Effect of waterlogging on soil biochemical properties and organic matter quality in different salt marsh systems

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Chiara Ferronato[a], Sara Marinari[b], Ornella Francioso[a], Diana Bello[c], Carmen Trasar-Cepeda[c], Livia Vittori Antisari[a]

[a] Dipartimento di Scienze e Tecnologie Agro-Alimentari, Alma Mater Studiorum – Università di Bologna, Via Fanin 44, 40127 Bologna, Italy

[b] Dipartimento per l' Innovazione dei Sistemi Biologici, Agroalimentari e Forestali, Università della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy

[c] Departamento de Bioquímica del Suelo, Instituto de Investigaciones Agrobiológicas de Galicia-Consejo Superior de Investigaciones Científicas, Apartado 122, 15780 Santiago de Compostela, Spain

Abstract

This study investigated the effects of hydroperiod on soil organic matter quality in three different salt marshes in the Baiona lagoon (N Italy) representing terrestrial, intertidal and subaqueous ecosystems in the area. The study specifically aimed to gain some insight into how soil waterlogging (hydroperiod) affects the chemical and biological properties of soils as well as the quality and structure of the soil organic matter (SOM). Total contents of selected nutrients, total organic carbon and carbon stable isotope (δC^{13}) were measured in all soil profiles. The results of these analyses enabled us to define the different origin of the SOM by discriminating between terrestrial and aquatic SOM sources. The findings also show that accumulation of nutrients and SOM is significantly magnified in intertidal systems, in which pedoturbation effects induced by water movements are particularly strong. In addition, DRIFT spectra of humic acids revealed the changes in the main

functional groups in relation to increased waterlogging, highlighting the lower aromaticity and complexity in subaqueous soils (SASs), which is possibly due to the effect of the soil water saturation on the chemical and biological SOM transformation processes. Microbial biomass carbon (MBC), microbial quotient (Qmic) and the activities of some soil enzymes were measured to estimate soil metabolic activity in the systems and to evaluate how the microbial pool contributes to transforming the SOM. In all systems, the enzymatic activities were generally higher in subsurface horizons than in the surface horizon. This unexpected behaviour can be explained by the combined effect of water movement, erosion processes and preservation of SOM under anaerobic conditions. This study represents an attempt to investigate and understand the ongoing degradation processes in salt marsh ecosystems. The findings emphasize the strong influence of water flow and erosional processes associated with soil waterlogging on chemical and biological reactions in intertidal and subaqueous systems.

Keywords: Subaqueous soils; Soil organic matter transformations; Soil waterlogging; Enzyme activity; Salt marshes

1. Introduction

Salt marshes and wetlands represent some of the largest and most efficient C sink systems in the world (the so called "blue carbon"). It has been estimated that wetland systems hold 45-70% of all terrestrial C (Mitra et al., 2005; Morrissey et al., 2014) and that salt marshes can store about 87.2 ± 9.6 Tg C yr^{-1} in comparison with the 53 ± 9.6 Tg C yr^{-1} stored by the most efficient upland terrestrial system (e.g. tropical rainforest, Macreadie et al., 2013). Salt marsh ecosystems have thus been globally defined as high-priority zones because of their fragile structure and their important role in providing ecosystem services (Barbier et al., 2011; De Groot et al., 2012). As recently highlighted by

76 the secretariat of the Convention on Biological Diversity, habitat disturbance, erosion and climate
77 change are threatening the functions of salt marshes as C sinks (Secretariat of the Convention on
78 Biological Diversity, 2010). In order to prevent the complete degradation of these ecosystems and to
79 enable implementation of appropriate protection strategies, further information must be obtained
80 about the chemical and biological processes associated with water-soil-plant relationships.
81 Salt marshes are mainly dominated by halophyte vegetation, in which plant zonation is driven by
82 changes in soil morphology, which modify edaphic conditions, competitive mechanisms and thus
83 development of plant communities (Ferronato et al., 2018; Laegdsgaard, 2006; Silvestri et al., 2005).
84 Tidal oscillations, salinity and waterlogging period (hydroperiod) affect plant zonation and form soil
85 hydrosequences ranging from terrestrial to intertidal and subaqueous systems (Ferronato et al., 2018).
86 The distribution of plant communities changes rapidly along hydrosequences and depends on the
87 ability of the plants to tolerate submergence of their roots in salt water (Bertness et al., 1992;
88 Ferronato et al., 2018; Silvestri et al., 2005). The residues of halophyte plant species (mostly annual
89 herbaceous species) contribute greatly to the C sink function of salt marsh ecosystems. Fresh biomass
90 is deposited on the soil surface every year and the rate of degradation depends on the availability of
91 oxygen in soil, persistence of waterlogging and pedoclimatic conditions (Chmura et al., 2003; Mcleod
92 et al., 2011; Sharifi et al., 2013). The tidal oscillations continually supply nutrients and allochthonous
93 suspended mineral matter and organic C, thus contributing to the overall C input in salt marsh soils
94 (Kennedy et al., 2010; Mcleod et al., 2011). The C stable isotope signatures can be used to identify
95 the origin of organic matter, by discriminating terrestrial plant residues (e.g. C3 plants −29 to −22‰
96 $\delta^{13}C$) from riverine phytoplankton (−34 to −26‰ $\delta^{13}C$) and marine phytoplankton (−23 to −17‰
97 $\delta^{13}C$) (Bristow et al., 2013; Middelburg and Nieuwenhuize, 1998; Peterson and Fry, 1987). Bristow
98 et al. (2013) recognized the importance of the input of marsh plants and seagrasses such as *Spartina*
99 spp. and *Zostera* spp. on the adjacent salt marshes and mudflats, as well as the presence of microalgae-
100 associated C deposited along the banks, while Santín et al. (2009) highlighted the contribution of the

101 phytoplankton to organic matter (OM) accumulation in a highly exposed area under *Spartina* spp.
102 close to the main tidal channel.

103 Long-standing theory states that soil organic matter comprises a labile pool (e.g. microbial biomass
104 carbon) and a recalcitrant pool (e.g. humic substances) (Ghabbour and Davies, 2001; Rodríguez-
105 Murillo et al., 2017; Schnitzer and Monreal, 2011). In both of these pools, humic substances are
106 commonly defined as complex molecules characterized by the presence of a number of functional
107 oxygenated groups such as carboxyl groups (-COOH) and phenolic hydroxyl groups (Ph–OH), which
108 are involved in soil aggregation, water retention, cation and anion exchange and chelation of mineral
109 elements (Ghabbour and Davies, 2001) and can even act as plant biostimulants (Canellas et al., 2002;
110 Nardi et al., 2002). Many studies have used infrared spectroscopy to analyze the structure of humic
111 compounds, in order to evaluate and predict structural modifications of soil organic matter in space
112 and time (Agnelli et al., 2000; Chai et al., 2007; Liu et al., 2008).

113 There has been some debate about the existence of humic substances in recent years (Lehmann
114 and Kleber, 2015). Thus, soil organic matter formation has been described by these authors "as a
115 continuum of progressively decomposing organic compounds" characterized by a wide range of
116 substances derived from plant residues and particularly enriched in carboxylic acids (Trumbore,
117 1997). Despite the application of advanced spectro-microscopic techniques for *in situ* investigation
118 of soil organic matter, there is no direct evidence for the existence of humic substances in natural
119 soils. Nonetheless, the study of humic substances remains very popular because of the impact of these
120 substances in a wide variety of disciplines (e.g. agronomy, environmental sciences and economy) and
121 the availability of standard samples isolated from soil, peat, leonardite and river water (http://humic-
122 substances.org/). In the present study, we chose to maintain the conventional approach to humic and
123 fulvic acids, as recommended by International Humic Substances Society (IHSS), in order to enable
124 comparison of our findings with those of similar studies on soils from hydromorphic environments
125 (Filip et al., 1988; Fooken and Liebezeit, 2003; Santín et al., 2008).

Although numerous studies have investigated the nature of humic substances in terrestrial soils, very few have considered the chemical composition of these molecules in marsh and salt marsh sediments (Keller et al., 2009; Santín et al., 2008; Lu and Xu, 2014). In anaerobic microenvironments where oxygen availability is a limiting element, degradation of SOM may also be mediated by reduction reactions. Therefore, the low levels or lack of molecular oxygen in hydromorphic and waterlogged soils may trigger a sequence of changes in chemical properties of the soil that could influence the chemical composition and the structure of humic substances (Rodríguez-Murillo et al., 2011, 2017). Important information can thus probably be obtained about the processes that transform dead plant material into refractory organic C.

The chemical diversity of stable SOM depends on the availability of oxygen, which influences the microbial community and the efficiency of soil microorganisms in processing organic carbon (OC) inputs (e.g. by plant cover) (Caravaca et al., 2005; Gleixner et al., 2001). Soil enzymes participate in SOM degradation processes by catalysing the conversion of complex high-molecular weight compounds into smaller compounds, either by redox reactions (catalysed by oxidoreductases), or by the breakdown of complex compounds (catalysed by hydrolases) (Burns, 1982; Sarathchandra et al., 1984). Soil enzyme activity can therefore be used to quantify and monitor changes in soil metabolic activity and SOM dynamics in different ecosystems and in soils disturbed by anthropogenic activities (Trasar-Cepeda et al., 2008). In hydromorphic/subaqueous soils, the restrictive environmental conditions (e.g. low redox potential, soil waterlogging, halinity and sulphide concentration, etc.) can influence the microbial community as well as enzyme synthesis and activities (Arbona et al., 2008; Pulford and Tabatabai, 1988). Soil salinity and waterlogging act as soil dispersant agents, inducing changes in ionic strength and in the molecular stability and sorption of enzymes (Morrissey et al., 2014; Rietl et al., 2016), thereby causing changes in the eco-physiological functions of the microbial community. The pathway of organic matter degradation and the end-products of the microbial metabolism may thus also be affected by both water quality and fluctuations (e.g. tidal oscillations).

These changes can be investigated by measuring the enzyme activities associated with biogeochemical cycles (Bello et al., 2015; Burns et al., 2013; Kandeler et al., 1996; Nannipieri, 1994).

