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INTERACTIONS BETWEEN SOIL AND PLANTS IN HALOPHILE SYSTEMS

PLANT ZONATION AND EFFECTS ON PEDOGENESIS

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STATEMENT OF ORIGINAL CONTRIBUTION

The research presented is an original contribution in the field of ecology, focusing on soil chemistry and plant physiology in saltmarsh environment. My supervisors, dott. Valentino Casolo and prof. Maria De Nobili, followed my work constantly and professionally, and their contribution was essential. Fruitful collaborations allowed the present achievements, two main groups of research in particular: the group of pedology of the University of Bologna with prof. Gilmo Vianello as referee and the freshwater biology group of the University of Copenhagen with prof. Ole Pedersen as referee.

The manuscripts included in this thesis are organised in five chapters:

- Chapter 1. Vittori Antisari L., De Nobili M., Ferronato C., Natale M., Pellegrini E., Vianello G. (2016) Hydromorphic to subaqueous soils transitions in the central Grado lagoon (Northern Adriatic Sea, Italy). *Estuarine, Coastal and Shelf Science*. 173, 39-48. doi: 10.1016/j.ecss.2016.02.004.
- Chapter 2. Vittori Antisari L., Ferronato C., Pellegrini E., Boscutti F., Casolo V., De Nobili M., Vianello G. (2016) Soil properties and plant community relationship in a saltmarsh of the Grado and Marano lagoon (northern Italy). *Journal of Soils* and Sediments. doi: 10.1007/s11368-016-1510-6.
- **Chapter 3.** Pellegrini E., Boscutti F., De Nobili M., Casolo V. Plant traits of key species shape the effects of tide flooding on soil and plant communities in saltmarshes. *Unpublished. Submitted to Plant Ecology*.
- **Chapter 4**. Pellegrini E., Konnerup D., Winkel A., Casolo V., Pedersen O. Internal tissue aeration in two halophytes of the Mediterranean region, during partial and complete submergence. *Plant Functional Biology.* In press
- **Chapter 5**. Pellegrini E., Contin M., Vittori Antisari L., Vianello G., Ferronato C., De Nobili M. Evaluation of a new paper sensor test for the field analysis of sulphides in waters, soils and sediments. *Unpublished. Submitted to Water Research.*

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SUMMARY

The increasing interest around the protection of coastal wetlands highlighted the scarce knowledge achieved about these systems and the consequent inability to operate efficiently during conservation and restoration programs. One crucial issue is related to saltmarsh systems, whose functioning appears highly complex and where a great connection between soil and halophytes drives the definition of the main characteristics.

For its complexity, the present work used a holistic approach with the aim to evaluate the main driving forces involved in saltmarsh formation and development, focusing on the effect of flooding, soil features and plant-soil feedback mechanisms.

Flooding drove the main changes on soil features, plant growth and plant distribution. The increasing flooding stress defined gley soils, with abrupt changes in colour between the submerged horizons and the upper less frequently inundated horizons of soil profiles. Clay soils at lower sites allowed the development of more severe anoxic conditions and the accumulation of sulphides, while higher sites seemed to be more influenced by plant competition instead of flooding.

Halophytes are plants extremely well-adapted to flooding involving several different strategies. Internal tissue aeration is the most common strategy based on the presence of high tissues porosity (up to 40% in *Spartina* genus), whereas other species could benefit from underwater photosynthesis or, less commonly, from fermentative processes when under water, keeping out temporarily internal oxygen diffusion (e.g. in *Limonium narbonense*).

Some plant species studied in this work actively affected pedogenesis, being able to change the original conditions defined by flooding. The tide oscillation and the presence of rooted plants led to the formation of redoximorphic features along soil profiles, which consequently determine changes on the soil redox status. These changes were mainly driven by the oxygen released from roots by some species like *L. narbonense*, able to tolerate great changes in flooding intensity and severe anoxic conditions in soil.

Sulphides are toxic for plants and for this reason can interfere with zonation but, currently, few works investigated this aspect and adequate field methods are not available, especially for the determination of the acid volatile sulphides (AVS) fraction, abundant in coastal environments. In contrast to their capability to alter the redox conditions in the rhizosphere, plants seemed not capable to diminish sulphides concentrations in soils, probably due to the restricted radial area affected by the oxygen released from roots.

The different plant communities, studied in the present work, were located according to different morphological and physicochemical soil properties, especially the degree of soil anoxia. Thus, vegetation represented a useful index of the hydrological and nutritional status of the underlying soils, providing several indirect information of the degree of flooding, the soil type and the possible plant feedbacks affecting a specific saltmarsh soil.

The feedback mechanism on rhizosphere triggered by plants diminishes the negative effect of flooding on soil features and plant communities, improving locally soil conditions and allowing in turn an easier establishment of other more demanding species. The development of soil (toward horizon formation and organic matter accumulation) and the increasing of plant communities richness could, nevertheless, regress to less developed states due to a more intense flooding or occasional storms. Therefore, flooding remains a primary factor affecting soil features and halophytes distribution, and that soil and vegetation together cooperate to the resulting complexity of saltmarsh environment, included all interactions between abiotic and biotic factors. This fact explains the existence of extremely different conditions about soil and plant zonation in sites of very similar elevation and flooding regime.

TABLE OF CONTENTS

STATEMENT OF ORIGINAL CONTRIBUTION	III
ACKNOWLEDGMENTS	IV
SUMMARY	V
MOTIVATION	1
INTRODUCTION	5
SOIL-PLANT INTERACTIONS	7
PLANT STRATEGIES AGAINST SALINITY AND FLOODING	9
SALTMARSH VEGETATION	10
SALTMARSH SOILS	13
THE RELEVANCE OF SULPHIDES IN SALTMARSH SOILS	15
THESIS' AIM	18
THESIS' STRUCTURE	19
CHAPTER 1	21
CHAPTER 2	
CHAPTER 3	51
CHAPTER 4	75
CHAPTER 5	
GENERAL DISCUSSION	125
CONCLUSIONS	
REFERENCES	135

MOTIVATION

Transitional ecosystems provide several economic, social and environmental services. The main ones are linked to coastal shoreline protection and human activities (e.g. fishery, recreational activities), water quality, nutrient cycling, and provision of habitats and food for migratory or resident birds (see e.g. Newton et al., 2014; Abbiati et al., 2010; McAlpine and Wotton, 2009). The high ecological value of these systems, which are able to support a very large productivity (Cronk and Fennessy, 2016; Reddy and DeLaune, 2008), is nowadays officially recognised and partially regulated. The European Habitat Directive (92/43/EEC) considers all transitional water ecosystems as priority habitats, and the European Water Framework Directive (2000/60/EC) strengthens prevention against further deterioration of the water resource and promotes sustainability of aquatic environments. Nevertheless, due to increasing population pressures and global climate change, coastal ecosystems appear severely compromised (Sun et al., 2015; He et al., 2014). Approximately 50% of the world's saltmarshes, 35% of mangrove covered areas, 29% of seagrass populations and 30% of coral reefs have been already lost or degraded (Barbier et al., 2008; Waycott et al., 2009). This scenario will be strongly enhanced in the future by the overexploitation of resources and pollution (Abbiati et al., 2010). Moreover, saltmarshes are among the most threatened ecosystems from climate change, being controlled by tidal flooding (Simas et al., 2001). In the next one hundred years, the mean sea level is attested to rise from 0.26 up to 0.82 m(IPCC, 2013), making semi-enclosed coastal systems sentinel hotspots of large-scale impacts (Newton, 2012). The concern around the fate of transitional water ecosystems has led to an increasing interest in preservation and restoration programs. Several efforts have been made worldwide towards the protection and reconstruction of these habitats (see e.g. Bayraktarov et al., 2015; Benayas et al., 2009) and new proposals are suggested for improving management applications (Liu et al., 2016).

Saltmarshes are highly dynamic environments, controlled by delicate equilibria where the driving forces involved in their formation are the same which can cause their decay (Townend et al., 2011). The survival of these environments is critically dependent on the balance of sedimentation-erosion rates (Adam, 2002) which

depends on sediment availability, sediment consolidation and the subsequent establishment of vegetation (Rinaldo et al., 2004). Recent studies highlighted the ability of marshes to maintain elevation despite the sea level rise (Elmore et al., 2016; Cadol et al., 2014), but accretion is not homogenous along the marsh profile and will not necessarily occur at all places. Sediments are transported inside the saltmarsh thanks to a complex tidal network (French and Stoddart, 1992) and the different redistribution of sediments defines the marsh topography. Elevation and tidal cycles define the so-called hydroperiod, that is the duration of water persistence over a surface (Reed, 1990). Very small differences in hydroperiod determine different soil features, microbial community composition, vegetation, availability of nutrients and presence of toxic substances etc. These compartments also interact each other and contribute to modify the starting conditions determined by the hydroperiod: plants influence soil characteristics e.g. releasing oxygen from roots; plants contribute to soil organic carbon and at the same time favour microbial decomposition processes; different plant species could compete for nutrients altering plant communities composition etc. (see for a review Cronk and Fennessy, 2016; Reddy and De Laune, 2008). These considerations, combining knowledge on soil features with that on vegetation, can provide a great amount of indirect information about the many interconnecting processes involved.

Soil and vegetation could be useful bioindicators of the main saltmarsh features and processes (Jenačković et al., 2016), being witnesses of past changes or possible predictors of future developments. Knowledge about saltmarshes is nowadays incomplete despite crucial, in particular for the mitigation of global environmental changes (Newton et al., 2014) and prediction of processes in lands newly invaded by the sea. Most of studies conducted in saltmarshes are detailed, but limited in scope, considering only few environmental compartments or focusing on a "one way" approach, that is, excluding the contribution of feedback mechanisms (e.g. Möller, 2006; Underwood, 1997). Moreover, several mechanisms are still poorly known and need additional data or more adequate methods for investigation. For example there are no universally accepted protocols available for anoxic soils, which are highly sensitive to oxygen during sampling, storage and laboratory analysis. Implementation of databases and sectorial experiments have to couple with a holistic approach, able to embrace the complexity of saltmarsh environment. Some preliminary steps in this direction have been proposed only in recent years (see e.g. Jjang et al., 2012; Cott et al., 2011) and an extensive research work has still to be carried out to progress toward a systematic application of integrated approaches.

INTRODUCTION



Algae on the peaty soil of a salt pond visible during low tides, *Le Mandragole* saltmarsh, Grado Lagoon.

SOIL-PLANT INTERACTIONS

Soil processes and plant feedbacks are key concepts to understand saltmarsh functioning and are fundamental to reach awareness of soil-vegetation relationships on a large scale.

A scheme of interactions at the soil-plant interface in a periodically submerged soil is represented in Figure 1.



Figure 1. Soil-plant interactions in saltmarsh environment. Consequences of flooding and soil anoxia.

In saltmarshes, soils are generally poorly developed due to regular waterlogging acting negatively on soil development (Elmore et al., 2016). Saturated conditions result in lower redox potential (Reddy and DeLaune, 2008) as a consequence of the establishment of hypoxic (less of 2 mg O_2 L⁻¹) or anoxic conditions in soil, slowing down microbial decomposition. Lack of oxygen implies accumulation of reduced inorganic and organic compounds, as a result of anaerobic microbial respiration and fermentation. Biological reduction processes can be monitored following the soil redox potential. Oxygen is already depleted in the soil gas phase at a redox potential of +330 mV and the reduction of nitrate (NO³⁻) begins at +250 mV, followed by the reduction of manganic ions (Mn⁴⁺) at +225 mV and ferric ions (Fe³⁺) at +120 mV. At negative values, from approximately -75 to -150 mV, sulphate (SO₄²⁻) is reduced producing sulphides. This process is particularly strong

in saltmarshes, due to the great availability of sulphate derived from seawater. On the contrary, the production of methane, coupled to the reduction of carbon dioxide which occurs even lower Eh values from approximately -250 to -350 mV, is not particularly intense. The reduction sequence strictly follows the thermodynamic order, so that the most energetically convenient terminal electron acceptors (TEA) are reduced first. In their oxidised forms, most of them are essential nutrients for plants (e.g. nitrate, iron, sulphate) but their reduction can result in loss or even toxicity (Crooks and Pye, 2000; Ponnamperuma, 1984). Denitrification removes nitrogen from soil limiting plant growth, reduced manganese interferes with enzyme structure, whereas reduced iron and sulphate diminish or inhibit photosynthetic activity (Cronk and Fennessy, 2016). Several studies demonstrate the intrusion and accumulation of sulphides in seagrasses with subsequent limitation in growth (Holmer et al., 2005; Pedersen et al., 2004) Moreover under complete anaerobiosis, fermentation produces soluble toxic organic compounds (e.g. acetic acids, butryc acids or ethanol) contributing to create an even more adverse environment for plants (Pezeshki, 2001).

Hydroperiods, combined to evapotranspiration, influence soil salinity which in turn limits plant growth already at about 100 mM in soil solution (Flowers et al., 1986). Under high salinity (in saltwater wetlands salinity can reach 1 M), plants are less efficient in acquiring water, excess Na⁺ ions inhibit K⁺ uptake and the uptake of CO₂ for photosynthesis is also reduced (Cronk and Fennessy, 2016).

Plants are conditioned by flooding and soil characteristics, but in turn can also actively modify soil properties. The main important feedback is the ability of some species to release oxygen from roots (radial oxygen loss - ROL) by diffusion, in order to restore an aerobic environment and oxidise toxic forms (Koretsky et al., 2008; Gribsholt and Kristensen, 2002). In addition, plants can alter the pH of soil releasing H⁺ and OH⁻ (or HCO₃⁻) ions, a necessary exchange to compensate for an unbalanced cation-anion uptake (Cocco et al., 2013; Burke et al., 2000; Riley and Barber, 1971). The change of redox status and pH of soil can strongly affect the availability of trace metals (Almeida et al., 2004; Windham et al. 2003) and similarly the release of root exudates can acting as complexing agents (Mucha et al., 2005).

Plants influence soil development contributing to soil organic matter accumulation (Laanbroek, 2010), especially at higher elevations where the litter structure is generally more dense and where plants exhibit high C:N ratios in aboveground biomass (Elmore et al., 2016). Aboveground tissues return to the detrital pool with senescence and undergo decomposition. Root exudation of organic compounds (e.g. ethanol, carbohydrates, amino-acids) contributes to soil organic matter and to modify the rhizosphere. Microbial decomposers use then detrital and soil organic matter to recycle energy and carbon, within and outside the saltmarsh system (Koretsky et al., 2008; Mucha et al., 2005; Nielsen et al., 2001).

In saltmarsh environments, all factors are highly variable, at both temporal and spatial scale, making the description and the clarification of interactions challenging.

PLANT STRATEGIES AGAINST SALINITY AND FLOODING

Halophytes inhabiting saltmarshes thrive in these systems thanks to adequate morphological and physiological adaptations to submergence and high soil salinity (Cronk and Fennessy, 2016; Colmer and Flowers, 2008; Flowers and Colmer, 2008).

High soil salinity could seriously affect plant survival. Halophytes tolerate salt concentrations that kill 99% of the other species (Flowers and Colmer, 2008) and at low salinity (50-250 mM) are even stimulated in growth (Flowers et al., 1986). Halophytes commonly develop succulent leaves to dilute internal salt concentration, accumulating Na⁺ and Cl⁻ in vacuoles (Wyn Jones and Gorham, 2002) while metabolically compatible solutes (e.g. sugars, sugar alcohols, amino acids etc.) are accumulated in the cytoplasm to balance the osmotic potential (Rhodes et al., 2002; Hasegawa et al., 2000). When salt concentration is high, some species can shed parts of the plant (leaves) or secret salts from salt glands sited on leaf margins (Flowers and Colmer, 2008).

Flooding tolerance in halophytes is even more important, considering that flooding stress occurs in saltmarshes on a daily base. When soil pores are filled with water, oxygen diffusion in soil is reduced about $3,2\cdot10^5$ times (Armstrong and Drew, 2002) and when plants are completely submerged, access of shoots to oxygen and

 CO_2 is limited too (10⁴ times slower diffusion rate in water, Colmer and Flowers, 2008). Only submerged plants benefit from thinner cuticle to contrast the lower gas diffusion rates under water, and from the use of HCO3⁻ for underwater photosynthesis (Pedersen et al., 2013). Upland plants could at least reduce the palisade tissue in leaves to improve gas exchanges, when acclimated under water (Mommer et al., 2007). Adventitious roots and shallow rooting commonly develop upon waterlogging and shoot elongation could be promoted under complete submergence, in order to restore the contact of shoots with air (Herzog and Pedersen, 2014; Voesenek et al., 2006). Flooding stress promote in both halophytes and glycophytes (not salt tolerant) the development of aerenchyma, which consists in gas spaces forming a virtual longitudinal pathway that facilitates oxygen diffusion from shoots to roots (Colmer, 2003). Aerenchyma production in stems could also provide buoyancy for floating-leaved plant (Kron and Visser, 2012; Colmer, 2003). Internal aeration could be also enhanced by barriers to radial oxygen loss (ROL) in the basal root zone, restricting oxygen losses to the rhizosphere and enhancing longitudinal oxygen translocation (Colmer, 2003). Some wetland species are able to avoid transient anoxia slowing down metabolic activities, while others have enough energy (ATP) for cell metabolism even under anaerobic conditions thanks to large rhizomes detaining reserves (Stunder and Braendle, 1987). In fact, some species move to fermentative metabolism, producing ethanol or lactate when submerged (Colmer and Flowers, 2008; Rivoal and Hanson, 1993). In better adapted species, fermentation pathways can sustain survival under water for many months, converting rapidly soluble sugars for anaerobic processes (Visser et al., 2003).

SALTMARSH VEGETATION

Saltmarsh vegetation is peculiar, considering that few species are able to tolerate the high salinity and the recurrent flooding of these environments. In saltmarsh plant communities, a single or few species usually are dominant (Adam, 1993) and the relative species abundance determines the attribution to a specific type of vegetation (*sintaxa*) (Cazzin et al., 2009). Vegetation composition is driven by the different ecological niches of each species. Plant species appear arranged along the flooding gradient, occupying ecological spaces based on their plasticity (see e.g. Silvestri et al., 2005; Bockelmann et al., 2002). *Sarcocornia fruticosa*, for instance, shows a broad distribution with higher covers in the high saltmarsh, less flooded and more saline (Batriu et al., 2011). *Halimione portulacoides* is an indicator of oxic conditions, preferring soils less frequently flooded, with a redox potential higher than 200 mV. *Spartina maritima* is instead a good indicator of anoxic often flooded environments, while *Puccinellia maritima* of anoxic-peaty soils (Lang et al., 2010; Sanchez et al., 1998).

Generally, species richness increases following the flooding gradient with saltmarsh accretion (Boorman, 2003). It moves from pioneer stages with annual species growing within bar soil (e.g. *Salicornia* sp. and *Suaeda* sp.) to perennial pioneer species like *Spartina maritima*, and even more demanding species like *Puccinellia* sp. and *Sarcocornia* sp. In more elevated areas, species less tolerant to salt, e.g. *Phragmites australis*, can be recorded (Lang et al., 2010; Boorman, 2003). Saltmarsh accretion and the presence of vegetation work with a positive feedback mechanism, where vegetation increases sedimentation which in turn facilitates plant growth (Van de Koppel et al., 2006).

Elevation (Rasser et al., 2013) and soil salinity (Jeppesen et al., 2007) are commonly used to explain the typical micromosaic pattern of saltmarsh vegetation, but literature shows contradictory results regarding both traits (Silvestri et al., 2005; Zedler et al., 1999). Niche species differentiation along these environmental gradients seems not well-defined (Batriu et al., 2011; Lang et al., 2010). Many edaphic factors (C org, pH, soil texture) as well as biotic factors and plant competition (Pennings and Callaway, 1992) may therefore also be important in defining species distribution at small scale (Reddy and DeLaune, 2008; Reed 1988). Bertness and Ellison (1987) proved that *Spartina alterniflora*, dominant in the low marsh, is capable to colonize the upper saltmarsh and its exclusion is determined only by the presence of other more competitive species. Similarly, Puccinellia marittima succumbs to competition when is moved to higher sites (Kiehl et al., 1997). Competition could therefore prevail on zonation (Ford et al., 2016), especially at higher elevations where environmental factors are less intense (Wang et al., 2006; Pennings and Callaway, 1992), and in plant communities with higher diversity (Wright et al., 2016).

Another interesting aspect of saltmarsh vegetation is that both progressive and retrogressive successions are common. Abiotic or biotic disturbances could determine a reverse process on vegetation where loss in cover involves a higher soil erosion and the reversion to a pioneer stage. Especially recurrent flooding determines a non-equilibrium state of vegetation (Tessier et al., 2002) which consequently appears extremely dynamic.

Experimental studies conducted in macrotidal saltmarshes reported the importance of competitive exclusion in determining plants distribution along environmental gradients (Pennings and Callaway, 1992). However, European coastal marshes seem to behave differently, showing coexistence of highly competitive species. European coastal marshes are usually less covered by vegetation compared to the American ones, despite being highly differentiated in terms of species richness (Cronk and Fennessy, 2016).

Different strength of environmental stresses, different origin of sediments, species with diverse ecological needs etc. could hide different mechanisms and relationships among variables. Therefore, studies conducted in saltmarshes cannot be fully generalised.

Mediterranean saltmarshes in particular are subject to irregular microtidal regimes (Costa et al., 2003), micro-scale variations in soil salinity (Adam, 1993; Silvestri et al., 2005) and a long history of anthropogenic disturbance (Curcó et al., 2002). In Mediterranean saltmarshes, the high variability of small scale topography with creeks, ponds and depressed areas, can determine changes of the vegetation pattern within 1 m² or less (Cazzin et al., 2009). Figure 2 shows a typical profile of a Mediterranean saltmarsh bar. Vegetation refer to four main classes: vegetation with annual succulents (*Thero-Suaedetea splendentis*), perennial pioneer grasses (*Spartinetea glabrae*), succulent perennial shrubs (*Sarcocornietea fruticosae*), and maritime grassland (*Juncetea maritimi*) (Cutini et al., 2010; Pandža et al., 2007; Chytrý and Otýpková, 2003).

12



Figure 2. Topography and vegetation pattern in a typical saltmarsh of the Mediterranean region. The red colour highlights the rhizosphere of plants able to release oxygen from roots. Zos=Zoostera marina, Spa=Spartina marittima, Lim=Limonium narbonense, Sar=Sarcocornia fruticosa, Sal=Salicornia patula, Puc=Puccinellia festuciformis, Inu=Inula chritmoides, Sua=Suaeda maritima, Ast=Aster tripholium, Jun=Juncus maritimus, Phr=Phragmites australis.

SALTMARSH SOILS

Anaerobic soils, determined by regular flooding, exhibit peculiar morphological and chemical properties resulting from the typical reduction processes carried out by facultative and obligated anaerobes. They are called hydric soils and can be divided into two main types: frequently waterlogged freshwater or saltwater marsh soils, and permanently flooded subaqueous soils (Reddy and DeLaune, 2008). The description of these soils is still poorly agreed and even the use of the term "soil" to describe the substrates colonized by saltmarsh plants or seagrasses has not been universally accepted as many biologists and marine biogeochemistry scientists still refer to them as "sediments". Pedologists started to describe submerged soils from the 1970s, but up to now most papers (approx. 65% related to emergent plant communities and up to 90% of submerged vegetation) refer still to "sediments" (Kristensen and Rabenhorst, 2015). In fact, even if the decomposition of soil organic matter is slow (Bradley and Stolt, 2006), processes like accumulation of organic carbon, alteration and translocation of materials can also occur under water (Erich and Drohan, 2012).

The classification of submerged soils was officially introduced with the second edition of Soil Taxonomy (Soil Survey Staff, 1999), only in 1999. Two new suborders were then included, *Wassents* for *Entisols* and *Wassists* for *Histosols*, and

defined by a positive water potential at the soil surface for more than 21 hours each day. However, periodically submerged soils of tidal environments are still not well differentiated.

The classification system of the Soil Taxonomy (Soil Survey Staff, 2014) includes most saltmarsh soils in the *Aquent* suborder (order *Entisol*). Regular flooding produces signs in the soil profiles described as morphological features that are used, in combination with physico-chemical properties, to describe hydric soils. Redoximorphic features include colour, odour, organic material content and presence of hydrogen sulphide, and are the most widespread morphological features in saltmarsh soils. These are linked to the mobilization and the oxidation of Fe and Mn, which determine the typical change from yellow-red to grey-black and gley colours when soil is reduced (Fiedler and Sommer, 2004).

Aquents must have one or more of the following:

1. Aquic conditions and sulfidic materials within 50 cm of the mineral soil surface;

2. Permanent saturation with water and a reduced matrix in all horizons below 25 cm from the surface;

3. Aquic conditions for some time in normal years (or artificial drainage), in a layer above a densic, lithic, or paralithic contact or at a depth between 40 and 50 cm below the soil surface, whichever is shallower;

and one or more of the following:

a. texture class finer than loamy fine sand in 50 % or more of the matrix, plus one or more of the following: (1) Neutral colours with no hue (N) and zero chroma; (2) Chroma ≤ 1 and a value, moist, of 4 or more; (3) Chroma ≤ 2 and redox concentrations;

b. texture class of loamy fine sand or coarser, in 50 % or more of the matrix plus one or more of the following:(1) Neutral colours with no hue (N) and zero chroma; (2) Hue of 10YR or redder, a value, moist, of 4 or more, and chroma = 1; (3) Hue of 10YR or redder, chroma ≤ 2 , and redox concentrations; (4) Hue of 2.5Y or yellower, chroma ≤ 3 , and distinct or prominent redox concentrations; (5) Hue of 2.5Y or yellower and chroma = 1; (6) Hue of 5GY, 5G, 5BG, or 5B; c. Enough active ferrous iron to give a positive reaction to alpha, alpha-dipyridyl at a time when the soil is not being irrigated.

Redoximorphic features are therefore a great indicator of soil type and history, being the resulting combination of flooding, soil features and plant feedback mechanisms (Lang et al. 2010, Hinsinger et al. 2003).

The World Reference Base system of soil classification is even less advanced. Recently, Lang et al. (2010) highlighted the lack of a suitable saltmarsh soil classification, which allowed them to classify only two different soil units (*Fluvisols* and *Histosols*) in the northern area of the Venice lagoon, despite the analysed soils differed strongly in redoximorphic features within the same soil unit. This deficiency in soil classification is also characterised by the no constant correspondence between soil type and vegetation, by which abrupt transitions between plant communities are apparently associated with small gradual changes in soil properties (Kristensen and Rabenhorst, 2015; Pennings and Callaway, 1992). However, Lang et al. (2010) successfully used redoximorphic features as a tool to explain changes in oxygen availability in soil and to predict plant species distribution. Different plant communities, grouped according to the most abundant species, were easily linked to different soil descriptions.

THE RELEVANCE OF SULPHIDES IN SALTMARSH SOILS

Sulphur is present in the environment in different oxidation states: as oxidised sulphur (sulphate, sulphite and thiosulphate) and as reduced sulphur (elemental sulphur and sulphide). Several factors regulate sulphur speciation: pH, Eh, temperature, presence of heavy metals or inorganic sulphur, required as electron acceptor, microbial communities present in soil, abundance of organic compounds as electron donors, presence of oxidants, toxic for sulphate reducing bacteria (SRB), soil bioturbation (Lamers et al., 2013; Reddy and DeLaune, 2008). These factors could vary considerably in saltmarsh soils, determining a huge spatial variability of the different sulphur species.

In saltmarshes, sulphur is mostly present as inorganic sulphur minerals, pyrite (FeS₂) in particular, formed by the precipitation of the dissolved sulphides species H₂S and HS⁻ derived from sulphate reduction. Amorphous iron sulphides (FeS), like

mackinawite, are also formed and could be transformed to greigite (Fe₃S₄) and pyrite (FeS₂). In this way, sulphide level in soils does not result in directly toxicity for organisms, considering the low solubility of sulphur minerals (Browuer, 1995).

Even if iron is abundant, free sulphides can strongly accumulate in saline saltmarsh soils, due to the higher sulphate availability. Compared to freshwater systems, sulphate concentration in marine and brackish soils is 10³ times higher (Marschner, 1995). Sulphate reduction is of large interest in saltmarsh environments, considering that the main product H₂S is toxic for fish, invertebrates, plants, algae (Dunnette et al., 1985). Anaerobic obligate bacteria like Desulfovibrio, Desulformanas, Desulfotomaculum find in these anoxic soils ideal conditions for their metabolism (Rickard and Morse, 2005) producing large amounts of sulphides through sulphate reduction. On the other hand, sulphides production may be limited especially at soil-root interface (Lamers et al., 2013; Rickard and Morse, 2005). Sulphides oxidizing bacteria, located inside plant roots or in the rhizosphere determine a detoxification 10^4 times quicker than the spontaneous chemical oxidation (Millero, 1986), and phototrophic bacteria could oxidise sulphide even under anaerobic conditions if light is present (Reddy and DeLaune, 2008). Nevertheless, oxidation is less influent than sulphate reduction, due to the severe conditions affecting marshes.

The presence of sulphides in saltmarsh soils is still rarely highlighted in literature, despite being crucial both for hydromorphic soils description (Soil Survey Staff, 2014) and halophytes growth and distribution (Holmer and Hasler-Sheetal, 2014; Borum et al., 2013).

The main fraction of sulphides is represented by the so-called Acid Volatile Sulphides (AVS), a complex of components ranging from dissolved sulphur species to metastable iron sulphide minerals (Rickard and Morse, 2005). AVS are commonly identified by the typical rotten-egg smell and the black deposits of insoluble metal sulphides, primarily FeS (Browuer, 1995), despite the black colour not necessarily reflects AVS distribution. The AVS fraction is operationally defined as the fraction of sulphides solubilised by soil treatment with cold 6N HCl (with or not SnCl₂) and consist in dissolved sulphur species (HS, H₂S), nanoparticles, FeS clusters in porewaters, mackaniwite (60-70%), greigite and probably small fractions of pyrite (Rickard and Morse, 2005).

The uncertainty around AVS composition is linked to the quick oxidation of iron sulphides when exposed to air and to the partial dissolution of pyrite during acidification of soil samples. Many field and laboratory methods were proposed (see e.g. Chen et al., 2014; Rabenhorst et al., 2010; Anderson and Wilson, 2000), also in response to the increasing interest around sulphides toxicity thresholds (Lamers et al., 2013). Seagrasses (Holmer et al., 2011) and some species of Spartina genus (Koch et al., 1990) seem to tolerate extremely high sulphides concentrations: up to 8 mmol·L⁻¹ in *S. alterniflora* (Lee, 1999). Literature demonstrated that sulphur in plants derived mostly from soil sulphide (Holmer et al., 2009; Frederiksen et al., 2006), which is transported as H₂S within plant tissues through the aerenchyma, especially during the night (Pedersen et al., 2004). Moreover, AVS are used to predict toxicity of metals, through the SEM (simultaneously extracted metals)-AVS approach, despite the limitation to not consider metal speciation during the extraction (McGrath et al., 2002). Therefore, a correct and quantitative measurement for AVS is still required, possibly a field methodology preventing as much as possible oxidation of soil samples, in order to improve data quality and saltmarsh system functioning comprehension.

THESIS' AIM

The aim of this PhD thesis was to obtain more precise and holistic information to explain the multiple interactions between soil and vegetation in saltmarsh systems along environmental gradients, with particular reference to the persistence of flooding periods. Existing literature, in fact, stresses the key role of the hydroperiod in determining soil characteristics and plant distribution in saltmarshes. From this starting point, I focused on soil and vegetation changes along the flooding gradient, with the aim to recognise recurrent relationships and to evaluate the relevance of feedback mechanisms. Secondarily, my research aimed to elucidate some specific aspects related to flooding tolerance in plants and to sulphides production in soil. In fact, literature lacks not only specific information but also suitable analytical methods to apply in these peculiar systems.

Different multidisciplinary approaches were adopted in order to give new insights on: i) saltmarsh soil classification, ii) plant zonation, iii) plant-soil interactions, iv) and feedback mechanisms mediated by vegetation.

The knowledge gained through my work will not only allow a better understanding of these highly biodiverse environments, which are threatened by the rising of sea level due to climate change, but will also be relevant for future restoration programs in coastal ecosystems.

THESIS' STRUCTURE

This thesis is composed of 6 chapters: 2 of them (Chapters 1 and 2) consist of papers already published, 2 of papers were submitted to journals and currently under revision (Chapters 3 and 4), and the last (Chapter 5) of a manuscript ready for the forthcoming submission.

Chapter 1 is a first contribute to link soil pedology and vegetation in saltmarsh systems. Here soil types and plant communities were described along a hydrosequence in order to collect additional data about saltmarshes and recurrent soil-plants relationships. Chapter 2 further proceeds along this insight, moving from a descriptive approach to a quantitative evaluation of consequences that flooding and soil drive on plants, especially those related to nutrient uptake ability. Chapter 3 concludes these stages focusing on a third essential aspect, the possible feedback mechanisms and the role of plants in driving changes towards soil and plant communities. Therefore, Chapter 1, 2 and 3 highlighted the soil-plants relationships based on three different points of view, and reaching interesting achievements based on a holistic approach of the system.

