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Research article

Tracing Anthropogenic Disturbances of a Wetland Through Carbon and Nitrogen Isotope Analyses in Sediments

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Abstract

In this study, we estimated the depth profiles of nutrients in the natural wetland "Ciénega Tamasopo" (Central Mexico) located in the Neotropic region. The concentration profiles of C_{org}, N_T, C_{org}/N_T ratios and isotopic composition ($\delta^{13}C$, $\delta^{15}N$) were determined in sediment cores collected at five sites throughout the wetland to estimate the contribution of nutrient sources to the sediments through analysis of profile shape. The results showed a recent enrichment in organic matter in the upper sediment layers at three sites (S1 to S3). Changes in the C_{org}/N_T ratios with the sediments depth suggested that organic matter is autochthonous at the pristine sites (upper part of the wetland) characterized by an abundant coverage of vegetation. The isotopic ($\delta^{13}C$, $\delta^{15}N$) profiles (-35 to -25.8 ‰ for $\delta^{13}C$ and 0.7 to 4.1 ‰ for $\delta^{15}N$) supported these conclusions and pointed to the entrance of allochthonous materials through local perturbations as a cause of changes in wetland productivity at the lower part. Analysis of isotopic composition can be used to evaluate trace productivity changes in tropical wetlands exposed to anthropogenic perturbations in this area.

Research highlights

Elemental and isotopic C_{org} and N_T depth-profiles are useful to trace OM in the "Ciénega Tamasopo" wetlands.

C_{org}/N_T ratio with δ^{13}C or δ^{15}N highlights changes in the nutrient sources in the wetlands.

Nutrients from external sources may alter OM deposition by increasing productivity

1. Introduction

In order to identify organic matter sources in sediments from aquatic systems such as wetlands, as well as to trace past productivity, to assess the impact of humans on local ecosystems and changes in the supply of nutrients to lakes, some studies have analyzed changes in the compositions of C_{org}, N_T, C_{org}/N_T ratios, and carbon and nitrogen isotopes (δ^{13}C and δ^{15}N) **[1]**-**[3]**. C_{org}/N_T ratios are widely used to distinguish the proportions of algal and land-plant organic matter **[1]**, **[4]**. δ^{13}C allows for the identification of carbon sources and photosynthetic pathways in plants (e.g. C3 and C4 plant differentiation) **[5]**, **[6]** and to determine enhanced algal productivity **[7]**. In freshwater systems, organic matter from algal production has a C_{org}/N_T ratio from 4 to 10 for cellulose poor and protein rich organic matter, 10 to 20 from aquatic/terrestrial sources and >20 from terrestrial sources (cellulose poor and protein rich organic matter) **[3]**, **[7]**. The δ^{13}C values of organic matter can range from −9 to −30 ‰ **[3]** and can differentiate between C3 (− 24 to − 30‰) and C4 plants (− 9 to − 15 ‰) in different environments (e.g. aquatic, terrestrial) **[3]**-**[5]**, **[7]**-**[9]**. The δ^{15}N values of organic matter from sediments can be used to differentiate between algal (aquatic) and land plant sources of organic matter and as evidence of nitrogen fixation and enhanced algal productivity. Environmental changes can affect the δ^{15}N in the sediments. As an example, Meyer **[3]** reported a 2 ‰ shift toward higher δ^{15}N values in Nevada Lake caused by water level variations and changes in the organic matter sources.

Brenner *et al*. **[10]** found isotopic signatures of δ^{13}C (-27.2 to -22.9 ‰) and δ^{15}N (-2.3 to 0.5 ‰) in sediments from a shallow lake dominated by macrophytes in Florida (USA), attributing the origin of the organic matter to the aquatic plants. δ^{15}N values in that lake correlated with an increased P concentration from human activities and forest clearance. Chang *et al.* **[11]** recorded isotopic signatures of δ^{13}C (-33.6 to -27.4 ‰) and δ^{15}N (-4.8 to 8.6 ‰) in the surficial sediment (~5 cm depth) of a Florida subtropical wetland attributed to changes in the water chemistry and wetland hydrology influencing the organic matter content as a result of modifications in the hydrophyte vegetation structure.

Nitrogen inputs from anthropogenic sources (fertilizers from agriculture, sewage, manures, etc.) can negatively affect the water quality of wetland ecosystems. This impact can be traced by measuring δ^{15}N as an indicator of the availability of nitrogen for biota **[12]**. The N-isotopic composition of settling organic detritus thus varies, depending on the extent of nitrogen utilization by organisms: low ^{15}N content indicates low relative utilization and high ^{15}N content indicates high utilization **[6]**.