The aim of the present study was to evaluate changes in SOM and enzyme activities in relation to the soil hydroperiod in three salt marshes in the Baiona Lagoon (Ravenna, North Italy). Soil hydrosequences consisting of terrestrial, intertidal and subaqueous ecosystems have previously been identified in this area, in a study focusing on the relationships between soil hydroperiod, soil physicochemical properties and vegetation pattern (Ferronato et al., 2018). In the present study, the same soils were used to investigate stable and labile carbon pools and also enzyme activities throughout the soil profiles, in order to gain some insight into SOM chemical and biological transformation processes in salt marshes.

2. Materials and methods

2.1. Study area and sampling survey

The Baiona lagoon (SPA/SAC IT4070004 of the Natura 2000 ecological network) is located in the S. Vitale Regional Park, south of the Po Delta on the northern coast of the Adriatic Sea (N Italy). The area is characterized by retrogradational back-barriers, shoreline and offshore transitional Holocene deposits (Buscaroli et al., 2011; Migani et al., 2015; Veggiani, 1974).

Soil profiles were collected in three different salt marshes in the lagoon, according to the terrestrial, intertidal and subaqueous areas defined by Ferronato et al. (2018). In particular, 5 terrestrial (TES), 4 intertidal (ITS) and 3 subaqueous (SAS) soil profiles were selected for this study (Figure 1). The soil profiles (0-100 cm depth) were collected using a beaker vibracore sampler equipped with a polyethylene core tube (diameter 6 cm). The genetic horizons were divided and described according to Schoeneberger et al. (2012) and McVey et al. (2012). Subsamples of soil were then prepared in

different ways for subsequent analysis: a) air-dried and sieved at 2 mm, for physical and chemical characterization; b) freeze-dried, for SOM fractionation and diffuse reflectance infrared Fourier transform (DRIFT) analysis; and c) stored at 4 °C, for the analysis of microbial biomass carbon and enzyme activities.

All soil profiles consisted of A-AC-C horizons (Table S1) and were classified according to the US Soil Taxonomy as *Typic psammaquent* to *endaquent* in the TES and ITS systems, and as *Fluventic psammowasssent* to *Typic fluviwassent* in the SAS system (Soil Survey Staff, 2010). All soil horizons (A, AC and C) were analysed separately and the average values for each genetic horizon were subsequently obtained for the TES, ITS and SAS systems.

2.2. Soil physical and chemical analysis

Soil particle size distribution was determined with the pipette method according to Gee and Bauder (1986). Soil pH (pHmeter, Crison, Spain) and electrical conductivity (EC; conductimeter Orion, Germany) were measured in a 1:2.5 (w:v) suspension of soil and deionized water, while total lime ($CaCO_3$) was quantified by the volumetric method (Loeppert and Suárez, 1996). The total Ca, Na, P and S contents were determined in finely ground soil samples by microwave-assisted acid digestion (Milestone, 1200) with aqua regia (HCl and HNO_3 suprapure, Carlo Erba, Italy, 3:1 v:v) and Inductive Coupled Plasma-Optic Emission Spectroscopy (ICP-OES, Ametek, Germany). The spectrometer was calibrated using international multi element standard (CPI 4400-12627-WG02), and a standard reference material (BCR 320R) was used to check the accuracy of the instrument (< 10%).

Soil total organic carbon (TOC) and total nitrogen (TN) contents were determined by Continuous Flow–Isotope Ratio Mass Spectrometry (CF–IRMS, Delta Plus Thermo Scientific) after pre-treatment of samples with 6 M HCl at 80 °C to eliminate carbonates.

The ^{13}C abundance was expressed in delta units ($\delta^{13}C‰$):

$$\delta^{13}C‰ = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where, R_{sample} is the isotope ratio $^{13}C/^{12}C$ of the sample and $R_{standard}$ is the $^{13}C/^{12}C$ ratio of the Pee Dee Belemnite carbonate standard (PDB) (Middelburg and Nieuwenhuize, 1998)

All analyses were performed in duplicate for each soil sample and the results shown are the mean values ± the standard error for the soil samples analysed for each of the soil systems.

2.3. SOM fractionation and DRIFT spectroscopy

Humic acid (HA) extraction was performed according to De Nobili et al. (2008). Samples were sequentially extracted at room temperature for 1 h under N2 flux (1:10 w:v). The HAs were first extracted with 0.5 M NaOH, to yield the fraction considered to consist of labile humic acids (free HA, HAF) consisting of both polysaccharides and humic acids, and then with 0.1 M NaOH + 0.1 M Na4P2O7, to yield the fraction considered to consist of humic acids bound to the soil mineral fraction (bound HA, HAB) via cationic bridges or strongly complexed by polycations (Parsons, 1988). The extracts were precipitated overnight at pH 1.5 with 6 M HCl, and then centrifuged to separate the compounds insoluble at pH 1.5 (humic acids, HA) from those soluble at this pH (fulvic acids and non-humic substances, FA). The HA fractions were then re-suspended in distilled water and freeze-dried.

The humic acids (HAF and HAB) were analysed by DRIFT spectroscopy. Spectra were recorded using a Bruker TENSOR series FT-IR Spectrophotometer (Bruker, Ettlingen, Germany) interfaced with a diffuse reflectance apparatus (Spectra-Tech. Inc., Stamford, CT, USA). HA samples were mixed with KBr powder (1:50 w:w) (Aldrich Chemical Co. Milwaukee, WI, USA), which was also used as a background reference. Spectra were recorded as Kubelk-Munk units, ranging from 4000 to 400 cm^{-1} and averaged over 100 scans with 4 cm^{-1} resolution and manipulated with OriginLab 7.0 software (USA). The percentage area under each band was calculated by curve-fitting analysis, implemented with Grams/386 spectroscopic software (version 6.00, Galactic Industries Corporation, Salem), as described by Mastrolonardo et al. (2015).

227 The main peaks and bands in the spectra were interpreted according to assignments used by
228 different authors (Fooken and Liebezeit, 2003; Mecozzi and Pierantonio, 2006; Stevenson, 1994) and
229 are shown in Table 1.
230
231 *2.4. Analysis of microbial biomass carbon and enzyme activities*
232
233 Soil microbial biomass C (MBC) was determined on previously conditioned (3 days at 25 °C) wet
234 subsamples of soil, by the chloroform fumigation-extraction method (Vance et al., 1987). This
235 method uses chloroform to kill soil microorganisms, before the soil C content is extracted with 0.5 M
236 K_4SO_4. The difference in the C content extracted from fumigated and unfumigated soil samples
237 (measured in a L-TOC analyser, Hypertoc Shimadzu) corresponds to the C released by the dead
238 microorganisms (C flush). This value was then used to estimate the microbial biomass C (expressed
239 in mg kg^{-1} of dry soil) by applying a transformation factor (Kc) of 0.38 (Joergensen and Brookes,
240 1990). Microbial biomass C was determined in triplicate for each soil sample, and the results reported
241 are the means ± standard error for the soil samples analysed for each of the soil systems. The microbial
242 quotient (Qmic) was calculated as the percentage of soil MBC relative to the TOC, as reported by
243 Anderson and Domsch (1989). This index was calculated as an indicator of the changes in organic
244 matter due to soil alteration processes and the availability of nutrients to soil microbiota (Sparling,
245 1997).
246 Different enzyme assays were performed in triplicate on fresh soil samples by using the traditional
247 methods described below. The results (mean ± standard error of the results for the soil samples
248 analysed for each of the soil systems) are reported as absolute values (µmol of released/consumed
249 compound per gram of oven-dried [105 ºC] soil per hour) and as specific activity, i.e. enzyme activity
250 per unit of soil organic carbon (Trasar-Cepeda et al., 2008), which enables comparison of different
251 types of soils under different soil use, and therefore with different organic matter contents (Barriuso
252 et al., 1988). Urease (URE) activity in the samples was determined using 1065.6 mM urea as

253 substrate, incubation for 1.5 h at 37 ºC and pH 7.0 (0.2 M phosphate buffer) and measurement of the
254 NH_4^+ released with an ammonia electrode (Nannipieri et al., 1980). Alkaline phosphomonoesterase
255 activity (ALK) was determined using 16 mM *p*-nitrophenyl phosphate as substrate, incubation for 0.5
256 h at 37 ºC with Modified Universal Buffer (MUB) pH 11.0 and spectrophotometric measurement (at
257 400 nm) of the *p*-nitrophenol (PNP) released (Tabatabai and Bremner, 1969). ß-glucosidase (GLU)
258 activity was determined using *p*-nitrophenyl-ß-glucopyranoside 25 mM as substrate, incubation for 1
259 h at 37 ºC with MUB pH 6.5 and measurement (at 400 nm) of the *p*-nitrophenol released (Eivazi and
260 Tabatabai, 1988). Arylsulphatase activity (ARYL) was determined using 5 mM *p*-nitrophenyl
261 sulphate as substrate, incubation at pH 5.8 (acetate buffer 0.5 M) and 37 ºC for 1 h (Tabatabai and
262 Bremner, 1970). Invertase activity (INV) was determined using 35.06 mM saccharose as substrate,
263 incubation for 3 h at 50 ºC and pH 5.5 (acetate buffer 2 M) and measurement of reducing sugars by
264 the Prussian-blue method (Schinner and Mersi, 1990). Catalase activity (CAT) was determined using
265 8.8 mM H_2O_2 as substrate, incubation of the soil samples for 10 min at 20 ºC and measurement of the
266 residual H_2O_2 at 505 nm after colorimetric reaction, and the results are expressed as mmol H_2O_2
267 consumed g^{-1} h^{-1} (Trasar-Cepeda et al., 1999). In order to obtain an indicator of the enzymatic activity
268 involved in C, N and P cycles, the Synthetic Enzyme Index (SEI) was calculated as the sum of three
269 activities measured using fluorogenic methylumbelliferyl (MUF)-based substrates. β-glucosidase
270 (GLU), chitinase (CHIT) and acid-phosphomonoesterase (ACP) activities were measured using MUF
271 substrates according to the method of Marx et al. (2001). The fluorogenic substrates, prepared with
272 acetate buffer 0.5 M pH 5.5, were 4-MUF-β-D-glucoside 4-MUF-N-acetyl-β-glucosaminide and 4-
273 MUF-phosphate. Fluorescence (excitation 360 nm, emission 450 nm) was measured in an automatic
274 fluorimetric plate-reader (Fluoroskan Ascent) and readings were taken after incubation of plates for
275 0, 30, 60, 120 and 180 minutes at 30 °C. The results are expressed as nmol of MUF released per g of
276 oven-dried soil per hour.
277
278 *2.5. Statistical analysis*

