PRELIMINARY NOTES ON C-N POOLS IN SUBAQUEOUS SOILS FROM THE SACCA DI GORO COASTAL LAGOON (PO DELTA, NORTHERN ITALY)

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Abstract

A very hot debate is currently focused on submerged substrates that can be classified either as sediments or subaqueous soils. In this paper, the controversy is contextualized on a specific case study, *i.e.* the Sacca di Goro coastal lagoon facing the North Adriatic Sea. The submerged substrate has been sampled along a a E-W transect and subsequently analyzed to investigate the C-N elemental and isotopic compositions. The recorded concentration of organic carbon (OC) and nitrogen is 0.26-0.50 and 0.02-0.06 wt%, respectively, and the spatial distribution of these parameters highlights a general decrease of concentration from W to E. The carbon isotopic composition of the organic matter ($\delta^{13}C_{OC}$) indicates the incorporation within the substrate of significant amount of seaweed and seagrass. Seaweed prevails in the west ($\delta^{13}C_{OC}$ down to -19‰) due to a nutrient-rich freshwater inflow (Po di Volano), whereas seagrass is preponderant eastward ($\delta^{13}C_{OC}$ up to -10‰) in a sector of the lagoon fed by riverine waters characterized by lower nutrient load (Po di Goro). The existence of these biological components plausibly implies a lush benthic vegetation, properly rooted on the submerged floor. For this reason, in our view the substrate of the investigated lagoon can be regarded as a subaqueous soil.

Keywords: *subaqueous soils, coastal lagoon, Sacca di Goro, C-N isotopes*

Introduction

In the recent Workshop "Plant-soil-water interfaces in hydromorphic-subaqueous ecosystems" held in Imola (June 2015) a very stimulating debate has been focused on submerged substrates that could be classified either sediments (mainly from the geological point of view) or subaqueous soils (from pedological perspective according to Soil Taxonomy). Controversial points are the possibility to evaluate if the considered substrates support rooted plant growth, which in turn allow additions, losses and transformations of energy and matter, that finally result in the development of soil forming (pedogenic) horizons. In order to provide fresh insights for the ongoing discussion we selected a key study case from a coastal lagoon (Sacca di Goro) located in the Po river delta (Northern Italy), where the submerged substrates has been sampled along a W-E transect to evaluate the

carbon (C) and nitrogen (N) elemental and their isotopic distribution. These nutrients are proxies of biological activities that are fundamental to unravel the extent of which the sedimentary matrices were transformed in hydromorphic soils in subaqueous ecosystems. The carbon isotopic data are useful to identify the nature of the investigated matrices in terms of different amounts of natural inorganic ($\delta^{13}C \sim 0‰$) and organic ($\delta^{13}C$ -10 to -30‰) compounds (Ryan, 2014), as well as anthropogenic contributions (i.e. synthetic fertilizers) that can be better discriminated with the nitrogen isotopic composition ($\delta^{15}N \sim 0‰$; Owens, 1987; Ryan, 2014 and references therein). The knowledge of these parameters are extremely important also because the investigated lagoon is intensively exploited for bivalve shell-fish farming (mainly manila clam) and therefore give a snapshot of the nutrient capacity of the ecosystem.

General outlines on the Sacca di Goro coastal lagoon

Sacca di Goro (Fig. 1) is a shallow coastal lagoon located in the southern part of the Po river delta (44.78–44.83 N and 12.25–12.33 S) with a surface area of 26 km^2 , an average depth of about 1.5 m that is variable in relation to river and seawater inflows (Simeoni et al., 2000).

Figure 1
Perspective view of the Sacca di Goro lagoon, reporting the sampling sites aligned along an E-W profile.

The main fresh water input comes from the Po di Volano canal (about $3.5 \cdot 10^8$ m^3 y^{-1}) that flows directly into the lagoon and from the Po di Goro deltaic branch which inflow into the lagoon is artificially regulated. Additionally, three irrigation canals, named Giralda, Romanina and Canal Bianco, with similar flows ($2.0–5.5 \cdot 10^7$ m^3 y^{-1}), contribute to overall fresh water input. On the other hand, the Sacca di Goro is connected by two mouths to the Northern Adriatic Sea which variably inflow according to the tidal dynamics. The floor of the lagoon is flat and the sediments mainly include clay/silt in the northern and central zones, prevalent sand near the southern shoreline and sandy mud in the eastern area (Carafa et al., 2007). It has been assessed that, the nutrients inputs conveyed by the local fluvial system, which drains a watershed mainly dedicated to agriculture, leads to eutrophication

