stressors and provide the scientific background for new management and conservation policies

Site description

Lake Estanya (0.19 km² surface area) is located in a small endorheic basin (2.45 km² drainage area) in the Pyrenees between the Cinca and Noguera Ribagorçana river catchments (Fig. 1). The lake sits over gypsum and clays (Keuper Formation), with Muschelkalk limestones and dolomites (Muschelkalk formation) outcropping in higher-relief areas, and it is classified as a “karstic lake on gypsum” under the European Habitat Directive (Morant et al., 2024). It belongs to the “Balsas de Estanya complex” that includes several sinkholes and has some environmental protection from the European and regional government as a Ramsar site. The lake waters are alkaline, moderate salinity and of carbonate-sulphate type (Morellón et al., 2009b, a) and the lake is classified as mesotrophic. The lake is composed of two subbasins with maximum depths of 9.5 and 17.5 m (+/- 2.5 depending on the lake level fluctuations) that become disconnected during drought periods. The karstic and endorheic complex is mainly fed by groundwater from the Muschelkalk associated aquifer. The hydrogeological model for the Estanya lake indicates that groundwaters and surface runoff are the main inputs, while groundwater fluxes and evaporation are the main outputs (Pérez Bielsa et al., 2013). There is a permanent spring situated at the north of Estanya village feeding the lake and several springs (e.g., Senara) acting as the outputs of the main aquifer outside the Estanya watershed (Fig. 1). While various limnological and paleolimnological studies have classified Lake Estanya as a predominantly monomictic system characterised by complete winter mixing (Ávila et al., 1984; Martínez-Alonso et al., 2008; Morellón et al., 2008), some authors have suggested that it may have experienced periods of meromixis, likely driven by cold episodes during the Little Ice Age, combined with intense human impact on the drainage basin (Morellón et al., 2011). During the last decades, long droughts (1991–1995, 2004–2005, 2015–2017 and 2023) have greatly affected the system, lowering lake level several meters and increasing salinity. Agricultural use of the watershed also increased in the late 20th century, and the lake is currently surrounded by winter cereal crop fields.

Previous paleolimnological reconstructions (Morellón et al., 2011) pointed to large changes in the lake during the last centuries in terms of sediment delivery, salinity and bioproductivity. Recent monitoring has documented an abrupt shift in mixing conditions. However, the roles of extreme evaporation, rainfall cycles, and salinity in driving transient meromictic phases have not been fully elucidated. Furthermore, evaluating the long-term resilience of a system requires a clear distinction between natural hydroclimatic variability and recent human-induced alterations. Increased nutrient loads from fertilisers and from increased Saharan dust episodes (Salvador et al., 2022) and stronger and longer periods of droughts (Reig et al., 2026) most likely have had a strong impact on the mixing lake conditions and the anoxic periods in the hypolimnion. Despite these paleolimnological inferences, a significant knowledge gap remains regarding the link between current hydroclimate and anthropogenic mechanisms that trigger and maintain chemical stratification. The Estanya Lake may be reaching a tipping point in the thermal

and mixing dynamics and the understanding of this new regime is essential for an adequate environmental management of this protected area.

Materials and methods

Lake monitoring

Continuous hourly resolution thermal monitoring was conducted between 2018 and 2023 using automated temperature data loggers (Hobo Water Temp Pro V2) deployed at 0, 10 and 15 m. Vertical water column profiles were obtained periodically using a multiparameter probe (YSI EX01) to characterise the physicochemical properties of the water column.

Water samples were collected at several depths using a Niskin bottle during the period 2018–2023 on a seasonal basis. Groundwater was sampled directly from the nearby Estaña and Senara springs. All samples were filtered through 0.45 µm filters for dissolved inorganic carbon and nitrate isotopic analyses, while particulate organic matter (POM) was recovered by filtering 1 L of water sample through pre-combusted glass fiber filters. Specifically, samples for DIC and NO_3^- stable isotopes analyses were collected in 12 mL borosilicate vials and poisoned with HgCl_2 to prevent microbial activity.

The concentrations of major anions (F^- , Cl^- , NO_2^- , Br^- , NO_3^- , SO_4^{2-} , HCO_3^- and CO_3^{2-}) and cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+} and NH_4^+) in the water samples were determined via ion chromatography using a 930 Compact IC Flex system (Metrohm). Analyses were performed at the Water Analysis Service of the Pyrenean Institute of Ecology (IPE-CSIC, Zaragoza, Spain). Stable isotope analyses of water ($\delta^{18}\text{O}_{\text{V-SMOW}}$, $\delta\text{D}_{\text{V-SMOW}}$), NO_3^- ($\delta^{15}\text{N}_{\text{AIR}}$, $\delta^{18}\text{O}_{\text{V-SMOW}}$), DIC ($\delta^{13}\text{C}_{\text{V-PDB}}$) and POM ($\delta^{13}\text{C}_{\text{V-PDB}}$, $\delta^{15}\text{N}_{\text{AIR}}$) were performed at the Andalusian Institute of Earth Sciences (IACT-CSIC). Isotopic analyses of oxygen and hydrogen in water were performed using a near-infrared laser analyser (Picarro L2140-i) based on the wavelength-scanned cavity ring-down spectroscopy (WS-CRDS) technique.

For the isotopic composition of DIC ($\delta^{13}\text{C}_{\text{V-PDB}}$), 0.5–2 mL aliquots were reacted with phosphoric acid in He-flushed vials for 24–36 h. The released CO_2 was analysed using a GasBench II system (ThermoFinnigan) coupled to a Delta XP isotope-ratio mass spectrometer (Thermo-Fisher-Scientific). Samples were analysed in triplicate and calibrated against internal standards (Na_2CO_3 previously standardised against NBS-18 and NBS-19). The analytical precision for $\delta^{13}\text{C}_{\text{V-PDB}}$ was better than 0.1‰.

The isotopic composition of dissolved nitrate ($\delta^{15}\text{N}_{\text{AIR}}$ and $\delta^{18}\text{O}_{\text{V-SMOW}}$) was determined following a modified protocol of Mcilvin and Altabet, 2005 involving a two-step reduction to N_2O and an extended 6-hour incubation at 50°C. Isotopic values were analysed using an automated purge and trap system (Gas Bench II) coupled to a Deltaplus XP IRMS (Thermo Fisher Scientific) and corrected using five international standards (EEZ-30, EEZ-31, USGS 34, USGS 35, and IAEA-NO3).

Sediment cores

In March 2023, two parallel short sediment cores were recovered using a UWITEC gravity corer. To ensure optimal density calculations and high-resolution records, core LEG23-1A-1G was subsampled in situ at 0.5 cm intervals. The parallel core, LEG23-1B-1G, was transported to the IPE-CSIC laboratory, where it was split longitudinally and imaged for stratigraphic descriptions.

The elemental geochemical composition of 82 discrete sediment samples was analysed by X-ray fluorescence (XRF) at a continuous resolution of 0.5 cm. Analyses were performed using a Counter Top XRF analyser (CTX; Bruker, USA) at the Stable Isotope Laboratory (CIEMAT), operating at 50 kV and 20 mA with a 90 s count time. The results of the XRF analyses for the selected elements and oxides used in the statistical evaluation (Ca, Sr, S, Fe, Mn, Rb, Ti, Zr, SiO₂, K₂O, Al₂O₃ and MgO) were expressed as mg*kg⁻¹.