This study reports the vertical patterns with depth of C_{org}, N_T, P, C_{org}/N_T ratios and carbon and nitrogen isotopes (δ^{13}C and δ^{15}N) in sediments of a natural wetland (Ciénega Tamasopo, Central Mexico). This wetland supplies water for agriculture, livestock, and towns and is impacted by the residues. The entrance of nutrients should favor increased productivity; thus, organic matter contributions to the wetland sediments could originate from internal sources (e.g. phytoplankton, aquatic macrophytes) and external sources (e.g. sewage, sediments from farmlands and agriculture). The aim is to trace changes in organic matter deposition and the impact of local human perturbations on the wetland. We consider that the perturbations caused by local inhabitants can be estimated from the variability of nutrient input to the wetland sediments, assuming an increased contribution of nutrients to the wetland from the surrounding agriculture and towns.

2. Materials and methods

2.1 Study site

Ciénega Tamasopo is a freshwater marsh (1364 ha) located in the Neotropic in Central Mexico [13]. Water inputs come from rainwater and springs in the upper part of the basin (twelve have been characterized). The average rainfall is 1500 mm/a with intensive precipitation from July to September. There is a main stream flowing from the upper (North) part that collects water from secondary streams and finally this stream forms the "El Trigo" river (see **Figure 1**). The main stream is not directly in contact with land runoff or sewage particularly at the upper part of the wetland, which has dense plant coverage. The wetland supplies water for 15 towns (~ 250 inhabitants per town) located along its margin and for sugar cane agriculture and livestock. Nearby houses do not have drainage systems and use latrines (wet or dry). The wetland is shallow (0.3 - 1.2 m depth).

During the dry season (May to June), the water depths decrease by 0.2-0.3 m Precipitation causes a significant increase in the water column level and floods the sugar cane fields and some livestock lands. The Ciénega Tamasopo wetland is surrounded by forest, mainly in the Northern part, and agricultural fields with sugarcane, towns and, to a lesser degree, livestock ranches mainly located to the South. Wetland vegetation is abundant and covers ~68 % of its surface. It is dominated by hydrophyte plants, water lilies (*Nymphaea sp*.), water lettuces (*Pistia stratiotes*), waterweeds (*Elodea sp*.), water hyacinth (*Eichhornia crassipes*), saw grass (*Cladium sp*.), and cattail (*Typha domingensis*).

Soils in the northern and highest part of the wetland range from the rendzina type (forest dominated) to litosol in the southern part of the watershed. The soils in the wetland are vertisols. From field observation at the time of sampling, the surficial sediments (~5 cm) are comprised of (dark) unconsolidated and fine-sized grain, while

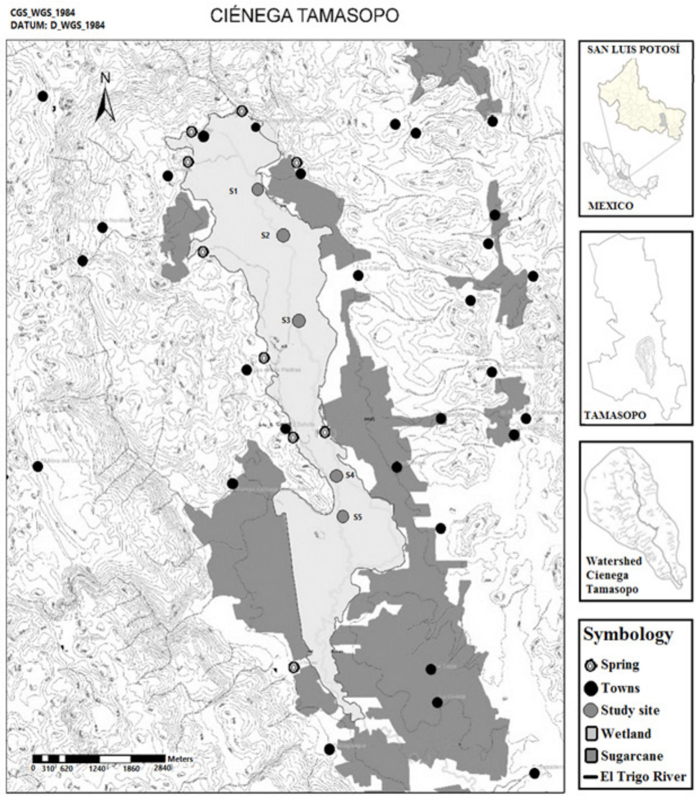

Figure 1: Location of the sampling stations in the Ciénega Tamasopo wetland, San Luis Potosí, México.

the deepest sediments (>10 cm) are more compacted. The sediments are mostly anoxic and there is limited oxygen availability in the underlying water (DO 0-1 mg/L; September 2012); the sulfide concentrations in porewater ranges from 2.2 to 300 µM at the sediment-water interface (0-3 cm depth), except at site S2, where the sulfides were detected in deeper sediments (>5 cm).