279

280 Statistical analysis of the data was performed using R free software (R Core Team, 2015), with
281 the Kruskal function of the *"agricolae"* package. As the basic assumptions required for the ANOVA
282 test were not met (i.e. normal data distribution and homogeneity of variance), the data were analysed
283 by one-way nonparametric ANOVA or the Kruskal-Wallis test (Hollander et al., 2013). The latter is
284 a non-parametric method for testing a nominal variable and a continuous (or ordinal) variable by
285 ranking the observations. It tests whether the mean ranks are the same in all the groups, namely if the
286 samples originate from the same distribution. The Kruskal-Wallis test was applied to distinct *a priori*
287 groups formed by the three soil systems (TES, ITS and SAS) and the three different horizons
288 considered (A, AC and C). Holm-Bonferroni correction was applied for multiple comparisons.
289

290 **3. Results**

291

292 *3.1. Physical and chemical properties of salt marsh soils*

293

294 The mean values of the main physical and chemical properties of the diverse horizons in the TES,
295 ITS and SAS soil profiles are shown in Table 2, while Table 3 shows the results of the Kruskal-Wallis
296 test to highlight the significant differences between the three ecosystems (TES, ITS and SAS).
297 The main differences between the physicochemical characteristics of the three different salt marsh
298 systems were observed in the A horizon, in which the pH, EC, organic C and nutrients varied
299 significantly (Table 2). Generally, in all soil pedosequences, the EC decreased sharply from A to AC
300 and C horizons, and the same was observed for clay percentage and the concentrations of
301 macronutrient elements (e.g. TOC, TN, P and S), while Ca and $CaCO_3$ contents increased in the AC
302 and C horizons (Table 2).
303 Significant differences between the TES, ITS and SAS systems mainly involved the A horizon
304 (Table 3). In the A horizon of the ITS system, the pH was significantly lower than in the A horizons

305 of the other soil systems (Table 3). Moreover, the EC values were significantly higher in all horizons
306 of the ITS system than in all horizons of the TES and SAS systems (Table 3). Similarly, the TOC and
307 P contents were significantly higher in the ITS than in the TES and SAS systems. The Na and S
308 contents increased significantly from the TES to the ITS and SAS system, while there was no
309 significant difference between ITS and SAS systems (Table 3). The $\delta^{13}C$ signature revealed
310 significant enrichment of the lighter ^{13}C isotope in the surface horizon of TES relative to the same
311 horizon in SAS. Moreover, in the TES and ITS systems, the $\delta^{13}C$ ratio increased from -25.9 and -25.1
312 to -23.9 and -24.8‰, respectively, throughout the soil profile, while in the SAS system the opposite
313 trend was noted, as the $\delta^{13}C$ ratio decreased from -23.8 to -25.3‰, although these differences were
314 not statistically significant (Table 3).
315
316 *3.2. SOM fractionation and DRIFT spectra*
317
318 Humic acids were only extracted from the A horizon because the organic carbon content of the
319 AC and C horizons was too low to obtain accurate results. The DRIFT spectra of both free and bound
320 humic acids (HAF and HAB, respectively) extracted from the A horizon exhibited some
321 modifications along the hydrosequence in both the aliphatic and the aromatic regions (Figure 2).
322 Quantitative information was obtained from the FTIR spectra by Gaussian curve fitting procedure
323 applied to the region considered. The percentage area for each functional group is representative of
324 the structure of humic substances (Figure 3). In general, the peaks in the spectra of the TES system
325 were more variable than those in the spectra of the ITS and SAS systems.
326 In both free and bound HA (HAF and HAB, Figure 3a and 3b, respectively), the percentage area
327 for aliphatic chains (2954-2952 cm^{-1}) was relatively low in the TES and ITS soils, and was higher in
328 the SAS soils (Figure 3a and b).
329 The peak at around 1729-1720 cm^{-1}, attributed to C=O carbonyl stretching in acids or ketones, was
330 not present in HAF and HAB of the TES soils, while it accounted for 5 and 7 % of the area in the ITS

331 and SAS soils, respectively. The peak at around 1670-1653 cm^{-1} was mainly due to Amide I vibration
332 of proteinaceous material. This band was present in all samples, but it was particularly evident in SAS
333 soils (accounting for 22-24% of the area). Conversely, the peak at 1592-1580 cm^{-1}, assigned to
334 aromatic rings and asymmetric stretching of carboxylate, only appeared in the TES soil and accounted
335 for 19% of the area in HAF (Figure 3).
336 On the other hand, lignin derivatives (indicated by peaks at 1547-1515 cm^{-1}) increased according
337 to the level of soil waterlogging (TES < ITS < SAS). The 1223-1211 cm^{-1} band, attributed to phenol
338 compounds, was only observed in the ITS soils, and accounted for respectively 11.9 and 6.5% of the
339 area in HAF and HAB.
340
341 *3.3. Microbial biomass and enzyme activities*
342
343 To evaluate the soil microbial community and its activity, microbial biomass C (MBC) content,
344 the metabolic quotient (Qmic) and a synthetic enzyme index (SEI) were determined in all soil profiles
345 (Figure 4 a-c). Enzyme activities and specific enzyme activities were also determined (Table 4 and
346 Figure 5a-f, respectively). The results of the statistical analysis are shown in Table 5.
347 The soil biochemical properties and indexes generally varied widely between the different soil
348 systems (Fig. 4a-c). As found for the soil physical and chemical properties, the most significant
349 differences between the TES, ITS and SAS systems involved the A horizon (Table 5). The MBC
350 content (Figure 4a) was higher in the A horizon of both the TES and ITS than in that of the SAS soil
351 profiles and was much lower in the AC and C horizons. Moreover, in the A horizon, Qmic decreased
352 significantly with increasing hydroperiod (TES>ITS>SAS, Figure 4b). The values of this index were
353 similar in all horizons of the TES soil profiles, but increased throughout the ITS and SAS soil profiles.
354 By contrast, the differences in the SEI between the different horizons and systems were generally not
355 significant (Figure 4c), as a result of the high variability in the data. However, some trends were
356 noted. Thus, for the A horizon, the mean SEI values were lower in the SAS than in TES and ITS soil

357 profiles. Moreover, in the SAS soils, the SEI increased gradually with depth of the soil profile, while
358 in the TES and ITS soils the opposite pattern was observed (Figure 3c). In the AC and C horizons,
359 the MBC and Qmic values were generally lower in the SAS than in the TES and ITS soils, although
360 the differences were statistically significant only for MBC (Table 5). The values of SEI in the AC
361 and C horizons were similar in all three hydrosequences.

362 The values of the enzyme activities are shown in Table 4, and the results of the statistical analysis
363 of the data are reported in Table 5. No significant differences in the enzyme activities were observed
364 between the TES, ITS and SAS systems for the different horizons, with exception of ALK and INV
365 in the A horizons and URE activity in the C horizons. These enzyme activities were significantly
366 lower in the SAS than in TES and ITS soils (Table 5).

367 The enzyme activity generally decreased with depth in the soil profile in the three systems,
368 although the differences were not always statistically significant (data not shown). The CAT, URE,
369 INV and ALK activities were significantly higher in the A horizon than in the deeper horizons of the
370 TES soils. The same trend was observed for CAT, INV and ALK activities in the ITS soil and for
371 CAT and INV in the SAS soils.

372 Generally, the specific enzyme activity, which allows comparison of the enzyme activity between
373 soils with different organic matter content, increased throughout the soil profiles. Notably, in all
374 horizons (A, AC and C), the CAT, URE, GLU and ARYL (Figure 5a, 5b, 5e and 5f) enzyme activities
375 were lower in the ITS soils than in the other soils. Considering the A horizon, CAT/C was lower in
376 the ITS system than in the TES and SAS soils (Figure 5a), although the differences were only
377 statistically significant for the comparison between the ITS and SAS systems (Table 5). Furthermore,
378 INV/C was significantly lower in the SAS than in the TES and ITS (Figure 5d), while GLU/C was
379 significantly higher in the TES system than in the other two systems (Figure 5e).