Chapter 4 and 5 are specific contributes aimed to answer some missing knowledge about flooding tolerance in halophytes and to the lack of adequate methods for sulphides evaluation in soils, respectively. These two last chapters highlighted the combined need to move across different scales, in order to elucidate different aspects contributing to saltmarsh soil development and plant zonation.

CHAPTER 1



The flooding effect on plant zonation and the complexity of saltmarsh environment.

PREFACE

This work was carried out with the aim to investigate the mutual effects of soil and vegetation cover along soil hydrosequences, from more elevated areas to subaqueous saltmarsh environment.

Physicochemical characteristics of soils were investigated following changes in plant communities and soil types were described and classified following Soil Taxonomy guidelines. Here, vegetation was considered a consequence of different soil features as well as a primary factor affecting pedogenesis. The key role of flooding and soil texture was confirmed only partially. Results highlighted the relevant contribution of vegetation in differentiating soil types, in particular regarding the ability of some plants to release oxygen from roots, estimated through the description of red-mottles along soil profiles, and the litter deposition, contributing to soil organic carbon.

This paper, published in *Estaurine, Coastal and Shelf Science,* is the result of a collaboration with the pedology group of the Agricultural Science Department of the University of Bologna. My contribute referred mostly to the vegetation analysis, to considerations arising from plant communities pattern and in writings.

Estuarine, Coastal and Shelf Science 173 (2016) 39-48



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Hydromorphic to subaqueous soils transitions in the central Grado lagoon (Northern Adriatic Sea, Italy)

CrossMark

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ABSTRACT

The Grado lagoon is among the largest in the Mediterranean sea and is characterized by salt marshes, where tides influenced the development of a complex micromorphology coupled to a micromosaic of vegetation covers. This study represents the first contribution to the understanding of the main processes governing formation, development and spatial transitions between hydromorphic and subaqueous soils in an Adriatic lagoon ecosystem. Physicochemical characteristics and development of soils were investigated in three salt marshes differing for their proximity to the open sea, textural composition and age of formation. Soils of back barrier salt marshes had A/C profiles and were mostly characterized by a sandy coarse texture that allows rapid drainage and subsurface oxygen exchanges. Soil sequences from the inner salt marsh to its submerged border slope or to a brackish waterhole do not simply represent a hydrosequence, but also reflect erosion/sorting/accumulation processes.

The soils in the central part of the lagoon have finer texture and in displayed transition or cambic horizons. Silty clay loam textures and low positions allowed the development of more severe anoxic conditions and accumulation of sulphides. The tide oscillation strongly contributed to formation of redoximorphic features, intensity of anaerobic conditions but also colonization by different plant communities. Discriminant analysis was performed to identify physicochemical properties which discriminate the different soils according to geo-morphological position and prevailing plants. It confirmed that differentiation of plant communities occurred according to distinct morphological and physicochemical soil properties, but also acted as a primary affecting factor of pedogenesis.

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1. Introduction

Submerged sediments that originate from deposition of transported materials have been considered for a long time as amorphous deposits not affected by pedogenesis. However, accumulation of organic C, alteration and translocation of materials occur also under submerged conditions and in ways that closely resemble terrestrial soil-forming processes (Erich and Drohan, 2012; McCall and Tevesz, 1982). The concept that sediments are capable of supporting rooted plants, and undergo transformation and horizon differentiation, has led soil scientists to consider the action of subaqueous pedogenetic processes (Bakken and Stolt, 2010; Ellis et al., 2002) and rethink the concept of soil.

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Accumulation of nutrients and biogenic CaCO₃, formation of structural aggregates (Barko et al., 1991; McCall and Tevesz, 1982), marine humus bioturbations, chemical transformation of sulfur and iron in anoxic environments were intensively studied (Bradley and Stolt, 2003; Payne, 2007). Pedologists therefore confirmed that superficial sedimentary deposits do evolve into aquatic soil horizons, leading to formation of subaqueous soil profiles (Demas et al., 1996; Demas, 1998; Demas and Rabenhorst, 1999). On the basis of Jenny's state factor equation, a new model for describing subaqueous soil (SAS) genesis was therefore proposed by Demas and Rabenhorst (2001). These observations have been incorporated by the Soil Taxonomy (Soil Survey Staff, 2014) by introducing two new suborders (*Wassents* and *Wassists*) to classify subaqueous soils (SASs), defined as soils submerged for at least 21 h each day by up to 2.5 m of water.

Estuaries and shallow, semi-enclosed coastal lagoons are

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L. Vittori Antisari et al. / Estuarine, Coastal and Shelf Science 173 (2016) 39-48

transitional depositional environments, that cover the boundary between the mainland land and sea (Newton et al., 2014). These landforms represent a continuum of soils with different degrees of hydromorphism, together with large areas of SASs. The combination of these features is fundamental for animal and plant biodiversity, and contributes to the maintenance of equilibrium as well as to a number of ecosystem services, spanning from protection of coastal water quality, to recreation and fish farming (De Groot et al., 2012). SASs may represent important sinks of nitrates and P (Ponnamperuma, 1972) and contribute to C sequestration (Homann and Grigal, 1996), since, under anaerobic conditions, the decomposition of soil organic matter is slow and thick organic-rich horizons may develop (Bradley and Stolt, 2006; Richardson et al., 2001; Stolt and Rabenhorst, 2011). Improving knowledge about SASs can play an important role in devising strategies for the sustainable management of water and coastal resources (Erich and Drohan, 2012). Soil survey may be a very useful key for understanding the formation and evolution of different land units in these very fragile environments (Erich and Drohan, 2012).

The Italian lagoons, and in particular those of the Northern Adriatic Sea, are characterized by salt marshes, sandbanks and subtidal mudflats, which represent important ecosystems for land management and wildlife protection (Ferrarin et al., 2010). The salt marshes, which undergo partial regular flooding at high tide, harbor several halophyte plant species and embody the habitat of several aquatic and migratory birds. Their importance has been highlighted by the European community through the Directive 92/ 43/CEE "Habitat" and the development of Natura 2000, in which the lagoon is considered as special protection area (SPA) and special area of conservation (SAC).

To our knowledge the investigation and classification of salt marsh soils in Italian lagoons has not been carried out so far.

In this work, we examined physicochemical characteristics of hydromorphic and subaqueous soils profiles in the salt marshes of Grado and Marano lagoon. The lagoon (160 km²) spans between the Tagliamento and the Isonzo rivers estuaries and is separated from the open sea by a barrier of islets and sandbanks. It is among the largest European shallow lagoons, second only to the Venice Lagoon, in the Mediterranean.

The aim of this study was to investigate how landscape morphology and vegetation influenced transitions from hydromorphic to subaqueous soils. In this ecosystem, salt marshes are characterized by a complex micromorphology coupled to a micromosaic of vegetation covers that is an intrinsic part of the huge biodiversity of these endangered habitats. For this reason, changes of soil properties were investigated in transects between hydromorphic and subaqueous ecosystems, in different salt marshes of the lagoon and under different plant communities. Extending knowledge about the pedogenesis of submerged soils can provide new concepts on the functioning of intertidal and subtidal habitats that can be useful to evaluate and predict future modifications in a perspective of climate change (Erich et al., 2010).

2. Materials and methods

2.1. Study area

This study focused on three salt marsh transects (Fig. 1), in the middle section of the lagoon (Buso and Grado basins). The salt marsh bars differ for their proximity to the open sea, textural composition and age of formation. The Marina di Macia site (MM) was located on a back barrier salt marsh, which was part of the old longshore bar that separated the Grado basin from the sea. The sampling transect was traced from the inner salt marsh, nowadays protected by a shallow sand ridge (MM-Lim1 and 2, 60 to 50 cm

height above mean sea level), to the marsh border slope (MM-Zos, -50 cm below m.s.l.). The Isole della Gran Chiusa site (GC) was located in the Buso basin, on a channel fringing marsh that has evolved from an ancient enclosed fish farm that had been abandoned long ago, re-invaded by waters and reverted to wilderness. The transect was sampled between a prevalently emerged (GC-Sar 1, 40 cm above m. s. l.) and a more frequently submerged part of the salt marsh (GC-Sar 2, 30 cm above m. s. l.). The third study site (MD) was located in a salt marsh parallel to the Mosconi dike road, which formed from the dredged sediments derived from the excavation of the dike and adjacent Belvedere channel (1902–1920). The transect was sampled in a mostly submerged area (MD-Sp1, 15 cm above mean sea level) and in a permanently submerged internal waterhole (MD-Sp2, 5 cm above men sea level). Altimetric data and ortophotos were obtained from LiDAR cloud points.

2.2. Vegetation structure analysis

Vegetation communities types were individuated observing presence and coverage of prevalent species. Nomenclature of plant species follows the latest Italian check list of vascular plants (Conti et al., 2005).

2.3. Climate and tides

Mean annual temperature is 13.1 °C, while annual precipitation is 1106 mm yr⁻¹. According to the Köppen-Geiger system, the area's climate is classified as "temperate/mesothermal" (Peel and Bloschl, 2011), characterized by the moderating effect of the sea (Michelutti et al., 2003). Climatic data were obtained from the meteorological station of Grado (0 m m.s.l.; about 5–10 km from the study sites). In the lagoon, the tidal mean range is 65 cm, with a spring range of 105 cm. The average salinity of sea water ranges from 20 mg L⁻¹ near the mainland to 35 mg L⁻¹ near the sea (ARPA, 2008). Water temperatures are comprised between 5 and 7 °C in winter and 28–30 °C in summer (Covelli et al., 2009; D'Aietti and Altobelli, 2007; Ferrarin et al., 2010).

2.4. Sampling

Sampling was carried out in summer 2013, during low tides periods. Soil profiles were excavated and genetic horizons described according to Schoeneberger et al. (2012). Samples were sealed in polyethylene bags and stored at 4 °C until analysis.

SASs samples (MM-Zos and MD-2) were collected using a vibracore Beeker sampler, (Eijkelkamp, NL), equipped with a 6 cm polyethylene tube (McVey et al., 2012). The cores were sealed with a tight stopper to avoid oxygen infiltration and stored at 4 °C.

2.5. Analyses of soil profiles

2.5.1. Water analysis

Analysis of sea water were carried out in the field for pH, dissolved oxygen (DO), salinity (SAL) and temperature (T) with portable electrodes (Hach-Lange Instruments). Water samples were collected in pirex glass bottles and analysis where replicated in the laboratory.

2.5.2. Physical and chemical analysis of soils samples

Soil columns were extracted on a suitable support and each genetic horizon was described for its depth, boundary, Munsell color, coats/films and redoximorphic features, organic fragments, fluidity class. Accumulation of sulphides (sulfidization) was observed through color change after adding 3% H₂O₂ (McVey et al., 2012) and odor description (Fanning and Fanning, 1989; Fanning et al., 2002).

L. Vittori Antisari et al. / Estuarine, Coastal and Shelf Science 173 (2016) 39-48



Fig. 1. Geographical location of study area and altimetric profiles of sampled transects in the three different salt mash bars examined: MM = Marina di Macia, GC = Gran Chiusa, MD Mosconi dike. Altimetric data and ortophotos were obtained from LiDAR cloud points.

Effects of reducing conditions on soil color (gleyfication) were investigated by field observations (H_2O_2 test and Munsell color recording). Measurement of pH after soil incubation (16 weeks) was carried out on SASs to detect pH lowering due to acid sulfate oxidation (Bradley and Stolt, 2003; Soil Survey Staff, 2014).

Electrical conductivity (EC) and pH were measured on 1:2.5 w:v (hydromorphic samples) or 1:1 w:v (SASs) (McVey et al., 2012) on moist samples. Soil samples were air-dried and sieved (<2 mm) when required to carry out the following analysis. Particle size distribution was determined by the pipette method (Gee and Bauder, 1986) and total carbonates quantified according to the Dietrich-Fruhling method. Total Organic Carbon (TOC) and Total Nitrogen (TN) were measured with an EA 1110 Thermo Fisher CHN elemental analyzer after dissolution of carbonates with 2 M HCl. Total Fe and S were quantified by ICP-OES (Ametek, Germany) after aqua regia digestion in a Millestone 1200 microwave oven (Vittori Antisari et al., 2011). The cation exchange capacity (CEC) was determined by shaking 2.5 g of soil for 2 h with 50 mL of 0.05 N [Co(NH₃)₆)Cl₃ (Ciesielski and Sterckeman, 1997). Samples were filtered (Watmann 42 filter paper) and CEC (cmol kg⁻¹) was estimated measuring the Co2+ remaining in solution by ICP-OES (Aran et al., 2008).

2.6. Statistical analysis

Discriminant Function Analysis (DFA) was performed following a forward stepwise approach to discriminate soils according to their location and prevalent vegetation cover. The statistical significance of the discriminant function was checked with Wilk's lambda test. The standardized canonical discriminate coefficients (SCDC) were used to rank the importance of each variable and the canonical score plot displayed the different samples according to the two dimensions that best separate the three groups.

41

3. Results

3.1. Soil climatic and morphological characterization

For permanently emerged soils in this area (not examined in this study), the water balance (Black, 2007), shows a limited deficit from July to August. Processing of climatic data by the Newhall Simulation Model (Cornell University, 1991; Newhall, 1972; Van Wambeke, 2000) defines a *Mesic* temperature regime (annual average soil temperature between 8 and 15 °C, with a difference between summer and winter above 5 °C) and a *Udic* soil moisture

L. Vittori Antisari et al. / Estuarine, Coastal and Shelf Science 173 (2016) 39-48



Fig. 2. Textural triangle representation of the textural distribution of soil profiles in the three different salt marshes, GC = Gran Chiusa, MD = Mosconi dike, MM = Marina di Macia.

Table	1									
Inon	÷									
Mean	chemical	properties	of the	Marina	di Macia	salt	marsh	(MM) soil	profiles	÷

Profile	Horizon	Depth cm	pH		EC dSm ⁻¹	CaCO ₃	TOC	TN	S	Fe	CEC mol+ kg-1	C/N	OC/S	Fe/S
			Initial	Final		g kg ⁻¹								
MM-Lim1	Oe	3-0	nd	6.7		35	99.8	7.6	nd	nd	nd	13.0	nd	nd
	AI	0-5	nd	7.7	16.5	374	12.3	1.4	5.2	26.2	38.0	8.6	2.4	5.0
	A2	5-10	nd	7.8	30.9	108	48.1	3.8	1.2	10.0	10.7	12.5	39.1	8.1
	Ab	10-17	nd	8.0	8.5	366	15.5	1.9	2.8	26.0	15.0	8.2	5.5	11.0
	C	17-30	nd	8.3	6.1	759	2.7	0.8	0.4	2.2	5.8	3.4	6.6	5.4
	Cg1	30-80	nd	8.3	5.3	800	1.6	0.2	0.4	1.7	7.1	8.4	4.1	4.3
	Cg2	80-100	nd	8.1	4.7	443	2.5	0.7	2.4	7.3	4.7	3.5	1.1	2.6
MM-Lim2	0e	1.5-0	nd	7.2		nd	98.8	7.6	nd	nd	nd	13.0	nd	nd
	AI	0-3	nd	7.6	35.0	227	46.6	5.2	2.9	13.0	24.6	8.9	16.1	4.5
	A2	3-6	nd	7.5	16.5	664	5.2	1.1	0.6	3.9	8.5	4.6	8.8	6.7
	Ab	6-20	nd	8.0	8.5	357	13.5	1.9	1.3	9.6	14.4	7.1	10.6	7.6
	C	20-35	nd	8.3	6.1	759	2.4	0.8	0.4	2.3	5.7	2.9	6.5	6.3
	CgI	35-70	nd	8.3	5.3	800	1.3	0.2	0.4	1.7	6.2	6.2	3.6	4.7
	Cg2	70-100	nd	8.1	4.7	422	2.7	0.7	0.4	1.8	4.9	4.1	6.6	4.4
MM_Sar1	AI	0-0.5	nd	7.7	41.3	195	73.2	6.1	5.1	17.1	18.5	10.3	14.3	3.3
	A2	0.5-1.5	nd	7.6	37.6	299	30.3	3.4	4.3	14.6	10.0	8.9	7.1	3.4
	C	1.5-15	nd	8.4	5.9	348	0.7	0.6	0.3	3.2	3.4	1.3	2.3	10.3
	AC	15-20	nd	7.5	27.7	141	26.5	2.6	2.3	20.4	17.4	10.3	11.5	8.9
	ACse	20-35	nd	7.7	22.0	151	22.8	2.6	3.2	17.5	14.2	8.9	7.1	5.4
	Cg	35-65+	nd	7.9	9.3	699	1.9	0.5	0.7	3.0	6.3	4.1	2.9	4.5
MM-Sar2	AT	0-0.5	nd	7.0	46.4	294	74.1	6.3	4.7	8.0	19.2	11.7	15.9	1.7
	A2	0.5-1.5	nd	7.5	39.7	324	47.3	4.2	3.5	10.3	7.5	11.1	13.3	2.9
	C	1.5-20	nd	8.4	3.5	802	1.3	0.2	0.3	1.8	5.8	5.4	4.8	6.8
	ACse	20-32	nd	7.3	28.3	170	34.4	2.7	3.7	12.9	17.7	12.6	9.2	3.4
	Cg1	32-35	nd	8,2	6.0	750	1.7	0.4	0.4	2.8	6.7	3.9	4.0	6.8
	Cg2	35-70+	nd	8.2	6.0	750	1.4	0.2	0.5	2.9	6.6	6.4	2.5	5.2
MM-Zos	Oig	0.2-0	nd	nd		613	97.8	8.1	nd	nd	nd	12.0	nd	nd
	Ase	0-12	8.3	7.0	6.14	618	33.0	5.1	2.1	4.9	7.0	6.5	15.9	2.4
	ACse	12-22	8.2	7.1	3.42	170	40.0	4.3	3.1	5.7	8.2	9.3	12.9	1.8
	O/Cg1	22-37	8.6	7.5	3.30	80	53.2	7.3	3.9	22.9	17.1	7.3	13.8	5.9
	O/Cg2	37-52	8.5	7.6	4.05	90	66.1	8.5	3.9	28.3	20.0	7.8	17.1	7.3
	Cg1	52-63	8.4	7.5	4.60	609	71.0	9.1	3.5	20.7	17.6	7.8	20.2	5.9
	Cg2	63-67+	8.1	7.4	4.12	52	22.0	7.2	0.8	4.7	8.2	3.1	25.9	5.5

CEC = cation exchangeable capacity.

regime (Soil Survey Staff, 2014) with more than 90 days of noncumulative rainfall.

The soils were poorly developed, being characterized by A/C profiles, except for the soils at the Gran Chiusa (GC-Sar1 and GC-Sar2), in which transition or cambic horizons (Bg) were also recognized. All soils were characterized by organo-mineral A horizons, ranging from 8 to 22 cm, which in some cases were covered by thin organic horizons. The morphological characterization of hydromorphic and submerged soil profiles are shown respectively in Tables 1S and 2S of Supplementary Materials.

Clay content was below 40% and silt below 60% at all sites in all soil horizons (Fig. 2). Sand predominated in the deeper horizons of the MM hydromorphic soils, even if layers enriched with silt were observed at different depths. In these soil profiles (MM-Lim1-2 and MM-Sar1-2), the sand content was larger than in the submerged profiles (MM-Zos) of the same transect, in which deposited silt and clay associated with accumulation of organic materials, overlay the deeper carbonatic sand layer (Table 1). The Gran Chiusa and Mosconi Dike soils had silty clay textures, with sand intercalations in some of the horizons (Fig. 2).

At Marina di Macia (MM), proceeding along a transect from the inner part of the bar to the edge of the sea inlet, the vegetation cover is represented by three different plant communities. The MM-Lim1 and MM-Lim2 soil profiles were collected in areas flooded only twice a day, during the high tides, where *Limonium narbonense* was the dominant species, having the largest percentage cover. On the contrary, *Sarcocornia fruticosa* shows higher coverage in MM-Sar1 and MM-Sar2 sites, situated in slightly lower areas (see profile in Fig. 1). In the adjoining submerged area (MM-Zos), the eelgrass *Zostera noltii* was dominant, in association with few
L. Vittori Antisari et al. / Estuarine, Coastal and Shelf Science 173 (2016) 39-48

Table 2						
Mean chemical	properties of	the Gran	Chiusa salt	marsh (0	GC) soil	profiles.

Profile	Horizon	Depth cm	pH (H ₂ C))	EC dSm ⁻¹	CaCO ₃	TOC	TN	5	Fe	CEC mol* kg-1	C/N	OC/S	Fe/S
			Initial	Final		g kg ⁻¹								
GC-Sar1	Oe	1-0	nd	7.2	10000-00	52	61.1	5.1	nd	nd	nd	12.0	nd	nđ
	AI	0-5	nd	7.0	26.7	23	43.5	3.9	2.6	42.0	22.1	11.2	17.0	16.4
	A2	5-10	nd	7.6	16.9	72	14.0	1.4	1.0	33.1	14.6	10.2	14.3	33.6
	Bg	10-25	nd	7.8	12.9	124	7.1	1.3	0.8	34.8	17.0	5.3	9.3	46.0
	AC	25-50	nd	7.9	12.5	106	7.4	1.0	1.0	35.3	16.9	7.6	7.5	35.7
	Cg1	50-60	nd	7.8	13.9	103	9.1	1.2	2.3	34.6	21.8	7.4	3.9	15.0
	Cg2	60-80	nd	7.7	15.0	75	11.8	1.3	5.7	35.9	20.8	9.2	2.1	6.3
	Cse3	80-110	nd	7.8	17.5	72	12.3	1.3	9.4	42.1	24.0	9.1	1.3	4.5
	Cse4	110-120+	nd	7.4	16.6	71	21.5	1.8	11.9	38.4	23.7	11.7	1.8	3.2
GC-Sar2	AI	0-0.5	nd	7.3	33.4	29	37.1	3.6	3.9	37.0	15.7	10.3	9.5	9.5
	A2	0.5-5	nd	7.2	17.8	38	28.5	3.0	1.6	40.5	25.4	9.6	17.6	24.9
	Bg	5-10	nd	7.7	12.0	48	13.2	1.6	0.8	42.3	21.4	8.4	17.1	54.5
	ACT	10-18	nd	7.8	9.5	76	8.1	1.2	0.6	43.2	19.8	6.9	12.5	66.3
	AC2	20-55	nd	7.9	9.9	100	6.2	1.1	0.6	39.5	17.3	5.8	10.3	65.6
	Cg1	55-75	nd	7.8	13.9	100	8.7	1.0	1.6	31.5	20.9	8.6	5.3	19.2
	Cse2	75-85	nd	7.7	15.0	81	11.1	1.2	4.1	32.8	21.3	9.3	2.7	7.9
	Cse3	80-95+	nd	7.8	17.5	79	12.2	1.3	9.3	42.7	24.1	9.0	1.3	4.6

CEC = cation exchangeable capacity.

Table 3

nation of

Mean chemical properties of the Mosconi Dike Road salt marsh (MD) soil profiles.

Profile	Horizon	Depth cm	pH (H ₂ C))	EC dSm ⁻¹	CaCO ₃	TOC		S	Fe	CEC mol+ kg-1	C/N	OC/S	Fe/S
			Initial	Final		g kg ⁻¹								
MD-Sp1	0/Ag1	0-8	7.0	6.3	4.72	38	56.1	5.4	6.6	27.7	25.7	13.7	8.5	4.2
	Ag2	8-13	7.6	7.2	9.69	194	15.9	1.9	4.6	17.6	19.0	8.3	3.4	3.8
	Cg1	13-30.5	7.6	7.3	8.10	248	13.1	1.5	8.7	17.7	18.6	8.6	1.5	2.0
	Cg2	30,5-41	7.6	7.3	5.91	270	13.5	1.5	6.9	16.5	17.6	9.0	2.0	2.4
	Cg3	41-46	7.9	7.5	7.35	323	9.1	1.0	7.6	14.7	14.0	8.8	1.2	1.9
	Cse4	46-66.5	7.7	7.5	4.73	199	13.7	1.6	10.1	18.8	19.4	8.8	1.3	1.8
	Cse5	66.5-80	7.7	7.5	6.74	181	18.4	1.8	12.9	22.0	22.8	10.1	1.4	1.7
	Cse6	80-87+	7.7	7.6	7.60	200	21.3	2.2	12.4	21.0	23.9	9.6	1.7	1.7
MD-Sp2	Oig	0.5-0	nd	nd		nd	92.7	7.9	nd	nd	nd	11.7	nd	nd
0.0000000000000000000000000000000000000	Ag1	0-1.5	7.9	7.1	9.58	111	29.3	3.1	10.4	37.8	8.6	9.5	2.8	3.6
	Ast2	1.5-4.5	7.9	6.9	9.38	56	55,7	5.0	12.5	42.6	17.0	11.2	4.4	3.4
	Ag3	4.5-8	7.7	6.5	9.93	68	52.8	4.7	17.2	47.6	17.0	11.2	3.1	2.8
	A/Cg	8-11	8.0	7.3	6.88	196	6.0	0.9	5.4	20.0	12.7	6.7	1.1	3.7
	Cse1	11-15.5	8.8	7.3	4.57	150	14.6	2.0	10.8	36.1	19.0	7.4	1.3	3.3
	Cse2	15.5-27.5	7.9	7.3	6.34	173	13.5	1.9	11.7	36.7	19.0	7.0	1.1	3.1
	Cse3	27.5-60+	7.9	7.3	6.55	198	15.5	1.7	12.3	39.4	19.0	9.1	1.3	3.2

CEC = cation exchangeable capacity.

species of both red (e.g., Gracilaria genus) and green algae (e.g. Ulva genus).

At the Gran Chiusa salt marsh (GC), *L. narbonense* was the dominant species in the GC-Sar1 site, whereas the GC-Sar2 showed a slight different species composition, with a similar abundance of both *L. narbonense* and *S. fruticosa*.

At the Mosconi dike salt marsh (MD), sampling concerned areas having the lowest maximum height with respect to the mean sea level among all the hydromorphic soils examined. The dominant species on the MD-Sp1 soil was *Spartina maritima*, while the MD-Sp2 profile refers to a confined brackish waterhole (*chiaro*). In this site with some *S. maritima* individuals and below shallow water, a thin (0–0.2 cm) dense orange-red film of organic fibers (fibric material) covered the surface.

3.2. Physicochemical characterization

The physicochemical characteristics of hydromorphic and SASs soils profiles of MM, GC and MD salt marshes are respectively shown in Tables 1–3.

In all soils, pH at sampling ranged between 6.7 and 8.6; in SASs the pH values determined after 16 weeks of wet incubation

decreased up to 1.3 pH units. Electrical conductivity decreased with depth in both hydromorphic and submerged soils. In the deeper horizons (C and Cg) of the hydromorphic soils low EC is determined by infiltration of non-saline waters, whereas in the upper horizons of hydromorphic soils accumulation of salts is driven by surface evapotranspiration. Total carbonates content was larger in MM soils, which are located nearer to the lagoon inlets, being associated with the carbonatic sands deposited by the Adriatic sea. Generally, total organic C and N decreased with depth, but in MM soils, intercalations and accumulations of organic matter were observed at different depths along the soil profiles. In the MM-Lim profiles, a consistent accumulation of organic materials derived from the decomposition of roots and biomass residues (Tables 1 and 1S) was observed in the A2 and 2Ab horizons at depths between 5 and 20 cm, whereas in the MM-Sa profiles it occurred in past surface horizons (AC and ACse horizons of a buried soil) between 15 and 32 cm. In the MM-zos soil, TOC peaked between 52 and 63 cm below the surface. The C/N ratio ranged from 10 to 14 in all superficial horizons, independently from the soil vegetation cover. Generally the C/N ratio decreased with depth, although not always in a regular way.

In the GC and MD soils, dark sulphide bearing materials were



Fig. 3. Scatterplot between total sulfur (S) and iron (Fe), expressed as g kg⁻¹, considering all soil profiles examined.

detected, upon treatment with H2O2, in horizons laying below the mean low tide level. Their depth varied with soil elevation and in the submerged MM-Zos, they were also present in the top soil. Total S content ranged from 17.7 to 1.1 g kg⁻¹ in the present superficial layers (e.g. A, Ag horizons) and in those belonging to buried surfaces of old soils (Ase horizons) and from 12.2 to 0.2 g kg⁻¹ in deeper ones (e.g. C, Cg and Cse horizons). C/S ratios varied with depth and were larger at the surface in hydromorphic soil profiles, whereas in the MD-Spa2 and MM-Zos pedons larger values were found deeper along the profile. Plotting Fe and S contents of soil horizons (Fig. 3) yielded a strong linear correlation ($R^2 = 0.72$) for the MM and MD soils (including both submerged soils) which highlights a link in the accumulation of these two elements. The strongest S accumulation was found in MD soil horizons. A large excess of iron (Fig. 3) was detected in the GC pedons, which displayed Fe concentrations ranging between 32 and 43 g kg⁻¹.

3.3. Classification of hydromorphic and SASs profiles

According to their water saturation regimes, the soil profiles ranked into the Aquents and Wassent suborders (Soil Survey Staff, 2014). Nearer to the open sea, MM-Lim1 and 2 soils displayed the same sequence of horizons (O/A/Ab/C) and were classified as Typic Psammaquents, because of their sandy texture and presence of less than 35% of rock fragments. MM-Sar1 and Sar2 were characterized by accumulation of sulphides within 50 cm of the mineral soil surface as testified by their strong rotten egg smell and soil color change tests carried out in the field. These soils did not show any pH change upon exposure to oxygen, due to the buffering of carbonates, so they had to be classified as Typic Psammaquents, in spite of the large presence of sulphides. On the same salt marsh bar, the MM-Zos soil was classified as a Typic Fluviwassent because of a positive water potential during more than 21 h per day. This soil was characterized by the presence of a 41 cm thick buried layer enriched in organic materials (Table 1).

At the Gran Chiusa salt marsh, both GC-Sar1-2 pedons were classified as *Typic Fluvaquents* due to an irregular TOC profile within a depth of 25 cm. They were also characterized by the presence of sulphide bearing materials within 50 cm from the mineral soil surface (Table 2).

In the inner part of the Mosconi dike salt marsh bar, soil MD-Sp1 displayed an irregular decrease in organic C between 25 cm and a depth of 100 cm, and sulphide bearing materials with a combined thickness of at least 15 cm within 50 cm of the surface, was classified as *Typic Hydrowassents* because of its longer daily submergence. In the lowest part of the transect, MD-Sp2 was again classified as *Typic Hydrowassents*, since it has an irregular decrease of organic C. This soil has horizons with a combined thickness of a least 15 cm within 100 cm of mineral surface that contain sulphide bearing materials (Table 3), which do not exhibit pH changes upon exposure to oxygen.

3.4. Characterization of salt marshes ecosystems

Discriminant function analysis was performed to identify the continuous variables related to the physicochemical properties which could discriminate the different soils according to their geomorphological position in the lagoon and their prevailing vegetation cover.

Differences among salt marsh soils were investigated, according to two independent functions and both standardized coefficients included in the model and the canonical score plot: Fig. 4 provides an evaluation of the separation of the different salt marsh soils according to their position.

Function 1, was driven by TOC and N as positive SCDC and by EC and clay as negative factors. This allows to discriminate the older MM pedons, more rich in TOC, from those of the CG and MD salt marsh bars, which were characterized by larger total S and clay contents. Function 2 discriminated CG pedons from MD ones: total S content and EC were respectively identified as negative and positive coefficients. The analysis underlines the strong differentiation among the three salt marshes.

The second discriminant analysis highlights the influence of vegetation (Fig. 5). Function 1, mainly driven by EC in the positive

L. Vittori Antisari et al. / Estuarine, Coastal and Shelf Science 173 (2016) 39-48



Fig. 4. Canonical score plot of discriminant function analysis (DFA) among the main physicochemical variables of the representative soil profiles and profile locations.



Fig. 5. Canonical score plot of discriminant function analysis (DFA) among the main physicochemical variables of soil profiles and dominant plant species.

sector and by TOC as the negative one, outlined the separation of a great group characterized by the dominant coverage of *S. fruticosa* and *L. narbonense* from soils with either submerged or prevalently submerged species. Moreover, according to Function 2, the soil covered by *Z. noltii* (MM-Zos) was discriminated from the sites with dominant *S. maritima* in the MD salt marsh bar by EC in the positive sector and by S and TOC contents in the negative one.

Again according to Function 2 a distribution of soils both above and below the central line appears associated to the dominant presence of *S. fruticosa*, whereas *L. narbonense* was mainly confined in the positive according to Function 2 (EC) and in the negative according to Function 1 (TOC).