Total nitrogen (TN) concentrations (considered representative of organic nitrogen given the negligible inorganic nitrogen fraction in these sediments), along with their isotopic compositions, were measured on untreated samples using a Carlo Erba NA1500 elemental analyser coupled with a Delta Plus XL mass spectrometer. The stable isotopes of organic matter $\delta^{13}\text{C}_{\text{V-PDB}}$ were measured using the same technique on samples that had been treated multiple times with 1 M HCl to completely eliminate inorganic carbon in the form of carbonate.

For the analysis of total sulphur isotopes ($\delta^{34}\text{S}$ relative to V-CDT), micro samples containing approximately 10 µg of sulphur were mixed with 1 mg of WO₃ to ensure complete combustion. The samples were then combusted with O₂ at 1030°C using a Carlo Erba NC1500 Elemental Analyzer coupled to a Delta Plus XP isotope ratio mass spectrometer (EA-IRMS).

The Total Inorganic Carbon (TIC) concentration was measured at CIEMAT and determined by the combustion of dry samples in a Shimadzu SSM 5000A carbon analyser. The samples were treated with 28% phosphoric acid (H₃PO₄). The Total Organic Carbon (TOC) concentration was determined by the difference between TC and TIC.

Principal Component Analysis (PCA) was performed on centred and scaled variables using the FactoMineR package, with missing data imputed via the missMDA package.

The chronology of core LEG23-1A is based on its stratigraphic correlation with the previously dated Estanya core 1A-1M (Morellón et al., 2011) and a new ¹³⁷Cs profile obtained via gamma spectrometry using a coaxial-type Extended-Range HPGe detector (Canberra) at 0.5 cm resolution. Climate data, specifically daily precipitation records (2016–2025) from the Benabarre meteorological station, were retrieved from the open data repositories of the Spanish State Meteorological Agency (AEMET).

Results

Thermal structure and mixing regime

High-resolution thermal monitoring (2018 to 2023) documented the onset of meromictic conditions during the 2018 stratification season and their persistence throughout the remainder of the monitoring period. The upper water column (up to 5 m depth, mixolimnion) exhibited high seasonal variability, ranging from a summer maximum temperature of 28.5°C to a winter minimum of 3.7°C. In contrast, the permanently isolated bottom waters (monimolimnion, > 15 m) remained stable throughout the monitoring period, with an annual temperature range of only 2.6°C (between 5.8 and 8.4°C). Furthermore, an additional measurement taken on May 25, 2026, using a multiparameter probe confirmed the continuation of this stable stratification, revealing a salinity difference of 0.15 PSU between surface and deep waters.

A notable aspect of lake dynamics during this period is the absence of winter isothermal conditions. During peak cooling events (e.g., January 2021 and February 2023), surface waters dropped below 5°C, whereas the deep monimolimnion remained significantly warmer (7–8°C) than the surface waters. Thermal records at 6.6 and 7.9 m closely followed surface seasonal trends and the temperature at 10 m displayed intermediate behavior but remained consistently above the temperature at 15 m.

The isotopic composition of the local source water (Estanya spring) shows mean values of $\delta D_{V-SMOW} = -52.62\text{‰}$, $\delta^{18}O_{V-SMOW} = -7.70\text{‰}$ and $d = 8.91\text{‰}$. From autumn 2018 (the start of the isotopic record) to summer 2022, the deep waters exhibited more negative deuterium excess (d) values than the surface waters. The maximum isotopic difference between the surface and deep waters was recorded in late-spring 2020. In summer 2022, a drastic decrease in the d -excess of surface waters occurred. Subsequently, the most evaporated signals in the entire time series were detected in autumn 2023 and spring 2024.

Sedimentary facies and units

The sedimentary units of the sequence recovered in 2023 (41 cm) can be easily correlated with those described in the literature (Morellón et al., 2011).

The top Unit 1 (0–15 cm) is composed of alternating dark grey and black, massive, organic-rich silts, organised in cm-thick bands. The unit 2 (15–35 cm) groups variegated, mm-thick laminated silts, alternating light grey, massive clays, dark brown, massive organic-rich laminae, and grey/brown massive silts. The basal unit 3 (35–41 cm) corresponds to dark grey, brownish, laminated silts with organic matter, organised in cm-thick bands (Fig. 2).

Carbon dynamics

Dissolved Inorganic Carbon (DIC) concentrations and isotopic signatures ($\delta^{13}C_{V-PDB}$ values) for water samples exhibit a pronounced vertical stratification within the lake, reflecting a meromictic regime

(Fig. 3). The mixolimnion (0–5 m depth) is characterised by lower DIC concentrations (averaging 28 ppm) and less negative $\delta^{13}\text{C}_{\text{V-PDB}}$ values (-4.5‰ on average, with maximum up to 1.88‰) than the monimolimnion (-18.05‰ on average, with minimum values of -23.29‰). The nearest Estanya spring provides the average groundwater signal, with higher DIC (average concentration of 37.6 ppm) and lower $\delta^{13}\text{C}_{\text{V-PDB}}$ (average -9.87‰). In contrast, the monimolimnion (> 15 m depth), displays substantially higher DIC concentrations (mean 75.1 ppm) and very negative $\delta^{13}\text{C}_{\text{V-PDB}}$ values (mean -18.78‰ , reaching down to -23.29‰). Samples from 10 m displayed intermediate and highly fluctuating values (ranging from -21.41‰ to -5.23‰).

The $\delta^{13}\text{C}_{\text{V-PDB}}$ of particulate organic matter also displayed a clear vertical zonation (Fig. 3). In the mixolimnion, acid-treated POM samples (to remove carbonates and isolate the organic fraction) yielded an average $\delta^{13}\text{C}_{\text{V-PDB}}$ value of -27.8‰ . Notably, the untreated bulk POM in these surface layers was systematically enriched by approximately $1.5\text{--}2.0\text{‰}$ compared to the acid-treated samples. In the monimolimnion (15 m depth), the organic POM signature became significantly lighter, averaging -31.7‰ (with a range of -31.7 to -32.9‰).

The sediment core (41 cm long, ~ 300 years old) displayed large changes in carbon content and isotope values (Figure 2). Total Organic Carbon (TOC) profiles (Fig. 2) exhibited three well-defined intervals corresponding to the sedimentological units. Unit 3 displayed the lowest and most stable values. A pronounced and continuous increasing trend characterised Unit 2, peaking at the base of Unit 1. In the uppermost section (Unit 1), TOC concentrations remained consistently high without a clear trend.

The $\delta^{13}\text{C}_{\text{V-PDB}}$ organic matter record parallels the sedimentary units (Fig. 2). Unit 3 exhibited the most enriched values, followed by a sharp negative excursion at the boundary with Unit 2. Values remained relatively stable throughout Unit 2 and the lower part of Unit 1, before displaying a pronounced negative excursion in the uppermost 5 cm of the core.