2.2 Sediment sampling and analyses

Some anthropogenic and major economic activities (agriculture and cattle livestock) affect the natural resources in the basin due to the extraction of water, the infiltration of wastes from agriculture, animals and wastewater, and the loss of biological diversity (flora and fauna). For this study, we have taken sediment cores at five sites in the wetland (S1, S2, S3, S4 and S5). Sites S1 and S2 are located in the (Northern) upper part of the wetland considered as the more pristine part. Site S3 may be affected by the construction of a channel that was initiated in 1995 (~12 km long and at least 1 m depth) to dry this part of the wetland and use the land for agriculture. The authorities stopped this work and the construction of the channel was not completed. Plants now cover the site affected by the construction. Site S3 was considered because it is located in the vicinity of this area. The shallowest sites at S4 (0.3 m) and S5 (0.7 m) have been the most perturbed for anthropogenic actions, since S4 is close to a secondary stream where people have cut the vegetation to access the wetland and drain water to the sugarcane fields at the right margin of the wetland (East side). At the left margin (West side), the most important influence appears to be from water extracted to supply rural towns.

Two sediment cores of ~20 cm in length were collected (gravity sampler; Wildco 2404-A14) at each of the five sampling sites (S1 to S5, **Figure 1**) in September 2012 in zones not covered by plants. Cores at sites S1 to S3 and S5 were collected in the main stream. Cores at site S4 were collected in a secondary stream because the main stream was densely covered by vegetation making access difficult. *In situ*, sediments were sliced at 0.5, 1 and 2 cm intervals for 0-5, 6-10 and >10 cm depth, respectively. Samples were preserved at 4 °C and then oven dried (60 °C, 12 h) in the laboratory. Dry sediments were acid digested to determine the total phosphorus using a colorimetric method **[14]** (molybdenum blue method; SD ± 0.2 %). Dry sub-samples (0.5 - 1.0 g) of homogenized sediment were treated with 0.1 N H_2SO_4 (at 60 °C, 1 h) to dissolve carbonates. The slurry was filtered, and the solid was dried (60 °C, 12 h) for C analysis. C_{org} (%), N_T (%), $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) were determined at the Environmental Isotope Laboratory (University of Arizona) with a continuous flow Isotope Ratio Mass Spectrometer (IRMS; Finnigan Delta PlusXL) coupled to an Elemental Analyzer (Costech); samples were combusted in the elemental analyzer. Instrument calibration was based on acetanilide for elemental concentration (SD ± 0.1 %), NBS-22 and USGS-24 for $\delta^{13}C$, and IAEA-N-1 and IAEA-N-2 for $\delta^{15}N$. Precision was better than ± 0.1 ‰ for $\delta^{13}C$ and ± 0.2 ‰ for $\delta^{15}N$ (1σ) and was based on repeated internal standards. The delta values (parts per thousand; ‰) were in relation to the standard (Equation 1).

$$\delta[‰] = (R_s/R_x - 1) * 1000 \quad (1)$$

where R_x and R_s are the ratios of heavy and light isotopes (e.g., $^{13}C/^{12}C$ and $^{15}N/^{14}N$) in the sample (R_x) and the standard (R_s).

A statistical analysis of multiple comparisons of means (Tukey test; GraphPad inStat Software Inc. v 3.06, 2003) was applied to identify significant differences in the contributions of C_{org}, N_T, $\delta^{13}C$ and $\delta^{15}N$ between sites (S1 to S5; each core is an experimental unit) and at different sediment depths (0-5 cm, 6-10 cm and >10 cm). This criterion was established by the variability in the pattern of vertical profiles at different depths. The data were normalized with ANOVA during the Tukey test.

3. Results

The vertical patterns of the C_{org} (%), N_T (%), P (%) and isotopic $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) determined from the sediment cores from the "Ciénega Tamasopo" wetland are shown in **Figure 2**. From the analysis of these patterns, it is possible to highlight changes in the proxies when comparing the upper part (0-5 cm), the middle part (6-10 cm) and the lower part (>10 cm) of the sediment cores by site and between sites.

C_{org} in the upper part of the sediment cores (0-5 cm; **Figure 2**) shows concentrations at sites S1 to S3 (23.3 ± 1.44, 25.5 ± 2.23 and 30.4 ± 5.95 %, respectively) higher than the concentrations at sites S4 and S5 (15.5 ± 9.81 and 16.5 ± 4.11 %, respectively). A slight enrichment is observed at the sediment – water interface at sites S1 and S2 and especially at S3 (0-3 cm depth). The sediments in the middle part of