380 Regarding the AC horizon, CAT/C was significantly lower in the ITS system than both in the TES
381 and SAS systems (Figure 5a), while GLU/C was significantly lower both in the TES and ITS systems
382 than in the SAS system (Figure 5e). This specific activity therefore increased with the intensity of

383 waterlogging. Considering the C horizon, URE/C, ARYL/C, GLU/C and INV/C were lower in the
384 ITS than in the TES and SAS systems (Figure 5b, 5f, 5e and 5d), and the differences were generally
385 statistically significant, while ALK/C and CAT/C (Figure 5c and 5a) were significantly higher in the
386 SAS system than in the TES and ITS systems, in which the activities were similar (Table 5).
387
388 **4. Discussion**
389
390 The soils in the Baiona lagoon area are developed on carbonate sandy substrate, resulting in C
391 horizons with relatively high carbonate content and sandy loam (TES) or sandy clay loam texture
392 (ITS and SAS), in concordance with previous findings (Amorosi et al., 2005; Ferronato et al., 2014;
393 Giambastiani, 2007). The main differences between the three salt marsh systems regarding their
394 organic matter and nutrients content and EC were observed in the A horizon, while the differences
395 between the AC and C horizon of the three salt marsh soils were less pronounced.
396 The $\delta\ ^{13}C$ signature indicates the different nature of organic C in the soils considered. In the TES
397 and ITS, the soil surface is enriched by lighter C isotopes ($\delta\ ^{13}C$= -25‰ c.a), which are generally
398 associated with organic material derived from terrestrial vascular plants (Bristow et al., 2013). In the
399 deeper horizons, the decrease in C isotopic ratio indicates buried organic C forms and marine plankton
400 material (average value of $\delta\ ^{13}C$= -23‰ c.a) (Dickens et al. 2004; Santín et al. 2009). Conversely, the
401 SAS profiles showed the opposite trend, highlighting the presence of C derived from marine plankton
402 on the soil surface and the presence of vascular plants in the deeper layer, probably associated with
403 the salt marsh erosion and subsidence phenomena characteristic of the study area (Ferronato et al,
404 2016; Teatini et al., 2005).
405 The difference between the terrestrial and aquatic soil organic matter was determined by analysis
406 of humic substances at the soil surface. Transformation of plant debris is known to be driven by
407 oxygen availability, and in terrestrial systems the organic molecules extracted with alkaline solution

(IHSS, 2015) are typically rich in aromatic compounds and poor in aliphatic ligands (Filip and Alberts, 1994). The structure of humic substances is more complex in terrestrial than in intertidal and subaqueous systems, where degradation of SOM may be mediated by reduction reactions, resulting in humic substances with more aromatic compounds and a higher degree of polymerization than in terrestrial systems (Bronick and Lal, 2005; De Nobili et al., 2008). As the soil waterlogging increased (from ITS to SAS systems), the structure of humic substances became more enriched in aliphatic compounds, and the differences between free HA structure and those bound to the soil mineral fraction (i.e. via cationic bridges or via complexation) became less evident (Figure 2 and 3). Enrichment of aliphatic compounds was mainly observed in the HA extracts from the SAS and can be attributed to the effect of the water movement on the soil surface. Sea water acts as a powerful dispersant of soil aggregates, directly breaking them up and contributing to repulsive charges that disperse clay particles (Bronick and Lal, 2005). The capacity of the soil to retain organic colloids, clay particles and other cementing agents is thus reduced, resulting in enhanced soil fluidity (Surabian, 2007). The shift in some characteristic bands observed in the HA extracts from the SAS profiles (Figure 2) is consistent with the findings of Filip et al. (1988) and Fooken and Liebezeit (2003), who reported that marine humic acids are distinguished by similar bands, mainly attributed to amide linkages of proteins (Ertel and Hedges, 1983). These authors also observed that as the soil hydroperiod increases there is usually an increase in the presence of bands at 1665-1660 cm^{-1}, and at 1725 cm^{-1}. These bands typically increase in the direction fresh plants < dead plants < mud. As expected, the bands at around 1547-1515 cm^{-1}, corresponding to the most characteristic aromatic skeletal vibrations of lignin, increased with the level of soil waterlogging. The persistence of lignin derivatives is probably due to the low level of oxygen, which may decrease the rate of decomposition (Filip et al., 1988). We can conclude that the structure of the organic molecules shifted toward a low complexity along the soil hydrosequences as a consequence of the anaerobic conditions. Nevertheless, we can infer that the water movement (both along the soil hydrosequence and

433 throughout the soil profiles) can produce additional perturbation such as the transport and
434 accumulation of nutrients in different parts of saltmarsh soils.

435 The TOC and MBC contents were lowest in the SAS profiles, while both Qmic and SEI increased
436 significantly with depth of the soil profile, indicating enhancement of biochemical activity in deep
437 horizons. This observation may be related to some pedoturbation due to water movements at the soil
438 surface. Movement of water at the soil surface washes and greatly depletes the soil (Allen, 2000),
439 while in the deeper horizons, water movement is limited and therefore may cause less intense
440 disturbance. This could somehow induce a new equilibrium of microbial communities in AC and C
441 horizons, and a different C metabolic pathways. However, there is another possible explanation for
442 the biochemical characteristics of the SAS. The presence of terrestrial-like C forms in deep SAS
443 horizons (indicated by the isotopic analysis) may be due to past erosion processes, e.g. detachment
444 and deposition of clumps of saltmarsh soils on the sea floor and transportation of fine terrestrial
445 material by water during tidal cycles. The enhanced enzymatic activities detected in the deeper
446 horizons may therefore be associated with past accumulation of upland terrestrial soil material
447 transported by erosion processes. Nevertheless, under altered environmental conditions, the
448 biochemical activity may reach a new equilibrium with different effects in relation to function. This
449 hypothesis suggests that the enhanced enzymatic activity observed in the SAS profiles will be related
450 to the quantity or availability of SOM and also to the type of SOM present in the organo-mineral
451 complex, as under anoxic conditions, the SOM is preserved from degradation and retains immobilized
452 enzymes. The specific enzyme activities (i.e. the activities expressed relative to organic C mass) are
453 often calculated in order to decouple the changes in soil enzyme activities from the changes in organic
454 matter content (Marinari et al., 2012; Trasar-Cepeda et al., 1999; 2008; Vittori Antisari et al., 2011)
455 and to enable comparison of soils under different types of use or subjected to diverse types of
456 disturbance. In terrestrial soils, the activity of hydrolytic enzymes usually decreases with depth
457 (Harrison, 1983; Kuprevich and Shcherbakova, 1971; Trasar-Cepeda and Gil-Sotres, 1987), while
458 the specific enzyme activity generally increases as a result of the lower organic C content in deeper

459 horizons where enzyme activity remains high (Marinari and Vittori Antisari, 2010). This enrichment
460 may represent an ecological mechanism for retaining soil metabolic activity or making organic matter
461 more available to degradation by enzymatic reactions (Boerner et al., 2000; Burns, 1982; Trasar-
462 Cepeda et al., 2008). In this study, the specific enzyme activities generally increased with soil profile
463 depth in all systems (TES, ITS and SAS) and were particularly high in the SAS system (Figure 5).
464 The high specific activity in these soil profiles was particularly noteworthy, as the biochemical
465 activity was expected to be lower in the submerged than in the terrestrial soils. However, as previously
466 hypothesized, the specific enzyme activity in deep horizons of the SAS soils may be due to different
467 types of pedoturbation operated by the combined effects of water movement and soil organisms on
468 the surface horizons, and to the presence of allochthonous terrestrial SOM in deeper horizons. The
469 organic matter derived in the past from terrestrial systems and accumulated in the lagoon sediments
470 may be enriched in immobilized enzymes, which would remain active in AC and C horizons (Burns,
471 1982; Nannipieri, 1994; Nannipieri et al., 1980). Given the lack of reference studies and the
472 complexity of enzyme variability under such environmental conditions, further research is required
473 to confirm this hypothesis.

474 The key focus area between the terrestrial and aquatic environment is the intertidal area, which
475 represents the real transitional system. In this part of the salt marsh hydrosequence, the accumulation
476 of C residues and the occurrence of alternating aerobic/anaerobic soil conditions enable growth of
477 terrestrial plants and mineralization of SOM, as in TES systems. However, the HAs in the ITS soils
478 are relatively poor in aromatic compounds and enriched in phenols and aliphatic compounds
479 (highlighting the lower degree of polymerization than in the TES soils). As in aquatic-like
480 environments, the biochemical and biological processes associated with SOM degradation in the ITS
481 soils are strongly limited by the effect of continuous and provisional waterlogging (Chendrayan et
482 al., 1980). The continuous wet and dry cycles associated with the tidal oscillations contribute greatly
483 to phenomena such as transportation of particulate and soluble organic matter (Fagherazzi et al.,
484 2013) and accumulation of nutrients and marine salts (Ferronato et al., 2016). Accumulation of high

amounts of nutrients and organic C content, as well as frequency of flooding and length of waterlogging period, affected both microbial communities and the production and activity of soil hydrolytic enzymes (Geng et al., 2017; Kang and Stanley, 2005), which often decreases in ITS soil horizons. Moreover, the alternation of aerobic/anaerobic conditions due to soil water saturation will affect the metabolic and biochemical pathway of SOM degradation in subaqueous systems, which may therefore differ from those in terrestrial systems, so that a biochemical equilibrium may not be reached.

5. Conclusions

Soil hydroperiod plays a fundamental role in the degradation of organic matter, inducing a decrease in the oxygen level in soil and thus inhibiting the overall oxidative processes. Water movement also acts as an important pedoturbation agent inducing profound changes in soil physico-chemical and biochemical processes. The biochemical reactions leading to SOM degradation are also influenced by the water flow dynamics, which hinder the activation of enzymes and microbial stabilization. The study findings demonstrate the importance of the origin of the SOM in explaining the enzyme activity in subaqueous soil environments. The high specific enzyme activities in deep horizons of subaqueous environments may be explained by the combined effect of water movement, erosional processes and preservation of SOM under anaerobic conditions. Considering the lack of similar approaches to studying the pathway of SOM degradation in salt marsh ecosystems, further studies are required to test the hypothesis presented in this study at different scales.

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Figure captions:

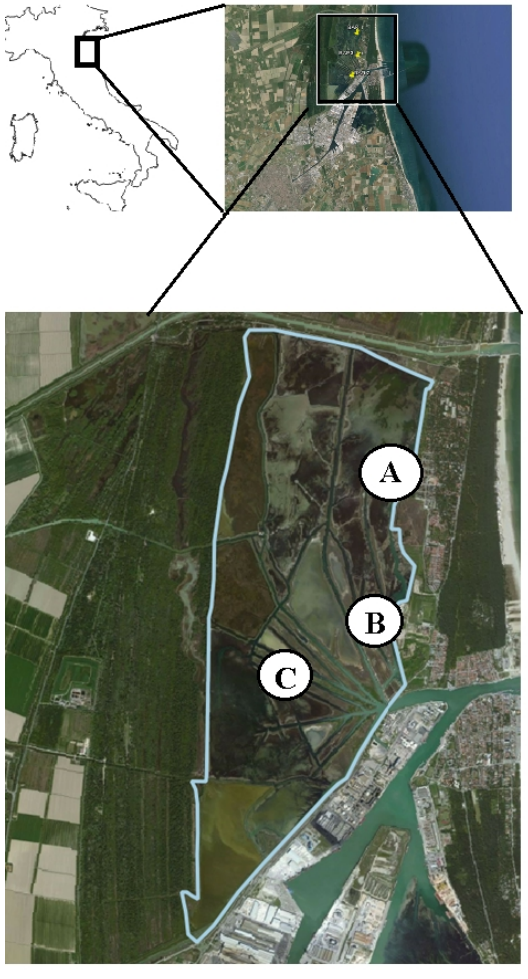
Figure 1: Study area, sampling sites and WGS-84 UTM 33T coordinates.