Nitrogen dynamics

Nitrogen analyses of the Estanya Lake revealed a clear distinction between catchment sources and lacustrine processes. The Estanya spring, representative of the primary groundwater input, showed a mean NO_3^- concentration of $13.91 \text{ mg}\cdot\text{L}^{-1}$ ($\delta^{15}\text{N}_{\text{AIR}} = 3.68\text{‰}$). In contrast, the Senara spring (located outside the immediate catchment but impacted by livestock manure) exhibited significantly higher values of both concentration ($88.92 \text{ mg}\cdot\text{L}^{-1}$) and isotopic signature ($\delta^{15}\text{N}_{\text{AIR}} = 14.07\text{‰}$). Within the lake, dissolved nitrate concentrations were frequently below the detection limit. However, isotopic measurements from two available samples collected at 5 m on July 2, 2022, and at the surface on August 7, 2023 revealed nitrate $\delta^{15}\text{N}_{\text{AIR}}$ values ranging from -1.97‰ to 1.61‰ .

Regarding the particulate organic matter (POM), $\delta^{15}\text{N}_{\text{AIR}}$ values displayed a marked vertical dichotomy. While the isotopic values remained stable in the monimolimnion (15 m; $3.00 \pm 1.55\text{‰}$), surface POM showed high variability (mean 6.80‰ , ranging from 0.64‰ to 12.88‰).

In the sediment core LEG23-1A-1G, Total Nitrogen (TN) increased progressively from the base (0.18%) to the core top (0.53%), with a more pronounced increase in the upper 25 cm. The strong correlation with TOC ($r = 0.97$) confirms that the sedimentary nitrogen is predominantly of an organic origin. The $\delta^{15}\text{N}_{\text{AIR}}$ values in the core range from 2.03‰ to 6.82‰ . The sequence showed distinct $\delta^{15}\text{N}_{\text{AIR}}$ enrichment in the uppermost 5 cm of the record. Below 10 cm, values remained stable between 2.5‰ and 3.5‰ , consistent with the signature of deep-water POM. Finally, C/N ratios (ranging from 8 to 12.5) indicate that the organic matter is mainly of algal origin, with a minor contribution of terrestrial material (Meyers and Teranes, 2001).

Sulphur dynamics

In the water column, dissolved sulphate $\delta^{34}\text{S}_{\text{V-CDT}}$ values remained relatively homogeneous across the different depths, showing enriched isotopic signatures ranging between $+16\text{‰}$ and $+20\text{‰}$ (Fig. 3). The TS content along the sequence reflects the gypsum content of the sediments (Fig. 2). The $\delta^{34}\text{S}_{\text{V-CDT}}$ record revealed a significant excursion throughout the sediment sequence. From the base of the core up to approximately 25 cm, (Units 3 and 2) the values of $\delta^{34}\text{S}_{\text{V-CDT}}$ remained relatively stable, oscillating between -19.4‰ and -15‰ . Between 25 and 15 cm (top of Unit 2), the record shows a significant positive excursion, with values rising sharply from -14.7‰ to -3.8‰ . Above 15 cm (Unit 1), the isotopic signal stabilised again, with values averaging -4‰ towards the top of the core. Notably, the surface sample (0 cm) had a positive value of $+2.4\text{‰}$. Isotopic analysis of sediment collected by traps revealed a wide variety of $\delta^{34}\text{S}_{\text{V-CDT}}$ values associated with different deployment periods and water column conditions. The sample from the longest deployment period (November 2019 to February 2022) when the monimolimnion remained under stable anoxic conditions, yielded a $\delta^{34}\text{S}_{\text{V-CDT}}$ value of -2.78‰ . In contrast, sediment trap samples from shorter deployments showed more positive signatures: $+3.49\text{‰}$ for the February - March 2022 period near the chemocline and $+14.08\text{‰}$ for the October 2022 - January 2023 interval in shallow waters. The last represents the most enriched signal of ^{34}S recorded in modern particulate matter.

Sediment geochemistry

A Principal Component Analysis (PCA) of the geochemical dataset identified two dimensions that accounted for 90.8% of the total variance.

Dimension 1 (Dim1) was the dominant factor, explaining 76.2% of the variance, and clearly differentiated two distinct groups of geochemical variables: the positive pole of Dim1 was strongly associated with lithogenic elements such as Fe, Rb, Ti, SiO_2 , K_2O , Al_2O_3 , MgO and Zr. The negative pole of Dim1 groups

authigenic, organic and isotopic proxies, including Ca, TIC, Sr, TOC, TN, S, and the isotopic signatures of $\delta^{13}\text{C}_{\text{V-PDB}}$, $\delta^{15}\text{N}_{\text{AIR}}$ and $\delta^{34}\text{S}_{\text{V-CDT}}$. Dimension 2 (Dim 2) explains a smaller fraction of the variance (14.6%) and is primarily driven by manganese (Mn) on the positive axis, contrasting with TIC and $\delta^{13}\text{C}_{\text{V-PDB}}$ on the negative axis.

The projection of individual sediment samples onto the PCA biplot revealed a clear stratigraphic evolution. Samples from the lower section of the core (from 40.5 cm up to approximately 26 cm) clustered strongly on the positive side of Dim1. A major transition occurred at approximately 25 cm, where the samples progressively shifted towards negative Dim1. The upper 22.5 cm of the record are entirely positioned in the negative domain. This temporal evolution mirrors the significant excursions observed in $\delta^{34}\text{S}_{\text{V-CDT}}$ and TN sedimentary records.

Age model

The chronology for core LEG23-1A was established through stratigraphic correlation with the proximate and previously dated Estanya core 1A-1M (Morellón et al. 2011). TOC (%) content was utilised as the primary geochemical proxy to correlate both records. The ^{137}Cs peaks identified in the LEG23-1A profile were compared with the reference ^{137}Cs profile from core 1A-1M to ensure correlation consistency. Correlation of lithological, geochemical, and ^{137}Cs records allowed the transfer of the chronological framework and the construction of an age-depth model using the R package clam (Blaauw, 2010). Full details of the chronological framework, including the correlation, radionuclide analyses, and age modelling procedures, are presented in Rodríguez-Veiga et al., 2026 (this volume).

Discussion

Our ability to detect shifts from alternative states in lake dynamics - even before their onset - relies on both, detailed monitoring and paleolimnological studies (Hessen et al., 2024). The identification of tipping points in lake evolution, defined as critical thresholds that, when exceeded, can lead to a significant, usually no reversible change in the state of the system, requires long enough time series and a clear understanding of the driving processes. In this section, we discuss the major changes observed in the Estanya Lake and determine whether the system has shifted to a new alternative state, the identification of possible precursors, and the consequences for management and conservation of the lake.

A recent tipping point: from monomixis to stable chemical stratification

Lake Estanya is one of the most thoroughly studied karstic lake ecosystems on the Iberian Peninsula. Various limnological and paleolimnological studies have historically classified this lake as a predominantly monomictic system characterised by complete winter mixing. This was observed during the winters of 1979 and 1980 (Ávila et al., 1984), 2002 (Martínez-Alonso et al., 2008) and 2008 (Morellón

et al., 2009b). However, some authors have suggested that the lake may have experienced periods of meromixis in the past, resulting from the combined effects of cold phases during the Little Ice Age and intense human impact on the catchment (Morellón et al., 2011), although the role of other factors as extreme evaporation and precipitation was not explored.