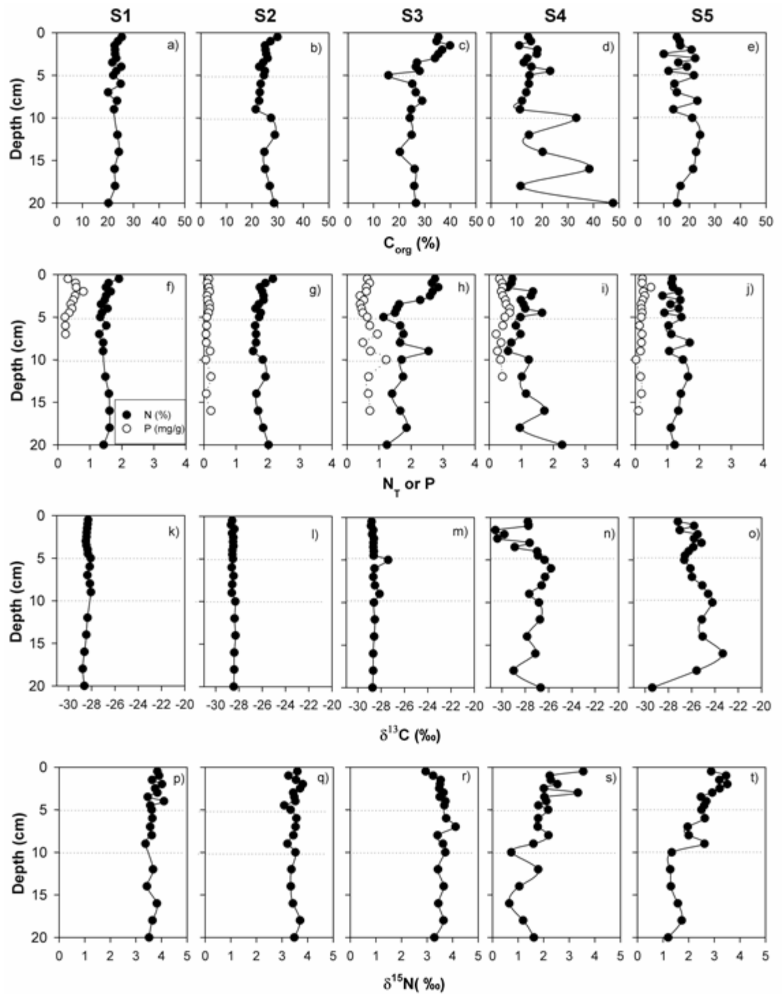

Figure 2: Vertical patterns of organic C, TN, P, δ^{13}C, and δ^{15}N with the depth of the sediments collected in September 2012 at sites S1-S5 in the "Ciénega Tamasopo" wetland (San Luis Potosi, Mexico).

the cores (6-10 cm) showed low variability at S1 (22.4-25 %) and S3 (24.2 %-25.1 %) and a decreasing concentration at S2 (from 27.4 to 23.4 %) and S4 (33.3 to 14.8 % with a minimum of 11.3 %). At the bottom of the sediment cores (>10 cm), the organic C concentrations were higher than those at the upper part of sediment cores, particularly at S4 (26.5 ± 15.7 %) and to a certain extent at S2 (26.9 ± 1.9 %) and S5 (20.1 ± 3.9 %).

NT shows the highest variability at S3 (1.1-2.9 %) in the upper sediments (0-5 cm) and low variability in the sediments in the middle part of the cores at S1 (1.3-1.5 %), S2 (1.6-1.8 %), S3 (1.6-1.7 % with a peak of 2.6 % at 9 cm) and S4 (0.6-1.2 %). Low variability was observed in the lower part of the cores (>10 cm) at S1 (1.4-1.6 %), S2 (1.6-2.0 %), S3 (1.3-1.9 %) and S4 (1.0-2.3 %). At S5, the NT concentrations (1.4 ± 0.2 %) were not significantly different to those in the upper sediments (0.5 cm). The highest P concentrations occurred in upper layer of the sediment (0-5 cm) at all sites (except at S3), ranging from 0.2 to 0.8 % (0-3 cm). At S3 the highest P concentrations (0.5-1.2 %) were observed in the middle part of the sediment core (6-10 cm). While, at the bottom of the cores, P was detected only at S3-S5, and the concentrations were similar to the concentrations found in the middle part of the sediment cores.

δ^{13}C (‰) values did not show substantial changes at sites S1 to S3 (-28.4±0.2, -28.5±0.1, -28.6±0.3, respectively) contrasting with those at S4 (-27.7±1.4) and S5 (-25.8±1.3). At sites S4 and S5, δ^{13}C varied as follows: in the upper sediments at S4 (-26.4 to -30.6 ‰) and at S5 (-26.6 to -25.2 ‰); in the middle part of the cores, at S4 values increased from -27.7 to -25.8 ‰; and at S5 the δ^{13}C increased from -24.2 to -26.1 ‰. Finally, from the bottom up to 10 cm, δ^{13}C varied at S4 from -29.0 to -26.7 ‰ and at S5 from -29.4 to -23-3 ‰. δ^{15}N (‰). The values did not show substantial changes at sites S1-S3 (3.69±0.20, 3.47±0.17, 3.54±0.24, respectively) with respect to S4 (1.92±0.73) and S5 (2.36±0.36). The δ^{15}N values from 0 to 5 cm, showed low variability at S1 (3.6-4.1 ‰), S2 (3.6-3.8 ‰) ‰), and S3 (3.7 to 3.0 ‰) compared with S4 (from 1.8 to 3.6 ‰)

and S5 (from 2.5 to 3.5 ‰). In the middle part of the cores, $\delta^{15}N$ values found at S4 (from 0.7 to 1.8 ‰, a peak of 2.2 ‰ at 8 cm) showed a sharp change between 8 and 10 cm. A similar pattern was observed at S5 (from 1.3 to 2.6 ‰). The $\delta^{15}N$ values were lower at S4-S5 with respect to S1-S3. At the bottom (>10 cm), values for the $\delta^{15}N$ at S4 (1.6 to 0.78 ‰) increased to 1.8 ‰. At S5, the $\delta^{15}N$ value varied from 1.3 to 1.7 ‰. $\delta^{15}N$ values in sediment >10 cm were lower at S4 and S5 with respect to S1-S3.