4. Discussion

The formation of salt marshes in the Grado and Marano Lagoon, occurred since the 4th century AD and originated from different marine and riverine sedimentation processes (Brambati, 1970). During time, some areas became permanently emerged, while others are now constantly submerged, contributing to the formation of tidal channels and subtidal zones (Ferrarin et al., 2010). In the study area, which lays far from riverine input sources, the distance from the open sea influences soil texture and soil development as highlighted by the first discriminant analysis (Fig. 4).

The back barrier MM salt marsh lies parallel to the sea and was formed by accumulation of calcareous sand deposits of marine origin, which are the main components of the parent material in C horizons. Based on discriminant analysis, the low Fe content and the high EC in the superficial horizons confirm both the influence of marine water intrusion and the accumulation of salts near the soil surface due to evaporation (Cidu et al., 2013; Rose and Waite, 2003). This trend is enhanced at the surface of soils located at higher position in the salt marsh microrelief.

The soil development from the SAS (MM-Zos) to the hydromorphic soils (MM-Sar and MM-Lim) is linked to an increasing accumulation of TOC on the topsoil, which ended with the formation of a thin organic O horizon in the upper soil profiles covered by *L narbonense*. However, the discontinuous distribution of TOC, the presence of mottles, coats and organic fragments in deep horizons of MM soils, suggest that an intense combination of erosionsedimentation events has occurred in the past (Bellucci et al., 2007).

This hypothesis is confirmed by C/N ratios which, in all soils, are little affected by plant cover and decrease irregularly along the soil profile. This index, which is a typical indicator of organic matter transformation, highlights that in these soils microbial activity is intense and that humification occurs more similar to well aerated soils (C/N = 10) than to anaerobic freshwater organic soil environments (C/N > 30). Even the deeper horizons of the MM-Zos soil display low C/N ratios which suggest that organic matter underwent decomposition before the occurrence of some dramatic subsidence or erosion/translocation event.

This hypothesis is further supported by CEC values of organic materials in mineral horizons, calculated from linear regressions (see Fig. 1S of supplementary materials) between CEC and TOC of the hydromorphic soils of the MM saltmarsh, where soils contain very little clay, Regression ($r^2 = 0.83$) for the MM-zos soil yielded a CEC of 274 cmoles_{c+}/kg TOC very close to that (283 cmoles_{c+}/kg TOC calculated for the hydromorphic soils of the same saltmarsh ($r^2 = 0.72$).

The strongly reduced (gley) C horizons of MM and GC hydromorphic soils as well as all the horizons in MD-Sp1-2 are characterized by C/S ratios below 5. Ivanov et al. (1989) pointed out that soils under recurrent or permanent anaerobic conditions have low C/S ratios that indicate accumulation of reduced sulfur forms. At present Soil Taxonomy does not provide for a way to recognize soils that have accumulated mineral iron sulfide phases, but which also contain substantial carbonates that would neutralize the acidity generated during oxidation. For this reason, in spite of the fact that sulphides accumulation reflected important pedogenic pathways, within these profiles, the GC-Sar1-2 pedons could not be classified in the sulfic great groups nor the MD-Sp2 pedon in the *Sulfiwassent* suborder.

The submerged MM-Zos, was the only pedon which featured large C/S ratios in horizons with bluish black to bluish gray color (Gley 2) in the deeper part of the profile. This feature can be explained by occurrence of footslope accumulation of upslope eroded materials, rich in organic matter and by the soil's mostly coarse sandy texture which permits diffusion of fresh water from the phreatic zone. The sequence from the inner MM salt marsh to its edge may not simply represent a soil hydrosequence, but reflects erosion/sorting/accumulation processes as in the Milne's second type of catena (hillslope with more than one type of parent rock). The evaluation of these phenomena, however, is made difficult by the on-going process of allochthonous sand sedimentation from the sea running from the MM-Zos surface to the MM-Sar and MM-Lim profiles (see Supplementary materials).

The silty clay loam texture and the high Fe content in GC soils suggest their formation was more influenced by sedimentation of re-suspended silt deposits of riverine particulates (Poulton, 2002) and soils are enriched in Fe due to weathering processes (Krachler et al., 2005). These soil sequences show pronounced accumulation processes linked not only to the reduction of sulfate, but also to the reductive dissolution of iron. The clay-silty texture of these soils, in fact, affects the diffusion of oxygen, allowing the onset of more severe anaerobic conditions, which are testified by accumulation of dark bluish-black sulphide bearing materials. These conditions, allow the formation of cambic horizons of at least 15 cm characterized by an increase of the Fe/S ratio, due either to a depletion of S above Cg and Cse horizons (in GC-Sar1) or to coatings of sesquioxides linked to the presence of redoximorphic features without any apparent Fe losses (GC-Sar2). The presence of many redoximorphic features up to 50 cm highlights the effect of the tide oscillation which induce the alternation of oxic/anoxic conditions, whereas in the deepest and permanently anoxic horizons, a stronger accumulation of sulphides occurs as a consequence of the reaction between sulphides and different reduced forms of Fe (Rickard and Morse, 2005).

At the MD salt marsh sites, the long submergence periods and finer clay-silty texture promote permanently anoxic conditions and accumulation of sulphide bearing materials in horizons deeper than respectively 46 cm (MD-Sp1) and 11 cm (MD-Sp2). MD-Sp2 in fact lays in one of the micro closed round basins (waterholes, *chiari*), which originate from subsidence due to structural failures associated to ground water fluctuations (Wysocki et al., 2012) and are typical of this type of salt marsh bars. The transition from the MD-Sp1 to the MD-Sp2 soil, highlights a much finer stratification derived from repeated accumulation of sorted materials, carried by surface flow of tidal or rain water. Permanently submerged conditions lead to enhanced TOC, Fe and S accumulation in the submerged profile compared to the surrounding hydromorphic soil.

It is well known that submerged mineral soils develop redoximorphic features due to the reduction, translocation and/or oxidation of iron and manganese oxides. Gley Munsell colors are associated to pale green reduced forms of Fe(II) in silicate minerals (Vepraskas and Fulkner, 2001) and are present in all soils starting from the level of the high tide. Redoximorphic features, associated with living roots, were observed in some gleyed horizons, confirming the great power of some plants to release oxygen and prevent anoxic conditions in the rhizosphere (Génin et al., 1998; Richardson et al., 2001).

The presence of several fragmented vegetation patterns in salt marshes is attributed to the degree of soil hydromorphism and sea water level (Silvestri et al., 2005).

Discriminant function analysis (Fig. 5) points out TOC and N as strong positive driving factors in the differentiation of soil properties following plant communities distribution. This highlights the action of feedback mechanisms among salt marsh soils and vegetation cover. Colonization by *L. narbonense* allows the development of well-structured organic and C-rich organo-mineral superficial layers, and strongly affects hydromorphic soil conditions through evapotranspiration, improved drainage and oxygen translocation to the root system. In soils submerged by shallow water, *S. maritima* contributes to differently characterize the submerged environment. Contrary to *L. narbonense*, the root system of *S. maritima* favors oxygen diffusion only near the surface because of its shallow root development (Pedersen et al., 2013; Zhang et al., 2006; Zuo et al., 2012).

On the other hand, its felt root system still allows for accumulation of poorly humified organic matter (Ding et al., 2010; Vann and Megonigal, 2003) and, according to its lower ability to accumulate C (Tables 1 and 3) and to diffuse oxygen (Pedersen et al., 2013; Zhang et al., 2006; Zuo et al., 2012), trigs a slower pedogenetic process.

5. Conclusions

Distinct pedogenetic processes affect the formation of closely located micro-environments on salt marshes due to a complex interaction of morphological, physical, chemical and biological factors.

The sandy coarse texture, which characterizes some back barrier saltmarshes, allows a more rapid drainage and subsurface oxygen exchange, and leads to less developed profiles in comparison to the silty-clay textured soils located farther from the sea. The genesis of soils in the Grado and Marano lagoon depends only in part on sediment type and length of submergence periods: the particular salt marsh morphology and the specific contribution of the vegetation are strong effecting factors that contribute to differentiate soils. Transitions from hydromorphic to submerged soils are particularly made complex by erosional/depositional processes that are acting also at micromorphological levels. Accumulation of sulphide bearing materials is a relevant pedogenetic process in these soils, but at present the Soil taxonomy, which focuses on the acidification potential of these materials, does not provide satisfactory ways to differentiate soils which also contain substantial carbonates.

Our study shows that vegetation plays an important role in the development of soils, also in these environments, by affecting organic matter accumulation and its distribution along the soil profiles. At the same time, a clear relationship exists between vegetation species and hydromorphology, confirming that these are non-independent variables of the system. The biodiversity

46

observed within the same salt marsh is due to a very complex feedback mechanism: hydromorphic features influence the colonizing vegetation, which is subsequently modified by biologically driven factors as evapotranspiration, accumulation of C, transfer of oxygen to roots, stabilization and improvement of soil structure.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http:// dx.doi.org/10.1016/j.ecss.2016.02.004.

References

- Aran, D., Maul, A., Masfaraud, J.-F., 2008. A spectrophotometric measurement of soil cation exchange capacity based on cobaltihexamine chloride absorbance. Comptes Rendus Geosci. 340, 865-871. http://dx.doi.org/10.1016/ Comptes Rendu
- JATE, 2008/07/07, PA, 2008. Sistema georeferenziato GIS e monitoraggio delle barriere artificiali sommerse. Agenzia Regionale per la Protezione dell'Ambiente del Friuli Venezia Giulia: Gestione sostenibile delle risorse alieutiche marine e lagunari. AR
- ken, T., Stolt, H., 2010. Freshwater subaqueous soil survey investigations and applications. In: Annual Meeting of the Soil Science Society of America, Soil Science Society of America, International Annual Meeting, November 1-3, Long Bakk each CA
- Barko, J.W., Gunnison, D., Carpenter, S.R., 1991. Sediment interactions with sub-mersed macrophyte growth and community dynamics. Aquat. Bot. 41, 41–65. http://dx.doi.org/10.1016/0304-3770(91)90038-
- http://dx.doi.org/10.1016/0304-3770(31.960038-7.
 Bellucci, L.C., Frignani, M., Cochran, J.K., Albertazzi, S., Zaggia, L., Cecconi, G., Hopkins, H., 2007, 210Pb and 137Cs as chronometers for salt marsh accretion in the Venice Lagoon links to flooding frequency and climate change. J. Environ. Radioact. 97, 85–102. http://dx.doi.org/10.1016/j.jenvrad.2007.03.005.
 Black, P.E., 2007, Revisiting the Thornthwaite and Mather water Balance1. JAWRA J. Am. Water Resour. Assoc. 43, 1604–1605. http://dx.doi.org/10.1111/j.1752-1688.2007.00132
- 007.00132
- Bradley, M.P., Stolt, M.H., 2003. Subaqueous soil-landscape relationships in a Rhode Island estuary. Soil Sci. Soc. Am. J. 67, 1487. http://dx.doi.org/10.2136/ sssaj2003.1487.
- Bradley, M.P., Stolt, M.H., 2006. Landscape-level seagrass-sediment relations in a coastal lagoon. Aquat. Bot. 84, 121-128, http://dx.doi.org/10.1016/ j.aquabot.2005.08.003.
- Jaquabot.2005.05.005. Brambati, A., 1970. Provenienza, trasporto e accumulo dei sedimenti recenti nelle lagune di Marano e di Grado e nei litorali tra i fiumi Isonzo e Taglia della Soc. Geol. Ital. 9, 281-329.
- della Soc. Geol. Ital. 9, 281–329. u, R., Vittori Antisari, L., Biddau, R., Buscaroli, A., Carbone, S., Da Pelo, S., Dinelli, E., Vianello, G., Zannoni, D., 2013. Dynamics of rare earth elements in water-soil systems: the case study of the Pineta San Vitale (Ravenna, Italy), Geoderma 193–194, 52–67. http://dx.doi.org/10.1016/j.geoderma.2012.10.009. sielski, H., Sterckeman, T., 1997. Determination of cation exchange capacity and exchangeable cations in soils by means of cobalt hexamine trichloride. Agronomie 17, 1–7. Cidu

- Agionomie 17, 1–7.
 Conti, F., Abbate, G., Alessandrini, A., Blasi, C., 2005. An Annotated Check-list of the Italian Vascular Flora. Palombi ed., Roma.
 Cornell University. 1991. Newhall (Soil Climate) Simulation Model. (BASIC Version).
 Covelli, S., Acquavita, A., Piani, R., Predonzani, S., De Vittor, C., 2009. Recent contamination of mercury in an estuarine environment (Marano lagoon, Northern Adriatic, Italy). Estuar. Coast. Shelf Sci. 82, 273–284. http://dx.doi.org/ 10.1016/j.ecesp.2009.01.021 10.1016/i.ecss.2009.01.021.
- D'Aietti, A., Altobelli, A., 2007. Fish farming in Grado Lagoon: impacts and dynamics of two fishfarm. In: Guidelines and Case Studies for the Management of Natura 2000 Sites in Transitional Environments. TRIESTE, EUT., Grado (GO) pp. 224–238. (GO),
- pp. 224–238.
 De Groot, R., Brander, L., van der Ploeg, S., Costanza, R., Bernard, F., Braat, L., Christie, M., Crossman, N., Ghermandi, A., Hein, L., Hussain, S., Kumar, P., McVittie, A., Portela, R., Rodriguez, L.C., ten Brink, P., van Beukering, P., 2012. Global estimates of the value of ecosystems and their services in monetary units. Ecosyst. Serv. 1, 50–61. http://dx.doi.org/10.1016/j.cosers.2012.07.005.
 Demas, G.P., 1998. Subaqueous Soil of Sinepuxent Bay, Maryland. Dep. Nat. Resour. Sci. Landsc. Archit. Univ. Maryland, Coll. Park. MD.
 Demas, G.P., Rabenborzh M.C., Stewaron, I.C., Streat, B., Hill, S. 1996. Subaqueous
- Demas, G.P., Rabenhorst, M.C., Stevenson, J.C., Street, B., Hill, S., 1996. Subaqueous
- soils: a Pedological approach to the study of shallow-water habitats. Estuaries

19, 229. http://dx.doi.org/10.2307/1352228.

- Demas, G., Rabenhorst, M.C., 1999. Subaqueous soils: pedogenesis in a submersed environment. Soil Sci. Soc. Am. I. 63, 1250–1257.
- Demas, G.P., Rabenhorst, M.C., 2001. Factors of subaqueous soil formation: a system of quantitative pedology for submersed environments. Geoderma 102, 189-204. http://dx.doi.org/10.1016/S0016-7061(00)00111-7.
- Ding, W., Zhang, Y., Cai, Z., 2010. Impact of permanent inundation on methane emissions from a Spartina alterniflora coastal salt marsh. Atmos. Environ. 44, 3894-3900, http dx.doi.org/10.1016/j.atmosenv.2010.07.025
- 022-0981/01)00355-0
- Biological Control (1993) Solution (1993) Erich. E., Drohan, P.J., 2012. Genesis of freshwater subaqueous soils following flooding of a subaerial landscape. Geoderma 179–180, 53–62. http://dx.doi.org/ erma.2012.02.004.
- Brich, E., Drohan, P.J., Elis, L.R., Collins, M.E., Payne, M., Surabian, D., 2010. Sub-aqueous soils: their genesis and importance in ecosystem management. Soil Use Manag. 26, 245–252. http://dx.doi.org/10.1111/j.1475-2743.2010.00278.x.
- Fanning, D.S., Fanning, M.C.B., 1989. Soil: Morphology, Genesis, and Classification. John Wiley & Sons, New York.Fanning, D.S., Rabenhorst, M.C., Burch, S.N., Islam, K.R., Tangren, S.A., 2002. Sulfides and sulfates. In: Soil Mineralogy with Environmental Applications. SSSA,
- Madison WI Ferrarin, C., Ungiesser, G., Bajo, M., Bellafiore, D., De Pascalis, F., Ghezzo, M., Mattassi, G., Scroccaro, I., 2010. Hydraulic zonation of the lagoons of Marano
- and Grado, Italy. A modelling approach. Estuar. Coast. Shelf Sci. 87, 561–572. http://dx.doi.org/10.1016/j.ecss.2010.02.012, c. G.W., Bauder, J.W., 1986. Methods of Soil Analysis: Part 1—Physical and Mineralogical Methods. In: SSSA Book Series. Soil Science Society of America, Gee, American Society of Agronomy. http://dx.doi.org/10.2136/ er5.1.2ed.c1
- Sabourset J.LeGUED. Génin, J.-M.R., Bourrié, G., Trolard, F., Abdelmoula, M., Jaffrezic, A., Refait, P., Maitre, V., Humbert, B., Herbillon, A., 1998. Thermodynamic equilibria in aqueous suspensions of synthetic and natural Fe(II)–Fe(III) Green rusts: occurrences of the mineral in hydromorphic soils. Environ. Sci. Technol. 32, 1058–1068. http://dx.doi.org/10.1021/es970547m.
 Homann, P.S., Grigal, D.F., 1996. Below-ground organic carbon and decomposition
- potential in a field-forest glacial-outwash landscape, Biol. Fertil. Soils 23, 207–214. http://dx.doi.org/10.1007/BF00336065, nov, M.V., Yu, A., Reeburgh, M.S., Skyring, G.W., 1989. Interaction of sulphur and carbon cycles in marine sediments. In: Evolution of Global Biogeochemical
- Sulphur Cycle, John Wiley & Son Ltd.
 Krachler, R., Jirsa, F., Ayromlou, S., 2005. Factors influencing the dissolved iron input by river water to the open ocean. Biogeosciences 2, 311–315. http://dx.doi.org/ 0.5194/bg-2-311-2005.
- MCGall, PL, Tevesz, MJS, 1982, Animal-sediment Relations. In: The Biogenic Alteration of Sediments, 100, Plenum Press, New York, p. 336, McVey, S., Schoeneberger, PJ, Turenne, J., Payne, M., Wysocki, D.A., 2012, Sub-aqueous soils (SAS) description. In: Field Book for Describing and Sampling
- Soils. National Soil Survey Center Natural Resources Conservation Service U.S. Sona variota son survey center natural resources conservation service os, Department of Agriculture, Michelutti, G., Zanolla, S., Barbieri, S., 2003, Suoli e paesaggi del Friuli Venezia
- Giulia, ERSA,
- Newhall, F., 1972. Calculation of Soil Moisture Regimes from the Climatic Record. Soil Conservation Service. USDA. Revision 4. Washington, DC. Newton, A., Icely, J., Cristina, S., Brito, A., Cardoso, A.C., Colijn, F., Riva, S.D., Gertz, F.,
- Hansen, J.W., Holmer, M., Ivanova, K., Leppäkoski, E., Canu, D.M., Mocenni, C., Mudge, S., Murray, N., Pejrup, M., Raznikovas, A., Reizopoulou, S., Pérez-Ruzafa, A., Schernewski, G., Schubert, H., Carr, L., Solidoro, C., Zaldívar, J.-M., 2014. An overview of ecological status, vulnerability and future perspectives of European large shallow, semi-enclosed coastal systems, lagoons and transi-tional waters. Estuar. Coast. Shelf Sci. 140, 95-122. http://dx.doi.org/10.1016/
- Payne, M.K., 2007, Landscape-level Assessment of Suba ous Soils and Water Ouality ts in Southern New England, University of Rhode Island, in Shallo w Embaymer
- Pedersen, O., Colmer, T.D., Sand-Jensen, K., 2013. Underwater photosynthesis of submerged plants recent advances and methods. Front. Plant Sci. 4, 140. http://dx.doi.org/10.3389/fpls.2013.00140.
- Peel, M.C., Bloschl, G., 2011. Hydrological modelling in a changing world. Prog. Phys. Geogr. 35, 249–261. http://dx.doi.org/10.1177/0309133311402550.
 Ponnamperuma, F., 1972. The chemistry of submerged soils. Adv. Agron. 24, 29–95.
 Poulton, S.W., 2002. The low-temperature geochemical cycle of iron: from conti-cently diverse to more an environment of the second source of the second sourc Bolton, S.W., 2002. The low-emperature geochemical cycle of non-non-non-nental fluxes to marine sediment deposition. Am. J. Sci. 302, 774–805. http:// dx.doi.org/10.2475/ajs.302.9.774. Richardson, J.L., Vepraskas, M.J., Craft, C.B., 2001. Wetland Soils: Genesis, Hydrology.
- Landscapes, and Classification. Lewis Scientific Publ, Boca Raton, FL. Rickard, D., Morse, J.W., 2005. Acid volatile sulfide (AVS). Mar. Chem. 97 (3), 141–197. http://dx.doi.org/10.1016/j.marchem.2005.08.004. Rose, A.L., Waite, T.D., 2003. Kinetics of iron complexation by dissolved natural
- organic matter in coastal waters. Mar. Chem. 84, 85–103. http://dx.doi.org/ 10.1016/S0304-4203(03)00113-0.Schoeneberger, P., Wysocki, D.A., Benham, E.C.J., 2012. Field Book for Describing and Sampling Soils, Version 3.0. Natural Resources Conservation Service. National

L. Vittori Antisari et al. / Estuarine, Coastal and Shelf Science 173 (2016) 39-48

- Soil Survey Center, Lincoln, NE.
 Silvestri, S., Defina, A., Marani, M., 2005. Tidal regime, salinity and salt marsh plant zonation. Estuar. Coast. Shelf Sci. 62, 119–130. http://dx.doi.org/10.1016/j.jeccss.2004.08.010.
 Soil Survey Staff. 2014. Keys to Soil Taxonomy, twelfth ed. United States Department of Agriculture, Natural Resources Conservation Service.
 Stolt, H., Rabenhorst, M.C., 2011. Introduction and historical development of sub-aqueous soil concepts. In: Huang, PM., et al. (Eds.), Handbook of Soil Science. CRC Press, LLC, Boca Raton, FL, ISBN 978-1-4398-0305-9.
 Van Wambeke, A.R., 2000. The Newhall Simulation Model for Estimating Soil Moisture & Temperature Regimes. Department of Crop and Soil Sciences, Cornell University, Ithaca, NY USA.
 Vann, C.D., Megonigal, J.P., 2003. Elevated CO₂ and water depth regulation of methane emissions: comparison of woody and non-woody wetland plant species. Biogeochemistry 63, 117–134. http://dx.doi.org/10.1023/A: 102339/7032331.
 Vepraskas, M.J., Fulkner, S., 2001. Redox chemistry of hydric soils. In:

- Richardson, J.L., Vespraskas, M.J. (Eds.), Wetland Soils: Genesis, Hydrology, Landscapes, and Classification. Lewis, Boca Raton, FL.
 Vittori Antisari, L., Carbone, S., Ferronato, C., Simoni, A., Vianello, G., 2011. Charac-terization of heavy metals atmospheric deposition for urban environmental quality in the Bologna city (1taly). EQA Int. J. Environ. Qual. 7 (7), 49–63. http:// dx.doi.org/10.6092/issn.2281-4485/3834.
 Wysocki, D.A., Schoeneberger, P., Hirmas, D., La Garry, H., 2012. Geomorphology of soil landscapes. In: second ed.Huang, P.M., et al. (Eds.), Handbook of Soil Sci-ence: Properties and Processes. CRC Press, Taylor and Francis Group, LLC, Boca Raton, FL, ISBN 978-1-4398-0305-9.
 Zhang, D., Yang, M., Li, J., Chen, X., 2006. Vegetative dispersal ability of Spartina *ulterniflora* in Eastern end of Chongming Island(Chinese). J. East China Norm. Univ. 130–135.
 Zuo, P., Zhao, S., Liu, C., Wang, C., Liang, Y., 2012. Distribution of Spartina spp. along China's coast. Ecol. Eng. 40, 160–166. http://dx.doi.org/10.1016/ j.ecoleng.2011.12.014.

48

CHAPTER 2



Juncus maritimus bushes established on saltmarsh soils in the Martignano saltmarsh, Marano Lagoon.

PREFACE

This work was published in the special issue "Biogeochemical dynamics of sediment-water systems: processes and modelling" of the *Journal of Soils and Sediments*, upon the 22nd International Symposium on Environmental Biogeochemistry (ISEB).

The work focused deeper on soil-vegetation relationship along a flooding gradient, again using a multidisciplinary approach. The uptake ability for the main macronutrients of the different plant communities was evaluated. The novelty of this work is based on the use of the bioaccumulation factor (BF) to evaluate the nutritional status of soils covered by these different plant communities. Vegetation revealed itself a great tool to describe soil hydric and nutritional conditions. The nutritional status varied within plant communities, depending on the soil status, and therefore could be considered a useful bioindicator of soil properties.

Sampling and writing of the paper was conducted in collaboration with the pedology group of the Agricultural Science Department of the University of Bologna. My contribute referred to experimental design, vegetation surveys and data analysis, including the coordinate discussion about the obtained achievements with the other authors, and writings.



ISEB 2015: BIOGEOCHEMICAL DYNAMICS OF SEDIMENT-WATER SYSTEMS: PROCESSES AND MODELLING

Soil properties and plant community relationship in a saltmarsh of the Grado and Marano lagoon (northern Italy)

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Abstract

Purpose The relationship between soil properties and plant communities was investigated in a saltmarsh of the Grado and Marano lagoon (northern Italy), where hydrology and micromorphology strongly influence the features of the ecosystem. A multidisciplinary approach was used to assess the change of soil properties and plant communities in relation to the submergence of soil.

Materials and methods The plant community and soil profile surveys were both carried out along a transect in six sampling sites of the Gran Chiusa saltmarsh (Grado and Marano lagoon). The morphological and physicochemical parameters of soil profiles were investigated, and soils were classified according to Soil Taxonomy. The concentration of macronutrients in both soils and plants was analysed by inductively coupled plasma-optical emission spectrometry. Cluster and linear discriminant analysis were used to assist the interpretation of the data of plant communities and soil properties, respectively. The bioconcentration factor explored the macronutrient relationship between plant community and soil. *Results and discussion* A high, middle and low zone were identified by clustering the different plant communities along

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² Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, Università di Udine, via delle Scienze, 206, 33100 Udine, Italy the studied transect. Discriminant analysis showed how the increase in soil submergence supported the accumulation of S and Ca content and depletion of Fe and Na. The development of different plant communities was linked to both soil water saturation and to the capacity of halophytes to tolerate anoxic conditions or salinity, by extrusion or bioconcentration strategies.

Conclusions This study demonstrates that tide level plays an important role in the pedological development and chemical transformations along a soil hydrosequence. The micromosaic vegetation pattern may therefore represent a useful index of the hydrological and nutritional status of the underlying soils and could be used to predict changes in coastal ecosystems.

Keywords Bioconcentration factor · Hydromorphic soil · Plant community · Saltmarsh · Subaqueous soil · Submergence · Transition

1 Introduction

Coastal ecosystems are complex and fragile systems whose high ecological value is recognized worldwide (Reddy and DeLaume 2008; Barbier et al. 2011). They harbour high biodiversity levels, providing important habitats for a large number of both migratory and resident birds and support important ecosystem services such as regulation of the biogeochemical cycles of nutrients and trace elements (Ponnamperuma 1972; Homann and Grigal 1996; Gedan et al. 2010; De Groot et al. 2012).

The global trend of sea level rise is increasingly manifest in most estuarine and costal ecosystems and affects their functions and conservation (Simas et al. 2001; Van Wijnen and Bakker 2001; Nicholls and Cazenave 2010; Watson et al. 2015). The rising of the sea level (Church and White 2006;

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IPCC AR4 SYR 2007; Wong et al. 2015) and the overexploitation of coastal areas have led to an increase of erosion processes and a considerable reduction and degradation of coastal habitats, in particular those of saltmarshes and mudflats (Lotze et al. 2006; Halpern et al. 2008). The response of saltmarshes to such pressures can lead either to their adaptation (e.g. accretion) or extinction (Warren and Niering 1993; Cahoon et al. 1995; Orson et al. 1998; Silinski et al. 2016), but in both cases, these changes may lead to serious problems linked to land management (Fontolan et al. 2012), protection and maintenance of the inner shore line and for wildlife conservation (Beaumont et al. 2007; Ferrarin et al. 2010).

The issue of coastal habitat degradation is of great concern, since about 10 % of the global population live in these areas (UNEP 2006; McGranahan et al. 2007), and local economies are often based on fishery, farming and tourism, which largely involve saltmarsh habitats (Worm et al. 2006; Beaumont et al. 2007). For this reason, the understanding of saltmarsh functioning, their preservation and consolidation is an increasing priority for coastal authorities.

It is well known that the consolidation of saltmarshes and mudflats can be considerably improved by halophytes, which foster soil pedogenesis by retaining sediments with their roots, affecting the redox conditions in the rhizosphere and providing an important source of organic carbon for microbial communities and soil development (Nyman et al. 1993; Reddy and DeLaume 2008; Laanbroek 2010). The halophytic vegetation in saltmarshes appears to be characterized by a micromosaic distribution that can be linked to the properties of the soils beneath (Van Wijnen and Bakker 1999; Álvarez Rogel et al. 2001). In these environments, soil hydrology has a profound effect on plant colonization, and the inability to define a general evolutionary trend testifies to the complexity of the system (Silvestri et al. 2005).

According to Demas's theory on subaqueous soils (Demas and Rabenhorst 1999), Ferronato et al. (2016) considered the formation and development of a soil continuum from a subaqueous to hydromorphic system. In this context, the bathymetry, as a proxy of waterlogging, can be considered as one of the main soil forming factors (Demas and Rabenhorst 2001), which influences several abiotic and biotic factors linked to the hydrodynamics of saltmarshes, such as the variability of soil properties and the occurrence of different pedogenetic processes along a soil hydrosequence. The number of soil profiles that need to be excavated is a key issue in soil surveys. Decisions are based on the well-known connections between topography and soil substrate, on the one hand, and ecological communities on the other (Wysocki et al. 2012). However, the assessment of variations in a saltmarsh environment is much more challenging. In such contexts, subtle variations in height (microrelief) have a profound effect not only on the soil hydrology and consequently on the soil properties but also on the formation and composition of plant communities. Many

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studies have investigated the species-specific mechanisms of nutrient adsorption from soils (Otero et al. 2000; Karlsons et al. 2008; Wong et al. 2015), but to our knowledge, the study of the relationship between soil and vegetation pattern, based on the capacity of plants to interact and compete with one another for the nutrients, has not been largely investigated. In this paper, we suggest the evaluation of a bioconcentration factor (BCF) as a powerful tool able to depict the relation between soil and vegetation. This approach could improve our knowledge of the functioning of saltmarsh ecosystems and help to predict any potential modifications in plant zonation, according to the soil property changes.

In accordance with this view, a soil hydrosequence and the related vegetation cover were studied in the saltmarsh of the Gran Chiusa Isles (Grado and Marano Lagoon, northern Italy). Soil property and plant community changes were investigated along an increasing gradient of flooding and anoxic stress conditions (Van Wijnen and Bakker 1999; Álvarez Rogel et al. 2001). A cross section of the island, traced from the inner part to the submerged zone, was surveyed. A survey of the plant community distribution and of the soil was carried out with the aims of (i) describing the change of soil properties according to the frequency and length of the soil submergence and (ii) defining the relationship between soil properties and the plant community nutrient composition according to the position along the hydromorphic sequence, through the evaluation of the BCF applied to plant communities.

2 Materials and methods

2.1 The study area

The Grado and Marano lagoon (SPA/SAC Nature 2000 IT3320037) is one of the largest lagoons in Italy. It extends for 160 km² between the Tagliamento and the Isonzo river mouths in the Northern Adriatic Sea (Fig. 1). The lagoon is delimited by seven natural barriers, which are separated by lagoon inlets (Brambati et al. 1998). The climate of the area, belonging to the biogeographical Mediterranean regions (Ibáñez et al. 2013), is "temperate/mesothermal" (Peel and Bloschl 2011). It has a mean annual temperature of 15.1 °C and a mean annual rainfall of 969 mm (OSMER 2016). Water temperatures range between 5 and 7 °C in winter and between 28 and 30 °C in summer, and the tide oscillation ranges between 65 and 105 cm (Ferrarin et al. 2010).

The soil survey and sampling was performed in July 2014 on the main saltmarsh area of the Gran Chiusa Isles, a large complex of saltmarshes of about 0.87 km², located in the central part of the lagoon, in the Buso basin. According to the LiDAR models, this area reaches elevations of about 70 cm a.m.s.l. on its abrupt canal margin and presents a gradual depression in the inner part of the saltmarsh. However, the



Fig. 1 a Grado and Marano Lagoon and location of the studied transect. b Position of the sampling points; the coordinates (WGS84/ EPSG 3857) are GC-a—5,737,165.6 N 1,481,450.3 E; GC-b—5,737,252.0 N 1,481,455.3

increase of the sea level (ICCP 2007) and periodical field observations makes difficult to establish the exact 0 m a.m.s.l. and the absolute altitudes of the tidal area. Several channels branch out from the saltmarsh in all directions and some of them, with straight and regular features, testify the former use of this saltmarsh as a fishing valley.