Thanks to the intensive limnological monitoring carried out between 2018 and 2024, we were able to answer this question. The data obtained allowed us to document the transition from a monomictic (observed in 2018) to a non mixing state in real time. The monitoring data indicate that the transition occurred rapidly. The lake still experienced effective winter mixing at the beginning of 2018, but stable anoxic conditions developed during the subsequent stratification season, leading to the isolation of the monimolimnion. Persistent meromixis was then maintained throughout the remainder of the monitoring period (six years). The monitoring data show that meromixis in Lake Estanya is heavily controlled by hydroclimatic variability (Fig. 4).

The chemical stratification was triggered by prolonged periods of highly negative water balance in the lake caused by severe droughts. During these phases, the intense evaporation of surface waters, particularly in summer, caused a drop in the lake level and a strong concentration of dissolved salts, especially carbonates and sulphates. Consequently, the water's deuterium excess signal ($d = \delta D_{V-SMOW} - 8 * \delta^{18}O_{V-SMOW}$) becomes notably negative. This interpretation is supported by isotopic monitoring data, which showed that the most evaporated signals of the entire record occurred during autumn 2023 and spring 2024.

Although this extreme evaporation primarily affects the surface waters, the entire water column becomes homogeneous through winter mixing (monomixis), acquiring a higher salinity and density and a strongly evaporated isotopic signature.

The hydrodynamic tipping point is reached when a drought is followed by a significant period of higher rainfall. Large volumes of freshwater enter the system, with its signature of lower density and salinity and less negative deuterium excess signal values. During these wetter periods, the lake level rises, the $\delta^{18}O_{V-SMOW}$ increases, and surface waters dilute. The isotopic record revealed that, between autumn 2018 and summer 2022, surface waters displayed systematically higher d-excess values than deep waters, indicating contrasting hydrological histories between the two compartments.

However, the decrease in salinity is not uniform: the freshwater accumulates in the epilimnion, while the deep waters remain concentrated. This decoupling could be more intense if the influx of freshwater coincides with the onset of thermal stratification (e.g. during spring rainfall), and prevents vertical mixing.

This interpretation is supported by the carbon isotope composition of the dissolved inorganic carbon. Surface waters had lower DIC concentrations and less negative $\delta^{13}C_{V-PDB}$ values than deep waters, which is likely due to the preferential uptake of ^{12}C during photosynthesis and higher atmospheric CO_2

exchange in the mixolimnion. By contrast, the monimolimnion accumulated higher DIC concentrations and markedly depleted $\delta^{13}\text{C}_{\text{V-PDB}}$ values, behaving as a long-term carbon reservoir isolated from surface processes. As a result of this process, a pronounced density gradient was established and the lake became chemically stratified, isolating the deep waters (monimolimnion).

The intermediate thermal behaviour recorded at 10 m suggests that the chemocline oscillated seasonally around this depth, while remaining well above the monimolimnion (> 15 m). The highly variable $\delta^{13}\text{C}_{\text{V-PDB}}$ values measured in DIC samples collected at 10 m further support this interpretation, as they likely reflect repeated seasonal displacement of the chemocline through the intermediate water column. Thermal monitoring demonstrated that the lake never reached winter isothermal conditions and that surface waters cooled below 5°C while bottom waters remained between 7 and 8°C, confirming that water-column stability was controlled by density differences rather than temperature alone.

These deep waters act as a 'time capsule', preserving the high chemical concentration and extremely evaporated isotopic signature acquired by the lake during the previous drought period. The largest isotopic offset between surface and deep waters was recorded in late spring 2020, when the monimolimnion still preserved a markedly evaporated signature while the mixolimnion had already been diluted by freshwater inputs.

This mechanism explains the apparent paradox observed throughout most of the monitoring period, where stronger evaporation indicators were present in the deep waters than in the surface waters.

This state of chemical meromixis is stable and would only change with the onset of a new severe drought. During this new drought, intense surface evaporation again will concentrate the salts in the epilimnion until their density equals or exceeds that of the deep waters. Then the chemical stratification will break, allowing the water column to mix completely again during the winter. As of May 2026, however, this homogenization has not occurred; a persistent salinity gradient between surface and bottom waters continues to inhibit vertical mixing, locking the monimolimnion in a state of ongoing anoxia.

A past tipping point during a climate transition: the end of the Little Ice Age

The paleolimnological record of Estanya Lake provides a long-term perspective for evaluating past rapid shifts in limnological conditions, the resilience of the lake system and its response to multiple stressors over the last centuries. The sediment sequence allows the identification of critical thresholds or “tipping points” driven by both natural climate variability and recent global warming and human activities.

The end of the Little Ice Age (LIA) constitutes a major climatic tipping point, providing an opportunity to investigate the lake system's primary response to natural climate forcings prior to the massive alterations of the modern Industrial Era.

Our data confirm previous studies (Morellón et al., 2011) demonstrating significant changes in the organic carbon accumulation at the end of the LIA and the onset of the Industrial Era (ca. 1850 CE). Both sequences show a notable increase in the organic carbon concentration and a rise in the C/N ratio indicating a more terrestrial signal in the organic inputs, but starting earlier (ca. 1750) than the end of the LIA (ca. 1850) and continuing till the end of the 20th century. This change is attributable to an increase in agricultural use in the surrounding areas during the 19th and mid 20th century (Riera et al., 2004). Furthermore, despite the regional rural abandonment in the 20th century, the remaining arable lands in the lake's immediate catchment underwent intense mechanization, causing an accelerated increase in allochthonous organic matter input (Corella et al., 2011; García-Ruiz, 2010).

To better understand the LIA-IR transition, the study of the sulphur cycle is highly valuable despite its complexity. The sediment sequence shows a large $\delta^{34}\text{S}_{\text{V-CDT}}$ positive excursion between 1850 and 1950, (from Total Sulphur values of $\approx -18\text{‰}$ in Unit 3 to $\approx +2.4\text{‰}$ in Unit 1) suggestive of a large change in the sulphur dynamics of the lake.

In Estanya, evaporites from the Triassic Keuper formation are the main sulphur source for the lake, showing $\delta^{34}\text{S}_{\text{V-CDT}}$ values between $+10$ and $+17\text{‰}$ in the literature (Claypool et al., 1980; Mazurek and de Haller, 2017), which is consistent with our values measured in the basin's rocks ($\delta^{34}\text{S}_{\text{V-CDT}} \approx +16\text{‰}$). In oxygenated waters, sulphur is present as dissolved sulphate from gypsum dissolution or the oxidation of deep-water reduced species. Under reducing conditions, sulphate-reducing bacteria reduce SO_4^{2-} to produce S^{2-} . This process involves significant isotopic fractionation, resulting in a more negative $\delta^{34}\text{S}_{\text{V-CDT}}$ of S^{2-} than that of SO_4^{2-} . The most negative isotopic values occur in environments undergoing repeated oxidation-reduction cycles; that is, when the lake experiences periods of complete and effective mixing and oxygenation alternating with periods of anoxia and reduction, allowing for further isotopic depletion through bacterial disproportionation (Canfield and Thamdrup, 1994). Total sulphur in the sediment therefore represents a mixture of all these signals. The positive $\delta^{34}\text{S}_{\text{V-CDT}}$ values ($+2.4\text{‰}$) at the sediment-water interface reflect the heavy isotopic signature of the original sulphate source (Keuper gypsum, $+18.5\text{‰}$). The recent sediments at this interface have not yet experienced the repeated cycles of microbial sulphate reduction and sulphide oxidation that typically drive isotopic values toward more negative numbers.