The results from the Tukey test (Table S1 in Supplementary Material) show the most significant differences ($p < 0.001$) in organic matter content and isotope signature. For the upper part of the sediment cores (0-5 cm) the most significant differences are observed between sites S2 - S3 vs. S4 - S5 (C_{org}, NT, $\delta^{13}C$ and $\delta^{15}N$), S1 vs. S5 ($\delta^{13}C$ and $\delta^{15}N$), S1 vs. S3 (organic C) and S1 vs. S4 (for $\delta^{15}N$). In the middle section (6-10 cm), significant differences are observed between S2 - S3 vs. S4 - S5 and S1 vs. S5 for $\delta^{13}C$ and $\delta^{15}N$. In the deepest sediments (>10 cm) the significant differences are observed between sites S1 - S3 vs. S4 - S5 for $\delta^{15}N$.

4. Discussion

Organic matter from several sources can be deposited in the sediments. To trace the contributions of the various organic matter sources, first, we compared the vertical patterns of C_{org}, N_T, P, C_{org}/N_T, $\delta^{13}C$ and $\delta^{15}N$ between sites to highlight changes or similarities in the nutrient content and isotopes in the sediments (**Figure 2** and **Figure 3**; Table S1). Then, by comparing the $\delta^{13}C$, $\delta^{15}N$, and C_{org}/N_T ratios measured in the sediments with values from the literature (e.g., terrestrial vegetation, emergent macrophytes, plankton, sewage, etc.), we intended to distinguish the main contributions of organic matter to the sediments.

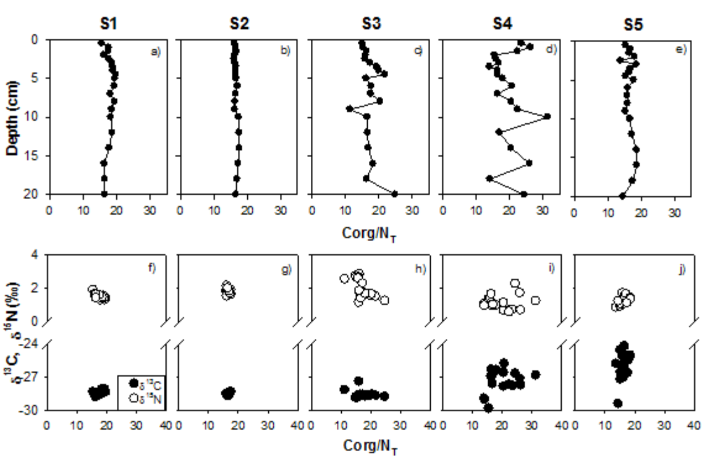

Figure 3: Ratios of Corg/NT with depth, $\delta^{13}C:C_{org}/N_T$ and $\delta^{15}N:C_{org}/N_T$ in the sediments collected in September 2012 at sites S1-S5 in the Ciénega Tamasopo wetland (San Luis Potosi, Mexico).

The results of Tukey tests (Table S1 in Supplementary Information) support the similarity in the organic matter sources at S1-S3 and differences with S4-S5 in the upper part of the sediment cores. Less significant differences were found in the middle and lower parts of the sediment cores but still suggest similar organic matter sources at S1-S3 compared with S4-S5. There is no information on sedimentation rates in Ciénega Tamasopo and we assume that the differences in the nutrient profiles between sites S1-S3 vs S4-S5 and in the profiles at each site reveal the differences in the sources of organic matter deposited in the sediments. The statistical analysis showed significant differences between the sediments in the upper part of the cores (0-5 cm depth) compared to the lower part (>10 cm depth) for $\delta^{15}N$ at S4 ($p<0.01$) and S5 ($p<0.001$), suggesting that the contribution of organic matter to the sediment column comes from different sources at these two sites; these differences were not observed at sites S1-S2.

Values of C_{org}, N_T, the P concentrations in the upper part of the sediments at S1-S3 suggest that an increasing productivity in these sites could occur even if the source of the nutrients has not changed due to the very low variability in the C and N isotopes profiles. In contrast, at sites S4 and S5, two possibilities arise: 1) there is a contribution of organic matter from a different source (e.g. from allochthonous materials) because the $\delta^{13}C$ values are higher and the $\delta^{15}N$ values are lower than the values at S1-S3; or 2) there is low mineralization of the organic matter as the $\delta^{15}N$ values increase at both sites in the upper sediments. Low variability of $\delta^{13}C$ and $\delta^{15}N$ values and different pattern profiles between S1-S3 vs. S4-S5 for these isotopes suggest differences in the productivity or in the sources of organic matter deposited in the sediments.