accompanied by blooms of seaweed and phytoplankton, in turn leading to summer anoxia episodes (Viaroli et al., 2001; Viaroli et al., 2006). The lagoon is one of the most important shellfish aquaculture systems in Italy and about 10 km^2 of the aquatic surface are exploited for the Manila clam (*Tapes philippinarum*) farms supporting an annual production between 8000 and 15000 t of clams (Carafa et al., 2007).

Materials and methods

Sample collection and preparation

Samples have been collected in May 2015 along a W-E longitudinal transect starting from Punta Volano to the main Po di Goro inflow south of Gorino village. 10 samples (2 to 3 kg) have been collected at approximately equal distance one to each other along this transect, dredging the floor of the lagoon down to approximately 5 cm depth. Samples have been placed in PTE bags to be transported to the Laboratories of the Department of Physics and Earth Sciences of the University of Ferrara, then dried in oven at T<60°C, separated by hand picking from shells and vegetation detritus and finally powdered in agate mill.

C-N elemental and isotopic analyses

The elemental and isotopic carbon composition of the different carbon and nitrogen pools have been carried out by the use of an Elementar Vario Micro Cube Elemental Analyzer in line with an ISOPRIME 100 Isotopic Ratio Mass Spectrometer operating in continuous-flow mode. The system allows variations of the combustion module temperature up to 1050°C; this theoretically permits extraction of different components having distinctive destabilization temperatures. The analysis of C, N are expressed in wt%, and $^{13}C/^{12}C$, $^{15}N/^{14}N$ isotopic ratios are notionally expressed as $\delta(‰)= (1000*[R_{sample}-R_{standard}]/ R_{standard})$ relative to the international isotope standards Pee Dee Belemnite (PDB) for carbon and AIR for nitrogen (Gonfiantini et al., 1995).

Powdered samples are introduced in tin capsules that are wrapped and weighed; these capsules, that allow loading up to 40 mg of sample, are subsequently introduced in the Vario Micro Cube autosampler to be analyzed. Flash combustion takes place in a sealed quartz tube filled with copper oxide grains (padded with corundum balls and quartz wool) which acts as catalyst, in excess of high purity (6 grade purity) O_2 gas. Freed gaseous species are transferred through a reduction quartz tube (at 550°C) filled with metallic copper wires that reduce the nitrogen oxides (NO_x) to N_2. The formed analyte gases (N_2, H_2O and CO_2), carried by dry He (5 grade purity) gas, pass through a water-trap filled with Sicapent™ ensuring complete removal of moisture, are sequentially separated by a temperature programmable desorption column (TPD) and quantitatively determined on a thermo-conductivity detector (TCD). Sample N_2 goes directly to the interfaced IRMS for isotopic composition determination, while the TPD column, kept at room temperatures 20–25 ° C., holds CO_2. When N_2 isotopic analysis is over, CO_2 is desorbed from the TPD column raising the temperature to 210°C, and finally

reaches the IRMS compartment for the determination of carbon isotopic ratios. The detection of the distinct isotopic masses of the sample are sandwiched between those of reference N_2 and CO_2 (5 grade purity) gases, which have been calibrated using a series of reference materials, in turn calibrated against IAEA international standards, such as the limestone JLs-1 (Kusaka and Nakano, 2014), the peach leaves NIST SRM1547 (Dutta et al., 2006), the Carrara Marble (calibrated at the Institute of Geoscience and Georesources of the National Council of Researches of Pisa), and the synthetic sulfanilamide provided by Isoprime Ltd. Mass peaks were recalculated as isotopic ratios by the Ion Vantage software package. SIAD Ltd. provided reference and carrier gases of certified purity.

The elemental precision estimated by repeated standard analyses, and accuracy estimated by the comparison between reference and measured values, were in the order of 5% of the absolute measured value. Uncertainties, increase for contents approaching the detection limit (0.001 wt %). Carbon and nitrogen isotope ratios are expressed in the standard (δ) notation in per mil (‰) relative to the international Vienna Pee Dee Belemnite (V-PDB) and atmospheric air (AIR) isotope standards (Gonfiantini et al., 1995). Isotopic values have been taken into consideration only for signals above 1.5nA based on standard reproducibility. The $\delta^{13}C$ and $\delta^{15}N$ values were characterized by an average standard deviation of ±0.1‰ and ±0.3‰, respectively as defined by repeated analyses of the above mentioned standards.