2.2 Sampling method and field analysis

Subsequent to previous work in the Grado and Marano lagoon (Vittori Antisari et al. 2016), the Gran Chiusa saltmarsh was selected as a representative site for this investigation. A 70 m long transect was traced from the inner part to the edge of the salt marsh, and sampling sites (form GC-a to GC-f) were selected according to six different zones characterized by site-specific topography, hydroperiod (hours per day, ISPRA 2013) and vegetation cover (Fig. 1). Considering the sitespecificity of the saltmarsh ecosystem (Wong et al. 2015), only one catena was investigated. However, accurate observation of the saltmarsh morphology, triplicate sampling and analytical replicates assured the quality and representativeness of the data. In each site, the vegetation surveys were conducted in three plots of 4 m² (2 \times 2 m). Plant communities were described referring to the most abundant species (percentage of cover), following the nomenclature reported in Biondi et al. (2014). Nomenclature of plant taxa followed the latest Italian check list of vascular plants (Conti et al. 2005). In each site, samples of the most representative and abundant species were collected in plastic cases and stored at 4 °C until analysis.

In addition, a soil profile was sampled in each site using a Beeker vibracore sampler (Eijkelkamp, NL) equipped with a

E; GC-c-5,737,184.2 N 1,481,462.3 E; GC-d-5,737,189.6 N 1,481,443.3 E; GC-c-5,737,204.0 N 1481447.6 E; GC-f-5,737,249.6 N 1,481,475.0 E. c Elevation profile of the studyed transect

polyethylene tube with a diameter of 6 cm. Soil profiles were described in the field according to the guidelines of McVey et al. (2012) and of Schoeneberger et al. (2012), including the H_2O_2 , and colour change and soil incubation test for the detection of sulphidic materials (Fanning et al. 2002; McVey et al. 2012). Each soil profile was further classified according to the USDA Soil Taxonomy (Soil Survey Staff 2014). In order to avoid oxygen infiltration, the samples were immediately sealed with a tight stopper and stored at 4 °C, until laboratory analysis.

2.3 Soil and plant samples analysis

Soil samples were air-dried and sieved at 2 mm. Soil particle size distribution was determined by pipette method (Gee and Bauder 1986). The pH (pH meter, Crison, Germany) and the electrical conductivity (EC; conductometer Orion, Germany) were measured on 1:2.5 (w/v) soil/distilled water suspension. Total carbonates (CaCO₃) were quantified by volumetric method (Loeppert and Suarez 1996). Total organic carbon (OC) and total nitrogen (TN) were measured by Dumas combustion with a CHN elemental analyser (EA 1110 Thermo Fisher, USA) after dissolution of carbonates with 2 M HCI (Tonon et al. 2010).

For the detection of total macroelements content, the samples were finely grounded and digested with *aqua regia* solution in a microwave oven (Stard D, FKV). Inductive coupled plasma-optic emission spectroscopy (ICP-OES, Ametek, Germany) processed the mineralized soil solution.

Plant samples were washed in deionized water in order to remove soluble salts and sediments deposited on the tissues,

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and then oven-dried at 60 °C for 48 h. The content of the macroelements was determined by ICP-OES (Ametek, Germany) after digestion of the finely ground dry biomass in a microwave oven with a solution of H_2O_2 and HNO_3 (2:6 v/v) according to Vittori Antisari et al. (2011).

All analyses were performed in duplicate; reference materials (BCR-320R and BCR 062) and reagent blanks were used to check the accuracy of the measurements, and the agreement was typically 10 %.

2.4 Data analysis

Differences between the chemical composition of the soil samples were evaluated with one-way analysis of variance (ANOVA) and post hoc tests (Tukey test, p < 0.05), whereas a Student's *t* test was applied to check the differences between plant sample compositions. Normality of data was verified using Shapiro's test (p > 0.05), while the homogeneity of variances was tested using Bartlett's test (p > 0.05).

A multivariate approach was used to assist the interpretation of the dataset. A hierarchical cluster analysis was performed to describe the plant communities using the Bray-Curtis dissimilarity as distance matrix and the complete link method for the clustering. A discriminant analysis (DA) with forward stepwise method was performed to identify the key variables that discriminate the different soil profiles according to their vegetation cover (Wilk's lambda < 0.0009; p < 0.001). The standardized canonical discriminant coefficients were evaluated to rank the importance of each variable, while the structure matrix was used to assign meaningful labels to the linear discriminant functions (LDs). The soil-plant relationships were investigated through the calculation of the BCF of macronutrients in each plant community. The BCF was calculated as the ratio between the total element concentration in the plant (mg kg⁻¹) and in the soil (g kg⁻¹) according to Li and Zheng (2011).

3 Results

3.1 Vegetation pattern along the hydrosequence

The characterization of plant communities along the hydrosequence was performed by cluster analysis. The analysis of the dendrogram (Fig. 2) showed the presence of two cut levels (i.e. 0.98 and 0.70). The first indicated three main clusters (clusters 1, 2, 3), corresponding to the high (cluster 2), middle (cluster 3) and subaqueous (cluster 1) parts of the saltmarsh. The latter was characterized by green algae cover (cluster 1), whereas in clusters 2 and 3, a further five different subclusters were identified (namely, A, B, C, D, E).

Vegetation surveys (Table 1S, Electronic Supplementary Material) were consistent with those reported in the literature

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(Poldini et al. 1999; Biondi et al. 2014), showing a clear zonation between plant communities along the hydrosequence, which is well represented by the dominance of diagnostic species such as Juncus maritimus, Sarcocornia fruticosa, Limonium narbonenese, Spartina maritima and Salicoria patula. Species composition and abundance (percentage of cover) allowed us to define four main vegetation classes, as described by Poldini et al. (1999) for the Marano and Grado lagoon. Within cluster 2, subcluster B referred to the vegetation class of Juncetea maritimi, representing the Mediterranean perennial salty and brackish grasslands; subcluster C encompassed both the GC-b and GC-c zones, referred to the Sarcocornietea fruticosae class, which includes pioneer, perennial, hyperhalophilous, succulent and woody and semi-woody plant communities. In subcluster B, the most abundant plant, occurring in all plots, was Juncus maritimus (80 % on average), followed by Limonium narbonense (27 %), Sarcocornia fruticosa (23 %) and Atriplex portulacoides (12 %), while in subcluster C (GC-b and GCc sites), the most abundant species were Limonium narbonense (65 %), Sarcocornia fruticosa (13 %) and Spartina maritima (13 %).

Similarly, also cluster 3 can be interpreted as a combination of two different vegetation patterns found in the middle zone (Fig. 2, subclusters D and E). Subcluster D showed a mean cover of 95 % of *Spartina maritima* and was ascribed to the *Spartinetea glabrae* class, which includes all pioneer vegetation of perennial formations that grow on muddy brackish soils inundated for a long period. Subcluster E showed the prevalence of *Salicornia patula* (72 %) and referred to the class *Thero-Suaedetea splendentis*, encompassing all pioneer communities of annual species of the genus *Salicornia*, in temporarily inundated saltmarsh sites and in saltpans.

3.2 Classification and properties of soil hydrosequence

The morphological description of the soil profiles (Table 2S, Electronic Supplementary Material) highlighted that all soils were little developed with a silty or silty loam texture and generally showed an A/AC/C pedosequence. The presence of a BC horizon was detected only in the GC-a profile, while the GC-f soil surface consisted of an L layer (limnic) (Table 2S, Electronic Supplementary Material).

Figure 3 summarizes some of the morphological features of the soil profiles. The pedons in the high zone (GC-a, GC-b and GC-c, cluster 2) presented many root sheaths in the topsoil and some shell fragments in the deeper C horizon. Conversely, the pedons located in the submerged zone (GC-d, GC-e and GC-f, clusters 3 and 1, respectively) presented common shell fragments in the A horizons and organic films and concentrations in the deeper one. Sulphidic materials were detected in the topsoil of the highest pedons and in the subsoil of the submerged profiles and increased from the inner part of the J Soils Sediments

Fig. 2 Hierarchical clustering based on vegetation surveys (Bray-Curtis, complete link method). Three main clusters (cut level = 0.98, clusters 1, 2, 3) represent the main saltmarsh areas based on height; a further separation (cut level = 0.70, subclusters A, B, C, D, E) regards vegetation classes characterization



saltmarsh to its edge (Fig. 3). Despite the accumulation of these materials in the investigated pedons, *sulphuric* horizons were not detected because of the lack of pH failure during aerobic incubation (McVey et al. 2012).

All the soil profiles collected in the GC-a, GC-b, GC-c and GC-d zones showed evidence of aquic

conditions, due to periodic saturation and signs of reduction processes, while the GC-e and GC-f pedons had a positive water potential at the soil surface for more than 21 h day⁻¹ (ISPRA 2013; Soil Survey Staff 2014) and were thus ranked in the *Wassent* suborder. Soil profiles in the high part of the saltmarsh (GC-a, GC-b and



Fig. 3 Scheme of the morphological description of the soil profiles, displaying the intensity of sulphidic material and biological concentration (codes according to McVey et al. 2012). S=sulphorous;

N=none; SL=slight; MD=moderate; ST=strong. Biological concentration: RSB=root sheaths; SFB=shell fragments; OSF=organic strains; f=few; c=common; m=many

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Profile	Master	Depth	pH*	EC* mS cm ⁻¹	$\begin{array}{c} CaCO_3 \\ g \ kg^{-1} \end{array}$	OC*	TN	К*	Р	S*	Al*	Fe*	Mn	Mg	Ca*	Na*	C/S*
GC-a	А	0-12/13	7.6	22.1	107.2	43.8	4.9	11.7	0.50	2.3	35.9	20.2	0.5	23.6	30.4	20.5	19
	A2	12/13-21/22	7.8	16.8	131.3	25.4	2.2	11.4	0.40	1.3	35.3	21.4	0.7	25.9	33.3	15.3	20
	BC	21/22-36	7.9	16.2	150.3	21.8	2.1	12.2	0.33	1.6	37.8	17.8	0.2	24.6	31.4	14.3	14
	C1	36-45/47	7.9	14.1	178.6	13.1	1.1	11.8	0.30	1.3	37.0	18.1	0.1	24.9	32.3	11.7	10
	C2	45/47-54	7.9	15.1	142.9	nd	nd	11.8	0.23	6.3	35.7	17.1	0.3	24.4	50.0	12.3	nd
	Mean		7.8	16.8	142.1	26.0	2.6	11.8	0.4	2.5	36.3	18.9	0.4	24.7	35.5	14.8	15.8
	SD		0.1	3.1	26.2	12.9	1.6	0.3	0.1	2.1	1.0	1.8	0.2	0.8	8.2	3.5	4.6
	Pairwise	post hoc	ab	ab	ns	ab	ns	b	ns	a	b	b	ns	ns	a	ab	Ь
GC-h	AL	0-18/20	75	23.9	1547	41.5	3.0	12.5	0.41	23	37.4	18.9	0.3	22.6	28.5	20.9	18
de v	AC	18/20-24/30	7.9	14.2	110.5	19.2	18	10.5	0.24	00	31.0	17.4	0.2	26.2	54.5	11.1	2
	Clse	24/30-49/50	82	14.3	44.2	16.4	1.5	9.7	0.23	10.2	27.9	173	0.4	24 1	57.1	11.4	2
	C2se	49/50-60	83	22.6	147.4	16.3	1.6	10.6	0.26	10.1	31.5	18.5	0.5	24.5	54.8	12.2	2
	Mean	10120 00	8.0	18.8	114.2	23.3	2.2	10.8	0.3	8.1	32.0	18.0	0.4	24.4	48.8	13.9	5.8
	SD		0.4	5.2	50.5	12.2	1.1	1.2	0.1	3.9	3.9	0.8	0.2	1.5	13.5	4.7	8.3
	Pairwise	post hoc	ab	ab	ns	ab	ns	ab	ns	a	ab	ab	ns	ns	ab	ab	ab
GC-c	Δ	0-8/10	60	40.2	10.3	94.0	8.6	11.3	0.45	4.2	20.4	16.1	0.2	15.3	15.7	374	22
out	A2g	8/10-15/16	52	39 3	1191	80.8	65	12.8	0.38	62	36.0	16.0	0.1	13.5	10.5	38.8	13
	AClee	15/16_22/23	76	19.2	218.8	28.6	2.5	10.9	0.24	13.9	31.2	19.5	0.2	24.2	45.0	18.2	2
	AC2se	22/23-41/42	81	16.4	229.5	26.6	1.5	10.3	0.21	7.6	29.8	16.1	0.3	24.2	50.2	12.7	3
	Cla	41/47-58/50	8.0	15.3	269.6	34.0	13	03	0.20	9.6	26.2	15.6	0.4	24.3	65 3	11.1	4
	C29	58/59-76	82	11.9	197.0	33.0	0.6	73	0.16	7.0	18.8	11.1	0.3	28.8	83.2	74	5
	Mean	20123 10	73	23.7	175.6	40.6	35	103	03	81	28.6	157	03	217	450	20.9	82
	SD		12	12.6	913	20 7	33	19	01	33	57	27	01	60	281	13.8	80
	Pairwise	post hoc	a	Ь	ns	b	ns	ab	ns	ab	ab	ab	ns	ns	ab	Ь	ab
GC-d	Δσ	0-11	7.9	15.9	223.3	23.3	19	10.6	0.24	15.9	31.5	22.8	0.2	24.0	45.9	12.8	1
oc a	ACo	11-24	8.0	15.6	173.4	18.9	1.8	11.5	0.27	4.5	35.7	19.5	0.1	223	29.7	13.2	4
	ACse	24-32/33	81	12.7	44.7	16.0	1.4	11.1	0.22	6.7	32.6	17.4	0.2	25.8	56.9	10.2	2
	Cle	32/33-60/63	8.1	12.0	35.7	13.1	1.1	9.1	0.18	8.5	24.5	14.3	0.3	27.3	74.8	8.1	2
	C2g	60/63-68	8.2	12.6	55.8	nd	nd	9.5	0.19	10.4	26.4	16.1	0.3	26.3	67.6	9.4	nd
	Mean		8.1	13.8	106.6	17.8	1.6	10.3	0.2	9.2	30.1	18.0	0.3	25.2	55.0	10.7	2.4
	SD		0.1	1.8	85.9	4.3	0.4	1.0	0.0	4.3	4.6	3.3	0.1	2.0	17.9	2.2	1.3
	Pairwise	post hoc	ab	ab	ns	a	ns	ab	ns	ab	ab	ab	ns	ns	ab	ab	ab
GC-e	Ao	0-4	8.0	18.7	58.1	39.4	54	11.4	0.34	9.2	33.6	17.9	0.2	19.5	48.1	15.8	4
000	A20	4-16/17	8.0	20.0	154.7	32.8	3.4	11.6	0.28	11.5	33.4	18.2	0.3	20.9	57.6	16.3	3
	A2se	16/17-23	8.1	13.0	154.7	19.1	2.1	10.6	0.23	11.0	31.3	18.4	0.3	25.1	55.4	11.4	2
	ACse	23-39/40	8.1	14.5	31.3	16.8	1.6	12.7	0.27	5.0	40.5	20.3	0.2	24.7	33.7	12.8	3
	Clg	39/40-49/50	8.1	12.9	88.4	13.6	1.2	10.0	0.20	7.6	28.2	15.9	0.3	26.8	63.7	9.8	2
	C2g	49/50-60	8.3	12.1	75.2	13.9	1.3	10.1	0.19	10.2	28.0	16.0	0.3	27.6	68.0	8.8	1
	Mean		8.1	15.2	93.7	22.6	2.5	11.1	0.3	9.1	32.5	17.8	0.2	24.1	54.4	12.5	2.6
	SD		0.1	3.3	51.0	10.9	1.6	1.0	0.1	2.4	4.6	1.7	0.1	3.2	12.3	3.1	1.1
	Pairwise	post hoc	ab	ab	ns	a	ns	ab	ns	ab	ab	ab	ns	ns	ab	ab	ab
GC-f	L se	0-4	84	11.8	189.6	193	2.0	00	0.28	13.5	29.2	16.8	03	24.1	61.7	9.5	1
	Ase	4-16/18	8.2	11.0	221.1	15.8	14	9.9	0.24	15.2	28.5	15.8	0.3	26.4	62.1	8.0	i
	AC 1g	16/18-30	8.1	10.8	204.1	24 3	1.4	10.4	0.23	18.1	29.9	16.3	0.3	263	63.3	7.9	1
	AC 2g	30-40	8.2	10.4	200.9	21.4	1.1	9.3	0.22	17.1	26.4	15.8	0.4	26.3	65.7	7.6	i
	C2g	40-63	8.4	10.1	57.5	23.6	1.0	7.6	0.21	13.7	21.1	13.3	0.4	28.2	72.0	7.0	2
	2 ACIg	63-82/84	8.5	9.5	128.8	21.5	1.0	8.3	0.21	13.1	22.6	12.9	0.3	27.6	72.9	6.5	2
	2 AC2g	82/84-91	8.5	9.2	176.9	23.3	1.0	8.9	0.22	12.8	24.6	13.5	0.3	27.3	72.2	6.8	2
	Mean		8.3	10.4	168.4	21.3	1.3	9.2	0.2	14.8	26.0	14.9	0.3	26.6	67.1	7.6	1.5
	SD		0.1	0.9	57.0	2.9	0.4	1.0	0.0	2.1	3.4	1.6	0.0	1.3	5.1	1.0	0.3
	n	mant has	6	*						4				***	6		

Table 1 Physicochemical characterization of soil profiles. Pairwise post hoc Tukey test results are reported with different letters on the statistically

GC-c, cluster 2) were classified as Typic Endoaquent, while in the middle part (cluster 3), the classification of GC-d and GC-e fell into the Typic Hydraquent and Typic Hydrowassent great groups respectively. In the lowest part of the saltmarsh (cluster 1), the GC-f pedon was classified as Typic Fluviwassent.

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Table 1 reports the main physicochemical characteristics of each genetic horizon and the significant differences among the mean values of the investigated pedons.

Among the different profiles, the subaqueous GC-f soil presented significantly higher pH values and lower EC values than the GC-c pedon did (p = 0.018 and 0.006, respectively). The irregular CaCO3 distribution along the soil profile was mainly due to intercalation of shell fragments in several layers (Table 1). The OC and TN content decreased along the soil profiles, with the exception of the subaqueous Fluviwassent profile (GC-f), where organic-enriched layers were present in deeper parts of the profile. Moreover, the post hoc test highlighted a significantly higher content of OC in GC-c (Typic Endoaquent) than in pedons located in the lower part of the saltmarsh (p < 0.05). A significantly higher EC value and Na content in the GC-c pedon compared to GC-f was also detected (p = 0.011). Among the investigated profiles, the macronutrients content (e.g. K, Fe Al) in the subaqueous soils was significantly lower than that in the GC-a pedon. Conversely, Ca and S contents were significantly higher (Table 1).

A discriminant analysis (DA) was performed on three a priori groups of soils, chosen according to the results of the cluster analysis (cut level = 0.98), using the soil physicochemical parameters described above as independent variables (Fig. 4). The canonical structure matrix (Table 2) was used to define the most relevant variables which constitute the discriminant functions (greater than 0.3).

The first linear discriminant function (LD1) separated the subaqueous *Fluviwassent* (GC-f) soil properties from those of the pedons in the middle and high zones of the saltmarsh (clusters 2 and 3, respectively). Notably, cluster 3 included both hydromorphic (GC-d, *Hydraquent*) and subaqueous soils (GC-e, *Hydrowassent*), while cluster 2 was only composed of hydromorphic *Endoaquent* soils. The canonical structure



Fig. 4 Canonical score plot of discriminant analysis (DA). Wilks' Lambda $0.0009 \ p \le 0.001$

tructure matrix of LD1		LD 1	LD 2
nd LD2 showing the correlation between the	pН	-0.330	0.390
alues of the explanatory	CE	0.410	-0.430
ariables and those of the	CaCO ₃	-0.280	-0.340
mortant variables were	OC	0.140	-0.420
reater than 0.300	TN	0.270	-0.250
	CSC	0.360	-0.230
	К	0.510	-0.120
	Р	0.260	-0.370
	S	-0.640	0.370
	C/S	0.290	-0.570
	C/N	-0.230	-0.240
	AI	0.470	-0.100
	Fe	0.470	0.060
	Mn	-0.170	-0.270
	Mg	-0.330	0.210
	Ca	-0.430	0.370
	Na	0.380	-0.400

matrix (Table 2) showed that among the relevant variables of LD1, EC, CEC, Na, Fe and Al increased from the subaqueous to the hydromorphic system, while the S and Ca contents followed the opposite trend.

The variables involved in LD2 separated the soils of cluster 3 in the middle zone of the saltmarsh (covered by *Spartinetea glabrae* and *Thero-Suaedetea splendentis* classes) from those of cluster 2 in the highest zone (covered by *Juncetea maritimi* and *Sarcocornietea fruticosae*). The driving variables were pH, S and Ca amount, which were higher in cluster 3 soils (*Typic Hydraquent* and *Typic Hydrowassent*); conversely, EC, Na, P, OC content and C/S ratio decreased from the *Endoaquent* pedons (cluster 2) to the *Hydraquent* and *Hydrowassent* pedons (cluster 3).

3.3 Soil-vegetation relationship

Table 3 presents the mean concentration of macroelements in the plants of the two groups obtained from the cluster analysis (cut level = 0.98, clusters 2 and 3). Cluster 3 (GC-d/e sites located in the middle zone of the transect), mostly covered by *Spartina maritima* and *Salicornia patula*, had a significantly higher content of Ca (p = 0.050), K (p = 0.011) and Fe (p = 0.008) than that determined in the plant tissues of cluster 2 (GC-a/b/c sites, located in the highest zone). Notably, these last two plant communities were characterized by the dominance of *Juncus maritimus* (GC-a) in the first case and of *Limonium narbonense* and *Sarcocornia fruticosa* (GC-b/c) in the second. In addition, the content of the other elements

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Table 3Mean macronutrient concentration found in the different plantcommunities. Minimum (min) and maximum (max) values and standarddeviation (SD) are also reported. Data are expressed as g kg-1, withexception of P (mg kg-1). *=p<0.05

		Cluster 2 (GC-a/b/c)	Cluster 3 (GC-d/e)
Ca*	Mean	2.8	5.4
	SD	1.3	2.3
	Min	1.0	3.1
	Max	3.9	8.1
K*	Mean	5.4	6.0
	SD	0.3	0.3
	Min	5.0	5.7
	Max	5.8	6.2
Mg	Mean	4.6	6.5
	SD	3.5	2.0
	Min	0.9	3.6
	Max	10.4	8.1
Na	Mean	41.6	88.7
	SD	38.3	57.0
	Min	3.8	44.4
	Max	105.2	167.8
Fe*	Mean	0.3	2.9
	SD	0.1	0.9
	Min	0.1	1.6
	Max	0.4	3.4
Р	Mean	0.6	0.8
	SD	0.3	0.5
	Min	0.3	0.4
	Max	1.1	1.4
S	Mean	3.6	4.0
	SD	2.6	1.4
	Min	0.6	2.4
	Max	6.7	5.3

in plant tissues (Mg, Na, P, S) was slightly higher in cluster 3 than in cluster 2, but no significant difference was detected.

In order to assess the different capacities of plant communities to absorb nutrients from the soil, the bioconcentration factor (BCF) of the elements, with respect to the soil, was calculated for all the plant samples of clusters 2 and 3. As shown in Fig. 5, some statistically significant differences were found between the two groups. K (p = 0.004) and Fe (p = 0.016) BCFs were significantly higher in the plant communities adapted to survive in frequently submerged areas (cluster 3, GC-d/e sites) than those located in the high zone of the saltmarsh. On the contrary, plants of these communities (*Spartinetea glabrae* and *Thero-Suaedetea splendentis*)

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classes) showed a noticeable lower BCF for S (p = 0.150) in comparison to specimens referring to the *Juncetea maritimi* and *Sarcocornietea fruticosae* classes (cluster 2).

The other elements did not show any significant differences; however, the BCFs of P, Mg and Na appeared higher in cluster 3, while the BCF of Ca showed an opposite trend.

4 Discussion

The vegetation survey performed in the Gran Chiusa saltmarsh identified three main vegetation groups and a high variability of features within the soil sequence, which highlighted the system's transition from the subaqueous to the hydromorphic environment.

The discriminant analysis allowed us to discriminate soil profiles on the basis of the most important physicochemical parameters. This separation was consistent with the vegetation pattern shown by cluster analysis, which highlighted a high, middle and low zones along the transect.

The highest soil profiles of the hydrosequence (GC-a, b, c) showed relatively weaker signs of reduction than the other soils, due to a shorter period of submergence, and they were soluble salt-enriched. A well-known horizontal gradient of salinity exists in the Grado lagoon that is typically observed in saltmarshes (Rogel et al. 2000; Ferrarin et al. 2010), but our data suggests that in these environments, there is also a surface accumulation of Na and soluble salts in the superficial soil horizons. This phenomenon, more evident in the GC-c pedon, is probably linked to the alternation of flooding/evaporation cycles (Salama et al. 1999; Cidu et al. 2013; Ferronato 2015). Data from OSMER (2016) on evapotranspiration rates along the Friuli sedimentary coast (Fig. 1S, Electronic Supplementary Material) suggest, in fact, that in summer, the increased temperature and the on-average lower tide excursions cause higher evaporation rates and a consequent salt accumulation at the saltmarsh soil surface. Furthermore, the C/S ratio, which is an indicator of anoxic pedogenesis (Ivanov et al. 1989; Ferronato et al. 2016), is larger in anoxic environments and thus increases in these soils with respect to the profiles in the lower part of the saltmarsh.

Indeed, the low C/S ratio of the submerged soils and soil horizons, and the detection of a strong sulphidic odour during the profile description, indicate that in the permanently saturated zones of the saltmarsh, the soils are characterized by an accumulation of reduced S compounds, OC enrichment and anoxic pedogenesis (Demas and Rabenhorst 1999; Ferronato et al. 2016). Sulfidization is a typical pedogenetic process of marsh soils (Fanning and Fanning 1989; Fanning et al. 2002) where the chemical and microbiological reduction of $SO_4^{2^-}$ into HS⁻ leads to the formation of iron-sulphide compounds, which define the sulphidic horizons (Fanning and Fanning 1989; Demas and Rabenhorst 1999). In the soil transect of the Gran Chiusa

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Fig. 5 Boxplots representing macronutrient bioconcentration factors (BCFs) in the two main clusters (*p < 0.05). BCF was calculated as the ratio between nutrient concentration in the plant samples and in the soil (mg kg⁻¹)

saltmarsh, the formation of these horizons was not possible, because although layers with accumulation of sulphides were observed in permanently saturated zones (e.g. in the C horizons of the GC-a/b/c highest profiles and in the A horizons of the GC-d/ e/f lowest pedons), the expected acidification did not occur. For this reason, according to the soil taxonomy (Soil Survey Staff 2014), these horizons were not considered as diagnostic *sulfic* horizons. Given that sulphide accumulation reflects important pedogenic pathways in the saltmarsh pedons, the lack of acidification during the aerobic pH detection must be ascribed to the buffering action of carbonates in the investigated soil (Vittori Antisari et al. 2016).

In the middle position of the investigated hydrosequence, *Typic Hydraquent* (GC-d) and *Typic Hydrowassent* (GC-e) coexist, reflecting a typical transition zone in micromorphologically depressed areas within the saltmarshes.

According to the DA, the physicochemical features of these soils are similar to those of *Endoaquents* mainly due to the increase of EC and other elements but also due to water saturation, to those of *Fluviwassents* for their moderate content of S and Ca. *Spartina maritima* and *Salicornia patula* cover 95 and 72 % of these transition pedons respectively, suggesting that these species are particularly adapted to grow under severe conditions, such as low oxygen availability, high soil salinity, water saturation and higher sulphide content in the soil (Koch et al. 1990; Stribling 1997; Zhang et al. 2006; Zuo et al. 2012). In particular, the soil under *Spartinetea glabrae* community showed on average the highest S content. This is probably due to the capacity of *Spartina maritima* to colonize such environment, also thanks to the oxidation capacity of the root system, as suggested by Gribsholt and Kristensen (2003). Species adaptation in

saltmarshes depends on a number of edaphic factors that act at both macromorphological and micromorphological scale, such as the bathymetry, the oxygen availability, the redox status of the soil, the life cycle of the species and their capacity to adapt to and tolerate severe environmental conditions (Génin et al. 1998; Pedersen et al. 2013). We suggest that the nutrient contents of the plant tissues and the BCF could be useful tools to depict the interaction between soil-vegetation cover systems. As demonstrated by our findings, in fact, the BCF is largely used in metal accumulation studies (Pandey and Tripathi 2010; Souza et al. 2015) but it could be further considered as an indirect indicator of plant ecological tolerance and distribution. In this paper, this method successfully allowed us to discriminate plant communities based on the main elements analysed, leading to meaningful considerations on the ecological requirements and constraints of each community.

The higher content of Fe, K and Mg in the plants living in low saltmarsh communities (*Spartinetea glabrae* and *Thero-Suaedetea splendentis* classes), with respect to other species established in higher saltmarsh areas, can be ascribed to the anaerobic conditions, which determine the reduction and mobility of several nutrients, e.g. Fe, K and Ca (Julie and Siobhan 2001).

At microedaphic scale, the results also confirm that plant uptake depends on both soil properties (e.g. soil salinity and water saturation) and on species-specific factors linked to the plants metabolism and adaptation capacity (Silvestri et al. 2005). For example, the highest Na content in plant tissues was recorded in the class *Thero-Suedetea splendentis*, where the salt-resistant *Salicornia patula* was the most abundant species (Ushakova et al. 2005; Sajna et al. 2013).

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Conversely, plant communities located in the higher zone need to face a high Na concentration and adopt various mechanisms to tolerate Na, such as the development of epidermal glands able to secrete salt from leaves (e.g. in *Limonium* genus), or the increase of leaf or stem succulence (e.g. *Sarcocornia* genus). Furthermore, the capacity of *Spartina maritima* and *Salicornia patula* to colonize transition areas and anoxic soils, where sulphides and other reductive species

are found, can be ascribed to their capacity to avoid passive S2-

uptake (Havill et al. 1985), as confirmed by the low BCF of S.

5 Conclusions

This paper points out the existence of a significant relationship in saltmarshes between soil types and plant communities along soil hydrosequences. The micromosaic vegetation pattern may therefore represent a useful index of the hydrological and nutritional status of the underlying soils. Indeed, the soil classification scheme and the vegetation clustering were able to reflect the micromorphology of the area and the evolution of different soil-forming processes linked to the lack of oxygen in the soil, such as the accumulation of sulphides and nutrients mobilization. The distribution of the vegetation pattern along the hydrosequence largely depends on the capacity of plants to adopt resistance strategies in order to colonize the different environmental and soil conditions.

This study demonstrates that tide level plays an important role in pedological development and chemical transformations along a soil hydrosequence. A multidisciplinary approach was successfully used to describe the pedogenetic and vegetation diversity and variability in such a peculiar environment. Furthermore, this interdisciplinary approach could represent a powerful tool to predict future changes in case of subsidence or increase of flooding events due to the rise in the mean sea level subsequent to ongoing climate changes.