Several processes explain the variability in the organic matter concentrations in the sediments. Enhanced aquatic productivity as a consequence of changes in the nutrient supply (from internal or external sources) is one of the considered explanations. Consequently, C_{org} concentration increases **[3]** and this could be the case at S1-S3. In wetlands, the residues of plants provide organic matter and the nutrients are recycled through the mineralization of those residues. In this case, the increase in organic matter arises from internal sources. In addition, high C_{org} content in the sediment is due to low mineralization of the organic matter during sedimentation because the microorganisms preferentially consume N from the organic matter, increasing the C_{org}/N_T ratio **[9]**, **[15]**-**[17]**. C_{org}/N_T ratios are used to distinguish the origin of the sedimentary organic matter because those ratios generally survive sinking and sedimentation **[7]**; C_{org}/N_T ratios from 4 to 10 are associated with organic matter from algal production, from 10 to 20 with aquatic/terrestrial organic matter and > 20 with terrestrial origins **[7]**. **Figure 3** shows the C_{org}/N_T ratios estimated from the sediments and their relationship with $\delta^{13}C$ or $\delta^{15}N$. There is low variability in the C_{org}/N_T ratio at S1-S2 in contrast with the higher variability observed at S3-S4. In the upper part of the sediments (0-5 cm) the C_{org}/N_T decreased to the surface (organic C and N_T increased) at S1 (from 20 to 15.6) and S3 (from 23 to 15.6). Low variability was observed at S2 (~15.6; C_{org}, P and N_T increased). At S4, the C_{org}/N_T ratios decreased (C_{org}, N_T and P decreased) from 20 to 13.8 and increased to 27.5 at the sediment surface. Finally, at S5, C_{org}/N_T varied from 13.8 to 18.8.

The C_{org}/N_T ratios suggest aquatic plants as the main source of organic matter at sites S1 – S3 and S5, the contribution of external sources at S4 and periods with increased productivity at S3 (0-10 cm), S4 (2-4.5 cm) and S5 (0-5 cm). Brenner *et al.* **[10]** proposed C_{org}/N_T values for submerged and floating-leaved aquatic macrophytes (14 - 22) and for emergent vegetation (~24), and C_{org}/N_T values for river phytoplankton (5.2–14.6) were proposed by Cunha *et al.* **[9]**. An increase in the C_{org}/N_T ratio is associated with changes in N concentrations due to organic matter mineralization **[17]** in which the transformation of nitrogen is dependent on the redox conditions of the sediments **[18]** or enhanced productivity **[15]**. As described in Methods, sediments are mostly reduced with limited dissolved oxygen (0-1 mg/L) in the overlying water. This condition should limit nitrification processes. Variations of stable isotopes of C and N in the sediments can help to elucidate these processes.

Regarding the C_{org}/N_T ratios at each site, an increased productivity in the wetland could occur at S1, S3 and S4 (2 - 5 cm depth) where the most important P concentrations were also detected and the C_{org}/N_T decreased. Two possibilities could explain the higher C_{org}/N_T ratios at S4 (0-1.5 cm): low mineralization of the organic matter or recent contributions from external sources (C_{org}/N_T ratios > 20). The vertical patterns of the C_{org}/N_T ratios from depths of 6-10 cm at S3 (11.3-20.4) and S4 (>20) suggest changes in productivity or the contribution of external sources. At S1, S2, S5, there is low variability in the C_{org}/N_T ratios, suggesting that the contribution of organic matter is from the same source (values varied from 15 to 19). In the lower part of the cores (>10 cm), there is a high variability in C_{org}/N_T (from 14.4 – 26) at S4 and this suggests a contribution of organic matter from terrestrial sources (C/N >20 for terrestrial sources; **[3]**, **[7]**); as indicated in Materials and Methods, this site is affected by the extraction of water for the agriculture.