Reflecting these dynamics, the most negative values in $\delta^{34}\text{S}_{\text{V-CDT}}$ found in unit 3 are related to sulphides and reduced sulphur species that have undergone several oxidation-reduction phases, whereas positive or less negative values are strongly correlated with the sulphate signal and sulphides that have experienced fewer oxidation-reduction cycles and, consequently, are less fractionated.

Principal component analysis (PCA) provided further evidence of this environmental transition. Dim1's positive side is dominated by lithogenic elements (Fe, Ti, Rb, Al_2O_3 , K_2O , SiO_2 , MgO and Zr), which are interpreted as indicators of detrital inputs associated with runoff and catchment erosion. In contrast, the negative side is characterised by the presence of Ca, Sr, TIC, TOC, TN and sulphur, as well as isotopic

variables, which reflect increased authigenic precipitation, evaporation processes and biological productivity within the lake.

The progressive migration of sample scores from positive to negative Dim1 values indicates a reduction in detrital dominance and a long-term shift towards increasingly restricted, evaporation-dominated limnological conditions.

The end of the Little Ice Age (LIA) around 1850 CE marks a definitive tipping point and a point of no return in the lake's hydrochemical and physical dynamics. Prior to this date, the lake was characterized by a more diluted state, sustained by LIA climatic conditions such as lower summer temperatures and reduced evaporation. Furthermore, colder winters likely induced a dimictic regime in some years, implying that the lake surface froze, an interpretation supported by historical documents recording the freezing of the Ebro River in lower-altitude cities such as Zaragoza and Tortosa during winter (Oliva et al., 2018). However, the climatic shift around 1850 CE irreversibly disrupted this equilibrium. From this point of no return onward, the lake could not revert to its former conditions and entered a century-long transitional phase (1850–1950 CE) of progressive environmental changes. During this period, the cessation of winter freezing and higher evaporation rates drove the system to permanently transition into a brackish and monomictic state.

Anthropogenic impacts: Alterations in the N cycle and primary production

Nitrate pollution of groundwater worldwide has become a major concern, and groundwater-dependent ecosystems, such as karstic lakes in the Mediterranean area, are not exempt from this problem (Abascal et al., 2022; Menció et al., 2016). The upper part of the sequence, which covers the last 25 years, records a recent tipping point in nitrogen dynamics likely caused by anthropogenic activity. The isotopic signature of nitrogen in the surface sediment (after ca. 1990 CE) showed very positive values, reaching up to + 6.82‰. This highly positive signal is consistent with inputs derived from slurry or wastewater and provides clear evidence of recent organic contamination. Locally, this isotopic evidence is strongly corroborated by governmental spatial data. The regional government (Gobierno de Aragón) officially designates this sector (Sinclinal de Graus) as a Nitrate Vulnerable Zone (NVZ, 'Zone E. Litera Alta') due to severe agricultural pollution (Gobierno de Aragón, 2021). Furthermore, specific geospatial analysis of livestock facilities within a 5 km radius of the study site, using the Instituto Aragonés de Gestión Ambiental (Gobierno de Aragón, 2026) database, reveals critical nitrogen oversaturation. While no farms are located in the immediate perimeter (1.1 km), the cumulative regional pressure is overwhelming. The estimated nitrogen load from intensive livestock farming reaches 245.56 tons·year⁻¹, exceeding the legal assimilation capacity (192.00 tons·year⁻¹) by nearly 28%. This interpretation is further supported by the strong relationship between TN and TOC in the sediment sequence, indicating that most nitrogen accumulation is associated with organic matter burial rather than inorganic nitrogen phases.

The impact of N pollution on groundwaters is corroborated by hydrochemical data from the nearby Senara spring, which is known to be impacted by livestock manure, and exhibits elevated nitrate concentrations ($88.92 \text{ mg}\cdot\text{L}^{-1}$) and a heavily enriched $\delta^{15}\text{N}_{\text{AIR}}$ signature (14.07‰). Although nitrate concentrations within the lake were frequently below analytical detection limits, the available isotopic measurements suggest substantial biological processing of dissolved nitrogen, including nitrate uptake and isotopic fractionation within the water column.

An enhancement of biological activity is clearly reflected at the top of the sedimentary record by a negative excursion in the $\delta^{13}\text{C}_{\text{V-PDB}}$ of the Organic Carbon and a decrease in the C/N ratio, both suggesting a major contribution of algal autochthonous organic matter during the most recent decades (Meyers and Teranes, 2001).

A similar period of low $\delta^{13}\text{C}_{\text{V-PDB}}$ has been documented in the longer sedimentary sequence (Morellón et al., 2011) when $\delta^{13}\text{C}_{\text{V-PD}}$ values were approximately -25‰ , and progressively became more negative throughout the 16th century, reaching values close to -29‰ , similar to the modern values post 2000 CE. Therefore, in the last millennium Lake Estanya exhibited two major historical negative $\delta^{13}\text{C}_{\text{V-PD}}$ excursions: one in the 16th century and another in the 21st century. However, the 16th-century negative excursion was not associated with any changes in other primary productivity proxies (diatom assemblages, C/N ratios). Consequently, Morellón et al. (2011) interpreted the 16th-century shift as being driven by natural hydroclimatic changes during the Little Ice Age (LIA), specifically a wetter phase with an increase in runoff that raised the input of land-derived organic matter and altered the vegetation type within the catchment, rather than changes in the trophic state and productivity of the lake. This clearly establishes that the earlier 16th-century shift was a natural, climatically driven event.

The $\delta^{13}\text{C}_{\text{V-PDB}}$ values in our sequence (Fig. 5) show three periods of negative excursions: 1700–1750, 1870–1930 and post 2000 CE. Unlike the hydroclimatic forcing of the 16th century, the changes during the periods of 1700–1750 and 1870–1930 are likely to be explained by anthropogenic alterations to land use. Specifically, they reflect an increased input of allochthonous terrestrial organic matter, as supported by a progressive increase in the C/N ratio (Fig. 5), driven by agricultural expansion in the watershed from the eighteenth century onwards and by the increasing intensification and mechanization of farming during the nineteenth and early twentieth centuries (García-Ruiz, 2010; Riera et al., 2004), which likely enhanced the transfer of terrestrial organic matter to the lake. Similar processes have been documented in Iberian karstic lakes affected by agricultural intensification (Corella et al., 2011).

In contrast, there have been no notable changes in land use or vegetation in the 21st century. Furthermore, the modern negative excursion is accompanied by a decrease in the C/N ratio, which points to a greater influence of lacustrine organic matter (Meyers and Teranes, 2001), alongside a marked positive excursion in the nitrogen isotopes, consistent with a shift in nutrient inputs. These indicators confirm that the current increase in primary productivity is driven by anthropogenic factors.