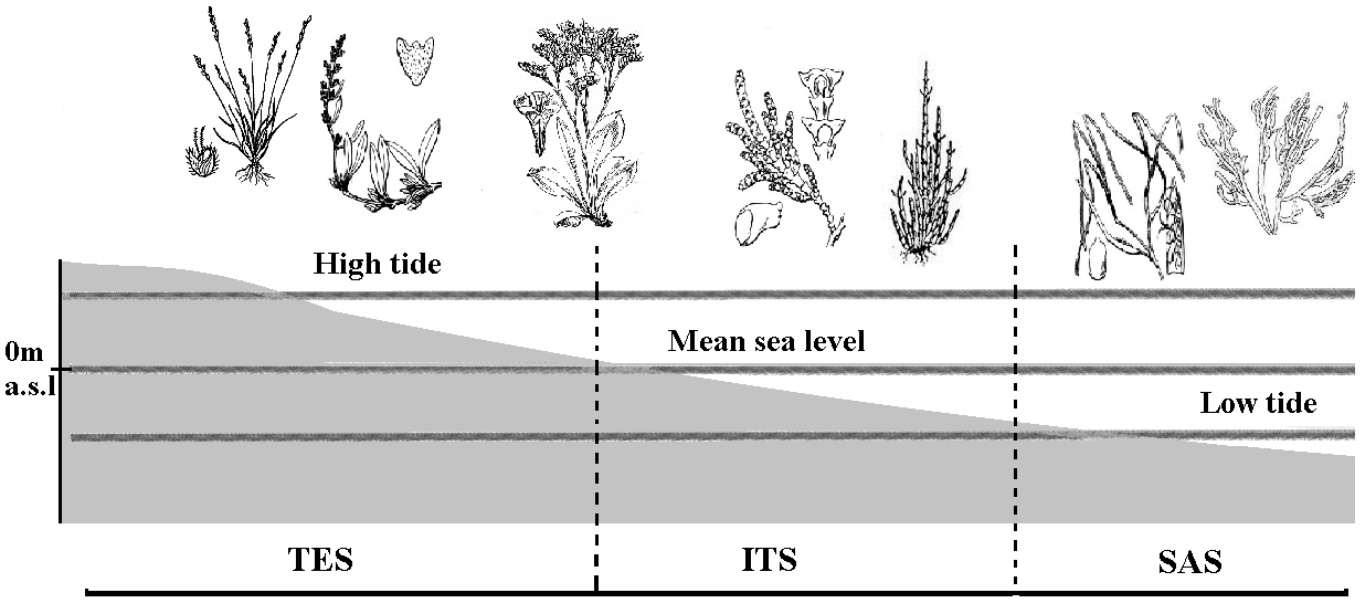
Figure 2. DRIFT spectra of free (F) and bound (B) humic acids (according to conventional theory) extracted from the A horizons of the TES, ITS and SAS systems.

Figure 3. Percentage of the main peak areas on a) free HA and b) bound HA (according to conventional theory) extracted from the TES, ITS and SAS soils.

Figure 4. Mean values ± standard error (SE) of microbial biomass carbon (MBC), microbial quotient (Qmic) and Synthetic Enzyme Index (SEI) for A, AC and C horizons of the TES, ITS and SAS profiles.

Figure 5. Mean values ± standard error (SE) of specific enzyme activities for A, AC and C horizons of the TES, ITS and SAS soil profiles. Data are expressed as mmol H_2O_2 consumed g C^{-1} h^{-1} (CAT/C), μmol NH_3 g C^{-1} h^{-1} (URE/C), μmol PNP g C^{-1} h^{-1} (ALK/C, ARYL/C, GLU/C), μmol glucose g^{-1} h^{-1} (INV/C).