The $\delta^{15}N$ or $\delta^{13}C$ against C_{org}/N_T in the sediments helps to distinguish between the aquatic or terrestrial origins of the organic matter **[4]**, **[9]**. The plots $\delta^{13}C$ or $\delta^{15}N$ vs. C_{org}/N_T (Fig. 3f-j) show that C and N contributions at S1 and S2 have the same origin, which is related to internal recycling of nutrients by the aquatic macrophytes; $\delta^{13}C$ or $\delta^{15}N$ indicate that the C and N sources remained the same. In contrast, from S3 onwards there is variability in the content and/or sources of N and organic C. At site S3, $\delta^{13}C$ is constant with depth, organic C and N_T increase in the recent sediments while $\delta^{15}N$ decreases. An external contribution with nutrients is possible at this site because it is close to a deforested area with intensive sugar cane agriculture and cattle breeding. In 1995 a drain was constructed near to this site and could disturb the sediments at the shoreline, suspending the particles and/or favoring the entrance of allochtonnous matter. These perturbations affect the productivity of the wetland ($\delta^{15}N$ decreased also the C_{org}/N_T ratio and C_{org} increased; 0-5 cm depth). Routh *et al.* **[15]** explained internal changes in productivity through C/N ratios, $\delta^{13}C$ and $\delta^{15}N$ in the sediments as follows: the C/N ratio remains constant, while the values of $\delta^{13}C$ and $\delta^{15}N$ vary during periods of increased productivity. Low productivity periods are characterized by relatively constant compositions of $\delta^{13}C$ and $\delta^{15}N$, and variable values of C/N. The latter could partially explain the productivity in the upper sediments at S3 due to the low variability of $\delta^{13}C$; however, this study considers that the decreasing content of $\delta^{15}N$ and marked increase in N_T concentration in the upper sediments are related to external sources.

Sites S4 and S5 show differences; both C and N isotopic signatures showed changes in the sediment cores. The C_{org}/N_T at S4 is not constant, while this ratio showed low variability with depth at S5. At these sites, it is more complex to explain the sources of the organic matter and the process affecting the productivity in this part of the wetland. The most significant findings are related to the marked decreases in concentrations of $\delta^{13}C$ and increases in $\delta^{15}N$ (0-10 cm), suggesting the entrance of substances enriched in ^{15}N (e.g., sewage, fertilizers, etc.) or changes in productivity. Increased mineralization of the organic matter due to the shallow conditions at these sites is possible since the organic C profiles show decreasing concentrations (0-10 cm). However, the reducing conditions in the sediments could prevent nitrification and thus N enrichment at these sites although N_T sees slight decreases (0-10 cm).

Variations in the $\delta^{15}N$ values have been used to explain paleoenvironmental reconstructions, even if the multiple processes involved in the N biogeochemical cycles complicate the interpretation **[19]**. Application of $\delta^{15}N$ values to distinguish organic matter sources is founded on the difference between the $^{15}N/^{14}N$ ratios of the inorganic nitrogen pools available to plants in water. Nitrate is the most common form of dissolved inorganic nitrogen (DIN) used by not-N_2 fixing algae, whereas land plants receive N_2 from the atmospheric N_2 fixers in soil **[7]**. Thus, $\delta^{15}N$ is related to the N source and helps to investigate the source of organic matter based on the biogeochemical process affecting the $\delta^{15}N$ records in the sediments. Increased concentrations of $\delta^{15}N$ at the surface compared to deeper layers of soils from undisturbed forest ecosystems are related to high nitrification rates, which under humid conditions correlate with loss of N. In contrast, low ^{15}N abundance indicates N limitation and a low nitrification rate **[20]**. As explained previously, nitrification is likely to be limited due to the reducing conditions of the sediments.

External nitrate loading from agricultural runoff and sewage as nitrate derived from human and animal wastes is enriched in ^{15}N. Denitrification in anoxic basins will considerably enrich the residual DIN in ^{15}N. Both will increase $\delta^{15}N$. An increase in abundance of N-fixing cyanobacteria, which directly fix atmospheric N_2 ($\delta^{15}N_{air}$ = 0 ‰), would decrease $\delta^{15}N$ in the organic matter **[21]**. The results observed at sites S4 and S5 are probably more related to the contribution of external sources enriched in ^{15}N or denitrification processes promoted by the reduced conditions in the sediments that lead to loss of N.

There is a low variability in $\delta^{13}C$ with depth at stations S1-S3, contrasting with S4 and S5 (**Figure 2**). The statistical analysis also revealed differences between sites (Table S1). Lower values were found in the sediments at S1-S3 (~ -28 ‰) and in the upper part of the core at S4 (~ -30 ‰), and the highest values were found at S4 (~ -26 ‰; 5-7 cm) and S5 (-23.3 ‰; 16 cm), suggesting differences in the organic matter contributions to the sediments between S1-S3 vs. S4-S5. The dominant vegetation in the wetland is comprised of the

C3 plants *Typha domingensis*, *Cladium sp*., and *Nymphaea sp*., and the $\delta^{13}C$ values of these are -24, -28 and -25.2 ‰ (root), respectively **[10]**, **[21]**. River phytoplankton has more negative values than aquatic plants (-35 ‰ to -25 ‰) **[9]**. The $\delta^{13}C$ values correspond to the preferential C source taken by aquatic plants and phytoplankton or the available source. For example, organic matter from phytoplankton and watershed C3 plants in lakes can be indistinguishable if they use an identically dissolved C source, but $\delta^{13}C$ is different if the organic matter sources are C4 plants **[3]**, **[7]**.