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References

- Álvarez Rogel J, Ortiz Silla R, Alcaraz Ariza F (2001) Edaphic characterization and soil ionic composition influencing plant zonation in a semiarid Mediterranean salt marsh. Geoderma 99:81–98
- Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2011) The value of estuarine and coastal ecosystem services. Ecol Monogr 81:169–193
- Beaumont NJ, Austen MC, Atkins JP, Burdon D, Degraer S, Dentinho TP, Derous S, Holm P, Horton T, van Ierland E, Marboe AH, Starkey DJ, Townsend M, Zarzycki T (2007) Identification, definition and

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quantification of goods and services provided by marine biodiversity: implications for the ecosystem approach. Mar Pollut Bull 54:253-265

- Biondi E, Blasi C, Allegrezza M, Anzellotti I, Azzella MM, Carli E, Casavecchia S, Copiz R, Del Vico E, Facioni L, Galdenzi D, Gasparri R, Lasen C, Pesaresi S, Poldini L, Sburlino G, Taffetani F, Vagge I, Zitti S, Zivkovic L (2014) Plant communities of Italy: the vegetation Prodrome. Plant Biosyst 148:728–814
- Brambati A, De Muro S, Marocco R, Selivanov A (1998) Barrier island evolution in relation to the sea-level changes: the example of the Grado lagoon (northern Adriatic Sea, Italy). Boll Geofis Teor Appl 39:145–161
- Cahoon DR, Reed DJ, Day JW (1995) Estimating shallow subsidence in microtidal salt marshes of the southeastern United States: Kaye and Barghoom revisited. Mar Geol 128:1–9
- Church JA, White NJ (2006) A 20th century acceleration in global sea-level rise. Geophys Res Lett 33:L01602
- Cidu R, Vittori Antisari L, Biddau R, et al (2013) Dynamics of rare earth elements in water–soil systems: The case study of the Pineta San Vitale (Ravenna, Italy). Geoderma 193-194:52–67. doi:10.1016/j. geoderma.2012.10.009
- Conti F, Abbate G, Alessandrini A, Blasi C (2005) An annotated check-list of the italian vascular flora. Palombi ed, Roma
- Demas G, Rabenhorst MC (1999) Subaqueous soils : pedogenesis in a submersed environment. Soil Sci Soc Am J 63:1250–1257
- Demas GP, Rabenhorst MC (2001) Factors of subaqueous soil formation: a system of quantitative pedology for submersed environments. Geoderma 102:189–204
- Fanning DS, Fanning MCB (1989) Soil: morphology, genesis, and classification. John Wiley & Sons, New York
- Fanning DS, Rabenhorst MC, Burch SN, Islam KR, Tangren SA (2002) Sulfides and sulfates. Soil mineralogy with environmental applications. SSSA, Madison, WI, pp. 229–260
- Ferrarin C, Umgiesser G, Bajo M, Bellafiore D, De Pascalis F, Ghezzo M, Mattassi G, Scroccaro I (2010) Hydraulic zonation of the lagoons of Marano and Grado, Italy. A modelling approach. Estuar Coast Shelf Sci 87:561–572
- Ferronato C (2015) Water, sediment and soil physicochemical interaction in freshwater, brackish and saline systems. Dissertation, University of Bologna
- Ferronato C, Falsone G, Natale M, Zannoni D, Buscaroli A, Vianello G, Vittori L (2016) Chemical and pedological features of subaqueous and hydromorphic soils along a hydrosequence within a coastal system (San Vitale Park, northern Italy). Geoderma 265:141–151
- Fontolan G, Pillon S, Bezzi A, Villalta R, Lipizer M, Triches A, D'Aietti A (2012) Human impact and the historical transformation of saltmarshes in the Marano and Grado lagoon, northern Adriatic Sea. Estuar Coast Shelf Sci 113:41–56
- Gedan KB, Kirwan ML, Wolanski E, Barbier EB, Silliman BR (2010) The present and future role of coastal wetland vegetation in protecting shorelines: answering recent challenges to the paradigm. Clim Chang 106:7–29
- Gee GW, Bauder JW (1986) Methods of soil analysis: part 1—physical and mineralogical methods. Soil Science Society of America, American Society of Agronomy
- Génin J-MR, Bourrié G, Trolard F, Abdelmoula M, Jaffrezic A, Refait P, Maitre V, Humbert B, Herbillon A (1998) Thermodynamic equilibria in aqueous suspensions of synthetic and natural Fe(II)–Fe(III) green nusts: occurrences of the mineral in hydromorphic soils. Environ Sci Technol 32:1058–1068
- Gribsholt B, Kristensen E (2003) Benthic metabolism and sulfur cycling along an inundation gradient in a tidal Spartina anglica salt marsh. Limnol Oceanogr 48:2151–2162
- De Groot R, Brander L, van der Ploeg S, Costanza R, Bernard F, Braat L, Christie M, Crossman N, Ghermandi A, Hein L, Hussain S, Kumar P, McVittie A, Portela R, Rodriguez LC, ten Brink P, van Beukering P

(2012) Global estimates of the value of ecosystems and their services in monetary units. Ecosyst Serv 1:50–61

- Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R (2008) A global map of human impact on marine ecosystems. Science 319:948–952
- Havill DC, Ingold A, Pearson J (1985) Sulphide tolerance in coastal halophytes. In: Beeftink WG, Rozema J, Huiskes AHL (eds) Ecology of coastal vegetation. Springer, Dordrecht, pp. 279–285
- Homann PS, Grigal DF (1996) Below-ground organic carbon and decomposition potential in a field-forest glacial-outwash landscape. Biol Fertil Soils 23:207–214
- Ibáñez JJ, Zinck JA, Dazzi C (2013) Soil geography and diversity of the European biogeographical regions. Geoderma 192:142–153
- IPCC AR4 SYR (2007) Climate change 2007: synthesis report, contribution of working groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerldand
- ISPRA (2013) [Internet]. Available from: http://www.venezia.isprambiente. it/rete-meteo-mareografica
- Ivanov MV, Yu A, Reeburgh MS, Skyring GW (1989) Interaction of sulphur and carbon cycles in marine sediments. Evolution of global biogeochemical sulphur cycle John Wiley & Son Ltd., pp 61–78
- Julie KC, Siobhan FM (2001) Wetland plants: biology and ecology. CRC Press
- Karlsons A, Osvalde A, Nečajeva J, levinsh G (2008) Changes of nutritional status of coastal plants *Hydrocotyle vulgaris* and *Aster tripolium* at elevated soil salinity. Acta Univ Latv 745:165–177
- Koch MS, Mendelssohn IA, Mckee KL (1990) Mechanism for the hydrogen sulfide-induced growth limitation in wetland macrophytes. Limnol Oceanogr 35:399–408
- Laanbroek HJ (2010) Methane emission from natural wetlands: interplay between emergent macrophytes and soil microbial processes. A minireview. Ann Bot 105:141–153
- Li S-X, Zheng F-Y (2011) Effect of macronutrient enrichment on the size distribution, sorption, and bioconcentration factor of iron by coastal phytoplanktonic diatoms. Mar Environ Res 72:89–95
- Loeppert RH, Suarez DL (1996) Carbonate and gypsun. USDA-ARS/UNL Faculty
- Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. Science 312:1806–1809
- McGranahan G, Balk D, Anderson B (2007) The rising tide: assessing the risks of climate change and human settlements in low elevation coastal zones. Environ Urban 19:17–37
- McVey S, Schoeneberger PJ, Turenne J, Payne M, Wysocki DA (2012) Subaqueous soils (SAS) description. Field book for describing and sampling soils USA: Natural Resources Conservation Service, National Soil Survey Center, Lincoln, NE, pp 2–97
- Nicholls RJ, Cazenave A (2010) Sea-level rise and its impact on coastal zones. Science 328:1517–1520
- Nyman J, DeLaune RD, Roberts HH, Patrick WH Jr (1993) Relationship between vegetation and soil formation in a rapidly submerging coastal marsh. Mar Ecol Prog Ser 96:269–279
- Orson RA, Warren RS, Niering WA (1998) Interpreting sea level rise and rates of vertical marsh accretion in a southern New England tidal salt marsh. Estuar Coast Shelf Sci 47:419–429
- OSMER (2016) [Internet]. Available from: http://www.meteo.fvg.it/clima. php?ln=&m=0
- Otero XL, Sánchez JM, Macías F (2000) Nutrient status in tall and short forms of *Spartina maritima* in the salt marshes of Ortigueira (NW Iberian peninsula) as related to physicochemical properties of the soils. Wetlands 20:461–469

- Pandey P, Tripathi AK (2010) Bioaccumulation of heavy metal in soil and different plant parts of *Albizia procera* (Roxb.) seedling. Bioscan 5: 263–266
- Pedersen O, Colmer TD, Sand-Jensen K (2013) Underwater photosynthesis of submerged plants - recent advances and methods. Front Plant Sci 4:140
- Peel MC, Bloschl G (2011) Hydrological modelling in a changing world. Prog Phys Geogr 35:249–261
- Poldini L, Vidali M, Fabiani ML (1999) La vegetazione del litorale sedimentario del Friuli-Venezia Giulia (NE Italia) con riferimenti alla regione alto-adriatica. Stud Geobot 17:3–68
- Ponnamperuma F (1972) The chemistry of submerged soils. Adv Agron 24: 29–95
- Reddy RK, DeLaume D (2008) Biochemistry of wetlands. CRC Press, Science and applications
- Rogel JA, Ariza FA, Silla RO (2000) Soil salinity and moisture gradients and plant zonation in Mediterranean salt marshes of Southeast Spain. Wetlands 20:357–372. doi:10.1672/0277-5212(2000)020[0357 :SSAMGA]2.0.CO;2
- Salama RB, Otto CJ, Fitzpatrick RW (1999) Contributions of groundwater conditions to soil and water salinization. Hydrogeol J 7:46–64. doi:10.1007/s100400050179
- Sajna N, Regvar M, Kaligaric S, Škvorc Ž, Kaligaric M (2013) Germination characteristics of Salicornia patula Duval-Jouve, S. emerici Duval-Jouve, and S. veneta Pign. et Lausi and their occurrence in Croatia. Acta Bot Croat 72:347–358
- Schoeneberger P, Wysocki DA, Benham ECJ (2012) Field book for describing and sampling soils, version 3.0. Natural Resources Conservation Service, National Soil Survey Center, Lincoln, NE, USA, USA
- Silinski A, van Belzen J, Fransen E, Bouma TJ, Troch P, Meire P, Temmerman S (2016) Quantifying critical conditions for seaward expansion of tidal marshes: a transplantation experiment. Estuar Coast Shelf Sci 169:227–237
- Silvestri S, Defina A, Marani M (2005) Tidal regime, salinity and salt marsh plant zonation. Estuar Coast Shelf Sci 62:119–130
- Simas T, Nunes J, Ferreira J (2001) Effects of global climate change on coastal salt marshes. Ecol Model 139:1–15
- Soil Survey Staff (2014) Keys to soil taxonomy. United States Department of Agriculture, Natural Resources Conservation Service, Lincoln
- Souza I da C, LD R, Morozesk M, MM B, HP A, ID D, LM F, MV M, Mazik K, Elliott M, ST M, CRD M, DA W, MN F (2015) Changes in bioaccumulation and translocation patterns between root and leafs of Avicennia schaueriana as adaptive response to different levels of metals in mangrove system. Mar Pollut Bull 94:176–184
- Stribling JM (1997) The relative importance of sulfate availability in the growth of Spartina alterniflora and Spartina cynosuroides. Aquat Bot 56:131–143
- Tonon G, Sohi S, Francioso O, Ferrari E, Montecchio D, Gioacchini P, Ciavatta C, Panzacchi P, Powlson D (2010) Effect of soil pH on the chemical composition of organic matter in physically separated soil fractions in two broadleaf woodland sites at Rothamsted, UK. Eur J Soil Sci 61:970–979
- UNEP (2006) Marine and coastal ecosystems and human wellbeing: a synthesis report based on the findings of the millennium ecosystem assessment. Banson production, UNEP
- Ushakova SA, Kovaleva NP, Gribovskaya IV, Dolgushev VA, Tikhomirova NA (2005) Effect of NaCl concentration on productivity and mineral composition of *Salicornia europaea* as a potential crop for utilization NaCl in LSS. Adv Space Res 36:1349–1353
- Vittori Antisari L, Carbone S, Ferronato C, Simoni A, Vianello G (2011) Characterization of heavy metals atmospheric deposition for urban environmental quality in the bologna city (Italy). EQA - Int J Environ Qual 7:49–63
- Vittori Antisari L, De Nobili M, Ferronato C, Natale M, Pellegrini E, Vianello G (2016) Hydromorphic to subaqueous soils transitions in the Central Grado lagoon (northern Adriatic Sea, Italy). Estuar Coast Shelf Sci 173:39–48

O Springer

- Warren RS, Niering WA (1993) Vegetation change on a northeast tidal marsh: interaction of sea-level rise and marsh accretion. Ecology 74: 96–103
- Watson CS, White NJ, Church JA, King MA, Burgette RJ, Legresy B (2015) Unabated global mean sea-level rise over the satellite altimeter era. Nat Clim Chang 5:565–568
- Van Wijnen HJ, Bakker JP (1999) Nitrogen and phosphorus limitation in a coastal barrier salt marsh: the implications for vegetation succession. J Ecol 87:265–272
- Van Wijnen HJ, Bakker JP (2001) Long-term surface elevation change in salt marshes: a prediction of marsh response to future sea-level rise. Estuar Coast Shelf Sci 52:381–390
- Wong JXW, Van Colen C, Airoldi L (2015) Nutrient levels modify saltmarsh responses to increased inundation in different soil types. Mar Environ Res 104:37–46
- Worm B, Barbier EB, Beaumont N, Duffy JE, Folke C, Halpern BS, Jackson JBC, Lotze HK, Micheli F, Palumbi SR, Sala E, Selkoe KA, Stachowicz JJ, Watson R (2006) Impacts of biodiversity loss on ocean ecosystem services. Science 314:787–790
- Wysocki DA, Schoeneberger P, Hirmas D, La Garry H (2012) Geomorphology of soil landscapes. In: Huang PM et al. (eds) Handbook of soil science: properties and processes, 2nd edn. CRC Press, Taylor and Francis Group, LLC, Boca Raton, FL ISBN: 978– 1–4398–0305–9
- Zhang D, Yang M, Li J, Chen X (2006) Vegetative dispersal ability of Spartina alterniflora in eastern end of Chongming Island (Chinese). J East China Normal Univ 130–135
- Zuo P, Zhao S, Liu C, Wang C, Liang Y (2012) Distribution of Spartina spp. along China's coast. Ecol Eng 40:160–166

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CHAPTER 3



Zonation of saltmarsh vegetation in the *Marina di Macia* backbarrier saltmarsh, Grado Lagoon: visible the abrupt changes among plant communities.

PREFACE

This work focused on plant feedback mechanisms on soil and on possible effects on vegetation, mediated by plant traits.

In order to investigate the relationships in saltmarshes among some ecological, edaphic and biotic factors, a structural equation model (SEM) was applied. This recent technique allowed to combine multiple effects of multiple variables and to define the main factors affecting an ecosystem. In our study, the tested SEM revealed interesting relationships summarised in Figure 3. Flooding affected directly plant size and soil features, and only indirectly plant abundance and plant community diversity, through plant traits. Bigger plants and larger covers were able to mitigate the negative effect of flooding on soil, improving soil redox status. Plant communities seemed shaped by plant traits and not by flooding stress.



Figure 3. A schematic view of the relationships arisen from the structural equation model

This work was submitted to *Plant Ecology* and it is currently under review. My contribute referred to sampling design, field sampling, laboratory analyses, data analysis, discussion and writings.

1 Plant traits shape the effects of tide flooding on soil and plant communities in

- 2 saltmarshes
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9 Abstract

10 Saltmarshes are recognised to be among the worldwide most complex and fragile ecosystems. 11 Albeit pivotal, the feedback mechanisms between plant communities and soil are frequently 12 overlooked. The aim of this work was to get new insight into these interactions focusing on the 13 plant-soil interface and on its consequences on vegetation. Aboveground traits of two key 14 halophytes of Marano and Grado lagoon (northern Adriatic sea), Limonium narbonense and 15 Sarcocornia fruticosa, were considered to estimate plant growth, while their abundances were used 16 as a proxy of species competition. Structural equation modeling was applied to test relations 17 between predictors and response variables in a single causal network. Flooding period negatively 18 affected plant growth and soil properties, whereas plants improved the redox status of soil. Flooding 19 did not directly affect species abundance or diversity, whose changes were instead driven by plant 20 traits. The direct relationships between plant traits and species richness highlighted that species 21 competition could be even more important than environmental stresses in defining plant diversity 22 and zonation.

23

24 Key words

25 Plant diversity, redox potential, waterlogging, competitiveness, species richness

26

27 Introduction

Trait-based approaches are widely used in plant ecology with the aim to understand ecosystem functioning (Schurr et al. 2005; Ozinga et al. 2009). A trait is any morphological, physiological or phenological feature, measurable for individual plants, which potentially affects its fitness (Violle et al. 2007) or its environment (Lavorel and Garnier 2002). Based on functional traits, many conceptual models have been developed to explain spatial and temporal heterogeneity in plant distribution (Noe et al. 2001; Angiolini et al. 2013). Nonetheless, feedback mechanisms driven by plants and their indirect relationships were often overlooked (Belay and Moe 2015).

35 In saltmarshes, the main driving force is tide flooding and its pivotal role is widely accepted in 36 literature (Marani et al. 2004; Lang et al. 2010). Tide flooding varies in frequency and duration 37 following hypsometry and micro-topology (Wang et al. 2006): it influences crucial soil features, 38 which can affect plant growth, such as oxygen availability and consequently the concentration of 39 toxic microbial reduction products (Colmer and Flowers 2008). In particular, the redox status of soil 40 reflects oxygen availability and determines the concentration of these toxicants (Sánchez et al. 41 1998). In a saltmarsh system, the redox status is not solely affected by flooding or other 42 environmental conditions (Davy et al. 2011), but also by the ability of some plants to release 43 oxygen from roots and to promote an oxic rhizosphere (Howes and Teal 1994; Pedersen et al. 44 2006). In saltmarsh soils, sulphide production is particularly intense due to the relative abundance 45 of sulphate ions in brackish water and the severe anoxic conditions of soils determined by recurrent 46 flooding. Recent studies considered sulphide concentration a potential zonation factor (Koch and 47 Erskine 2001; Borum et al. 2005), but did not consider the existence of plant-soil feedback 48 mechanisms. In fact, many plants are actually able to modify the redox status of soil around roots, 49 and potentially to inhibit sulphide production in a relative large volume of soil. Finally, several 50 studies stressed the importance of species competition in zonation processes of saltmarshes, 51 especially in more elevated areas where environmental factors are not prohibitive (Huckle et al. 52 2002; Wang et al. 2006). The relevance of competition is also supported by gradual changes of 53 physiochemical parameters of soil, even when sharp boundaries among plant communities were 54 recorded (Ungar 1998). Recently, soil stabilisation and development has been attributed to plant 55 diversity and species richness (Ford et al. 2016), highlighting a feedback mechanism of the whole 56 community. All these mechanisms do not act separately, but co-occur and produce complex and 57 highly variable effects (Callaway and Walker 1997), especially in a sensitive ecosystem such as 58 saltmarshes. On the other hand, species-specific strategies could be even more relevant in 59 determining zonation. For instance, dispersal traits are effective indicators to assess species 60 establishment mechanisms in saltmarsh bare soils (Wolters et al. 2008).

61 A trait-based approach could represent a powerful tool to test relationships between soil features 62 and plant communities along ecological gradients. The response of one or multiple plant traits could 63 influence the structure of plant communities (De Bello et al. 2011), which, in turn, determines changes at higher levels (Díaz and Cabido 1997; Westoby and Wright 2006). With this approach, 64 65 the plant response can be quantified and modelled (Vile et al., 2006). In this light, we studied such 66 relations using a structural equation model (SEM) technique. We focused on the relations between 67 the main environmental predictors, i.e. hydroperiod and soil features, and plant communities. In 68 particular, we hypothesised that the hydroperiod affects the entire ecosystem functioning, whereas 69 plant traits of key species may shape their effects. We expected a strong and direct impact of 70 flooding on soil, plants and plant communities diversity, and a possible influence of plant traits on 71 soil chemico-physical properties. We expected also the combined influence of soil properties and 72 species cover in defining species richness, being vegetation the result of the interaction between 73 these two abiotic and biotic compartments.

74

75 Materials and methods

76 Sampling design

77 Data were collected in four saltmarshes of the Grado lagoon (45°42'50"N, 13°20'30"E Northern

78 Adriatic Sea). In each saltmarsh, based on LiDAR elevation model of 1 m resolution, 15 points

57

were randomly distributed along the hypsometric gradient (approx. every 2 ± 0.5 cm), for a total of 60 sampling points. At each point, a plot of 4 m² (2x2 m) was established.

81 Plant community and traits

82 Inside each plot, all occurring vascular species were recorded and their percentage cover was 83 estimated. Two target halophytes were considered, namely Limonium narbonense Mill and 84 Sarcocornia fruticosa (L.) A. J. Scott. These are perennial species displaying a similar distribution 85 along the flooding gradient in the Mediterranean region (Silvestri et al. 2005), but different growth 86 forms and flooding strategies. Ten basal leaves of L. narbonense (at least six, when few individuals 87 were present) and five shoots of S. fruticosa were randomly collected from different individuals in 88 each plot and stored in plastic cases. L. narbonense leaves were the youngest fully expanded leaves 89 of the basal rosettes, while S. fruticosa shoots were the longest annual ones. All samples were 90 afterwards pressed and scanned at a resolution of 300 dpi. Images were analysed using the software 91 ImageJ (Schneider et al. 2012). For each plot, the average leaf area for L. narbonense and the 92 average shoot length for S. fruticosa were measured.

93 Nomenclature of plant *taxa* followed the latest Italian check list of vascular plants (Conti et al.
94 2005).

95 Soil proprieties and hydroperiod

In each plot, three random measurements of soil redox potential were performed at 5 cm depth using a pH-Eh field probe (Crison pH25, Crison Instruments, SA, Spain). Sulphide concentration in soil was recorded once in each plot, using an ion-selective microelectrode (ISE, Lazar Research Laboratories, Los Angeles) and following the procedure described by Eaton et al. (1995).

The hydroperiod, that is the period of time during which the soil is flooded by tidal waters each day (Reed 1990), was calculated for each plot using the elevation of sites and tidal regime data recorded for the Grado station (http://www.venezia.isprambiente.it). Tidal regime data were based on a historical series of ten years (from 2005 to 2015), in order to estimate the hours of submergence each day for each sampling site.

105 Data analysis and priori hypothesised model

Species richness (number of *taxa*) was calculated for each plot. All the soil proprieties pseudo replicates were pooled for each plot.

108 All variables of hydroperiod, plant traits, soil properties and vegetation, were analysed by structural 109 equation modeling (Grace 2006; Lamb et al. 2011). Structural equation model (SEM) techniques 110 provided a structured view of relationships involved and combine multiple predictors and response 111 variables in a single causal network (Grace et al. 2007; Lefcheck 2016). SEM modelling enabled to 112 include hypotheses about how the system works, in order to test them using field data (Grace 2006). 113 Represented as a graphical path model (Vile et al. 2006), SEM resolved complex multivariate 114 relationships (Lefcheck 2016) attributing a weight to each unidirectional relationship and explaining 115 the influence of that variable in each single regression model tested, associated with a standardized regression coefficient (R²). 116

SEM analysis was performed using the "piecewiseSEM" (Lefcheck 2016). Prior to SEM analysis, 117 118 all variables were tested and transformed (when necessary) to achieve linearity (i.e. cover of both 119 species with a logarithmic function). Collinearity of variables was checked by Pearson r (r>0.7) and 120 by variance inflation factor using the 'vif' function (Fox and Weisberg 2011). Linear mixed models 121 were applied including the saltmarsh site (four in total) as random factor, to avoid scale-dependent 122 effects. In fact, it is demonstrated that flooding is a site-specific factor (see Silvestri et al. 2005). For 123 all variables, a polynomial term was also preliminary included to check for possible non-linear 124 relationships. None of these was included in the final SEM. All tested models were accepted when 125 Chi-square test resulted above the significance level (p>0.05) and compared each other using the 126 Akaike's Information Criterion (AICc), which increases oppositely to the relative likelihood of the 127 model (Burnham and Anderson 2004). The final model presented the low AICc among the possible 128 models generated by the SEM analysis.

- 129 The fit of the models was assessed using Shipley's test in order to evaluate possible missing paths
- 130 and include them in the hypothesised a priori model (Shipley 2009). The goodness of fit of the SEM
- 131 was evaluated using the Chi-square test (p>0.05) in the "lavaan" package (Rosseel 2012).
- 132 The analyses were performed using R statistical Software v. 3.3.2 (R Development Core Team
- 133 2016).
- 134 With this model (figure 1) we tested: i) the impact of flooding on all other variables considered, ii)
- 135 the feedback mechanisms of plants towards soil, iii) and the influence of flooding, soil and plant
- 136 traits on plant communities.



137

138 Figure 1. The hypothesized structural equation model.

139

143 Lundholm et al. 2014), while the percent cover of each species represented their abundance (i.e.

The hydroperiod was considered the driving independent factor and represents the flooding stress. It drove changes in edaphic features and plant growth influencing plant traits. *L. narbonense* leaf area and *S. fruticosa* shoot length were considered proxies of individual plant size (Navarro et al. 2010;

density) (Pottier et al. 2007). We used species abundance as an index of both intra- and interspecific competition. We hypothesized that both plant traits and cover could significantly influence soil features (Elmore et al. 2016), interfering with the effects of flooding. Finally, we tested if flooding, soil properties and plant traits or cover can affect the species richness of plant communities, as highlighted in other coastal wetlands (Grace and Pugesek 1997; Ford et al. 2016).

149

150 Results

151 Data obtained from the tested model are reported in table 1. The hydroperiod varied significantly 152 between sites with different elevations. Redox potentials and sulphide concentrations measured in 153 soil were also highly variable. Soil conditions were mostly anoxic, ranging from moderate oxic 154 conditions to severe anoxia and sulphide concentrations reached very high values. Species richness 155 was very low, with a total of 15 species recorded during vegetation surveys. L. narbonense 156 (frequency 1.00) and S. fruticosa (frequency 0.92) were the most frequent species. Plant traits (leaf 157 area and shoot length) and cover of these two target species appeared highly variable. Other 158 recurring species were Aster tripolium (frequency 0.42) in more elevated sites, or Puccinellia 159 festuciformis (frequency 0.42) and Spartina maritima (frequency 0.40) in lower sites.

160

161 Table 1. Mean, minimum and maximum values of the analysed variables included in the structural

162 equation model.

Variable	Average ± sd	Min	Max
Hydroperiod (h _{submergence} ·day ⁻¹ ·year ⁻¹)	3.4±2.0	0.5	7.2
Redox potential (mV)	-15±193	-407	221
Sulphides (mM)	5.03±9.90	0.07	58.65
Limonium narbonense leaf area (cm ²)	13.2±7.0	3.1	37.9
Sarcocornia fruticosa shoot length (cm)	18.4±6.3	9.1	39.3

Limonium narbonense cover (%)	34±25	3	85
Sarcocornia fruticosa cover (%)	26±23	0	75
Species richness (n. of species)	4.6±1.6	2	8

163

The tested structural equation model (figure 2) showed a good fit with a Chi-square test of 5.65 (4 df, p=0.22). The hydroperiod negatively affected the redox status of soil and the traits of both species. The reduction of plant size appeared more pronounced in *S. fruticosa* than in *L. narbonense*, showing a slightly stronger sensitivity of *S. fruticosa* to flooding. The hydroperiod showed, as expected, a positive correlation with sulphide concentrations in soil, where a great flooding stress corresponded to a large production of sulphides. The hydroperiod did not show any direct relationship with the cover of both species.

171 Plant traits strongly affected species covers but in opposite ways. Plants of S. fruticosa with longer 172 shoot length were associated with an increase of the overall cover of the species, while the increase 173 of L. narbonense leaf area was, on the contrary, associated with a lower overall cover of the species. 174 No direct relationships were found between the two species, in terms of either growth (plant traits) 175 or competitiveness (cover). The cover of both species and L. narbonense leaf area positively 176 influenced the redox status of soil. Plant traits and species cover showed therefore a direct influence 177 on soil properties. Species richness was positively correlated to L. narbonense leaf area, while no 178 direct relationships were recorded with the hydroperiod or soil features (redox status and sulphides).





Figure 2. Results of the tested structural equation model. Black arrows refer to statistical significant relationships ($p \le 0.05$). Arrow width is proportional to the size of the effect, reported as the standardized effect size in the text box (* $0.05 \le p < 0.01$, ** $0.01 \le p < 0.001$, *** $p \le 0.001$). Grey dashed arrows represent nonsignificant paths (p > 0.05). Conditional R² are also shown in the boxes.

185 Discussion

186 Results highlighted two interesting aspects: i) the direct effect of plant traits and cover on soil redox
187 status, and ii) the indirect influence of the hydroperiod on plant communities, mediated by plant
188 traits.

Our findings suggest that most abundant species can actively mitigate the anoxic conditions of soil determined by flooding, by means of their traits. Plant growth is affected by oxygen deficiency (Jackson and Colmer 2005), but plant traits allow changes of soil conditions within the rhizosphere, diminishing the negative influence of flooding. In our study, greater individuals of *L. narbonense* and larger covers of both considered species improved redox soil status. Specifically, soil anoxia 194 was reduced in sites with high plant densities and large individual size. It is well known that some 195 plants are able to release oxygen from roots (Colmer and Flowers 2008), a common trait for species 196 inhabiting flood-prone areas. This process was already observed in several halophytes, e.g. Spartina 197 alterniflora (Howes and Teal 1994), Juncus roemarianus (Koretsky et al. 2008) and L. narbonense, 198 for which indirect evidence is related to the presence of red-mottles along soil profiles (Vittori 199 Antisari et al. 2016). Large individuals display a well-developed root system and effectively modify 200 their soil environment. At the same time, a dense vegetation stand can produce an even more 201 complex and dense root system, which can promote soil aeration (van der Putten et al. 2013). The 202 re-establishment of aerobic conditions in the region of soil explored by roots allows plants to take 203 up nutrients more easily and to decrease the potential adverse effect of toxic products of soil 204 reduction, sulphides included (Koretsky et al. 2008). Moreover, oxygen diffusion favours not only 205 soil development, but also nutrient cycling, by enhancing decomposition processes (Reddy and 206 DeLaune 2008). This feedback mechanism allows to distinguish the effect of site elevation, in 207 which the flooding variable was calculated, from the effect of soil redox potential. Davy et al. 208 (2011) have found that the distribution of some species responds only to one of these two variables 209 and that the distinct effects of these could be separately evaluated. Nevertheless, the same authors 210 listed only soil texture and marsh microtopography as possible effecting factors, without 211 considering the relevance of plant-soil interactions. Our work suggests, instead, that plants can 212 modify significantly the redox status of soil, providing a new perspective for this debated 213 achievement.

Anoxia, derived from flooding, favours the establishment of anaerobic microbial communities in soil. At low values of redox potential (from -75 to -150 mV), when oxygen is completely depleted, sulphate is reduced to sulphides during a process carried out by sulphur reducing bacteria (Reddy and DeLaune 2008). Under these conditions, oxygen released from plant roots is rapidly consumed by reaction with reduced soil components and it is, therefore, not able to restore oxic conditions in a sufficient portion of the soil volume to inhibit sulphides formation (Reddy and DeLaune 2008). For
this reason, plants can not always limit sulphide production and probably can effectively interferewith soil redoximorphic properties only when conditions are not too severe.

Plant growth and distribution could be also affected by soil salinity, especially in inland saltmarsh habitats (Álvarez Rogel et al. 2000; Redondo-Gómez 2007). Nevertheless, the salinity variable was excluded from the present work, considering that some authors rejected its relevance (Noe et al. 2001; Piernik 2003) or highlighted the great spatial variability of the salinity stress and the large salt tolerance range of halophytes (Pennings et al. 2005).

227 The positive relationship recorded in the model between size and cover of S. fruticosa could be 228 related to the suffrutex form of this species, whose competitiveness is enhanced in big individuals. 229 The size of shrubs was already linked to species competition (Treydte et al. 2009) and shrub 230 encroachment was well-predicted using plant traits (Belay and Moe 2015). On the other hand, the 231 negative relation between L. narbonense size and population cover is a common trait in wetland 232 species, especially those forming highly dense or mono-specific stands (Conk and Fennessy 2001). 233 L. narbonense intraspecific competition determined stands with few bigger individuals covering 234 less than those with more individuals of smaller size. In literature, self-competition phenomena 235 were linked to clonal stands (van Kleunen et al. 2001; Herben et al. 2013) or to severe ecological 236 conditions (Klimešová et al. 2012). L. narbonense stands fulfil both conditions. Environmental 237 stresses in saltmarshes are extremely intense and could limit the development of vegetation. 238 Moreover, the pioneer stands are usually mono- or pauci-specific, with individuals of similar age 239 contributing to the overall cover. Life stage may also affect the interactions with other plant species 240 (Callaway and Walker 1997), where plant size and density are functions of life stages, able to 241 determine a larger or smaller competitive ability (Keddy and Shipely 1989).

The size of *L. narbonense* individuals affected also plant communities by acting directly and positively on species richness. A species could in fact have a negative or positive influence on the others (Callaway 2002), e.g. altering sediments or plant dynamics (Langlois et al. 2003). Big but few individuals of *L. narbonense* mean more ecological space for the establishment of other species 246 and the increase of species richness. Oppositely to the findings of Grace and Pugesek (1997), our 247 results highlighted the absence of direct influences of flooding or soil properties on plant 248 communities. The pivotal importance of biotic factors and competition, compared to environmental 249 stresses, was noted by other authors after an intense stress (e.g. fire, Grace et al. 2006) or in other 250 vegetation types (e.g. alpine shrubs, Callaway et al. 2002). A close research work has highlighted 251 that the distribution limit of some species is simply set by competition and not by physical stresses 252 (e.g. for Spartina alterniflora) or, on the contrary, only by physical factors (e.g. for the species 253 Juncus roemerianus) (Pennings et al. 2005).

Our results highlighted the prevalence of competition phenomena in defining plant community richness in saltmarsh environment, where plant traits of target species drive changes at the vegetation scale, modulating the effect of flooding.