Discrimination of inorganic and organic pools

The elemental and isotopic compositions of the of Total Carbon (TC), Organic Carbon (OC), Inorganic Carbon (IC) and the associated nitrogen fractions, have been carried out slightly modifying the method described by Natali and Bianchini (2014 and 2015). According to this analytical protocol:
- TC (and TN) was carried out by EA-IRMS combusting at 950°C the bulk sample;
- OC was carried out by EA-IRMS combusting at 470°C the bulk sample;
- IC was carried out by difference between TC and OC and the relative isotopic composition has been calculated according to the mass balance equation:

$$\delta^{13}C_{TC} = \delta^{13}C_{OC}*X_{OC} + \delta^{13}C_{IC}*X_{IC} \qquad [1]$$

where $X_{OC}+X_{IC} = 1$ and represent the organic and inorganic fractions, respectively.

Results

The carbon concentration (TC) in the substrata of the Sacca di Goro varies from 1.64 to 2.69 wt%, whereas nitrogen (TN) ranges between 0.02 and 0.06 wt% (Fig. 2). The distribution of these parameters is higher in the western side (samples 10 and 1) and generally tend to decrease eastward, with the exception of sample 7 which shows a countertendency. The same parameters investigated in the organic fraction reveal that OC varies between 0.26 and 0.50 wt% and N ranges between 0.02 and 0.06 wt%, showing the same trend in the spatial distribution of the bulk samples. As emphasized in Fig. 3, the C/N ratio is distinctly higher in the bulk samples respect to the relative organic fractions.
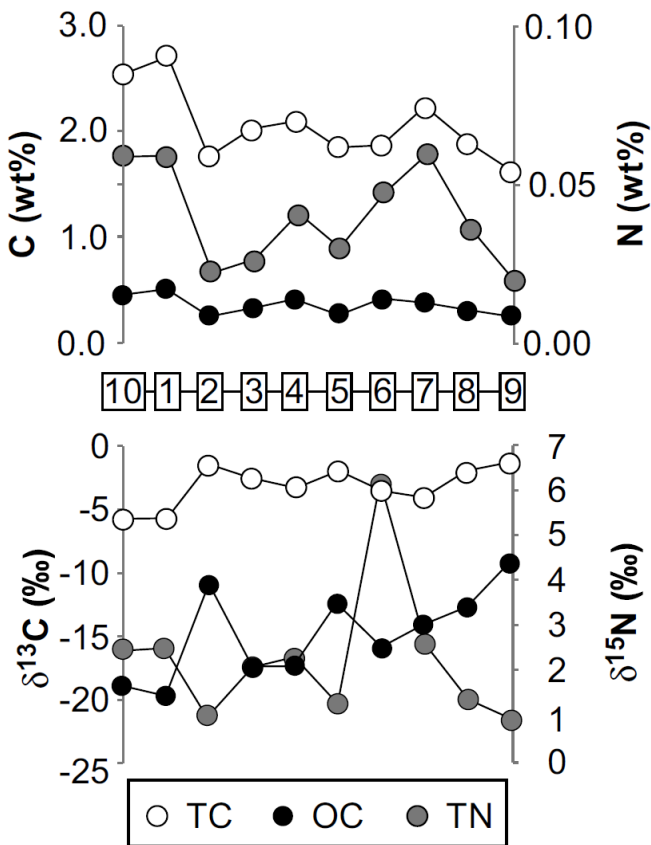
Figure 2
C-N elemental and isotopic variation along the transect depicted in Fig. 1.
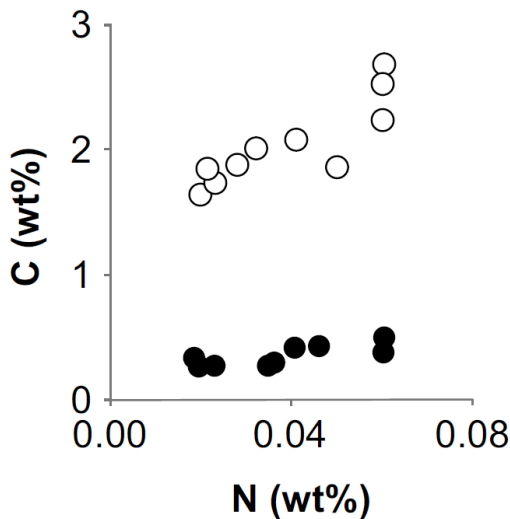
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Figure 3
C-N elemental distribution in the bulk (open circles) and in organic fraction (filled circles) of the Sacca di Goro substrates.