Based on the above and due to the lack of measured $\delta^{13}C$ in the organic matter sources possibly contributing to the sediments, in this study we compared our results with the values from the literature for the aquatic plants in the wetland and terrestrial plants. The values considered are $\delta^{13}C$ for aquatic C3 plants (-32 to -24 ‰) and for terrestrial C4 plants (-17.0 to -9.0 ‰) **[7]**, **[8]**; sewage ($\delta^{13}C$ -23(± 2.5) ‰) **[22]** and phytoplankton (-25 ‰ to -35 ‰) **[23]** to distinguish the main sources contributing to the organic matter deposited in the sediments in the wetland (**Figure 4**).

From this comparison, the values found in the sediments correspond to those reported for aquatic macrophytes (C3 plants). Sugarcane plants (C4; $\delta^{13}C$ -10.5 ‰, $\delta^{15}N$ 4.4 ‰) do not explain the organic matter sources in the sediment of the wetland, suggesting that the sugarcane agriculture may contribute through fertilizers entering the wetland due to land runoff ($\delta^{15}N$ of synthetic fertilizers -3 to 3 ‰) **[24]**; $\delta^{15}N$ is 5 ‰ for phytoplankton **[23]**. This is reasonable because the sugarcane production is processed far from the agricultural land. In addition, the results obtained were similar to the $\delta^{13}C$ values in sediment from wetlands in Florida **[11]**. However, other researchers attribute the origin of the organic matter in the sediment to the aquatic vegetation and suggest that changes in wetland hydrology and water chemistry affect the structure of the hydrophyte vegetation **[11]**.

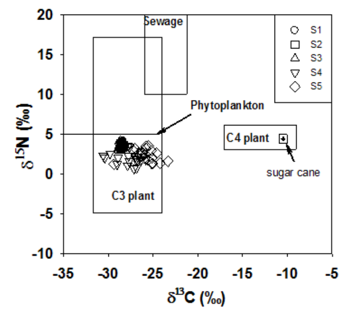

Figure 4: Stable Isotope values $\delta^{15}N$ vs $\delta^{13}C$ for samples from Ciénega Tamasopo at sites S1- S5, C3 and C4 plants, phytoplankton and sewage. Value ranges of $\delta^{15}N$ vs $\delta^{13}C$ from the literature are shown by the boxed areas.

5. Conclusions

The results of this study suggest that the spatial variability in the organic matter content is described mainly by the internal recycling of nutrients at S1-S3 with some impact from external sources (terrestrial plants, fertilizers from agriculture) that can affect the upper sediments at S3, S4 and S5 as can be deduced from the variability in $\delta^{13}C$ and $\delta^{15}N$. Anthropogenic perturbations cause changes in the contribution of the OM at S3 (0-5 cm) due to increased productivity, as shown by the $\delta^{15}N$, organic C and Nitrogen concentrations. Therefore, the main contributions of organic matter in the wetland come from the abundant vegetation covering 68 % of its area. The preservation of the wetland implies regulation of water extraction from the area, improvement of the irrigation systems, to consider an alternative crop to the sugar cane or re-evaluate the land use through provision of ecosystem services. Surely, such actions can affect the current socioeconomic organization of villagers and could complicate the preservation of the wetland.

Supplementary information

Table S1: P values obtained from the statistical analysis (Tukey test) obtained when comparing the upper part (0-5 cm), the middle part (6-10 cm) and the lower part (>10 cm) of the sediment cores for organic C, total N, $\delta^{13}C$ and $\delta^{15}N$ between sites.

SITE	Organic C (%)			Total N (%)			d13C			d15N		
	P value											
	0-5 cm	6-10 cm	>10 cm	0-5 cm	6-10 cm	>10 cm	0-5 cm	6-10 cm	>10 cm	0-5 cm	6-10 cm	>10 cm
S1 vs. S2	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
S1 vs. S3	< 0.001	> 0.05	> 0.05	< 0.01	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
S1 vs. S4	< 0.01	> 0.05	> 0.05	< 0.05	< 0.05	> 0.05	> 0.05	< 0.01	> 0.05	< 0.001	< 0.001	< 0.001
S1 vs. S5	< 0.01	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001	< 0.001
S2 vs. S3	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
S2 vs. S4	< 0.001	> 0.05	> 0.05	< 0.001	< 0.01	> 0.05	> 0.05	< 0.001	> 0.05	< 0.001	< 0.001	< 0.001
S2 vs. S5	< 0.001	> 0.05	> 0.05	< 0.01	> 0.05	> 0.05	< 0.001	< 0.001	< 0.01	< 0.05	< 0.001	< 0.001
S3 vs. S4	< 0.001	> 0.05	> 0.05	< 0.001	< 0.001	> 0.05	> 0.05	< 0.001	> 0.05	< 0.001	< 0.001	< 0.001
S3 vs. S5	< 0.001	> 0.05	> 0.05	< 0.001	< 0.05	> 0.05	< 0.001	< 0.001	< 0.01	< 0.05	< 0.001	< 0.001
S4 vs. S5	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	< 0.001	< 0.01	> 0.05	< 0.05	> 0.05	> 0.05

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