257

258 Conclusion

259 In this paper, the direct influence of some plants on soil redox potential was confirmed. The 260 relevant role of species competition in defining plant zonation was also highlighted. The indirect 261 effect of flooding was crucial: it determined the soil chemico-physical properties, the concentration 262 of reduced soil compounds and the plant growth. Moreover, it indirectly affected species richness 263 through plant traits. In the light of the ongoing global changes (Boesch et al. 1994; Zhang et al. 264 2004), our findings suggest that the expected sea level rise will impact directly on species growth 265 and only partially or indirectly on both soil and plant diversity. Therefore, plant acclimations at 266 individual level seem to shape the changes on soil and vegetation. In saltmarsh ecosystems, these 267 findings have important implications for ecological prediction, nature conservation and restoration. 268 As in wetlands, Perry et al. (2004) proposed to manipulate resources availability to both enhance 269 native species and control invasive plants; similarly, we may suggest to actively act on the growth 270 of target species, modifying their abundance, in order to enhance soil features and plant diversity.

271 The monitoring of vegetation will be a useful tool to supervise the success of management practices

- 272 and, if necessary, to plan more suitable programs for each specific wetland system.
- 273

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- 278

279 References

- 280 Álvarez-Rogel J, Ariza FA, Silla RO (2000) Soil salinity and moisture gradients and plant zonation
- 281 in Mediterranean salt marshes of Southeast Spain. Wetlands 20: 357-372
- 282 Angiolini C, Landi M, Pieroni G, Frignani F, Finoia MG, Gaggi C (2013) Soil chemical features as
- 283 key predictors of plant community occurrence in a Mediterranean coastal ecosystem. Estuar Coast
- 284 Shelf Sci 119: 91-100
- 285 Belay TA, Moe SR (2015) Assessing the Effects of Woody Plant Traits on Understory Herbaceous
- 286 Cover in a Semiarid Rangeland. Environ. Manage 56(1): 165-175
- 287 Boesch DF, Josselyn MN, Mehta AJ, Morris JT, Nuttle WK, Simenstad CA, Swift DJP (1994)
- 288 Scientific assessment of coastal wetland loss, restoration and management in Louisiana. J Coast
- 289 Res, Special Issue 20: 103
- 290 Burnham KP, Anderson DR (2004) Multimodel Inference: Understanding AIC and BIC in Model
- 291 Selection. Sociol. Methods Res 33: 261-304
- 292 Borum J, Pedersen O, Greve TM, Frankovich TA, Zieman JC, Fourqurean JW, Madden CJ (2005)
- 293 The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass,
- 294 Thalassia testudinum. J Ecol 93(1): 148-158

- 295 Callaway RM, Brooker RW, Choler P, Kikvidze Z, Lortie CJ, Michalet R, Paolini L, Pugnaire FI,
- 296 Newingham B, Aschehoug ET, Armas C, Kikodze D, Cook BJ (2002) Positive interactions among
- alpine plants increase with stress. Nature 417: 844-848
- 298 Callaway RM, Walker LR (1997) Competition and facilitation: a synthetic approach to interactions
- 299 in plant communities. Ecology 78(7): 1958-1965
- 300 Colmer TD, Flowers TJ (2008) Flooding tolerance in halophytes. New Phytol 179: 964-974
- 301 Conti F, Abbate G, Alessandrini A, Blasi C (2005) An annotated check-list of the italian vascular
- 302 flora. Palombi ed, Roma
- 303 Cronk JK, Fennessy MS (2016) Wetland plants: biology and ecology. CRC press LLC,
- 304 Washington, DC, 482 pp.
- 305 Davy AJ, Brown MJ, Mossman HL, Grant A (2011) Colonization of a newly developing salt marsh:
- 306 disentangling independent effects of elevation and redox potential on halophytes. J Ecol 99(6):
- 307 1350-1357
- 308 De Bello F, Lavorel S, Albert CH, Thuiller W, Grigulis K, Dolezal J, Janecek S, Lêps J (2011)
- 309 Quantifying the relevance of intraspecific trait variability for functional diversity. Methods in
- 310 Ecology and Evolution 2: 163-174
- 311 Díaz S, Cabido M (1997) Plant functional types and ecosystem function in relation to global
- 312 change. J Veg Sci 463-474
- 313 Eaton AD, Clesceri SL, Greenberg AE (1995) Standard methods for the examination of water and
- 314 wastewater. 19th ed. Am Public Health Assoc, Washington, DC.
- 315 Elmore AJ, Engelhardt KA, Cadol D, Palinkas CM (2016) Spatial patterns of plant litter in a tidal
- 316 freshwater marsh and implications for marsh persistence. Ecol App doi: 10.1890/14-1970
- 317 Ford H, Garbutt A, Ladd C, Malarkey J, Skov MW (2016) Soil stabilization linked to plant
- 318 diversity and environmental context in coastal wetlands. J Veg Sci 27(2): 259-268
- 319 Fox J, Weisberg S (2011) An {R} Companion to Applied Regression. Sage Publications

- 320 Grace JB, Keeley JE (2006) A structural equation model analysis of postfire plant diversity in
- 321 California shrublands. Ecol Appl 16(2): 503-514
- 322 Grace JB, Pugesek BH (1997) A structural equation model of plant species richness and its
- 323 application to a coastal wetland. Am Nat 436-460
- 324 Grace JB (2006) Structural equation modeling and natural systems. Cambridge Univ Press
- 325 Grace JB, Anderson MT, Smith MD, Seabloom E, Andelman SJ, Meche G, Weiher E, Allain LK,
- 326 Jutila H, Sankaran M, Knops J, Ritchie M, Willig MR (2007) Does species diversity limit
- 327 productivity in natural grassland communities? Ecology Letters 10: 680-689
- 328 Gribsholt B, Kristensen E (2003) Benthic metabolism and sulfur cycling along an inundation
- 329 gradient in a tidal Spartina anglica salt marsh. Limnol Oceanogr 48(6): 2151-2162
- 330 Herben T, Nováková Z, Klimesová J (2014) Clonal growth and plant species abundance. Ann Bot
- 331 114: 377-388
- 332 Howes BL, Teal JM (1994) Oxygen loss from Spartina alterniflora and its relationship to salt
- 333 marsh oxygen balance. Oecologia 97(4): 431-438
- 334 Huckle JM, Marrs RH, Potter JA (2002) Interspecific and intraspecific interactions between salt
- 335 marsh plants: integrating the effects of environmental factors and density on plant performance.
- 336 Oikos 96: 307-319
- 337 Jackson MB, Colmer TD (2005) Response and adaptation by plants to flooding stress. Ann Bot
- 338 96(4): 501-505
- Keddy PA, Shipley B (1989) Competitive hierarchies in herbaceous plant communities. Oikos
 54:234-241
- 341 Klimešová J, Doležal J, Prach K, Košnar J (2012) Clonal growth forms in Arctic plants and their
- 342 habitat preferences: a study from Petuniabukta, Spitsbergen. Polish Polar Research 33(4): 421-442
- 343 Koch MS, Erskine JM (2001) Sulfide as a phytotoxin to the tropical seagrass Thalassia testudinum:
- 344 interactions with light, salinity and temperature. J Exp Mar Biol Ecol 266:81-95

- 345 Koretsky CM, Haveman M, Cuellar A, Beuving L, Shattuck T, Wagner M (2008) Influence of
- 346 Spartina and Juncus on saltmarsh sediments. I. Pore water geochemistry. Chem. Geol. 255(1): 87-
- 347 99
- 348 Lamb EG, Shirtliffe S, May W (2011) Structural equation modeling in the plant sciences: an
- 349 example using yield components in oat. Can J Plant Sci 91: 603-619
- 350 Lang F, von der Lippe M, Schimpel S, Scozzafava-Jaeger T, Straub W (2010) Topsoil morphology
- 351 indicates bio-effective redox conditions in Venice salt marshes. Estuarine, Coastal and Shelf
- 352 Science 87(1): 11-20
- 353 Langlois E, Bonis A, Bouzillé JB (2003) Sediment and plant dynamics in saltmarshes pioneer zone:
- 354 Puccinellia maritima as a key species? Estuar Coast Shelf Sci 56: 239-249
- 355 Lavorel S, Gamier E (2002) Predicting changes in community composition and ecosystem
- 356 functioning from plant traits: revisiting the Holy Grail. Funct Ecol 16: 545-556
- 357 Lefcheck JS (2016) piecewiseSEM: Piecewise structural equation modelling in r for ecology,
- 358 evolution, and systematics. Methods in Ecology and Evolution 7: 573-579
- 359 Lundholm J, Heim A, Tran S, Smith T (2014) Leaf and life history traits predict plant growth in a
- 360 green roof ecosystem. PloS one 9(6), e101395
- 361 Marani M, Lanzoni S, Silvestri S, Rinaldo A (2004) Tidal landforms, patterns of halophytic
- 362 vegetation and the fate of the lagoon of Venice. J Mar Syst 51(1): 191-210
- 363 Navarro T, Oualidi JE, Taleb MS, Pascual V, Cabezudo B, Milla R (2010) Leaf patterns, leaf size
- 364 and ecologically related traits in high Mediterranean mountain on the Moroccan High Atlas. Plant
- 365 Ecol 210: 275-290
- 366 Noe GB, Zedler JB (2001) Spatio-temporal variation of salt marsh seedling establishment in
- 367 relation to the abiotic and biotic environment. J Veg Sci 12(1): 61-74
- 368 Ozinga WA, Romermann C, Bekker RM, Prinzing A, Tamis WLM, Schaminee JHJ, Hennekens
- 369 SM, Thompson K, Poschlod P, Kleyer M, Bakker JP, van Groenendael JM (2009) Dispersal failure
- 370 contributes to plant losses in NW Europe. Ecol Lett 12:66-74

- 371 Pedersen O, Vos H, Colmer TD (2006) Oxygen dynamics during submergence in the halophytic
- 372 stem succulent Halosarcia pergranulata. Plant Cell Env 29(7): 1388-1399
- 373 Pennings SC, Callaway RM (1992) Salt marsh plant zonation: the relative importance of
- 374 competition and physical factors. Ecology 73(2): 681-690
- 375 Penning SC, Grant MB, Bertness MD (2005) Plant zonation in low latitude salt marshes:
- 376 disentangling the roles of flooding, salinity and competition. J Ecol 93(1): 159-167
- 377 Perry LG, Galatowitsch SM and Rosen CJ (2004) Competitive control of invasive vegetation: a
- 378 native wetland sedge suppresses Phalaris arundinacea in carbon-enriched soil. J App Ecol 41: 151-
- 379 162
- 380 Piernik A (2003) Inland halophilous vegetation as indicator of soil salinity. Basic Appl Ecol 4(6):
- 381 525-536
- 382 Pottier J, Marrs RH, Bédécarrats A (2007) Integrating ecological features of species in spatial
- 383 pattern analysis of a plant community. J Veg Sci 18(2): 223-230
- 384 R Core Team (2016) R: A language and environment for statistical computing. R Foundation for
- 385 Statistical Computing, Vienna, Austria
- 386 Reddy KR, DeLaune RD (2008) Biogeochemistry of wetlands: science and applications. CRC
- 387 press, Washington, DC, 800 pp.
- 388 Redondo-Gómez S, Mateos-Naranjo E, Davy AJ, Fernández-Muñoz F, Castellanos EM, Luque T,
- 389 Figueroa ME (2007) Growth and photosynthetic responses to salinity of the salt-marsh shrub
- 390 Atriplex portulacoides. Ann Bot 100(3): 555-563
- 391 Reed DJ (1990) The Impact of Sea-level Rise on Coastal Salt Marshes. Prog Phys Geog 14(4): 465-
- 392 481
- 393 Rosseel Y (2012) lavaan: an R package for structural equation modeling. J Stat Software 48: 1-36
- 394 Sánchez JM, Otero XL, Izco J (1998) Relationships between vegetation and environmental
- 395 characteristics in a salt-marsh system on the coast of Northwest Spain. Plant Ecol 136(1): 1-8

396 Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis.

397 Nature methods 9(7): 671-675

- 398 Schurr FM, Bond WJ, Midgley GF, Higgins SI (2005) A mechanistic model for secondary seed
- 399 dispersal by wind and its experimental validation. J Ecol 93:1017-1028
- Shipley B (2009) Confirmatory path analysis in a generalized multilevel context. Ecology 90: 363368
- 402 Silvestri S, Defina A, Marani M (2005) Tidal regime, salinity and salt marsh plant zonation. Estuar
- 403 Coast Shelf Sci 62(1): 119-130
- 404 Treydte AC, Grant CC, Jeltsch F (2009) Tree size and herbivory determine below-canopy grass
- 405 quality and species composition in savannahs. Biodivers Conserv 18: 3989-4002
- 406 Ungar IA (1998) Are biotic factors significant in influencing the distribution of halophytes in saline
- 407 habitats?. Bot Rev 64(2): 176-199
- 408 van Kleunen M, Fischer M, Schmid B (2001) Effects of intraspecific competition on size variation
- 409 and reproductive allocation in a clonal plant. Oikos 94: 515-524
- 410 van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P,
- 411 Klironomos JN, Kulmatiski A, Schweitzer JA, Suding KN, van de Voorde TFJ, Wardle DA (2013)
- 412 Plant-soil feedbacks: the past, the present and future challenges. J Ecol 101: 265-276
- 413 Vile D, Shipley B, Garnier E (2006) A structural equation model to integrate changes in functional
- 414 strategies during old field succession. Ecology 87(2): 504-517
- 415 Violle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E (2007) Let the concept
- 416 of trait be functional!. Oikos 116(5): 882-892
- 417 Vittori Antisari L, De Nobili M, Ferronato C, Natale M, Pellegrini E, Vianello G (2016)
- 418 Hydromorphic to subaqueous soils transitions in the central Grado lagoon (Northern Adriatic Sea,
- 419 Italy). Estuar Coast Shelf Sci 173: 39-48

- 420 Wang Q, Wang CH, Zhao B, Ma ZJ, Luo YQ, Chen JK, Li B (2006) Effects of growing conditions
- 421 on the growth of and interactions between salt marsh plants: implications for invasibility of habitats.
- 422 Biological Invasions 8(7): 1547-1560
- 423 Westoby M, Wright IJ (2006) Land-plant ecology on the basis of functional traits. Trends Ecol Evol
- 424 21(5): 261-268
- 425 Wolters M, Garbutt A, Bekker RM, Bakker JP, Carey PD (2008) Restoration of salt-marsh
- 426 vegetation in relation to site suitability, species pool and dispersal traits. J App Ecol 45(3): 904-912
- 427 Zhang KQ, Douglas BC and Leatherman SP (2004) Global warming and coastal erosion. Climatic
- 428 Change 64: 41-58

CHAPTER 4



A plant individual of *Sarcocornia fruticosa* partially submerged during high tide, *Martignano* saltmarsh, Marano Lagoon.

PREFACE

Since *Sarcocornia fruticosa* and *Limonium narbonense* were recognized as key species in the saltmarsh community, equally able to colonise well drained and frequently flooded sites, the work was carried out in a greenhouse with the aim to clarify their strategies against flooding.

The main factor limiting plant growth in saltmarshes is the unavailability of oxygen. Therefore, the role of internal plant aeration during submergence of the two halophytes was investigated. Internal aeration seemed relevant only for *S. fruticosa*, which benefitted from snorkelling, transporting oxygen from shoots in contact with air to submerged tissues. *L. narbonense* flooding tolerance seemed instead more complex: the high tissue porosity did not support aeration even during partial submergence. These achievements suggested that other processes could be even more important, e.g. fermentative processes supporting plant metabolism during anoxia.

The work was performed thanks to the collaboration with the prof. Ole Pedersen, of the Freshwater Biological Laboratory, Department of Biology, of the University of Copenhagen (Denmark). My contribute referred to sampling, laboratory work, conducted in the Pedersen's laboratories, data analysis, discussion and the writings. The work was presented during the 12th conference of the International Society for Plant Anaerobiosis (ISPA) and *in press* in the journal *Plant Functional Biology*.

1

Contrasting oxygen dynamics in *Limonium narbonense* and *Sarcocornia fruticosa* during partial and complete submergence

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12

13 Abstract

14 Terrestrial saltmarsh plants inhabiting flood-prone habitats undergo recurrent and 15 prolonged flooding driven by tidal regimes. In this study, the role of internal plant aeration 16 in contrasting hypoxic/anoxic conditions during submergence was investigated in the two 17 halophytes Limonium narbonense Mill. and Sarcocornia fruticosa (L.) A.J. Scott. 18 Monitoring of tissue O₂ dynamics was performed in shoots and roots using microelectrodes 19 under drained conditions, waterlogging, partial and complete submergence, in light or 20 darkness. For both species, submergence in darkness resulted in significant declines in 21 tissue O2 status and when in light, in rapid O2 increases first in shoot tissues and 22 subsequently in roots. During partial submergence, S. fruticosa benefitted from snorkelling 23 and efficiently transported O_2 to roots, while the O_2 concentration in roots of L. narbonense declined by more than 90%. Significantly thinner leaves and articles were 24 25 recorded under high degree of flooding stress and both species showed considerably high tissue porosity. The presence of aerenchyma seemed to support internal aeration in S. 26 27 fruticosa while O_2 diffusion in L. narbonense seemed impeded, despite the higher porosity (up to 50%). Thus, the results obtained for L. narbonense, being well-adapted to flooding, 28 29 suggests that processes other than internal aeration could be involved in better flooding 30 tolerance e.g., fermentative processes, and that traits resulting in flooding tolerance in plants are not yet fully understood. 31

32

Additional keywords: aerenchyma, dark respiration, flooding, oxygen diffusion,
 photosynthesis

35 Introduction

36 Flooding tolerance in terrestrial plants is a topic widely discussed on crops (for a recent 37 review see Colmer and Pedersen 2008; Loreti et al. 2016). However, laboratory or field 38 investigations conducted on natural wetland species are less common and mostly related to 39 plants affected only occasionally or temporarily by submergence (see e.g. White et al. 40 2014). Terrestrial halophytes inhabiting saltmarshes represent successful strategies of 41 flooding tolerance, and these plants have to come with recurrent tissue anoxia occurring 42 during tidal inundation to tackle partial or complete submergence stress on a daily basis. 43 The very low elevations of saltmarshes, usually less than 2 m above the mean sea level 44 (McKee and Patrick 1998), entail a sequence of recurrent flooding events, linked to the 45 tidal cycle, which affect soil O_2 availability and consequently the growth, survival and 46 distribution of halophytes (Silvestri et al. 2005; Lang et al. 2010). Potential toxic 47 compounds can easily accumulate in flooded soils when O2 is depleted (Ponnamperuma 48 1984) and these compounds, sulphides in particular (Borum et al. 2013), can be 49 detrimental to plant survival. Therefore, roots of halophytes adopt strategies to resist O₂ 50 deficiency (see e.g., Colmer et al. 2013) and the intrusion of toxic compounds from anoxic 51 sediments. For this reason, flooding tolerance in plants is basically associated with the 52 capacity to take up and/or produce O_2 during submergence and to direct it from the shoots 53 to the roots (Colmer and Flowers 2008).

54 The presence of internal gas spaces in plant tissues (aerenchyma) is of great importance for 55 O₂ diffusion, supporting not only root aeration during partial (Herzog and Pedersen 2014) 56 or complete submergence (Colmer 2003), but also reducing tissue respiratory demand per 57 unit of volume (Armstrong 1979, Perata et al. 2011). During partial submergence, plants 58 could benefit from snorkelling (Herzog and Pedersen 2014) and when completely 59 submerged, shoot elongation could be stimulated in order to restore contact with the 60 atmosphere (Huber et al. 2012). Other adaptations could also be promoted e.g., the 61 acclimation of aquatic leaves with a reduction of palisade tissue and a thinner cuticle to 62 facilitate underwater net photosynthesis (P_N, Mommer et al. 2007). In fact, plants can 63 benefit considerably during light periods from O_2 produced by underwater P_N , using 64 respiratory CO₂ accumulated in the tissues during darkness (Pedersen et al. 2006, Colmer 65 and Pedersen 2008) or CO₂ from sediment that diffuses to shoots via aerenchyma (Perata et 66 al. 2011). The capacity to continue P_N when underwater enhances plant tolerance to 67 submergence, providing O2 for internal aeration and sugars for growth and plant 68 metabolism (Mommer et al. 2007).

69 Flooding is the main environmental factor affecting plant zonation in saltmarshes and the 70 majority of halophytes show a narrow ecological distribution (Silvestri et al. 2005). 71 Nevertheless, some well-adapted species, capable of being equally competitive in drained 72 and flooded soils, stimulated our interest in tolerance strategies adopted and the possible 73 acclimation to different degrees of flooding stress. Following this insight, the perennial 74 halophytes Limonium narbonense Mill. and Sarcocornia fruticosa (L.) A.J. Scott were 75 selected as model plants for the present study. These two saltmarsh species are well-76 adapted to flooding, sharing a similar distribution in the Mediterranean coastal region 77 (Pandža et al. 2007). The two species show different growth forms: L. narbonense has 78 basal fleshy leaves in rosettes and a deep root system connected to a vertical rhizome 79 storing carbohydrate reserves, while S. fruticosa is a semi-woody plant with modified 80 photosynthetic succulent stems and a superficial root system. Regarding photosynthesis, L. 81 narbonense uses the C₃ pathway (Eley et al. 2016) whereas S. fruticosa has been described 82 as a CAM species (Duarte et al. 2012). L. narbonense seems better adapted to 83 submergence compared to S. fruticosa, sharing with Spartina maritima the more frequently 84 flooded saltmarsh sites (Pandža et al. 2007).

85 The present study focused on the role of internal aeration in supporting flooding tolerance 86 in L. narbonense and S. fruticosa, when partially or completely submerged, and in 87 darkness or in light. Internal aeration is widely accepted in the literature as a crucial factor 88 promoting submergence tolerance (see e.g., Colmer and Flowers 2008) and internal 89 aeration is probably the main strategy employed by these two halophytes. We expected a 90 decline in internal O₂ upon submergence in both species, especially in roots. Based on field 91 observations, we hypothesised that S. fruticosa could be more severely affected by 92 submergence than L. narbonense, which shows numerous adventitious roots. The ability to 93 translocate O2 was evaluated by assessing O2 dynamics and measuring tissue porosity, 94 since high porosity should result in better internal aeration.

In addition, possible acclimation phenomena to different degrees of flooding stress (high and low stress) were investigated. Acclimation considers morphological features, tissue porosity for instance, or physiological traits, especially underwater P_N and underwater dark respiration (R_D) of photosynthetic tissues (leaves of *L. narbonense* or green articles of *S. fruticosa*). Underwater P_N could significantly enhance flooding tolerance in terrestrial plants (Pedersen *et al.* 2013), sustaining tissue aeration and producing carbohydrates, albeit

101 at much lower rate, to support growth during tidal submergence in light.

103 Materials and methods

104 Sampling sites and plant material

105 Plants were collected in two saltmarshes of the Grado and Marano Lagoon (approximately 106 30 km from Trieste, northern Adriatic Sea, Italy), where the average elevation range of 107 saltmarshes is about 0.25-0.30 m above the mean sea level and tides are semidiurnal, with 108 neap and spring tidal ranges of about 0.45 and 1 m respectively (Sarretta et al. 2010). The 109 first saltmarsh (BB - 45°43'04''N, 13°23'02''E) is a channel margin saltmarsh, located 110 parallel to the Mosconi dike road and partially artificial due to dredged sediments derived 111 from the excavation of the adjacent Belvedere channel. This saltmarsh is characterised by 112 pans and channels developing inside the marsh and a quite homogeneous muddy texture of soil. The second saltmarsh (BO - 45°40'52"N, 13°21'16"E) is a back-barrier saltmarsh, 113 114 located in the peri-lagoonal area, developed during a wash-over event which broke the 115 ridge of the external sand barrier (Banco d'Orio). For its origin the saltmarsh is quite small (0.12 km²) and characterised mostly by sandy coarse sediment. 116 117 Plants were collected during winter rest (November 2015) by digging up individuals in

118 intact blocks of soil and transferring them to squared pots (12 x 12 cm, 18 cm depth). Pots 119 containing plants were placed in a 22°C room and watered with diluted artificial seawater

115 containing plants were placed in a 22 c room and watered with ended autherial seawater

120 (see below) to facilitate vegetative recovery of plants until the start of the acclimation.

121

122 Acclimation system and plant tissue turnover

123 Plant acclimation setup was placed in a 25/15°C day/night greenhouse, simulating tidal variations using artificial seawater and exposing plants to light for 10 h a day (from 8.00 124 a.m. to 6.00 p.m., PAR approx. 250 µmol photons m⁻² s⁻¹ from 11.00 a.m. to 3.00 p.m.). 125 126 The simulated tides were imposed during the day (9.00 a.m.-3.00 p.m.) and night (10.00 127 p.m.-4.00 a.m.). Artificial seawater (salinity 35‰) was prepared with the following 128 composition: 23.926 g NaCl, 11.09 g MgCl₂ 6H₂O, 4.008 g Na₂SO₄, 1.55 g CaCl₂ 2H₂O, 129 0.677 g KCl, 0.196 g NaHCO₃, 0.098 g KBr, 0.026 g H₃BO₃, 0.003 g NaF for 1 L solution 130 (Kester et al. 1967).

Immersion pumps and plastic tanks with drainage holes at different heights were used to impose two different flooding treatments. Half of the pots were exposed to a relative low flooding stress (low stress), where conditions changed from well-drained (water level at 2 cm from the bottom of the pots) to waterlogged (approx. 0.5 cm above soil surface). The remaining half of the pots were exposed to a higher flooding stress where water level 136 changed from waterlogging to complete submergence of the plants, ensured by a 137 transparent net positioned on the top of the plastic tanks so that snorkelling was prevented.

138 Tissue turnover of plants was monitored by marking all leaves in L. narbonense and the

139 apex of all green articles in S. fruticosa with a permanent marker before the acclimation.

140 After two months, new leaves and new articles were counted and tissue turnover rates were

141 expressed as new leaves or articles divided by number of initial leaves or articles of the

142 same stem per month. None of the acclimated leaves of *L. narbonense* or articles of *S.* 143 *fruticosa* showed the presence of gas film. The other analyses regarding tissue O_2

dynamics, porosity, underwater net photosynthesis (P_N) and dark respiration (R_D) were conducted three months after the beginning of the acclimation.

146

147 Sediment O₂ dynamics

Partial pressures of O_2 (pO_2) and temperature changes in the sediment were monitored during the simulated tides in the acclimation system with optical oxygen sensors (Optodes, fibre diameter 230 µm, PyroScience GmbH, Torino, Italy), inserting needles approx. 4 cm from the soil surface (three in each pot). Optodes were connected to an optical oxygen meter (FireStingO₂, PyroScience GmbH) and pO_2 was recorded every 60 s using Pyro Oxygen Logger software (PyroScience GmbH).

154

155 Plant tissue O2 dynamics

Replicated 12 h flooding experiments were conducted at 20 °C using artificial seawater (see above) in a well-stirred aquarium. Tissue O_2 dynamics were monitored while manipulating light and submergence conditions, starting initially from waterlogged status.

159 A pot with one plant was placed in the aquarium at least 3 h before the experiment.

160 A basal leaf (of L. narbonense) or a succulent stem (of S. fruticosa) was mounted on a 161 mesh fixed onto the pot, and a superficial root (adventitious root in L. narbonense) was 162 exposed, after gentle excavation of the sediment, and fixed with clips. Two Clark-type O₂ 163 microelectrodes with guard cathode (Unisense A/S, Aarhus, Denmark) were inserted 164 simultaneously in the photosynthetic tissue (petiole or green article) and in the root using a micromanipulator (MM5; Märzhäuser, Wetzlar, Germany), in order to monitor O2 165 166 dynamics during submergence. The first O_2 microelectrode with a tip diameter of 50 μ m (OX-50, Unisense A/S) was inserted 300 µm or 500 µm into the leaf petiole (of L. 167 168 narbonense) or article (of S. fruticosa), respectively; the second O2 sensor with a tip

6

169 diameter of 25 μ m (OX-25, Unisense A/S) was inserted 250 μ m into the root. After 170 insertion, the exposed root was again carefully covered with sediment.

171 Concentrations of O_2 and water temperature were monitored during the experiment using a 172 Clark-type O_2 microelectrode with a tip diameter of 500 μ m (OX-500, Unisense A/S) and a 173 temperature probe (TP2000, Unisense). Microelectrodes and the temperature probe were 174 connected to a Microsensor pA Multimeter (v. 2.01, Unisense) and outputs were logged 175 every 60 s on a computer using the logger software provided by the Unisense SensorTrace 176 Suite software package (v. 2.7.0). Stirring of the water in the aquarium was maintained by an air pump, and light was provided underwater at a quantum flux density (PAR) of 110 177 µmol m⁻² s⁻¹ (lamp ALCX3100, Ceab Acquari, Brescia, Italy). 178 179 Starting from waterlogged conditions in darkness, the water level was gradually increased,

180 then the light was switched on and the water level was again lowered following the same 181 procedure in reverse. In detail, conditions were in the following order: waterlogging in 182 darkness (until stabilisation of the signal), partial submergence in darkness (1 h 30 min), 183 complete submergence in darkness (1 h 30 min), complete submergence in light (2 h), 184 partial submergence in light (3 h), waterlogging in light (3 h). This was repeated for three 185 replicate plants of each species. The petiole or article with the microelectrode inserted was 186 already under water during partial submergence. Separately, O2 levels of both roots and 187 petioles or articles were obtained under drained conditions, both in darkness and in light 188 (within approx. 1 h 30 min).

189

190 Tissue porosity

191 Tissue porosity of leaves, petioles and adventitious roots of L. narbonense, and articles, 192 stems and roots of S. fruticosa, was measured using the pycnometer method (Jensen et al. 193 1969). A 25 mL pycnometer (Isolab GmbH, Wertheim, Germany) was filled with 194 deionised (DI) water and weighed. Plant tissue (approx. 0.2 g fresh mass) was gently 195 washed and wiped before weighed. Roots were cut 1 cm from the tip (excluding high 196 density tissues in the non-expanded zone) and 2 cm from the base. The root tissue was then 197 inserted into the water-filled pycnometer and weighed again. The tissue was then retrieved 198 and ground with a mortar and pestle, and the resulting homogenate was weighed in the 199 pycnometer filled with DI water. The temperature of the water was maintained constant 200 during all measurements (20 °C). Tissue porosity was determined using the formula of 201 Jensen et al. (1969).

203 Porosity of L. narbonense rhizomes was measured by determining the difference in weight 204 before and after vacuum infiltration of tissue gas spaces with water (Raskin 1983). This 205 method was preferred in the case of the rhizome due to the greater size of samples, the 206 results being highly comparable with those of the pycnometer method (reproducibility > 207 95%, tested with smaller samples). The balance was placed on a stand with a hook 208 underneath the balance, reaching a beaker containing approx. 900 ml DI water. The water 209 level in the beaker was maintained constant during all measurements. The tissue sample 210 was weighed in air on top of the balance and then held in a clamp, hanged to the hook and 211 weighed underwater. Plant sample and clamp were transferred to a small beaker filled with 212 water, covering the tissue sample, and subjected to vacuum infiltration for 20 min after 213 which air was let back in to the system. This procedure was repeated 3 times to ensure 214 complete water infiltration of the internal gas spaces of the tissue. Finally, plant sample 215 and clamp were weighed again under the hook and porosity was calculated using the 216 equations of Thomson et al. (1990).

217

218 Underwater P_N, R_D and SLA

219 Underwater net photosynthesis (P_N) and dark respiration (R_D) of excised leaves (of L. 220 narbonense) or articles (of S. fruticosa) were measured using the approach described in 221 Pedersen et al. (2013). In brief, tissue samples of approx. 0.2-0.3 g fresh mass were 222 incubated for a known period of time (1 h 30 min for underwater $P_{\rm N}$, 4 h for underwater 223 $R_{\rm D}$) in closed transparent 25 mL vials containing artificial seawater (see above) with two 224 glass beads inside to ensure mixing, as the vials were positioned in a rotating wheel 225 according to the method, in light or darkness. Afterwards, the O_2 produced (P_N) or 226 consumed (R_D) by the tissue was measured. In detail, five replicates of leaves (youngest 227 fully expanded) or articles developed during the three months acclimation were harvested 228 from four individual plants of each species, removing the extremities (apex and base of 229 both leaves and articles) and the mid nerve in L. narbonense leaves. Five vials without leaf 230 segments served as blanks.

The dissolved inorganic carbon (DIC) of the artificial seawater was 2 mM, corresponding to 16 μ M CO₂ at pH 8.00 (see "pH-drift approach" in Pedersen *et al.* 2013). Free CO₂ was calculated from total alkalinity, salinity, temperature and pH, using the CO₂ constants proposed by Millero (2010). Photosynthetically active radiation (PAR) inside the vials during underwater P_N was 1000 μ mol photons m⁻² s⁻¹, provided from a horizontallypositioned light source (Gavita Pro 300 LEP, Gavita Holland BV, Aalsmeer, TheNetherlands).