This means that a notable C content is included the inorganic compounds (i.e. carbonates), whereas N is exclusively associated to the organic matter. The bulk carbon isotopic composition ($\delta^{13}C_{TC}$) reflects the relative contributions of the distinct compounds to the total C budget, varying between -5.7 and -1.3‰, confirming the significant presence of carbonates in the investigated matrices. The organic fraction shows a much wider variation in the carbon isotopic composition ($\delta^{13}C_{OC}$), which ranges between -9.3 and -19.6‰ (Fig. 2 and 4).
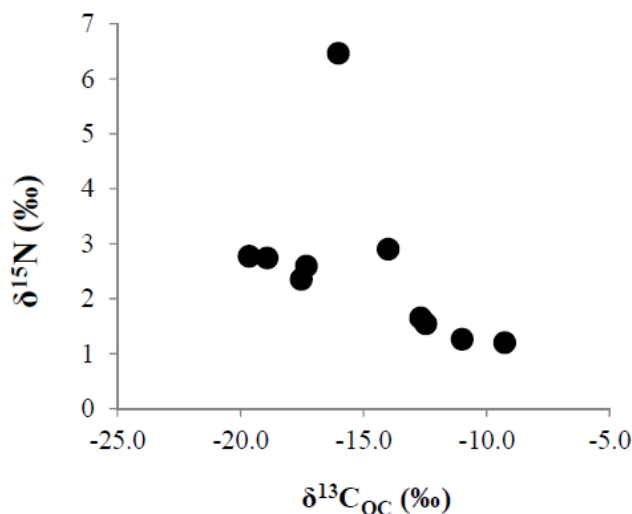

Figure 4
$\delta^{13}C_{OC}$ vs $\delta^{15}N$ (‰) of the Sacca di Goro substrates

The spatial distribution of $\delta^{13}C_{TC}$ shows a general W-E increase, i.e. a trend toward less negative values, which is also observed for $\delta^{13}C_{OC}$. The associated nitrogen isotopic composition ($\delta^{15}N$) varies from 0.9 to 2.6‰, with the exclusion of a single outlier (sample 6) having $\delta^{15}N$ = 6.2‰, generally showing a positive correlation with the elemental N content. Overall, the carbon data suggest that the investigated matrices cannot be simply explained as a mixing between two end-members (e.g. one organic and one inorganic component) because significant compositional variations are observed in the organic fraction, implying the presence of multiple biological compounds having distinctive isotopic compositions.

The nitrogen isotopic composition (average $\delta^{15}N$ 1.8‰) indicates that the nutrient load cannot be related only to a diffuse inflow of synthetic fertilizers from the neighboring croplands, but also reflects channel-driven wastewater effluents. Accordingly, the isotopic fingerprint of the investigated solid matrices is very similar to that from other wetlands stressed by intense anthropogenic activities (Bernot et al., 2009). However, it has to be noted that precise attribution of the nitrogen load to specific sources has to be taken with caution, since significant isotopic fractionations can be induced by trophic successive levels (DeNiro and

Epstein, 1981) and/or by denitrification processes in which ^{14}N enriched compounds volatilize preferentially (Swart et al., 2013 and references therein).