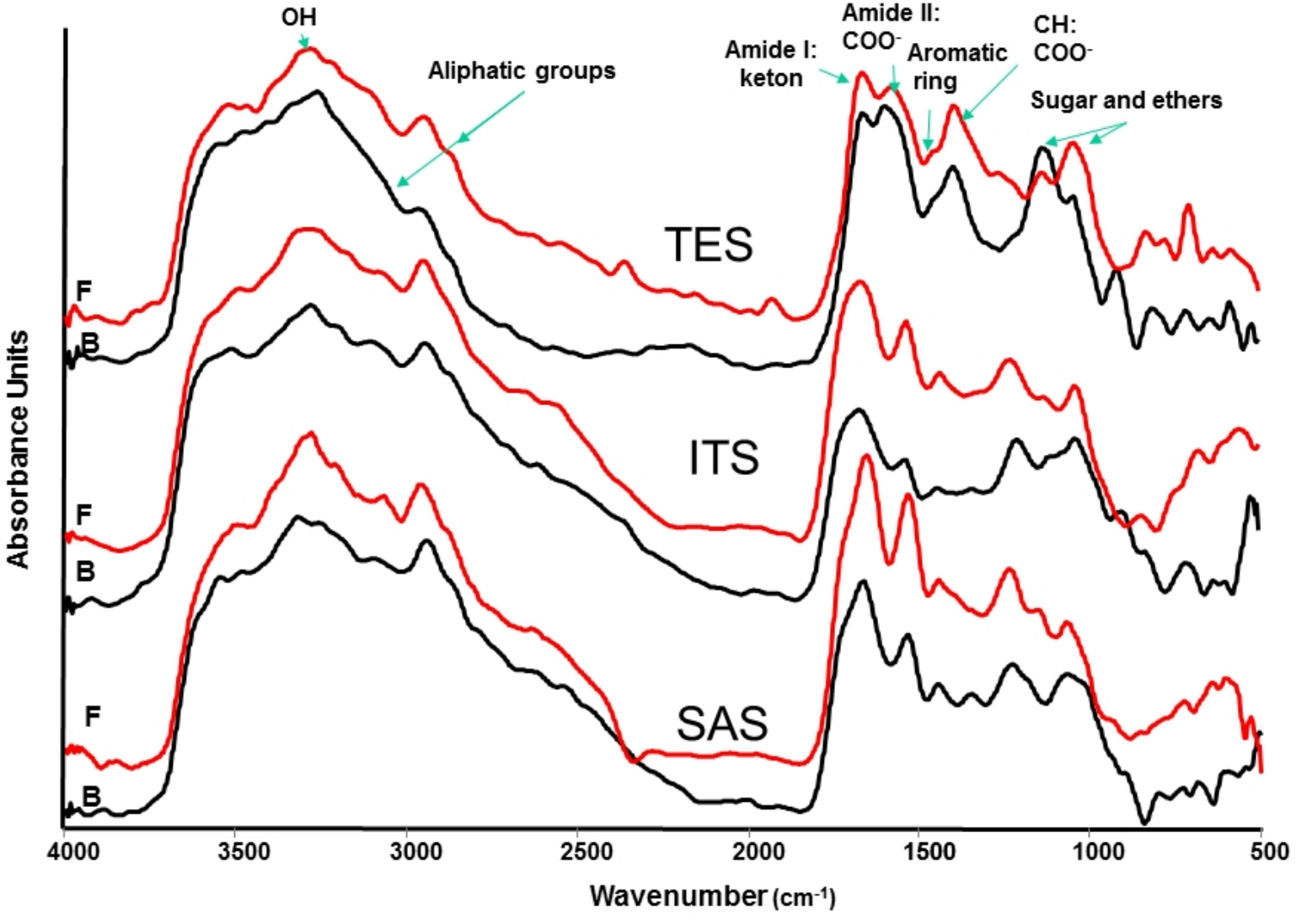


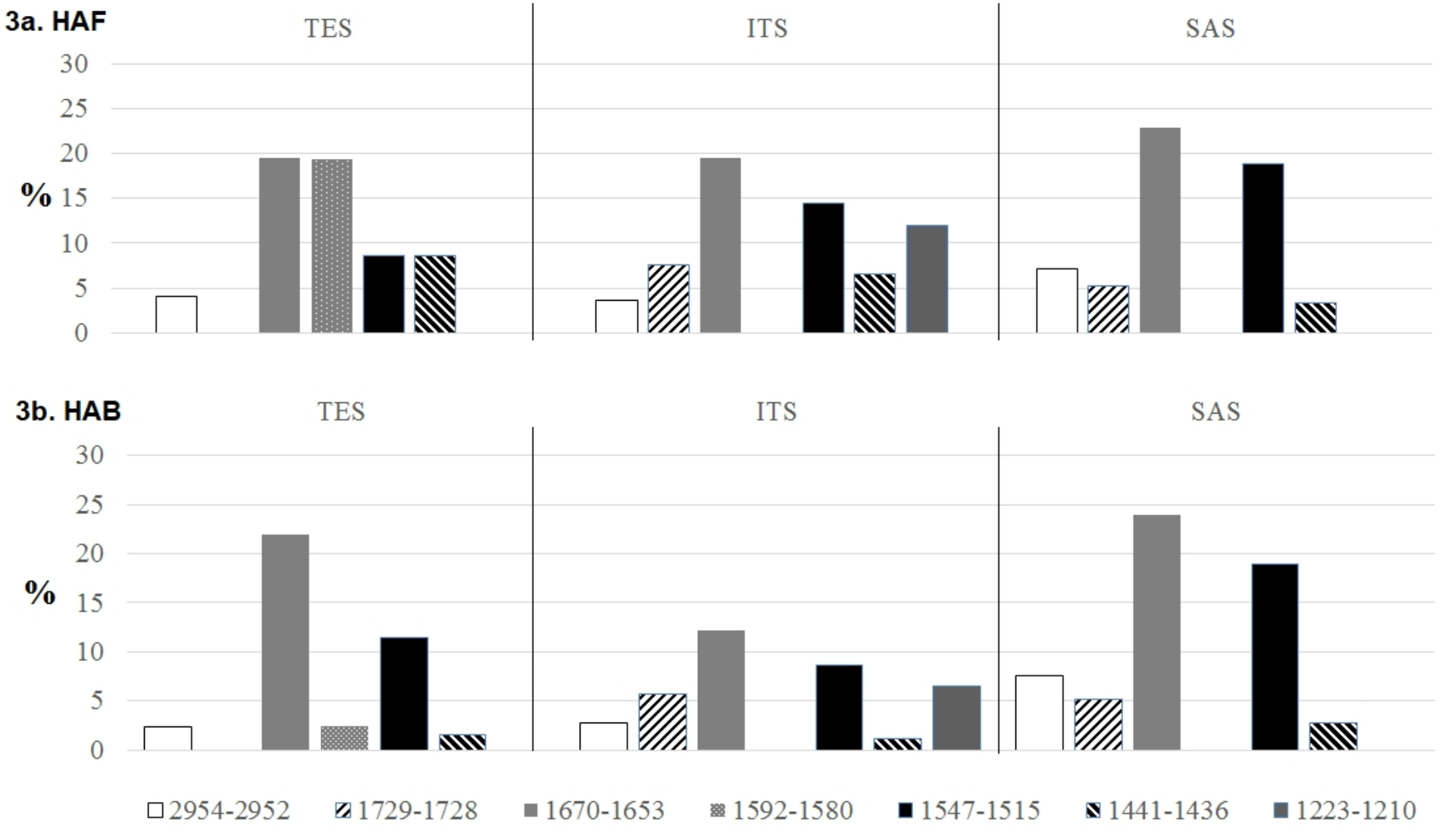
WGS84-UTM33

TES		
SITE	E (m)	N (m)
TES-1A	282468	4932596
TES-2A	282473	4932595
TES-3B	281276	4930301
TES-4B	281269	4930281
TES-5C	282412	4930932

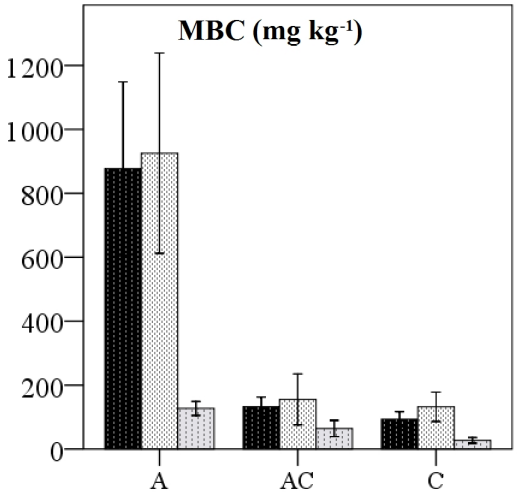
ITS		
SITE	E (m)	N (m)
ITS-1A	282478	4932594
ITS-2B	281264	4930269
ITS-3C	282411	4930926
ITS-4C	282412	4930921

SAS		
SITE	E (m)	N (m)
SAS-1A	282487	4932592
SAS-2B	281260	4930261
SAS-3C	282412	4930913

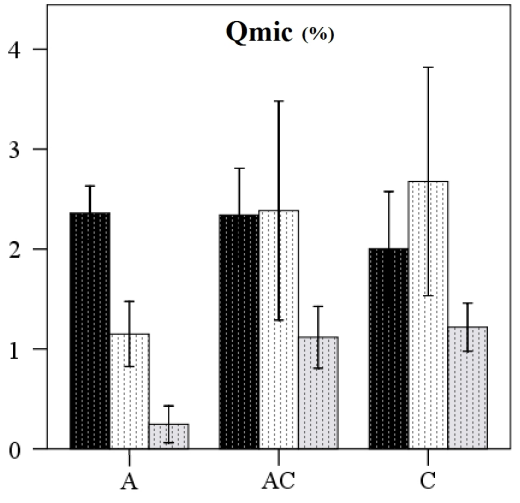

OH
Aliphatic groups
Amide I:
keton
Amide II:
COO-
Aromatic
ring
CH:
COO-
Sugar and ethers
TES
ITS
SAS
F
B
F
B
F
B
Absorbance Units
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)


3a. HAF
TES
ITS
SAS
30
25
20
%
15
10
5
0
3b. HAB
TES
ITS
SAS
30
25
20
%
15
10
5
0
2954-2952
1729-1728
1670-1653
1592-1580
1547-1515
1441-1436
1223-1210

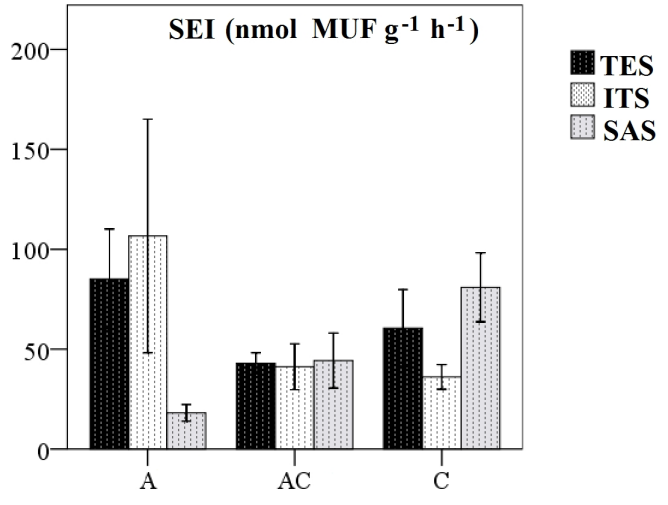
4 a


MBC (mg kg-1)
1200
1000
800
600
400
200
0
A
AC
C

4 b


Qmic (%)
4
3
2
1
0
A
AC
C

4 c


SEI (nmol MUF g-1 h-1)
200
150
100
50
0
A
AC
C
TES
ITS
SAS

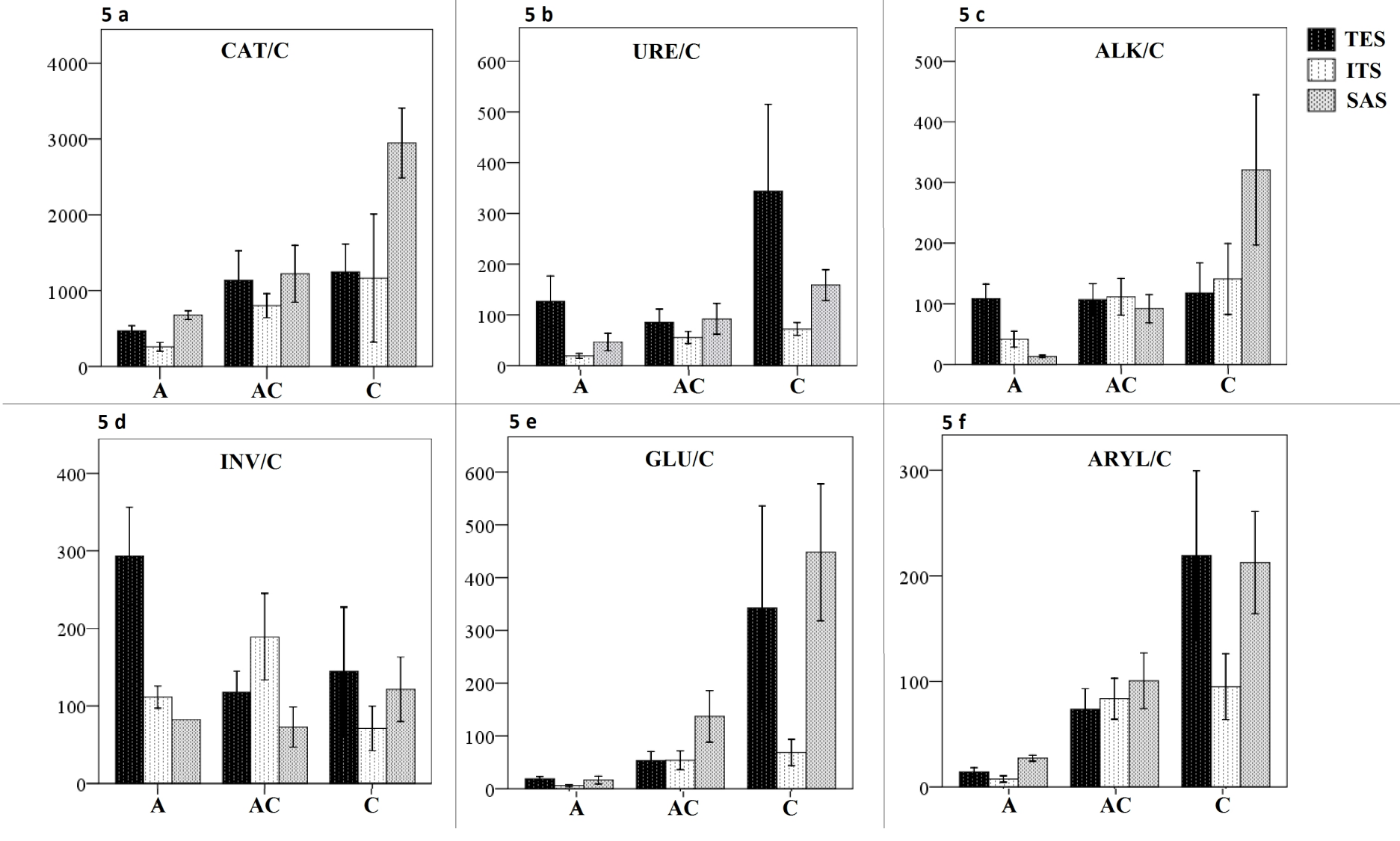

5 a
CAT/C
4000
3000
2000
1000
0
A
AC
C
5 b
URE/C
600
500
400
300
200
100
0
A
AC
C
5 c
ALK/C
500
400
300
200
100
0
A
AC
C
TES
ITS
SAS
5 d
INV/C
400
300
200
100
0
A
AC
C
5 e
GLU/C
600
500
400
300
200
100
0
A
AC
C
5 f
ARYL/C
300
200
100
0
A
AC
C