Following incubation, the dissolved O_2 concentration in each vial was measured using a microoptode with retractable needle (OP-430, fibre diameter 430 μ m, Unisense A/S) connected to a 1 channel pA meter (Oxymeter, Unisense A/S). Values of O_2 were logged on a computer using the software provided by Unisense SensorTrace Suite software package (v. 2.7.0).

- Projected area of *L. narbonense* leaves and half cylinder surface of *S. fruticosa* articles (approx. 200 mm² for both) were measured analysing scanned images in ImageJ software (Schneider *et al.* 2012). Samples were then oven-dried (65°C for 4 days), treated with 80% ethanol, boiled for 20 min 3 times and dried again in order to determine the ethanolinsoluble dry mass (DM), excluding sugars and salts from the dry mass. A relationship between DM and leaf/article area enabled the calculation of specific leaf area (SLA, m² kg⁻¹ DM) based on one-sided area (n = 8 for each species).
- 250

251 Data analysis

Student's *t*-test was applied to evaluate differences between means in plant growth between the two flooding treatments imposed (high and low stress), after verifying the normality of dataset distributions (Shapiro-Wilks test > 0.05) and the homogeneity of variances (Bartlett's test > 0.05). A logarithmic transformation was applied to *S. fruticosa* dataset to ensure normality of data.

257 ANOVA test was applied to the other datasets (O2 in soil and in plants, porosity,

underwater $P_{\rm N}$ and $R_{\rm D}$, SLA), after verifying the priori assumptions (normality of data

- 259 distributions and homogeneity of variances). Logarithmic transformations were applied to
- 260 porosity and SLA datasets.
- All analyses were computed in R software (R Core Team 2013).
- 262

263 Results

264 O2 dynamics in sediment

Sediment O_2 dynamics monitored during plant acclimation appeared highly reproducible. This was particularly true for muddy sediment samples (BB site) whereas the sandy sediments (BO site) showed higher variability (Figure S1). In drained-waterlogged conditions, O_2 dynamics in sediment showed a rapid increase in O_2 at the onset of draining (approaching 20 kPa pO_2 in some replicates) with a subsequent decline during waterlogging. 75% of the samples showed average pO_2 higher than 10 kPa during drained conditions and lower than 5 kPa during waterlogging. In contrast, when waterloggedsubmerged, steady anoxic conditions were assessed with consistently lower pO_2 (lower

- 273 than 0.5 kPa for 75% of samples).
- 274

275 O2 dynamics in plant tissues

Plant tissue O_2 dynamics were followed in drained conditions and in a series of replicated 12 h flooding experiments, in darkness or in light. Three replicate plant samples from pots with clear soil O_2 dynamics were used to obtain shoot and root tissue O_2 status of *L. narbonense* (Figure 1) and *S. fruticosa* (Figure 2) during submergence. Steady-state O_2 values were obtained from each original O_2 trace and summarised in supplementary material (Table S1).

For both *L. narbonense* and *S. fruticosa*, submergence in the dark resulted in significant declines in tissue O_2 status in both root and shoot tissues. The decline in tissue O_2 was more pronounced following complete submergence (Figure 1 and Figure 2). In contrast when in light, O_2 rose rapidly in photosynthetic tissues leading to subsequent gradual increases of O_2 in roots. In both species, O_2 responses were more rapid in dark and in photosynthetic tissues, reaching a new *quasi* steady-state within 1 h.



Figure 1. Tissue O_2 concentrations of *Limonium narbonense* petioles (grey curve) and adventitious roots (red curve) during waterlogging, partial or complete submergence, in darkness or in light. Mean (data point) and standard deviation (vertical bar) of the two curves are shown (n=3). Measurements were carried out in intact plants in pots containing

natural soil, submerged in artificial seawater (35‰, 20 °C) maintained at air equilibrium and moderately mixed. The water level was 0.5 cm above soil surface during waterlogging, and 6 cm above soil surface (approx. 50% of individual plant submerged) during partial submergence. The leaf of the analysed petiole was completely under water during partial submergence. Darkness is indicated by grey shading. The dashed line indicates zero O_2 while the dotted line indicates O_2 air equilibrium (231 µmol L⁻¹ at 20 °C and 35 ‰ salinity).



300

301 Figure 2. Tissue O₂ concentrations of Sarcocornia fruticosa articles (grey curve) and roots 302 (red curve) during waterlogging, partial or complete submergence, in darkness or in light. 303 Mean (data point) and standard deviation (vertical bar) of the two curves are shown (n=3). 304 Measurements were carried out in intact plants in pots containing natural soil, submerged 305 in artificial seawater (35‰, 20 °C) maintained at air equilibrium and moderately mixed. 306 The water level was 0.5 cm above soil surface during waterlogging, and 8 cm above soil 307 surface (approx. 50% of individual plant submerged) during partial submergence. The 308 branch sustaining the analysed article was completely under water during partial 309 submergence. Darkness is indicated by grey shading. The dashed line indicates zero O2 while the dotted line indicates O2 air equilibrium (231 µmol L⁻¹ at 20 °C and 35 ‰ 310 311 salinity).

312

313 Considering *L. narbonense* (Figure 1), O_2 decreased dramatically by more than 90% 314 already during partial submergence (new *quasi* steady-state of 9±6 and 6±4 µmol L⁻¹ in

315 petioles and adventitious roots, respectively). In adventitious roots, O2 declined to almost

316 zero upon complete submergence in darkness in all three replicates. In light, O₂ increased 317 rapidly in petioles, especially when de-submerged, and more sluggishly in roots, but 318 eventually both tissues reached the O2 status prior to submergence (163±3 and 72±21 µmol 319 L^{-1} in petioles and roots, respectively). The abrupt incline in tissue O₂ observed in petioles 320 at the end of each experiment, where values approached air equilibrium O2, was possibly 321 due to desiccation of the tissue at the point of microsensor insertion, which could result in 322 atmospheric O_2 entering the gap between tissue and sensor tip (Figure 1). Therefore, O_2 323 values obtained prior to the steep incline were used as new quasi steady-state. Similarly, 324 the increasing trend of petiole O_2 during complete submergence in darkness was caused by 325 a noisy sensor in one of the replicates and consequently for this stage, only two replicates 326 were considered for the data analysis.

327 Tissue O₂ status of S. fruticosa (Figure 2), in contrast, showed a noticeable O₂ level upon partial submergence, both in articles (107 \pm 28 µmol L⁻¹) and in roots (44 \pm 16 µmol L⁻¹). In 328 the case of roots, the quasi steady-state O2 concentration exceeded the tissue O2 329 330 concentration of adventitious roots in L. narbonense by 8-fold. In light during complete submergence, O₂ in articles increased immediately (180±14 µmol L⁻¹) reaching a similar 331 332 quasi steady-state of the initial condition (waterlogged in darkness). At the same time, the 333 O_2 concentration in roots was considerable too, being 4-fold higher than in roots of L. 334 narbonense. Also in the case of S. fruticosa, a noisy sensor required exclusion of one 335 replicate root sample in the light upon partial submergence (included in Figure 2 but 336 excluded from the subsequent data analysis).

337 The tissue O_2 status of petioles and adventitious roots of L. narbonense and articles and 338 roots of S. fruticosa were analysed according to the influence of both flooding and light 339 effects (Figure 3). The drained conditions and the waterlogging, in light or darkness, 340 appeared statistically similar in all tissues and differed significantly from all other stages, except for S. fruticosa roots (Figure 3d) where more gradual O2 changes were recorded, 341 342 despite the prevailing dominant role of flooding effect. O₂ dynamics in the adventitious 343 roots of L. narbonense were highly determined by submergence status leading to a non-344 influential role of light (Figure 3b, $p \le 0.043$ between a and b groups). On the contrary, the 345 role of light was prevalent in photosynthetic tissues, in particular in S. fruticosa articles 346 (Figure 3c), where partial or complete submergence in light were indiscernible from 347 waterlogging or drained conditions, both in darkness or light. In petioles of L. narbonense, flooding and light effects were both important in determining O2 dynamics, revealing a 348

- 369 Table 1. Porosity (% gas volume/tissue volume) in different tissues of Limonium
- 370 narbonense and Sarcocornia fruticosa species. Values reported are means ± standard
- deviation (n=8). Letters indicate significant differences ($p \le 0.05$) based on ANOVA and
- 372 Tukey's HSD post-hoc test.

Species	Tissue	Porosity (%)
L. narbonense	leaf	37.8±9.4 ^a
	petiole	48.9±12.5 ^a
	rhizome	20.1±3.5 ^b
	adventitious root	36.9±6.6 ^a
S. fruticosa	article	9.1±2.8 °
	stem	15.3±5.9 ^b
	root	18.2±4.1 ^b

373

S. fruticosa displayed considerably high tissue porosity both in lignified stems (15%) and roots (18%). *L. nabonense* tissues showed even higher tissue porosity, 2-fold higher in adventitious roots (37%) and up to 49% in petioles. Lower but still considerable values of tissue porosity were recorded for *L. narbonense* rhizomes (20%). In both species, the high porosity values supported a low-resistance internal pathway for O₂ diffusion to roots.

379

380 Tissue turnover

381 The two treatments imposed (drained-waterlogged or waterlogged-submerged) impacted 382 tissue turnover and thus plant growth (Figure 4). The high stress treatment (waterlogged-383 submerged) adversely affected L. narbonense leaf production resulting in significantly 384 lower tissue turnover (p=0.023) compared to the low stress situation (drained-385 waterlogged). On the contrary, high flooding stress stimulated the development of new 386 articles in S. fruticosa, with 4-fold higher tissue turnover compared to the low stress 387 situation (p < 0.0001). It is possible that a faster leaf replacement occurred under high stress 388 conditions in L. narbonense, determining the replacement of some new acclimated leaves 389 grown during the two months acclimation. Nonetheless, this replacement would not 390 consistently alter the observed results.



392

393 Figure 4. Tissue turnover of Limonium narbonense (a) and Sarcocornia fruticosa (b) 394 during 2 months of acclimation. For L. narbonense, the turnover was calculated as the 395 number of new leaves produced divided by the total number of initial leaves; in S. 396 fruticosa, the turnover was calculated as the number of new articles produced divided by 397 the total number of initial articles in a particular stem. Low stress represents the drained-398 waterlogged treatment, and high stress refers to the waterlogged-submerged treatment. In 399 the box-whisker plot, the horizontal line indicates the median, the box is 25 to 75% of the 400 observations and the error bars show minimum and maximum values.

401

402 Underwater net photosynthesis (P_N), underwater dark respiration (R_D) and specific 403 leaf area (SLA)

Underwater P_N and R_D were determined at air equilibrium of CO₂ (16 μ M) and expressed both as O₂ produced or consumed per area (one-sided area, Figure 5*a*) and per dry mass (Figure 5*b*), comparing the two treatments (drained-waterlogged or waterloggedsubmerged). Similarly, specific leaf area (SLA) was measured for plants being acclimated to high or low stress for 3 months on tissues that had been produced during acclimation (Figure 5*c*).

410 Considering tissue O_2 balance expressed per area (Figure 5*a*), no significant differences

411 were apparent between treatments. On the contrary, O2 production or consumption

412 expressed per tissue dry mass (Figure 5b) resulted in statistically significant differences

413 between treatments, both in $P_{\rm N}$ and $R_{\rm D}$. In the submerged treatment (high stress), $P_{\rm N}$ was

414 2.5-fold higher in both species and dark O_2 consumption (R_D) was 3.5-fold and 4-fold

415 higher in L. narbonense and S. fruticosa, respectively.



416

417 Figure 5. Underwater net photosynthesis (P_N) and dark respiration (R_D) of Limonium 418 narbonense excised leaves (white bars) and Sarcocornia fruticosa excised articles (grey 419 bars) expressed per photosynthetic one-sided area (a) or per dry mass (b), and specific leaf 420 area (SLA, c). Low stress represents the drained-waterlogged treatment and high stress 421 refers to the waterlogged-submerged treatment. Conditions in each treatment changed 422 every 6 h; plant tissues were harvested and analysed after 3 months of acclimation. Values 423 are means \pm standard deviation (n=4). Letters indicate significant differences ($p \le 0.05$) 424 based upon one-way ANOVA and Tukey's HSD post-hoc test applied separately for each 425 dataset and each species. * indicates p=0.09.

426

The different responses depending on how the O_2 balances were expressed are due to morphological acclimations in leaf or article thickness, resulting in significantly thinner tissues under submerged conditions (high stress). SLA was 2-fold higher in *L. narbonense* (*p*=0.002) and more than 3-fold higher in *S. fruticosa* (*p*<0.0001) in tissues produced under high stress.

432

433 Discussion

The present study of *L. narbonense* and *S. fruticosa* revealed different tissue O_2 dynamics in the two species when submerged and suggests that other adaptations than internal aeration could influence flooding tolerance. In fact, these two species are both welladapted to flooding, being distributed in the field along a flooding gradient and colonising 438 occasionally or daily flooded areas. Intraplant O_2 seemed to play a key role in S. fruticosa 439 flooding tolerance, considering the noticeable tissue O₂ concentration recorded during the 440 experiment. On the contrary, in L. narbonense, the abrupt O_2 collapse observed both in 441 petioles and roots when submerged suggested that internal aeration could not be considered 442 the main tolerance strategy adopted. In light, tissue O_2 dynamics showed a conspicuous O_2 443 peak in photosynthetic tissues after exposure to a period of darkness due to underwater $P_{\rm N}$, 444 both in L. narbonense and S. fruticosa, entailing a subsequent increase of O_2 in roots. In 445 fact, despite underwater $P_{\rm N}$ measured at air equilibrium of CO₂ from excised leaves (in L. 446 narbonense) and articles (in S. fruticosa) was close to zero, it appeared essential for 447 internal aeration during submergence, although the rate was approx. 100-fold lower 448 compared to P_N recorded in air in similar halophytes (Konnerup *et al.* 2015). Our results 449 did not indicate any influence of C_3 or CAM photosynthetic pathways on internal aeration 450 for the two species studied. O_2 dynamics in light were similar in both halophytes (a steep 451 O_2 peak followed by a slow decrease) and, when under water, S. fruticosa showed no 452 benefit from reduction in photorespiration as shown by Redondo-Gómez et al. (2007). The 453 steep increase of O_2 observed in both species when in light, likely resulted from CO_2 454 accumulation in plant tissues during $R_{\rm D}$ (Colmer and Pedersen 2008) and possibly also 455 from CO_2 entry from the CO_2 -rich sediment followed by diffusion via aerenchyma to 456 shoots (Perata *et al.* 2011). The possible use of CO_2 sourced from the soil has already been 457 demonstrated for several hydrophytic plant species (Winkel et al. 2009) and for the 458 halophytes Halosarcia pergranulata (Pedersen et al. 2006) and Suaeda maritima (Colmer 459 et al. 2013).

460 L. narbonense could possibly benefit from a quicker leaf renewal, similarly to other 461 saltmarsh species (Adam 1990). By contrast, the survival in flooded soils by S. fruticosa 462 could be promoted, when underwater, thanks to the superficial root system (e.g., in Suaeda maritima, Justin and Armstrong 1987) and by shoot elongation processes, as demonstrated 463 464 for the closely related species S. perennis (Adams and Bate 1994). Effective internal 465 aeration is commonly attributed to the development of aerenchymal tissue, interconnecting 466 longitudinally shoots with roots and adopted by wetland plants, halophytes included, to 467 support root aeration in flood-prone habitats (Colmer 2003). Considering the high 468 variability in root porosity recorded in wetland halophytes, from approx. 5% in S. maritima (Justin and Armstrong 1987) and up to 40% in the Spartina genus (Colmer and Flowers 469 470 2008), both L. narbonense and S. fruticosa showed a high tissue porosity to enhance O_2 471 diffusion from shoots to roots.

472 Even with the woody stem, S. fruticosa could easily translocate O₂ to the root system and 473 the tissue porosity of both stems and roots (approx. 20%) was moderate. This explains the 474 capacity to maintain aerated roots during submergence (approx. 45 and 30 μ mol O₂ L¹ 475 upon partial submergence in darkness and in light respectively). This capacity depends on 476 O_2 produced in shoots from underwater P_N in light (Pedersen *et al.* 2006) and on possible 477 benefits from snorkelling, which could increase root O2 during partial submergence 478 (Herzog and Pedersen 2014). L. narbonense, despite the even higher tissue porosity and the 479 possible contribution of green petioles to carry out photosynthesis (Aschan and Pfanz 2003), showed an inefficient O_2 supply to roots (only approx. 6 and 18 μ mol O_2 L⁻¹ upon 480 complete and partial submergence respectively in light). There are several potential 481 482 explanations for the observed O_2 deficiency in roots e.g., a longer pathway for O_2 diffusion from shoots to roots (Rich et al. 2013) or O2 consumption for root respiration (Armstrong 483 484 1971). In addition, if there is a poor connection (bottlenecks) between roots-rhizome or 485 rhizome-shoot junctions in L. narbonense it would restrict internal aeration. In fact, 486 unidentified bottlenecks for O2 diffusion in rhizome aerenchyma as well as layers of 487 cortical cells surrounding adventitious roots limiting O2 diffusion have already been 488 described in some rhizomatous wetland species (Armstrong and Armstrong 1988). The low 489 O₂ concentration recorded in L. narbonense could also be due to the absence of a barrier to 490 radial oxygen loss (ROL) in roots, resulting in a less efficient O₂ retention by the plant and 491 hence lower tolerance to anoxic conditions (Colmer 2003). Nevertheless, the potential 492 capacity to release O₂ from roots and rhizomes, suggested by the red mottles clearly visible 493 along soil profiles (Vittori Antisari et al. 2016), suggests an easier recovery of oxic 494 conditions after flooding of the rhizosphere.

495 Underwater $P_{\rm N}$ and $R_{\rm D}$ rates were comparable to those reported for other halophytes in the 496 literature, which ranged from 0.04 to 0.89 μ mol O₂ m⁻² s⁻¹ for underwater $P_{\rm N}$ and from 0.27

- 497 to 0.90 μ mol O₂ m⁻² s⁻¹ of O₂ consumption during R_D (comparison based on total surface
- 498 area; see e.g., Colmer *et al.* 2013, Konnerup *et al.* 2015). Moreover, underwater P_N and R_D
- 499 rates recorded in L. narbonense and S. fruticosa appeared only slightly higher compared to
- 500 Tecticornia medusa (approx. 0.04 µmol O₂ m⁻² s⁻¹, Konnerup et al. 2015) and slightly
- 501 lower than rates of Halosarcia pergranulata (Pedersen et al. 2006), Suaeda maritima
- 502 (Colmer *et al.* 2013) or *T. articulata* (approx. 0.89 μ mol O₂ m⁻² s⁻¹, Konnerup *et al.* 2015).
- 503 In agreement with Pedersen et al. (2006), L. narbonense and S. fruticosa did not show
- 504 acclimation in underwater $P_{\rm N}$ and $R_{\rm D}$ rates to improve CO₂ uptake after high degree of
- 505 flooding stress.

506 The acclimation in leaf thickness (SLA), observed in both species under high flooding 507 stress, agreed with observations recorded for other terrestrial plants, where the thickness of 508 aquatic acclimated leaves was from 1.5-fold to 3-fold thinner compared to terrestrial leaves 509 (Mommer et al. 2007). Despite the development of acclimated leaves could be of great 510 advantage to survival under water (Huber et al. 2012), leaf plasticity did not ensure 511 flooding tolerance, since the reduction in leaf thickness of flooding tolerant and non-512 tolerant species were very similar under submerged conditions (Mommer et al. 2007). 513 In field situations, L. narbonense seemed even more adapted to flooding compared to S.

514 fruticosa, sharing the lowest elevated saltmarsh areas with Spartina maritima, a species 515 well-adapted to the anoxic soils of tidal inundated areas (Castillo et al. 2000). In fact, even 516 in species with large volumes of aerenchyma in rhizomes and roots e.g., up to 33% of 517 tissue porosity recorded in several halophytes (Justin and Armstrong 1987), O₂ deficiency 518 can still locally occur in roots (S. patens, Burdick and Mendelssohn 1990). For this reason, 519 strategies other than porosity should be involved in flooding tolerance of L. narbonense 520 e.g., tolerance to transient anoxia/severe hypoxia in roots already suggested for the 521 halophyte Suaeda maritima (Colmer et al. 2013). Moreover, the activation of fermentative 522 processes has been demonstrated in several species differing in flooding tolerance (Visser 523 et al. 2003, Perata et al. 2011), species of the Limonium genus included (Rivoal and 524 Hanson 1993).

525 In conclusion, L. narbonense and S. fruticosa both benefit from low but influential rates of 526 underwater $P_{\rm N}$ to supply O₂ to roots during light periods and show a potentially well-527 constructed longitudinal pathway for gas diffusion as a result of high tissue porosity. 528 Despite these similarities, the overall tolerance strategy to flooding appeared different 529 between the two species. S. fruticosa seemed to tolerate flooding thanks to facilitating O_2 530 diffusion via aerenchyma, accompanied by snorkelling benefits when shoots were in contact with the atmosphere. On the contrary in L. narbonense, O₂ diffusion through 531 532 aerenchyma was insufficient to keep tissues well aerated, as the diffusion was probably 533 being impeded by some unidentified bottlenecks which led to very low O_2 status in roots 534 during submergence. However, this species could be tolerant to transient anoxia or could 535 benefit from possible fermentative processes to tolerate prolonged submergence stress.

536

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- 541

542 References

- 543 Adam P (1990) Salt marsh ecology. Cambridge University Press, Cambridge
- 544 Adams JB, Bate GC (1994) The effect of salinity and inundation on the estuarine 545 macrophyte Sarcocornia perennis (Mill.) AJ Scott. Aquatic Botany 47(3), 341-348.
- 546 Armstrong W (1971) Radial Oxygen Losses from Intact Rice Roots as Affected by
- 547 Distance from the Apex, Respiration and Waterlogging. Physiologia Plantarum 25, 192-548 197.
- 549 Armstrong W (1979) Aeration in higher plants. Advances in Botanical Research 7, 225-550 332.
- 551 Armstrong J, Armstrong W (1988) Phragmites australis - a preliminary study of soil-552
- oxidizing sites and internal gas transport pathways. New Phytologist 108, 373-382.
- 553 Aschan G, Pfanz H (2003) Non-foliar photosynthesis - a strategy of additional carbon
- 554 acquisition. Flora-Morphology, Distribution, Functional Ecology of Plants 198(2), 81-97.
- 555 Borum J, Raun AL, Hasler-Sheetal H, Pedersen MØ, Pedersen O, Holmer M (2013)
- 556 Eelgrass fairy rings: sulfide as inhibiting agent. Marine biology 161(2), 351-358.
- 557 Burdick DM, Mendelssohn IA (1990) Relationship between anatomical and metabolic 558 responses to soil waterlogging in the coastal grass Spartina patens. Journal of
- 559 Experimental Botany 41, 223-228.
- 560 Castillo JM, Fernández-Baco L, Castellanos EM, Luque CJ, Figueroa ME, Davy AJ (2000)
- 561 Lower limits of Spartina densiflora and S. maritima in a Mediterranean salt marsh
- 562 determined by different ecophysiological tolerances. Journal of Ecology 88, 801-812.
- 563 Colmer TD (2003) Long-distance transport of gases in plants: a perspective on internal
- 564 aeration and radial oxygen loss from roots. Plant, Cell & Environment 26, 17-36.
- 565 Colmer TD, Flowers TJ (2008) Flooding tolerance in halophytes. New Phytologist 179, 566 964-974.
- 567 Colmer TD, Pedersen O (2008) Oxygen dynamics in submerged rice (Oryza sativa). New 568 Phytologist 178(2), 326-334.
- 569 Colmer TD, Pedersen O, Wetson AM, Flowers TJ (2013) Oxygen dynamics in a salt-marsh
- 570 soil and in Suaeda maritima during tidal submergence. Environmental and Experimental
- 571 Botany 92, 73-82.

- 572 Duarte B, Santos D, Caçador I (2013) Halophyte anti-oxidant feedback seasonality in two
- 573 salt marshes with different degrees of metal contamination: search for an efficient
- 574 biomarker. Functional Plant Biology 40(9), 922-930.
- 575 Eley Y, Dawson L, Pedentchouk N (2016) Investigating the carbon isotope composition
- 576 and leaf wax n-alkane concentration of C3 and C4 plants in Stiffkey saltmarsh, Norfolk,
- 577 UK. Organic Geochemistry 96, 28-42.
- 578 Herzog M, Pedersen O (2014) Partial versus complete submergence snorkeling aids root
- aeration in *Rumex palustris* but not in *R. acetosa. Plant, Cell & Environment* 37, 23812390.
- 581 Huber H, Chen X, Hendriks M, Keijsers D, Voesenek LACJ, Pierik R, Poorter H, de
- 582 Kroon H, Visser EJW (2012) Plasticity as a plastic response: how submergence-induced
- 583 leaf elongation in *Rumex palustris* depends on light and nutrient availability in its early life
- 584 stage. New Phytologist 194, 572-582.
- 585 Jensen CR, Luxmoore RJ, van Gundy SD, Stolzy LH (1969) Root air measurements by a
- 586 pycnometer method. Agronomy Journal 61, 474-475.
- 587 Justin SHFW, Armstrong W (1987) The anatomical characteristics of roots and plant
- response to soil flooding. *New Phytologist* **106**(3), 465-495.
- 589 Kester D, Duedall IW, Connors DN, Pytkowicz RM (1967) Preparation of artificial
- 590 seawater. Limnology and oceanography 12, 176-179.
- 591 Konnerup D, Moir-Barnetson L, Pedersen O, Veneklaas EJ, Colmer TD (2015)
- 592 Contrasting submergence tolerance in two species of stem-succulent halophytes is not
- determined by differences in stem internal oxygen dynamics. *Annals of Botany* 115, 409-418.
- 595 Lang F, von der Lippe M, Schimpel S, Scozzafava-Jaeger T, Straub W (2010) Topsoil
- 596 morphology indicates bio-effective redox conditions in Venice salt marshes. Estuarine,
- 597 Coastal and Shelf Science 87(1), 11-20.
- 598 Liao CT, Lin CH (2001) Physiological adaptation of crop plants to flooding stress.
- 599 Proceedings of the National Science Council, Republic of China, B. 25(3), 148-157.
- 600 Loreti E, van Veen H, Perata P (2016) Plant responses to flooding stress. Current Opinion
- 601 in Plant Biology 33, 64-71.
- McKee KL, Patrick WH Jr (1988) The relationship of smooth cordgrass (*Spartina alterniflora*) to tidal datums: A review. *Esturaries* 11, 143-151.
- 604 Millero FJ (2010) Carbonate constants for estuarine waters. Marine and Freshwater
- 605 Research 61, 139-142.

- 606 Mommer L, Wolters-Arts M, Andersen C, Visser EJW, Pedersen O (2007) Submergence-
- 607 induced leaf acclimation in terrestrial species varying in flooding tolerance. New
- 608 Phytologist 176, 337-345.
- 609 Pandža M, Franjić J, Škvorc Ž (2007) The salt marsh vegetation on the East Adriatic coast.
- 610 Biologia 62(1), 24-31.
- 611 Pedersen O, Vos H, Colmer TD (2006) Oxygen dynamics during submergence in the
- halophytic stem succulent *Halosarcia pergranulata*. *Plant, Cell & Environment* 29, 13881399.
- 614 Pedersen O, Colmer TD, Sand-Jensen K (2013) Underwater photosynthesis of submerged
- 615 plants recent advances and methods. Frontiers in Plant Science 4(140), 1-19.
- 616 Perata P, Armstrong W, Voesenek LACJ (2011) Plants and flooding stress. New
- 617 Phytologist 190(2), 269-273.
- 618 Ponnamperuma F (1984) Effects of flooding on soils. In "Flooding and plant growth". (Ed.
- 619 Kozlowski T) pp. 9-45 (New York, NY, USA: Academic Press)
- 620 R Core Team (2013) R: A language and environment for statistical computing. R
- 621 Foundation for Statistical Computing, Vienna, Austria
- 622 Raskin I (1983) A method for measuring leaf volume, density, thickness and internal gas
- 623 volume. HortScience 18, 698-699.
- 624 Redondo-Gómez S, Mateos-Naranjo E, Davy AJ, Fernández-Muñoz F, Castellanos EM,
- Luque T, Figueroa ME (2007) Growth and photosynthetic responses to salinity of the saltmarsh shrub *Atriplex portulacoides*. *Annals of Botany* **100**(3), 555-563.
- 627 Rich SM, Pedersen O, Ludwig M, Colmer TD. (2013) Shoot atmospheric contact is of little
- 628 importance to aeration of deeper portions of the wetland plant Meionectes brownii;
- submerged organs mainly acquire O₂ from the water column or produce it endogenously in
- 630 underwater photosynthesis. Plant, Cell & Environment 36, 213-223.
- 631 Rivoal J, Hanson AD (1993) Evidence for a Large and Sustained Glycolytic Flux to
- 632 Lactate in Anoxic Roots of Some Members of the Halophytic Genus Limonium. Plant
- 633 Physiology 101, 553-560.
- 634 Sarretta A, Pillon S, Molinaroli E, Guerzoni S, Fontolan G (2010) Sediment budget in the
- 635 Lagoon of Venice, Italy. Continental Shelf Research 30(8), 934-949.
- 636 Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image
- 637 analysis. Nature methods 9(7), 671-675.
- 638 Silvestri S, Defina A, Marani M (2005) Tidal regime, salinity and salt marsh plant
- conation. Estuarine Coastal and Shelf Science 62, 119-130.

- 640 Thomson CJ, Armstrong W, Waters I, Greenway H (1990) Aerenchyma formation and
- 641 associated oxygen movement in seminal and nodal roots of wheat. Plant, Cell &
- 642 Environment 13, 395-404.
- 643 Visser EJW, Voesenek LACJ, Vartapetian BB, Jackson MB (2003) Flooding and Plant
- 644 Growth. Annals of Botany 91(2), 107-109.
- 645 Vittori Antisari L, De Nobili M, Ferronato C, Natale M, Pellegrini E, Vianello G (2016)
- 646 Hydromorphic to subaqueous soils transitions in the central Grado lagoon (Northern
- 647 Adriatic Sea, Italy). Estuarine, Coastal and Shelf Science 173, 39-48.
- 648 White AC, Colmer TD, Cawthray GR, Hanley ME (2014) Variable response of three
- 649 Trifolium repens ecotypes to soil flooding by seawater. Annals of Botany 114, 347-355.
- 650 Winkel A, Borum J (2009) Use of sediment CO₂ by submersed rooted plants. Annals of
- 651 Botany 103, 1015-1023.

652 Supplementary material



Figure S1. Oxygen dynamics, expressed as O2 partial pressure, in sediment monitored 654 655 during plant acclimation, in muddy sediment (BB) and sandy sediment samples (BO). In 656 each graph, continuous vertical lines refer to an increase of water level (up to waterlogging 657 for the low stress, up to complete submergence for high stress), dashed vertical lines refer 658 to a decrease of water level (down to drained condition for low stress, down to 659 waterlogging for high stress). The two species are identified by the abbreviations Lim for 660 Limonium narbonense and Sar for Sarcocornia fruticosa with a number (1-6) 661 corresponding to different plant individuals.
CHAPTER 5



Black coloured horizon with rotten-egg smell, testifying the accumulation of volatile sulphides in soil, *Martignano* saltmarsh, Marano Lagoon.

PREFACE

This work answered to the need of field methods for the evaluation of sulphides in soils, sediments and waters. This need arose especially considering the huge concentrations of sulphides in saltmarsh soils and the susceptibility of sulphides to oxidation, for which a field method is preferred. Moreover, sulphides were recently linked to plant zonation in saltmarshes, due to the phytotoxic effect of free sulphide ions on plants, especially on seagrasses inhabiting sulphides rich environments. For this reason, a quick field colorimetric procedure was assessed for a semi-quantitative determination of acid volatile sulphides (AVS). Sulphides evolved from acidification as H_2S (Figure 4) were trapped in a paper strip previously treated, precipitating as lead sulphide (PbS). The colour of the strip corresponded to the amount of sulphides in the sample, which was determined through comparison with reference charts.



Figure 4. Sulfur speciation based on pH (Reddy and DeLaune 2008)

The method was tested both in the laboratory and in the field, and validated against the response of a ion-selective microelectrode probe for S²⁻. This cheap method appeared simple, robust and highly reproducible. Moreover, the colorimetric approach can benefit from future implementation of smart-phone apps that, through image analysis, will enable to determine sulphides concentration in a sample instantly.

This work was submitted to *Water Research* and now is under revision. My contribution referred to sampling, laboratory analysis and writing of the methods.