Discussion and Conclusions

The C-N elemental and isotopic composition of the substrates of Sacca di Goro on the whole shows a geochemical fingerprint that seems deeply influenced by the inflow of the Po di Volano distributary channel. This deltaic branch is typically characterized by waters having a notable content of nutrients (e.g. Total Dissolved Solids – TDS often higher than 1 g/l, NO_3^- up to 22.0 mg/l, HCO_3^- up to 230 mg/l), higher than that provided by Po di Goro. This is in agreement with the observed spatial trends that show higher N concentrations close to the inflow of Po di Volano, and a general progressive depletion in organic fraction from west to east. However, the nature of the organic fraction recorded in the substrates of the Sacca di Goro, highlighted by $\delta^{13}C_{OC}$ between -9.3 and -19.6‰ appears significantly different from that typically observed in the local riverine systems ($\delta^{13}C_{OC}$ from -19.7 to -28.2‰; Tesi et al., 2013; Corazzari et al., 2015) and in the related alluvial deposits ($\delta^{13}C_{OC}$ from -21.9 to –25.5‰; Natali and Bianchini, 2014; 2015). At the same time, the observed anomalous ^{13}C-enriched compositions of Sacca di Goro organic matter cannot be ascribed to a marine influence where the organic carbon is dominated by phytoplankton having $\delta^{13}C_{OC} < $ -20‰. Therefore, the data necessarily require a peculiar organic ^{13}C-rich end-member that variously influence (and sometimes dominate) the $\delta^{13}C_{OC}$ of the investigated matrices; this end-member could be represented by seagrass ($\delta^{13}C_{OC} \sim$ -10.0; Komorita et al., 2014) that notoriously proliferate in the Sacca di Goro. As suggested by Viaroli et al. (2008) seagrass represented the pristine algal community of the Sacca di Goro, which is progressively substituted by more opportunistic seaweed in relation to the increased pollution load. Accordingly, we found a carbon isotopic signal suggesting the prevalence of seaweed end-member ($\delta^{13}C_{OC}$ down to -19; Magni et al., 2008) in the sites close to the Po di Volano inflow (that is the principle nutrient carrier) and a progressive eastward shift toward isotopic values conforming to those typical of seagrass.

Considering the significant amount of these algal components included in the substrata, we suspect that these aquatic plants (at least in part) growth directly on the floor of the lagoon, in turn suggesting that the investigated matrices could be classified as subaqueous soils. Beyond the semantic debate (sediments or subaqueous soils?), the presented data are also useful for practical reasons because the Sacca di Goro is renowned for the shell (manila clams) production. In this light, we hope that the current research could be useful to individuate sectors of the lagoon suitable to concentrate the working activities.

Further developments are represented by the C-N elemental and isotopic analyses of the produced Manila clams which could be important to establish relationships between the quality of the substrata and the obtained bivalves, as well as to delineate a geochemical fingerprint of the local production useful for its

traceability, i.e. to provide proxies which indicate the provenance/origin and unmask frauds.

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NOTE PRELIMINARI SUI POOLS DI CARBONIO ED AZOTO NEI SUOLI SUBACQUEI DELLA SACCA DI GORO (PO DELTA, ITALIA SETTENTRIONALE)

Riassunto

Un acceso dibattito scientifico è attualmente in corso sulla classificazione dei substrati sommersi che possono essere denominati sedimenti o suoli subacquei. In questo articolo la problematica è contestualizzata ad un caso di studio specifico, localizzato nella laguna costiera della Sacca di Goro prospicente il settore settentrionale del Mare Adriatico. Il substrato sommerso è stato campionato lungo un transetto E-W al fine di investigarne la composizione elementare ed isotopica del carbonio e dell'azoto. Le concentrazioni di carbonio organico (OC) ed azoto variano da 0.26 a 0.50 e da 0.02 a 0.06 wt%, rispettivamente con una distribuzione spaziale caratterizzata da una generale diminuzione delle concentrazioni da W verso E. La composizione isotopica del carbonio della materia organica ($\delta^{13}C_{OC}$) indica l'inclusione di una significativa quantità di materiale derivato da praterie sottomarine e macroalghe. L'apporto di macroalghe sembra prevalere nella zona occidentale ($\delta^{13}C_{OC}$ tendente a -19‰) caratterizzata dall'influsso di acque dolci cariche di nutrienti (Po di Volano), mentre il componente derivato da prateria sottomarina appare preponderante nel settore orientale ($\delta^{13}C_{OC}$ tendente a -10‰) alimentato da acque dolci relativamente povere in nutrienti (Po di Goro). L'esistenza di questi componenti bio-geochimici suggerisce la presenza di una lussureggiante vegetazione bentonica radicata al substrato. Per tale motivo è nostra opinione che sia corretto classificare il substrato della laguna investigata come "suolo subacqueo".

Parole chiave: *suoli subacquei, laguna costiera, Sacca di Goro, isotopi C-N*