This modern negative excursion can be explained by the assimilation of isotopically lighter carbon sources resulting from eutrophication. The massive phytoplankton bloom leads to higher accumulation of organic matter at the lake bottom. The subsequent bacterial decomposition of this biomass depletes the dissolved oxygen and releases large amounts of respired CO₂, which is highly depleted in ¹³C. As new generations of phytoplankton photosynthesise using this recycled, isotopically light CO₂, their isotopic signature becomes markedly negative. The global Suess Effect (the progressive decrease in atmospheric δ¹³C_{V-PDB} due to fossil fuel combustion) (Keeling, 1979) could also have contributed to the recent trend, but the range is too large to be explained just by this external forcing. Therefore, unlike the shifts in terrestrial organic matter sources observed in the 16th century, the current negative excursion reflects a highly productive system recycling its own depleted carbon.

Limnological resilience and future perspectives

Our data demonstrate a remarkable limnological plasticity of Lake Estanya, having transitioned through three different states in less than 200 years: the 16th century, the end of the LIA and post 2000 CE. The three transitions included changes in the N, C and S cycles and were forced by a combination of hydroclimate and watershed variability. However, the synergistic relationship between climate and human activities was different in the three transitions.

Both the 16th and mid-19th century transitions were induced by hydroclimatic changes; however, they displayed different biogeochemical responses. The 16th century transition was characterised by a marked negative excursion in organic matter δ¹³C_{V-PDB} values (close to -29‰). However, this negative shift was not related to an increase in lake productivity; instead, it was driven by increased runoff in a more humid phase during the LIA, which raised the input of land-derived organic matter and altered the vegetation type in the catchment area (Morellón et al., 2011). In contrast, the mid-19th-century transition (the end of the Little Ice Age) was characterised by changes in land use (Riera et al., 2004) and profound alterations in the lake's hydrochemistry and sulphur dynamics. This period displays a significant positive shift in δ³⁴S_{V-CDT} (from approximately -18‰ to -5‰) and a clear shift in Principal Component Analysis, moving from a system dominated by detrital inputs to one strongly influenced by evaporitic elements. These indicators reflect a natural tipping point at which the lake transitioned from a more diluted state with effective mixing periods to a brackish system with poor or absent mixing. Therefore, while past historical transitions reflect climate driven vegetation shifts or natural threshold crossings towards evaporitic concentration, the modern 21st century shift is distinctly unprecedented: it is a meromictic regime forced by the combined impact of severe anthropogenic nutrient loading and extreme evaporation during prolonged droughts.

The recent shift towards a meromictic regime in Lake Estanya can be contextualised within the broader evolution of other Iberian karstic lakes. While recent anthropogenic pressures, such as water extraction, forced Lake Arreo to transition from meromictic to monomictic conditions (Corella et al., 2011) (Corella et al., 2011), the dynamics in Lake Estanya closely mirror those of Lake Montcortès. Sedimentary records from Montcortès similarly indicate stability until the early 19th century, followed by hydroclimatic

instability and a significant 20th-century increase in hypolimnetic anoxia driven by warming and historical human activities (Vegas-Vilarrúbia et al., 2026, 2018). The parallel timing of these limnological reorganisations suggests that the end of the LIA and recent anthropogenic warming represent regional-scale thresholds affecting Mediterranean karstic lakes across north-eastern Iberia.

Beyond these regional implications, this novel meromictic state poses a severe threat to the lake's resilience. When the lake chemically stratifies and enters a persistent meromictic state, the deep waters become permanently isolated from the oxygenated surface layers. Concurrently, the system receives an excessive influx of nutrients derived from surrounding agricultural activities, which drives a significant increase in primary productivity. As this abundant organic matter sinks, its subsequent bacterial decomposition rapidly depletes the dissolved oxygen in the bottom waters. This process establishes persistent anoxic conditions in the monimolimnion, creating a highly reducing environment that favours the release of toxic compounds into the sediment. If these toxic byproducts accumulate and eventually diffuse upward or are released during an eventual mixing event, they could severely damage the entire lacustrine ecosystem.

Given the cumulative effects of permanent stratification and anthropogenic-derived eutrophication presented in this study, effective management of the Lake Estanya watershed is required to prevent irreversible ecological change. To preserve the lake's ecological integrity and mitigate the consequences of these stressors, conservation strategies should focus on controlling external nutrient inputs and implementing appropriate measures about land uses in the hydrogeological watershed.

Beyond its implications for Lake Estanya, the 21st century transition (post 2000 CE) confirms that climate change can lead to profound alterations in lake mixing regimes over the coming decades (Woolway and Merchant, 2019). Together with the global expansion of lake hypoxia (Jenny et al., 2016) and recent projections of increasing anoxia under future warming scenarios (Nkwilale et al., 2026), these findings place Lake Estanya within a broader global trend of freshwater ecosystem deoxygenation. Consequently, this has significant implications for our understanding of, and approach to managing, karstic lakes, particularly in regions subject to high hydroclimatic variability.

The climate-driven, chemical meromixis mechanism observed in Lake Estanya suggests that other relatively deep, evaporite-influenced karst lakes traditionally considered to be strictly monomictic during this epoch, such as Lake Zoñar in southern Spain (Martín-Puertas et al., 2011) or El Tejo (Morata et al., 2003) and La Parra (Barreiro-Lostres et al., 2014) lakes in the Serranía de Cuenca, could also experience similar, transient meromictic phases that have gone unnoticed. Understanding these dynamics is essential for interpreting palaeolimnological records, as sedimentary proxies may reflect these decoupled conditions within the water column.

Furthermore, these findings highlight the vulnerability of these ecosystems to projected climate change scenarios involving a greater frequency of extreme droughts followed by erratic rainfall, which could push more lakes beyond their hydrodynamic tipping points and severely alter their ecological resilience.

Conclusions

Until recently, Lake Estanya exhibited a predominantly monomictic regime, whereas monitoring data indicate that meromictic conditions became established during 2018, and evidence suggests that it may have been dimictic during the colder periods of the Little Ice Age (LIA). The recent meromixis identified in Lake Estanya is primarily driven by cycles of prolonged drought followed by intense rainfall episodes, which create a significant salinity differential between the surface and deep waters.

The evolution of the lake over the past 300 years reveals how climatic pressures increased the lake's salinity at the end of the LIA, representing a natural tipping point. Furthermore, starting in the 2000s, an unprecedented alteration in nitrogen dynamics has been observed, which is directly linked to the application of fertilisers in the drainage basin. This severe anthropogenic impact has consequently altered carbon dynamics and fuelled an increase in primary productivity.

The results of this study highlight that the combined effects of climate change and intensive agriculture are pushing these karstic ecosystems into unprecedented scenarios. Therefore, current management policies must urgently consider this unprecedented limnological evolution, implementing practices that control agricultural fertiliser use and limit nutrient inputs into the watershed to prevent irreversible ecological damage.