Table 1. Assignment of the main bands observed in the SOM FT-IR spectra according to Fooken and Liebezeit (2003), Mecozzi and Pierantonio (2006), and Stevenson (1994)

Wavelength (cm^{-1})	**Band assignments**
3500-3200	O–H stretching vibration in phenols, carboxylic acids and carbohydrates
2952-2850	Symmetric and symmetric C–H stretching
1730-1720	C=O stretching in acids or ketones
1670-1650	C=O stretching in Amide I; aromatic and aliphatic C=C stretching; C=N stretching; H-bonded conjugated ketones, carboxyls and quinones
1570-1515	Aromatic C=C stretching, aromatic skeletal vibrations, aromatic skeletal (lignin), amide II; carboxylates
1440-1340	C–H deformation of CH_2 and CH_3 groups and/or to symmetric stretching of COO^- groups
1260-1216	Amide III, C–O stretching and O–H deformation of COOH groups and to C–O stretching of aryl ethers and phenols

Table 2. Physical and chemical properties of soil horizons (A, AC and C horizons) in the different ecosystems (TES, ITS, SAS). Mean values and Standard Error (SE) for each group are shown**.** "na"= not available (because of lack of sufficient material).

		pH		EC dS m^{-1}		Sand %		Silt %		Clay %		$CaCO_3$ g kg^{-1}		Ca g kg^{-1}	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	TES	8	0.1	12	3	58.2	8.8	30.1	6.8	11.7	3.1	6.3	1.2	31.5	3.6
A	ITS	7.5	0.2	24.9	2.6	16.5	1.9	64.2	5.2	19.3	3.6	3.3	1.4	17.2	4.1
	SAS	8.3	0.2	8.1	1.4	na	na	na	na	na	na	na	na	36.7	13.2
	TES	8.4	0.1	6.1	0.6	57.3	11.8	36.3	10.6	6.4	1.3	12.9	0.8	51.4	1.8
AC	ITS	8.2	0.1	10.2	0.9	40.8	14.6	51.6	14.3	7.6	1	9.6	2.3	36	7.8
	SAS	8.3	0.1	7.2	0.8	39.3	15.9	53.6	14.4	7.1	1.5	15.1	1	57.1	2.2
	TES	8.5	0.1	5.7	0.9	65.5	13	28.2	12.5	6.3	1.1	15.1	0.9	59.6	2.7
C	ITS	8.5	0	8.1	1.5	53.7	15.9	42	15.8	4.4	0.7	13.4	0.7	52.8	0.9
	SAS	8.5	0.1	5.1	0.8	72.6	6.6	26.5	9.8	5.2	1.3	15.7	0.6	59.8	1.7

		TOC g kg^{-1}		$\delta^{13}C$ ‰		TN g kg^{-1}		Na g kg^{-1}		P g kg^{-1}		S g kg^{-1}	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	TES	51.6	15.1	-25.9	0.3	4.7	1.5	6.5	0.9	0.7	0.1	1.3	0.6
A	ITS	111.1	17.4	-25.1	0.6	11.1	1.9	9.9	1	1	0.1	4.6	1.2
	SAS	38.3	17.7	-23.8	0.9	6	3.1	13.9	1.3	0.5	0.1	8.2	1.2
	TES	5.9	0.9	-25	0.2	0.6	0.1	5.6	0.7	0.5	0.1	0.4	0.1
AC	ITS	6.1	1.4	-25	0.3	0.7	0.2	8.6	1.3	0.6	0.1	0.6	0.1
	SAS	5.1	1	-25.3	0.3	0.9	0.3	6.7	1	0.5	0.1	1.7	0.5
	TES	3.8	1.1	-24.8	0.1	0.4	0.1	5.2	0.9	0.5	0.1	0.3	0.1
C	ITS	4.7	0.7	-23.9	0.2	0.5	0.1	5.3	0.7	0.4	0.1	0.3	0.1
	SAS	2	0.4	-25.3	0.3	0.3	0	4.3	0.6	0.4	0	0.7	0.1

Table 3. Results of the Kruskal-Wallis test based on the grouping of A, AC and C horizons from the three salt marsh ecosystems (TE, IT and SA). nd= not determined; ns= not significant; * P <0.05, ** P <0.01.

		pH	EC	Sand	Silt	Clay	$CaCO_3$	Ca	TOC	$\delta^{13}C$	N	Na	P	S
A	**TES vs ITS**	*	*	nd	nd	nd	ns	*	*	ns	ns	*	*	**
	TES vs SAS	*	*	nd	nd	nd	ns	ns	ns	*	ns	**	ns	**
	ITS vs SAS	**	**	nd	nd	nd	ns	ns	*	ns	ns	ns	**	ns
AC	**TES vs ITS**	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	TES vs SAS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
	ITS vs SAS	ns	*	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
C	**TES vs ITS**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	TES vs SAS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	ITS vs SAS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 4. Mean values (± standard error, SE) of enzyme activities in the A, AC and C horizons of the TE, IT and SA soil profiles.

		CAT mmol H_2O_2 g^{-1} h^{-1}		URE µmol NH_3 g^{-1} h^{-1}		ALK P µmol PNP g^{-1} h^{-1}		GLU µmol PNP g^{-1} h^{-1}		ARYL µmol PNP g^{-1} h^{-1}		INV µmol glucose g^{-1} h^{-1}	
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
	TES	17.4	4.2	2.9	0.7	3	0.4	0.6	0.2	0.4	0.1	12.1	4.4
A	**ITS**	22.5	4.8	2.2	0.6	3.3	1	0.4	0.1	0.6	0.2	11.6	2.2
	SAS	13.9	0.7	1	0.3	0.3	0.1	0.3	0.1	0.6	0	1.6	0.1
	TES	7	2.8	0.5	0.1	0.7	0.2	0.3	0.1	0.5	0.1	0.7	0.2
AC	**ITS**	4.5	1.3	0.6	0.3	0.9	0.3	0.3	0.1	0.5	0.1	1.4	0.5
	SAS	10.1	2.5	0.4	0.1	0.5	0	0.6	0.1	0.5	0.1	0.5	0.1
	TES	4.9	2.3	0.6	0.1	0.3	0	0.6	0.1	0.5	0.2	0.3	0.1
C	**ITS**	6.7	5.3	0.4	0.1	0.6	0.2	0.3	0.1	0.4	0.1	0.4	0
	SAS	5.1	1.1	0.3	0	0.3	0.1	0.6	0.1	0.6	0.1	0.2	0

Table 5. Results of the Kruskal Wallis test based on the single A, AC and C horizons of three salt marsh ecosystems (TE, IT and SA). ns= not significant; * P <0.05, ** P <0.01, *** P <0.001.

		MBC	Qmic	SEI	CAT	URE	ALK	INV	GLU	ARYL	CAT/C	URE/C	ALK/C	INV/C	GLU/C	ARYL/C
A	**TES vs ITS**	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
	TES vs SAS	***	***	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	***	**	ns
	ITS vs SAS	***	*	ns	ns	ns	*	ns	ns	ns	*	ns	ns	*	ns	ns
AC	**TES vs ITS**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
	TES vs SAS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
	ITS vs SAS	*	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
C	**TES vs ITS**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
	TES vs SAS	*	ns	ns	ns	**	ns	ns	ns	ns	*	ns	ns	*	ns	ns
	ITS vs SAS	*	ns	ns	ns	**	ns	*	ns	ns	**	*	ns	ns	ns	ns

Table S1. Morphological soil classification (McVey et al., 2012; Schoeneberger et al., 2012).