Declarations

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Author Contribution

M.G.P: Investigation (fieldwork and laboratory analyses), Formal analysis, Writing-original draft preparation. G.P: Investigation (fieldwork), Writing-review & editing. A.D.H: Investigation (stable isotope laboratory analyses), Writing-review & editing. I.P.M: Investigation (fieldwork), Writing-review & editing. J.P.C: Conceptualization, Investigation (fieldwork), Writing-review & editing. I.R.V: Investigation (fieldwork), Formal analysis (age-depth modelling), Writing-review & editing. M.M: Conceptualization, Writing-review & editing. B.V.G: Conceptualization, Supervision, Project administration, Writing-review & editing.

Data Availability

The datasets generated during the current study will be available in a Zenodo repository.

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Figures



Figure 1

Location of Lake Estanya and its watershed in northeastern Spain. (Basemap and topography from Instituto Geográfico Nacional - IGN) The red polygon outlines the catchment area, and the red diamond indicates the coring site (LEG23-1A-1G). The position of the Estanya and Senara springs is also shown. The upper-right panel depicts the location of the study area within Europe, while the lower-right panel shows the bathymetry of Lake Estanya (contour interval in metres, based on Morellón et al., 2009b).

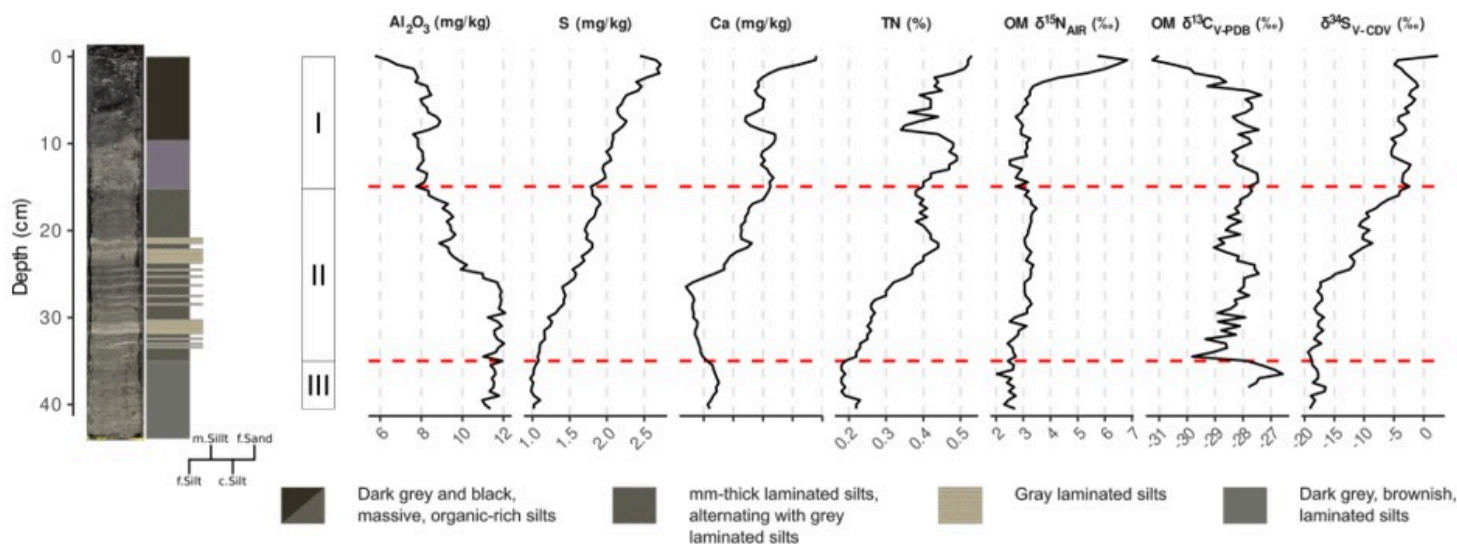


Figure 2

Sediment lithology and selected geochemical proxies from core LEG23-1A-1G. From left to right, the figure shows the core photograph, lithology, and sedimentary units (I-III) established through correlation with previously studied sediment sequences from Lake Estanya (Morellón et al. 2011). The geochemical proxies include Al_2O_3 as an indicator of detrital input, sulphur (S) and calcium content (Ca), total nitrogen (TN), stable isotopes of organic matter ($\delta^{15}\text{N}_{\text{AIR}}$ and $\delta^{13}\text{C}_{\text{V-PDB}}$), and total sulphur isotopes ($\delta^{34}\text{S}_{\text{V-CDT}}$).

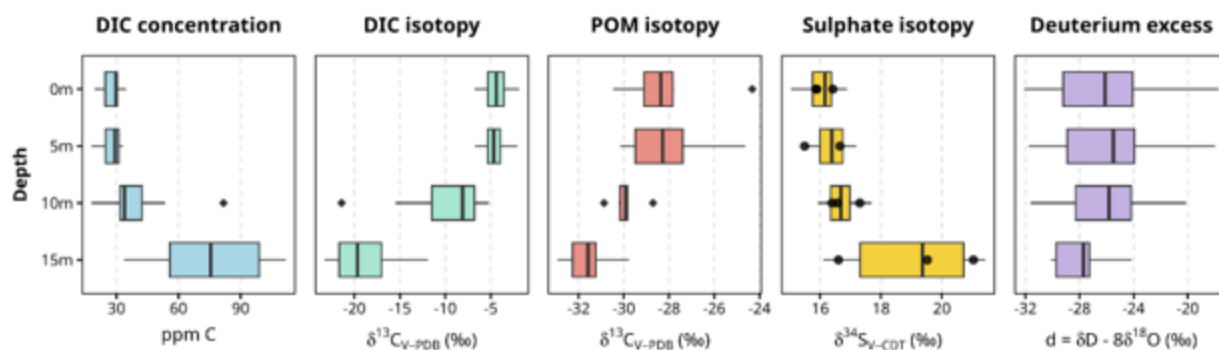


Figure 3

Depth profiles of dissolved inorganic carbon (DIC) concentration, stable isotope compositions, and deuterium excess along the water column of Lake Estanya (0-15 m). Panels from left to right show: DIC concentration (ppm C); DIC isotope ($\delta^{13}\text{C}_{\text{V-PDB}}$ (‰)); POM isotope (particulate organic matter $\delta^{13}\text{C}_{\text{V-PDB}}$ (‰)); sulphate isotope dissolved sulphate ($\delta^{34}\text{S}_{\text{V-CDT}}$ (‰)); and lake water Deuterium excess ($d = \delta\text{D} - 8\delta^{18}\text{O}$ (‰)). Box plots represent the median (center line), the 25th and 75th percentiles (box limits), and 1.5x the interquartile range (whiskers). Black diamonds indicate outlier values. For sulfate isotope, box

plots illustrate the distribution of analytical replicates, and superimposed solid black circles indicate the sample mean at each depth.

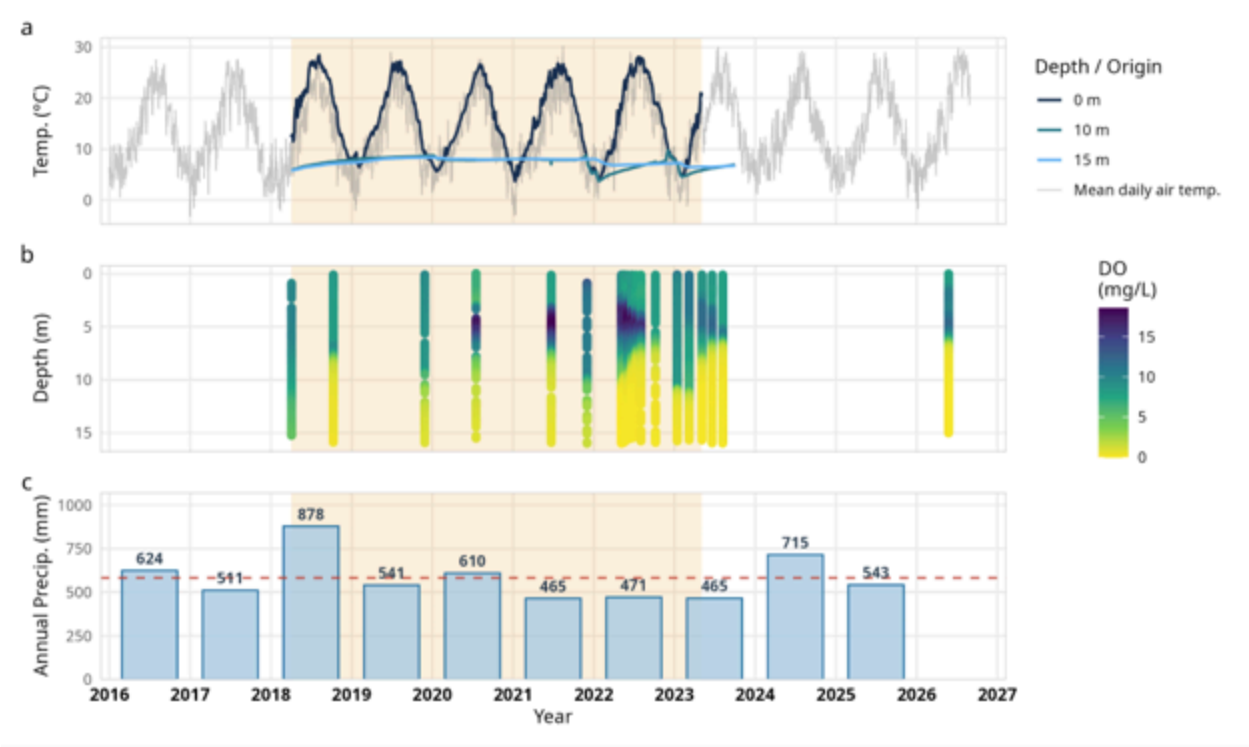


Figure 4

Hydroclimatic and limnological variability in Lake Estanya from 2016 to 2026: a. Lake thermal evolution at depths of 0, 10 and 15 metres, alongside regional mean daily air temperature (Benabarre station, AEMET). b. Dissolved oxygen (DO) profiles showing seasonal stratification and the evolution of bottom anoxia. c. Total annual precipitation (mm) at Benabarre (10 km NNW from the lake) with the long-term mean indicated by the red dashed line. The orange vertical band marks the thermal monitoring period.

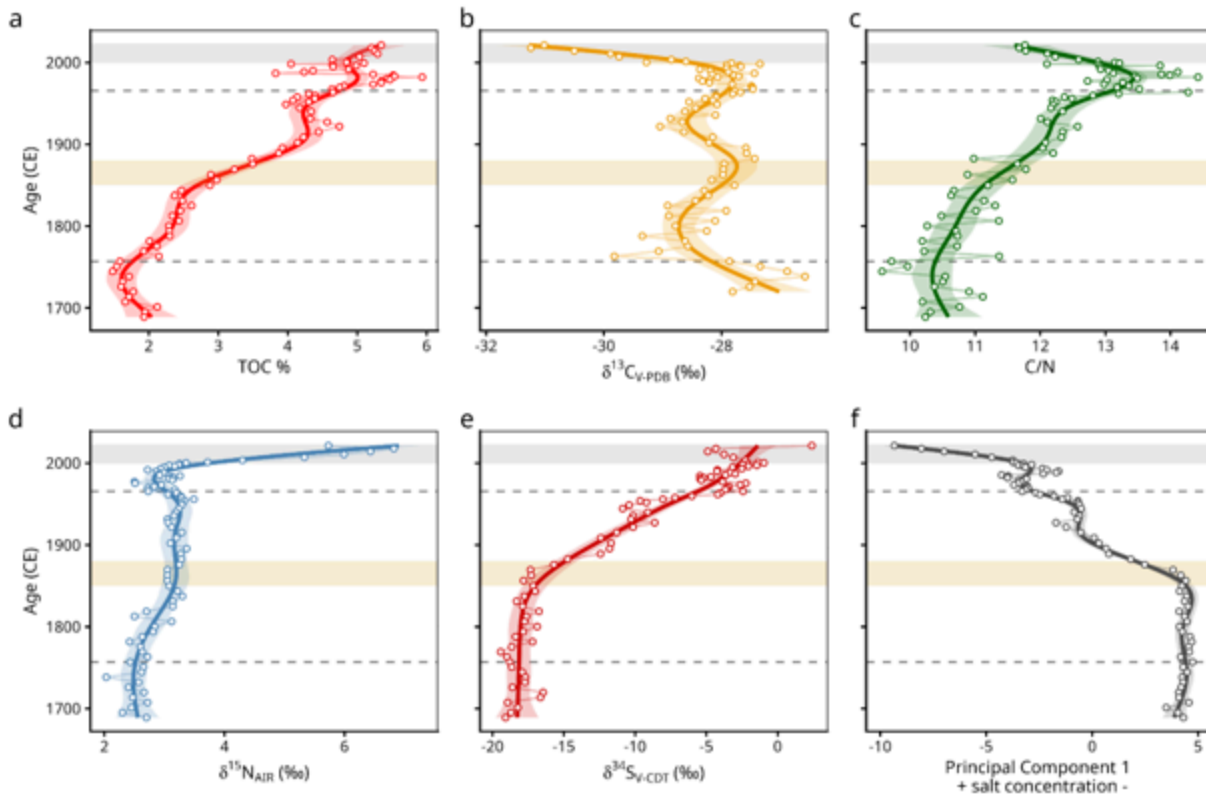


Figure 5

Geochemical and isotopic stratigraphy of the LEG23-1A-1G sediment core from Lake Estanya over the last three centuries. The panels display: a Total Organic Carbon (TOC, %); b organic matter $\delta^{13}\text{C}_{\text{V-PDB}}$ (‰); c C/N ratio; d total nitrogen $\delta^{15}\text{N}_{\text{AIR}}$ (‰); e total sulphur $\delta^{34}\text{S}_{\text{V-CDT}}$ (‰); and f Principal Component 1 (Dim1) scores, reflecting the transition from detrital (positive) to evaporitic/authigenic (negative) dominance. Solid lines represent a smoothed trend with confidence intervals. Dashed horizontal lines mark the boundaries between stratigraphic units 1, 2, and 3, consistent with Morellón et al. (2011). The orange shaded area highlights the new stage reached at the end of the LIA after crossing a natural tipping point associated with increasing salinity and hydroclimatic variability, while the grey shaded area indicates the recent anthropogenic tipping point driven by external nutrient inputs.