Soil Classification	Soil System	Master	Depth (cm)	Bound.	Color Munsell		Text.	HC (cm h^{-1})	Structure	Consistence /Fluidity	Concentrations						Sulphide detection			
					Moist	Dry					Mottles I	Mottles II	Roots	Bio	Other	RXF	H_2O_2	Odor	$pH_{in.}$	$pH_{fin.}$
Typic Psammaquent	**TES**	A1	0-5	A/W	10YR 3/2	10YR 4/3	SL	7.59	SBK/1/f	so/po			c/f-m				N	N	7.98	*nd*
		A2	5-15/19	C/W	10 YR 3/2	2,5Y 5/2	SL	7.29	SBK/1/f	so/po			c/f-m	c/SFB			N	N	8.14	*nd*
		AC	15/19-37	G/W	10YR 4/4	10YR 6/3	S	11.67	SG/0/f	so/po			f/f	f/SFB		f/F2M	N	N	8.63	*nd*
		Cg	37+	U	2.5Y 4/4	2.5Y 6/3	S	3.67	SG/0/f	so/po			f/f				N	N	8.65	*nd*
Typic Psammaquent	**TES**	A1	0-5	A/W	1oYR 3/2	10YR 4/1	SL	9.06	SBK/1/m	so/po			c/f-m	f/SFB			N	N	8.42	*nd*
		A2	5-17/20	C/W	10YR 3/2	10YR 5/2	SL	4.09	SBK/1/m	so/po			c/f-m				N	N	8.42	*nd*
		AC	17/20-28	G/W	10YR 4/2	10YR 6/3	S	10.02	SG/0/f	so/po			f/f	f/SFB	f/OAF		N	N	8.61	*nd*
		Cg	28+	U	5Y 5/1	10YR 6/3	S	12.83	SG/0/f	so/po	c/d - 10YR 5/6		Vf/f				N	N	8.61	*nd*
Typic Psammaquent	**ITS**	**Oi**	**2.5-0**	A/S	5Y 3/1	5Y 4/2	MK		*nd*	ss/p			m/f; c/d	m;c/RSF			Y	S/ST	7.47	7.40
		A	0-8/9	A/S	5Y 3/1	5Y 3/2	S	0.40					m/f-m							
		AC	8/9-19/20	A/W	10YR 3/1	10YR 4/2	S	2.65	SBK/1/m	so/po			m/f; c/d			c/F3M	N	N	7.98	7.35
		AC2g	19/20-30	A/W	5Y 3/2	2.5Y 6/2	S	8.44	SG/0/f	so/po			c/f	c/RFS	m2/OSF	m/FED	N	N	8.04	*nd*
		Cg	30+	U	2.5Y 4/2	2.5Y 6/3	S	11.32	SG/0/f	so/po			f/d				N	N	8.51	*nd*
Fluventic Psammowassent	**SAS**	A	0-5	AS	*nd*	*nd*	MK	10.83	*nd*	vf							Y	S/ST	*nd*	7.33
		C1	5-17	CW	N 2.5/0	5Y 5/2	S	11.97	*nd*	sf							Y	S/MD	8.62	7.49
		AC	17-60	GW	10Y 4/1	5Y 6/2		12.09	*nd*	nf					f/OSF	m/F3M	N	N	8.94	*nd*
		2C	60-84	AS	10Y 5/1	5Y 6/2	S	11.67	*nd*	nf				cSFB		RMX	N	N	8.85	*nd*
		2ACg	84-86	AW	???	???			*nd*	sf					m/OSF		Y	S/SL	*nd*	7.35
		3Cg	86-109	CW	10Y 5/1	5Y 6/2	S	8.78	*nd*	nf							N	N	*nd*	*nd*
		Cg	109-136+	U	N 5/0	5Y 6/1	S	8.44	*nd*	nf					f/OSF	m/F3M	N	N	8.60	*nd*
Typic Endoaquent	**TES**	OA	0-2	A/S	10YR 2/1	10YR 4/1	SL	2.31	GR/1/f	so/ps			m/vf	f/SFB			N	N	7.98	*nd*
		A1	2-4	A/S	10YR 3/1	10YR 4/1	SL	3.45	GR/1/f	so/ps			m/vf				N	N	7.88	*nd*
		A2	4-10	A/W	10YR4/3	10YR 5/2	LS	4.35	SBK/2/m	ss/p	f/f - 10YR 5/8		f/f				N	N	8.08	*nd*
		AC	10-22	C/W	10YR 4/2	2,5Y 6/3	LS	4.04	SBK/3/m	so/ps	c/d - 10YR 5/8	f/f - 2.5Y 5/1	f/f-m				N	N	8.45	*nd*
		Cg	22+	U	5Y 5/1	2.5 Y 6/3	LS	3.71	Massivo	s/p	c/f -10YR 5/6			m/SFB	f/OSF		N	N	8.42	*nd*
Typic Endoaquent	**TES**	Oi	1-0	A/S	2.5Y 2.5/1	2.5Y 3/2	MK	0.35	*nd*						m/OAF		N	N	7.55	*nd*
		A	0-3	A/S	7.5YR 3/3	7.5YR 4/2	MK	1.17	*nd*				m/vf	f/SFB		RMX	N	N	7.12	*nd*
		AC	3-15	C/W	2.5Y 5/3	2.5Y 6/3	LA	2.69	Massivo	s/p	c/d - 7.5YR 4/6	f/f - 2.5Y 5/1	c/f-m	f/RSB	f/OSF	c/FMM	N	N	8.13	*nd*
		Cg	15+	U	2.5Y 5/2	2.5Y 6/3	L	3.46	massivo	sv/pv	c/f 2.5Y 4/1						N	N	8.28	*nd*
Typic Endoaquent	**ITS**	Oi	0.5-0	A/S	10Y 3/2	2.5Y 3/2	MK	2.73	*nd*						m/OAF		N	N	7.84	*nd*
		A	0-4	A/S	7.5YR 3/3	7.5YR 4/1	MK	2.76	*nd*				m/vf		c/OSF		N	N	7.61	*nd*
		AC	4-23	C/W	5Y 5/2	5Y 6/2	LA	3.41	ABK/2/m	s/p	c/f - 5Y 4/1	f/d - 10YR 5/6	f/f	f/RSB		c/F3M	N	N	8.45	*nd*
		Cg	23+	U	N 3/0	5Y 6/2	LA	3.88	massivo	s/p	f/d - 5Y 5/6				c/OSF		Y	N	8.46	7.66
Typic Fluviwassent	**SAS**	A1	0-11	AW	10BG 2,5/1	*nd*	MK		*nd*	vf				c;m/SFB			Y	S/ST	*nd*	7.29
		A2	11-28	AW	10G 4/1	*nd*			*nd*	sf			c/d; m/f				Y	S/SL	7.93	7.38
		AC	28-66/67	AI	10Y 4/1	*nd*		3.38	*nd*	nf			f/f	f/RSB			N	N	8.40	*nd*
		1C	66/67-80	AW	10Y 4/1	*nd*	S	3.71	*nd*	nf					f/OSF	c/CLD	N	N	8.42	*nd*
		2C	80-110+	U	10Y 3/1	*nd*	S	5.37	*nd*	sf				f/SFB	c/OSF		N	N	8.17	*nd*
Typic Endoaquent	**TES**	OA	0-4	A/W	10YR 3/1	10YR 3/2	LS	3.03	GR/2/f	so/ps			m/f-m				N	S/SL	7.78	8.06
		A2	4-14	C/W	2.5Y 4/3	2.5Y 5/2	LS	3.20	SBK/3/m	ss/p			c/f-m	f/SFB			N	N	*nd*	*nd*
		AC	14-38	G/W	2.5Y 5/3	2.5Y 6/3	L	3.07	SBK/2/m	s/p	c/d - 10YR 5/6	f/d - 2.5Y 6/1	f/m	c/SFB			N	N	8.46	*nd*
		Cg	38-56+	U	5Y 5/3	2.5Y 6/3	LS	5.92	SBK/2/f-m	ss/ps	m/f - 5Y 5/6	c/d - 10YR 5/8		m/SFB			N	N	8.61	*nd*
Typic Endoaquent	**ITS**	Oi	0.5-0	AS	10Y 3/2	????	MK								m/OAF		Y	S/ST	*nd*	8.09
		A1	0-3	AS	10YR 3/2	10YR 4/4	MK	0.89					m/vf-f				N	N	6.96	*nd*
		AC	3-10	G/W	10Y 2.5/0	N 7/0	LA	3.87	massivo	s/p	f/d - 2.5Y 5/6		f/f	c/RSB; f/SFB		c/F3M	N	N	8.24	*nd*
		Cg	10+	U	10Y 5/1	5Y 6/2	SL	4.08	massivo	ss/ps					f/OSF		Y	S/SL	8.49	7.61
Typic Endoaquent	**ITS**	Oi	4-0	AS	10YR 3/2	1oYR 4/1	MK	2.12					m/vf-f				N	S/SL	7.01	7.50
		A1	0-3	AS	5Y 5/1	5Y 6/2		3.71	massivo	s/p	f/d - 10YR 5/6		f/f-m		c/OSF	f/FMM	Y	N	8.29	*nd*
		AC	3-20	G/W	5Y 4/1	5Y 7/2		4.12	massivo	s/p						c/F3M	N	N	8.41	*nd*
		Cg	20+	U	N 2.5/0	5Y 6/2	S	4.89	SG/0/f	so/po				f/RSB;	f/OSF		N	N	*nd*	*nd*
Typic Fluviwassent	**SAS**	A1	0-3/4	AS	N 2,5/0	*nd*	MK			sf			m/f	m/RSB			Y	S/ST	*nd*	7.26
		A2	3/4-18/19	AW	2,5Y 4/2	*nd*		3.23		nf							Y	S/SL	8.22	7.78
		1ACg	18/19-62	AW	2,5Y 3/2	*nd*		1.76		nf			c/d	c/RSB		m/FMM	N	N	8.46	*nd*
		2ACg	62-76	AI	10Y 4/1	*nd*		3.60		sf			f/f	f/RSB	c2/OSF	c/FMN	N	N	8.31	*nd*
		Cg	76-109+	U	10Y 3/1	*nd*	S	4.85		nf			f/f	f/RSB	f2/OSF	f/F3M	N	N	8.39	*nd*

Soil System: TES = Terrestrial soils; ITS = intertidal soils; SAS = subaqueous soils --- Horizon master (Master): g = strong gleying, se = presence of sulphides --- Horizon boundary (Bound.): Distinctness: A = abrupt, C = clear, G = gradual, D = diffuse / Topography: S = smooth, W = wavy, I = irregular, U = unknown --- Mottles Quantity: f = few, c = common, m = many / Contrast: f = faint, d = distinct --- Texture Field estimation (Text.): MK = mucky, L = Loam, LS = Loamy Sand, S = sand, SL = Sandy Loam, SICL = Silty Clay Loam, SIL = Silt Loam --- Hydraulyc Conductibility (HC) --- Structure (T) Type: GR = granular, ABK= angular blocky, SBK = subangular blocky, SG = single grain / (G) Grade: 0 = structureless, 1 = weak, 2 = moderate / (S) Size: vf = very fine, f = fine, m = medium --- Consistence: Stickiness: so = non-sticky, ss = slightly sticky, s = moderately sticky, sv = very sticky / Plasticity: po = non-plastic, ps = slightly plastic, p = moderately plastic, pv = very plastic / Fluidity: nf = non fluid; sl = slightly fluid; mf = moderately fluid; vf = very fluid; --- Mottles Quantity: f = few, c = common, m = many / Contrast: f = faint, d = distinct --- Roots. Quantity: vf = very few, f = few, c = common, m = many / Size: vf = very fine, f = fine, m = medium --- Biological concentrations (Bio). Quantity: f = few, c = common, m = many / (K) Kind: RSB = root sheaths, SFB = shell fragments. Other (Coats/films and redoximorphic features): Quantity: f = few, c = common, m = many / (K) Kind: CLD = cley depletion, FED = iron depletions, FEF = ferriargillans coats, F2M = reduced iron Fe2+ masses, FMM = iron-manganese masses, OAF = organoargillans concentrations, OSF = organic stains concentrations, RMX = reduced matrix --- H2O2 color change: Y= yes; N = no; Odour: (K) Kind: S = sulforous; N = none; (I) Intensity: SL = slight; M D = moderate; ST = strong.