- 12 Evaluation of a new paper sensor test for the field analysis of sulphides in waters,
- 13 soils and sediments
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22 Abstract

23 We propose an improved fast, non-expensive and accurate colorimetric paper sensor test 24 for quantitative field determination of free sulphides in waste waters and pore waters. This method can also be applied for the semi-quantitative determination of volatile 25 26 sulphides in anaerobic soils and sediments. Hydrogen sulphide produced by reaction 27 with hydrochloric acid is absorbed by a paper strip treated with 1.57 M PbNO₃. The colour developed on the paper is compared to a set of reference charts obtained by 28 29 exposing treated paper strips to the sulphides evolved from a set of standard solutions. The detection limit was 0.7 μ moles S²⁻ with a sensitivity of the method of 1 μ mole S²⁻. 30 31 The method was validated against the response of an ion-selective microelectrode probe 32 and successfully applied to the analysis of acid volatile sulphides (AVS) in coastal environmental soil samples. Field applicability for water samples ranges from 7.8.10⁻⁶ 33 to 10^{-2} M S²⁻, whereas for soils and sediments it spans from $6.2 \cdot 10^{-5}$ to $2.5 \cdot 10^{-4}$ 34 moles/dm³ S². Absence of matrix effects were confirmed and reproducibility of the 35 method determined. 36

37

38 Key words

- 39 sulphide analysis, AVS, field method, sediment, soil, water
- 40

41 1. Introduction

42 Under anoxic conditions, the activity of sulphate reducing bacteria leads to the 43 formation of sulphide ions in a variety of natural and as well as anthropogenic 44 environments, including sewers (Liu et al., 2016). Free dissolved sulphide in its 45 protonated acid form is corrosive for metal pipes and concrete, and is toxic for aquatic 46 and benthic organisms and vegetation. Its presence in wastewater reduces the efficiency 47 of wastewater treatment plants. Sulphate reduction in sediments leads to accumulation 48 of free sulphides in pore water, and to the formation of metal sulphide precipitates 49 (Billon et al., 2001; Rickard and Morse, 2005).

50 Coastal floodplain soils often contain subsurface sulfidic layers originated by 51 accumulation of bioauthigenic sulphide minerals. Drainage and reclamation of these 52 soils cause oxidation of sulphides and the formation of acid sulphate soils (Van 53 Breemen, 1982; Dent, 1986). Their global extent is widespread and about 12-13 million 54 hectares worldwide (Andriesse and Van Mensvoort, 2006). Upon exposure to air, 55 sulfidic materials release ferrous iron and sulphuric acid, and develop properties similar 56 to acid mine drainage, leading to severe environmental degradation and economic 57 damage (Burton et al., 2006; Wei et al., 2014) such as severe acidification processes and 58 release of toxic metals. It is therefore of great importance to identify buried sulfidic 59 layers, whose exposure to air, due to dredging or land upraise, may result harmful for 60 the environment. The Soil Taxonomy (Soil Survey Staff, 2014) defines sulfidic 61 materials as soil or sediment layers with a pH >3.5 that after wet oxidation, show a drop 62 in pH of more than 0.5 to a pH value of 4 or less within sixteen weeks. According to the 63 main international soil classification systems (e.g. Soil Taxonomy or World Reference 64 Base), potential acid sulphate soil conditions need to be identified during field surveys. 65 However, only qualitative empirical indicators are available: oxidation of monosulphides which quickly change colour upon exposure to peroxide (Stone et al., 1998); 66 offensive rotten egg odour, due to hydrogen sulphide release; pore water S2-67 68 determination, based on reduction of synthetic Fe oxides on the surface of plastic 69 tubings, known as IRIS tubing (Rabenhorst et al., 2010).

The U.S. Environmental Protection Agency (U.S. EPA, 2007) has recommended the use
of acid-volatile sulphides (AVS) and their relationship to simultaneously extracted
metals (SEM) as predictors of the bioavailability of toxic metals in sediments. AVS

refers to a fraction of sulfidic materials, which include dissolved sulfur species and metastable iron minerals, among which mackinawite (FeS) and greigite (Fe₃S₄) are the most representative (Rickard and Morse, 2005). The SEM:AVS approach has also been recommended for the establishment of sediment guidelines for the European Union through the Water Framework Directive (2000/60/EC). AVS:SEM ratios are often used in establishing benchmarks for toxicity in sediments.

79 Usually, quantitative analysis of sulphides are carried out in the laboratory by three 80 main methods (Lawrence et al., 2000): the iodometric, the methylene blue and the 81 potentiometric methods. The iodometric method, is the less sensitive, because it suffers 82 interference from solid and dissolved reducing substances that react with iodine, 83 including thiosulfate, sulphite, and several organic compounds. Formation of methylene 84 blue, by reaction between sulphide and N,N-dimethylphenyl-1,4- diamine, has widely 85 been used. Recent developments have improved its sensitivity and detection limits: flow 86 injection analysis and photoelectrocatalytic oxidation of sulphide at a poly-methylene 87 blue modified glassy carbon electrode, for instance, allows the determination of 88 sulphide at 0.5 μ M (Dilgin et al., 2012). The U.S. EPA recommends the well-89 established potentiometric method based on a silver sulphide ion-selective electrode 90 (ISE) probe, which can be part of a portable equipment and hence may be used for field 91 analysis. The ISE only responds to free sulphide dianions and will not detect complex forms. Therefore, it allows to estimate pore water S²⁻ (Rabenhorst et al., 2010) or, when 92 sediments are treated with a strong base to release S^{2} , total sulphides (Brown et al., 93 94 2011).

95 Inter-laboratory comparisons of AVS analysis, made with standard protocols, found that 96 results can vary by orders of magnitude among laboratories (Hammerschmidt and 97 Burton, 2010), confirming that reproducibility and accuracy of AVS measurements is 98 questionable. A crucial issue in AVS determination is the rapid oxidation of sulphides 99 during transport and storage of samples, as well as during the analysis in laboratory. 100 Sulphide measurement, in fact, is made difficult by its volatility, susceptibility to 101 oxidation, adsorption to glass and rubber and binding to organic molecules. For this 102 reason, fast and quantitative on-site analysis methods that can be performed at low cost 103 are needed, but are however lacking. Some rapid field methods, e.g. the lead acetate 104 paper and silver foil test, allow in fact only a qualitative estimate of sulphides (Holm et

al., 2000). Lead acetate paper tests were developed to detect sulphide concentrations in
ambient air as low as 50 ppb, but quantitation requires instrumental optical density or
reflectance readings (Bethea, 1973). Also the silver foil test, which is even more
sensitive, needs reflectance reading before and after exposure and suffers interferences
from mercaptans and disulphides (Bethea, 1973; Natusch et al., 1974). Field application
of these methods is therefore hampered by the need to carry expensive and heavy
equipment, as reacted paper is subject to fading (Natusch et al. 1974).

112 In this work, we describe and validate a rapid quantitative field method based on paper 113 strip sensors, to provide an easy to use, inexpensive, and portable tool for determination 114 of AVS in waste waters, effluents, pore waters, sediments and soils. Several paper 115 monitor sensors have been recently developed for screening of contaminants (Alkasir et 116 al., 2012) and toxic metals in water (Hossain and Brennan, 2011; Aragay et al., 2012; 117 Ratnarathorn et al., 2012). This new kind of easy-to-use technology will have much 118 success in the near future, thanks to the availability of smart-phone applications for on-119 site rapid quantification of colorimetric readouts, thus reducing the need of expensive 120 and complex equipments for image and data acquisition and processing (Sicard et al., 121 2015).

122 In this work, the efficacy of this new paper sensors method for analysis of waters, soils 123 and sediment samples was successfully tested and applied to the analysis of 124 environmental saltmarsh samples. The method was validated against ISE potentiometric 125 probe measurements in terms of efficacy, accuracy and repeatability. Our method 126 represents an improvement of the field test proposed by Anderson and Wilson (2000) 127 for the analysis of AVS in anoxic soils and sediments. With the proposed procedure, the 128 test is made more sensitive, much more rapid and quantitative by introducing the use of 129 reference colour charts.

130

131 2. Materials and methods

132 2.1. Reagents and solutions

133 All chemicals were of analytical grade and used as received. Solutions were prepared in

134 Milli-Q water (Millipore, Bedford, USA).

135 Stock solutions $(1 \cdot 10^{-4} \text{ to } 4 \cdot 10^{-3} \text{ M})$ of sodium sulphide Na₂S·9H₂O (208043 Sigma-136 Aldrich) were prepared by dissolving the required amount of salt in ultra-pure water

137 previously deoxygenated by flushing gaseous N₂.

138 Potentiometric standard solutions were prepared by dissolving Na₂S·9H₂O (208043

139 Sigma-Aldrich) in 50 mL of alkaline antioxidant buffer (SAOB), a variable amount of

140 standardized sodium sulphide solution and ultrapure water up to 100 ml.

141 The alkaline antioxidant buffer was prepared by dissolving 2 moles of NaOH (Carlo

142 Erba, Italy), 0.2 moles of disodium ethylendiaminetetraacetic acid (Sigma-Aldrich) and

143 0.2 moles of ascorbic acid (VWR International) per litre of solution (Eaton et al., 1995).

144 Due to the lack of certified reference materials, spiked soil samples were prepared by

145 adding increasing volumes of a sodium sulphide solution, and then brought up to 100 ml

146 with either water or buffer. Standard solutions were prepared weekly and refrigerated

- 147 when not used.
- 148 2.2. Paper sensors method

149 2.2.1. Preparation of reference colour charts

150 Reference colorimetric charts were prepared in the following way. Paper strips (3 cm x 151 8 cm) were cut from Whatman® n.1 filter paper and immediately treated before use 152 with 0.3 ml (6 drops) of 1.57 M PbNO₃ (52 g in 100 ml). Strips were placed in 153 polyethylene screw cap jars (250ml), containing 10 ml of freshly prepared Na₂S·9H₂O standard solutions, with concentrations ranging from 10^{-4} to $4 \cdot 10^{-3}$ M. To promote H₂S 154 155 evolution, 50 ml of 6M HCl were added and the jar was gently swirled for 15 sec. The 156 reaction is completed in about 2 min. Gaseous H₂S reacts with lead nitrate forming a 157 precipitate of lead sulphide (PbS). The colour of each paper strip reflects the amount of 158 PbS deposited, which is directly related to the total amount of H₂S evolved. The paper develops colours ranging from light yellow to brown, and eventually turns to a metal 159 grey (10^{-3} M), which acquires a metallic shining at higher S²⁻ concentrations (2 to 4 $\cdot 10^{-3}$ 160 161 M). 162 Reference paper strips were cut and immediately scanned to avoid changes in colour

reference puper strips were cut and miniculately scanned to avoid enanges in coroar

163 due to oxidation. The acquired image was printed and checked against the original. The

164 image was eventually corrected with Adobe Photoshop 6.0 till a faithful printed

reproduction was obtained. Copies of the printed charts were used for the field validation of the method (Figure 1).



167

168 Fig. 1 Colour reference charts obtained from a series of Na_2S standard solutions: 169 numbers represent sulphide concentrations in moles L^{-1} .

170

171 2.2.2. Analysis of sulphides by the colour strip method

172 The reaction was performed in 250 ml polyethylene screw cap jars, using lead nitrate 173 papers strips prepared as described above. Sulfidic acid was evolved adding 50 ml of 174 HCl 6 M to 5-10 ml of water or saltmarsh soil or sediment (collected with the aid of a 175 truncated disposable syringe). For saltmarsh soils, the optimum volume of sample 176 required was on average 8 ml (about 10 g). After adding the sample and the acid, the jar 177 was carefully swirled for about 15 sec and the reaction needed approximately 5 min to 178 be completed. Lower quantities of sample (5 ml) were used in high carbonate materials. 179 The scheme reported in figure 2 summarizes the main steps of the paper strip field test 180 procedure. 181 The AVS concentration in the sample is deduced from the comparison of reacted paper

182 strips with the reference colorimetric charts. Whenever the developed colour was

- 183 intermediate between two adjacent squares, the concentration of S^{2-} in the sample was 184 attributed to the mean value between the two reference concentrations.
- 185 The repeatability of the method was evaluated using 8 ml of soil or sediment and 10 ml
- of Na₂S·9H₂O standard solutions. The test was repeated six times with five different primary standard solutions in the range from $2 \cdot 10^{-4}$ to $3 \cdot 10^{-3}$ M and the limit of
- 188 repeatability was calculated following ISO 5725-2:1994 standard criteria.
- 189 Upper limits were evaluated by adding increasing volumes of soil (5 to 88 ml), to 10 ml 190 of 10^{-3} M Na₂S·9H₂O standard solution.
- 191 Errors due to adsorption to glass and rubber were avoided by the use of polyethylene
- 192 bottles and minimized by performing the preparation of the colorimetric reference
- 193 charts with the same type of vessels used for soil analysis. The matrix effects were
- 194 evaluated by adding increasing volumes, from 5 to 40 ml of 2.10⁻⁴ M Na₂S.9H₂O to 8
- 195 ml of soil or sediment samples. The sulphides content in the added soil was determined
- 196 by the same paper strip method and subtracted from the AVS readings.





197

200 2.3. Potentiometric ISE method

201 An ion-selective microelectrode (ISE, Lazar Research Laboratories, Los Angeles) was

202 employed according to the procedure described by Eaton et al. (1995). The electrode

was calibrated with freshly prepared $Na_2S \cdot 9H_2O$ standard solutions ranging from 0.1 M to 10^{-5} M, using separate linear functions in order to optimize the linearity of the relationship between the millivolt readings of the probe and the S²⁻ concentrations (see supplementary materials). The 5 linear functions ranged respectively from 100 to 10 mM, 5 to 1 mM, 0.9 to 0.5 mM, 0.5 to 0.2 mM and 0.2 to 0.01 mM.

Water, soil or sediment (10 ml) was added to 10 ml of SAOB (Eaton et al., 1995) and vigorously shaken immediately after sampling. Sulphides were measured twice in each sample: first in the field and then in the laboratory. In the laboratory, the measure were performed within few hours from sampling, in order to avoid eventual oxidation phenomena, and after centrifuging samples, by immersing the probe in the solution.

213 2.4. Sampling and sample preparation for validation test

Samples of widely different S^{2-} contents were collected in different saltmarshes of the Grado and Marano Iagoon (Northern Adriatic sea, Italy), considering variability in soil composition (e.g. carbonate content, redox potential status) and morphology of sites (frequency of waterlogging events).

- From 5 to 10 ml of fresh surface saltmarsh soil samples were collected with a cut disposable graduated syringe at depths between 0 and 12 cm (Brown et al., 2011) and immediately analysed in field (colorimetric method) or in the field and in the lab within the same day (potentiometric method), in order to avoid oxidation and loss of AVS.
- The soil and sediment samples taken to validate the method covered a sulphide concentration range from $3.75 \cdot 10^{-3}$ to 0.25 moles dm⁻³.
- 224 2.5. Data analysis

225 The paper sensor and the ISE methods were compared in order to validate the modified 226 method here proposed. Concentrations of AVS above field method evaluation limits 227 were excluded. Models were computed by linear regression techniques based on the 228 least mean squares method (checking scatter plots for absence of curvilinear 229 relationships) and by Kruskal-Wallis test, for data not normally distributed (Shapiro-230 Wilk test < 0.05) with equal variances (Bartlett's test > 0.05). The coefficient of determination (R^2) was calculated for assessing data fit linear models. Data analysis 231 232 were performed with R statistical software (R Core Team, 2013).

The Horwitz ratios were calculated and converted following the computation proceduredescribed by Mc Clure and Lee (2003).

235

236 3. Results

237 3.1. Colour charts range and analysis of water samples

The sensitivity of the method, as can be seen from colour charts reported in figure 2 is 1 μ mole S²⁻. The precipitation of lead sulphide on the paper strips responds to the total amount of sulphide in the sample, so the actual sensitivity, in terms of concentration, depends on the volume of sample used.

In the laboratory, the minimum amount of S^2 , that causes a unequivocally detectable 242 development of colour (LOD) is 0.687 μ moles S², corresponding to 21.7 μ g S². In the 243 244 field, under non optimal light conditions (e.g. very bright sunlight or thick clouds), the 245 colour developed by the paper strip, when exposed to vapours released by acidifying a sample containing this quantity of sulphide, was however very difficult to discern from 246 the blank. At the same time although papers exposed to more than 10 μ moles S²⁻ (315.9 247 $\mu g S^2$) could still be distinguished and evaluated in the lab by their increasing grey 248 metallic brilliance and disappearing brown hue, it was virtually impossible to discern 249 250 these differences in the field.

Therefore, in order to avoid ambiguous results and considering a soil sample volume of 10 ml, colorimetric reference charts prepared for field work should be limited to the range from 1 to 40 μ moles S²⁻ (32 to 1280 μ g S²⁻). This corresponds to a concentration range of 10⁻⁴ to 4·10⁻³ M. Considering the proposed volume and headspace of the jar, the maximum sample volume for the analysis of sulphides in water samples is 128 ml. Using larger volumes, in fact, it is impossible to swirl the acidified sample without contaminating the paper strip.

The calculated field limit of quantitation (LOQ) for liquid samples (max. sample volume 128 ml) is therefore $7.8 \cdot 10^{-6}$ M. On the other side, considering a minimum sample volume of 1 ml, the upper limit for quantitation in the field is 10^{-2} M.

262 3.2. Analysis of soil and sediment samples

Absence of matrix effects in soil and sediment samples was tested by comparing the 263 paper sensors readings obtained from known amounts of S²⁻ added with increasing 264 volumes of a 0.2 mM standard solution with readings obtained in the presence or 265 266 absence of soil. As shown in figure 3, regression lines obtained with or without soil do 267 not significantly differ one from the other and have slopes of respectively 0.91 and 0.81, with intercepts falling well within 95% confidence limits, confirming that matrix 268 effects are negligible. The small positive deviation at low sulphide additions, may be 269 270 due to the presence of a residual small amount of sulphide in the soil.



271

Fig. 3 Matrix effect test. Empty dots refer to results obtained by comparison with colour charts of paper sensors, reacted with H_2S evolved from different standard solutions without soil (slope=0.99, R²=0.99), filled dots refer to 8 cm³ of soil spiked with the different standard solutions (slope=0.81, R²=0.95).

The maximum volume of soil or sediment that can be used for the analysis was determined by measuring sulphides evolved by 10 ml of a 1 mM standard solution after addition of increasing volumes of soil (figure 4). The recovery was > 99% up to 16 ml of soil and decreased to 80% at 20 ml.



282 Fig. 4. Upper limit of soil sample volumes that can be used for field analysis

283

284 3.3. Method validation

To further evaluate the quantitative performance of the paper sensors in the field, data obtained from an extensive number of soil and sediment samples by the colour charts readings were compared with those obtained by the potentiometric ISE method.

288 ISE readings were taken in field after adding the SAOB buffer, hand shaking the sample 289 for 5 minutes and waiting for another 15 minutes. The samples treated with the SAOB 290 solution were then taken to the laboratory, where they were again measured with the 291 ISE probe, after centrifugation. Contrary to expectations, 63% of measurements carried 292 out in the lab were larger than those in the field and only 15% of measurements returned 293 the same field value (figure 5). This means that the SAOB solution was effective in 294 protecting sulphides from oxidation, but solubilisation was relatively slow and the 295 waiting time of 15 minutes, adopted in the field, was not long enough to achieve complete S²⁻ solubilisation. 296



Fig. 5 Comparison between sulphide measured by the ISE probe on SAOB treated soil samples in the field and in the lab.

300

301 To carry out the validation, the sample volume was kept constant and was the same for 302 the two methods, in order to avoid variations in the contribution of other types of 303 uncertainties. Results obtained by paper strip readings in the field were compared, once 304 again by the method of least mean squares, with results obtained from ISE readings carried out in the laboratory. The linearity of response was confirmed in the range 305 between 2 and 30 μ moles S²⁻ and the linear regression (figure 6) between the two sets of 306 data yielded a very high coefficient ($R^2 = 0.96$). Application of the Kruskal-Wallis test 307 308 showed that, at the 95% significance level, there is no evidence of stochastic dominance 309 between the samples and that the mean ranks of the two sets of data (ISE readings and 310 paper strip readings) are the same.





Fig. 6 Correlation of results obtained by the paper sensor field test and ISE probe results (slope=0.91, R²=0.96).

Repeatability was calculated from five replicate measurements taken on six replicate samples of solutions at concentrations ranging from $2 \cdot 10^{-4}$ to $3 \cdot 10^{-3}$ M, which were analysed in two different days. The limit of repeatability for a 95% confidence level (Hansen et al., 2003) attested by the calculation was $1.52 \cdot 10^{-3}$.

319

320 4. Discussion

321 In the original procedure by Anderson and Wilson (2000) the test was made semi-322 quantitative by repeating it with progressively smaller quantities of sediment until it no 323 longer yielded the desired darkening of the filter paper. This is a time consuming 324 procedure. On the contrary the use of charts allows a much simpler, rapid and accurate 325 quantitation, besides allowing to improve the sensitivity of the method. In fact, 326 Anderson and Wilson (2000) considered their test to be positive when the formation of 327 PbS caused a blackening of the paper strip, and negative for light grey-brown and 328 yellowish-brown staining. Following their procedure, the sensitivity given by the method is 3.10⁻⁵ moles of S²⁻ (1 mg). However, the pale brown to cream colours 329

330 developed on the paper exposed to smaller quantities, albeit not typical of PbS 331 precipitates, are both reproducible and univocally identifiable on the colour charts 332 prepared from standard solutions. This, together with the smaller volume of the jar 333 employed according to our procedure (250 ml versus 1 litre) improves the sensitivity of 334 the method and allows the direct quantitation of sulphide in water samples in the field down to a concentration of 0.78 · 10⁻⁵ M (sample volume 128 ml). This corresponds to a 335 field LOQ for waters of 25.10⁻⁴ ppm S²⁻. Considering that the largest volume of soil or 336 sediment, that does not interfere with the diffusion of H2S out of the slurry into the gas 337 338 phase, is on the contrary only 16 ml, the lowest concentration that can be quantified in the field in this type of samples is 0.62 μ moles S²⁻ per dm³ (19.8 ppm). 339

340 Field experience with the lead acetate impregnated paper (Sanderson et al., 1966) has 341 indicated that large errors may arise due to colour fading. Fading is due to the action of 342 light, sulphur dioxide, ozone or other substances capable of oxidizing lead sulphide and 343 impairs the possibility to obtain reliable results by carrying back the exposed paper 344 strips to the laboratory to be evaluated by light transmission measurements. Fading, 345 however, does not represent a problem if colours are immediately evaluated by 346 comparison with printed colour charts or eventually by the aid of applications that allow 347 a cell phone to perform image analysis of a test strip and a control strip producing a 348 quantifiable measurement (Sicard et al., 2015).

349 Validation with the potentiometric method in soils and sediments shows that the paper strip test responds to free sulphide ions as the ISE probe, but apparently, even under 350 laboratory conditions, it has a much narrower range of applicability $(10^{-4} \text{ to } 4 \cdot 10^{-3})$ 351 moles dm³) compared to the linearity range of the ISE (10⁻⁵ to 0.1M). However, we 352 353 must consider that the paper strip test, contrary to the ISE, responds to total amounts of 354 sulphides and not to concentrations and that the volume of samples can be actually 355 varied from 1 to 128 ml in the case of liquids and from 2.5 to 16 ml in the case of soils. The actual applicability range of the paper sensor method for liquid samples therefore 356 spans from 0.78 10⁻⁵ to 10⁻² M, whereas for solids samples, it is much narrower and 357 ranges from $0.625 \cdot 10^{-4}$ to $2.5 \cdot 10^{-3}$ M. A strong limitation to the volume of sample that 358 359 can be analyzed is linked to the potential presence of a large amount of carbonates in the 360 sample. Carbonates react with HCl to produce carbon dioxide, which not only increases 361 the final pH of the slurry, eventually interfering with the quantitative evolution of H_2S , but can also cause strong effervescence that may reach the paper strip and interfere withPbS deposition.

The accuracy of the method for solid or semisolid samples is affected by the fact that field analysis can provide concentrations referred only to soil or sediment volumes and not on a weight bases. It is not only impractical to accurately weight soil samples in the field, the sample's water content is also largely variable and utterly unknown. When necessary, however, additional samples can be taken in close proximity to the ones tested and they can be carried back to the laboratory to measure bulk densities and water contents in the laboratory, in order to convert results into the desired units.

The repeatability of the test is affected by the way the sample quantity is measured. In the worst possible scenario, the sample is solid and its volume (soil) is measured by way of a disposable plastic syringe, as suggested by WRB guidelines for field measurement of pH and EC. Yet, the corrected Horwitz ratio (Mc Clure and Lee, 2003), calculated between the found value for RSD_R to that calculated for this worst possible scenario (soil analysis), ranges between 0.41963 and 0.590071: well below limit values for this ratio which are typically up to 2.

378

379 5. Conclusions

Sulphide measurement in environmental samples is made difficult by its volatility, susceptibility to oxidation, adsorption to glass and rubber and binding to organic molecules. For this reason, elaborate analytical methods have been developed. The present method easily measures free sulphide plus AVS in the field and is simple and robust. Overall recoveries from spiked soil samples were close to 100%, thanks to the lack of adsorption or errors due to loss of H_2S during sample storage and preparation.

The good agreement between analysis of soil and sediment samples carried out in the field and laboratory by the paper strip test and those obtained by the standard potentiometric method, demonstrates that the results obtained by the ISE probe and the newly developed method do not differ significantly. The method is perfectly suitable for screening purposes, being rapid, low cost and providing at the same time good levels of sensitivity and accuracy. The application of the method in field surveys can be further simplified by smart-phone applications for on-site quantification of colorimetric 393 readouts, which are easily linked to GPS data, to produce real time sulphide map 394 outputs. All these characteristics make the paper sensor colorimetric method a very 395 good tool for use in field surveys for sulphide concentrations measurements in 396 anaerobic soils, sediments and waters.

397

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- 401

402 References

- 403 Alkasir, R.S.J., Ornatska, M., Andreescu, S., 2012. Colorimetric paper bioassay for the
- 404 detection of phenolic compounds. Anal. Chem. 84 (22), 9729-9737.
- 405 Anderson, E.F, Wilson, D.J., 2000. A simple field test for acid volatile sulphide in 406 sediments. J. Tenn. Acad. Sci. 7 (3-4), 53-56.
- 407 Andriesse, W., Van Mensvoort, M.E.F., 2006. Acid sulfate soils: distribution and 408 extent. Encyclopedia of soil science. 1, 14-19.
- 409 Aragay, G., Montón, H., Pons, J., Font-Bardía, M., Merkoçi, A., 2012. Rapid and highly
- 410 sensitive detection of mercury ions using a fluorescence-based paper test strip with an
- 411 N-alkylaminopyrazole ligand as a receptor. J. Mater. Chem. 22 (13), 5978-5983.
- 412 Bethea, R.M., 1973. Comparison of hydrogen sulfide analysis techniques. Journal of the
- 413 Air Pollution Control Association. 23 (8), 710-713.
- 414 Billon, G, Ouddane, B, Laureyns, J, Boughriet, A., 2001. Chemistry of metal sulphide
- 415 in anoxic sediments. Phys. Chem. 3, 3586-3592.
- 416 Brown, K.A., McGree, E.R., Taekema, B., Cullen, J.T., 2011. Determination of Total
- 417 Free Sulphides in Sediment Porewater and Artefacts Related to the Mobility of Mineral
- 418 Sulphides. Aquat. Geochem. 17 (6), 821-839.
- 419 Burton, E.D., Bush, R.T., Sullivan, L.A., 2006. Acid-volatile sulfide oxidation in
- 420 coastal flood plain drains: iron-sulfur cycling and effects on water quality. Environ. Sci.
- 421 Technol. 40 (4), 1217-1222.

- 422 Dilgin, Y., Canarslan, S., Ayyildiz, O., Erteka, B., Nisli G., 2012. Flow injection
- 423 analysis of sulphide based on its photoelectrocatalytic oxidation at poly-methylene blue
- 424 modified glassy carbon electrode. Electrochimica Acta. 66, 173-179.
- 425 Dent, D., 1986. Acid sulphate soils: a baseline for research and development.
 426 Wageningen, Netherlands. International Institute for Land Reclamation and
 427 Institute for Land Reclamation and
- 427 Improvement/ILRI. series 39, pp. 200
- 428 Eaton, A.D., Clesceri, S.L., Greenberg, A.E., 1995. Standard methods for the
- examination of water and wastewater. 19th ed. Am. Public Health Assoc., Washington,DC.
- 431 Hammerschmidt, C.R., Burton, G.A., 2010. Measurements of acid volatile sulfide and
- 432 simultaneously extracted metals are irreproducible among laboratories. Environ.
- 433 Toxicol. Chem. 29 (7), 1453-1456.
- 434 Hansen, T.L., Schmidt, J.E., Angelidaki, I., Marca, E., la Cour Jansen, J., Mosbæk, H.,
- 435 Christensen, T.H., 2004. Method for determination of methane potentials of solid 436 organic waste. Waste Manage. 24 (4), 393-400.
- 437 Hossain, S.M.Z., Brennan, J.D., 2011. b-Galactosidase-Based colorimetric paper sensor
- 438 for determination of heavy metals. Anal. Chem. 83, 8772-8778.
- 439 Holm, T.R., Fisher, R.P., Frant, M.S., Gagnon, C., Goodwin, L.R., 2000. Joint Task
- 440 Group 4500-S2. A, H, I, J. 20th Edition. S. Stieg, Bradford R. Fisher, O. B. Mathre, T.
- M. Wright. https://www.edgeanalytical.com/wp-content/uploads/Inorganic_SM4500 S2-.pdf
- 443 Liu, Y., Tugtas, A.E., Sharma, K.R., Ni, B.J., Yuan, Z., 2016. Sulfide and methane
- production in sewer sediments: Field survey and model evaluation. Water Res. 89, 142-150.
- Lawrence, N.S., Davis, J., Compton R.G., 2000. Analytical strategies for the detection
 of sulfide: a review. Talanta. 52, 771–784.
- 448 Mc Clure, F.D., Lee, J.K., 2003. Computation of HORRAT values. Journal of OAC
- 449 international. 86, 1056-1058.
- 450 Natusch, D.F.S., Sewell, J.R., Tanner, R.L., 1974. Determination of hydrogen sulfide in
- 451 air. Assessment of impregnated paper tape methods. Anal. chem. 46 (3), 410-415.

- 452 R Core Team., 2013. R: A language and environment for statistical computing. R
 453 Foundation for Statistical Computing, Vienna, Austria.
- 454 Rabenhorst, M.C., Megonigal, P.J, Keller, J., 2010. Synthetic Iron Oxides for
- 455 Documenting Sulfide in Marsh Pore Water. Soil Sci. Soc. Am. J. 74, 1383-1388.
- 456 Ratnarathorn, N., Chailapakul, O., Henry, C.S., Dungchai, W., 2012. Simple silver
- 457 nanoparticle colorimetric sensing for copper by paper-based devices. Talanta. 99, 552-458 557.
- 459 Rickard, D., Morse, J.W., 2005. Acid volatile sulphides (AVS). Mar. Chem. 97, 141-460 197.
- 461 Sanderson, H.P., Thomas, R., Katz, M., 1966. Limitations of the lead acetate
 462 impregnated paper tape method for hydrogen sulfide. J. Air Pollut. Control Assoc. 16
 463 (6), 328-330.
- 464 Sicard, C., Glen, C., Aubie, B., Wallace D., Jahanshahi-Anbuhi, S., Pennings, K.,
- Daigger, G.T., Pelton, R., Brennan, J. D., Filipe, C.D.M., 2015. Tools for water quality
 monitoring and mapping using paper-based sensors and cell phones. Water Res. 70,
 360-369.
- Soil Survey Staff, 2014. Keys to Soil Taxonomy, 12th ed. United States Department of
 Agriculture, Natural Resources Conservation Service.
- 470 Stone, J., Ahern, C.R., Blunden, B., 1998. Acid Sulfate Soils Manual 1998. Acid Sulfate
- 471 Soil Management Advisory Committee, Wollongbar, NSW, Australia.
- 472 U.S. Environmental Protection Agency, 2007. Framework for metals risk assessment.
- 473 EPA/120/R-07/001. Office of the Science Advisor, Washington, DC.
- 474 Van Breemen, N.V., 1982. Genesis, morphology, and classification of acid sulfate soils
- 475 in coastal plains. In: Kittrick, J.A., Fanning, D.S., Hossner, L.R., (eds) Acid Sulfate
 476 Weathering. Soil. Sci. Soc. Am. Spec. Publ. 10, 95-108.
- 477 Wei, S., Jiang, Z., Liu, H., Zhou, D., Sanchez-Silva, M., 2014. Microbiologically
- 478 induced deterioration of concrete: a review. Braz. J. Microbiol. 44 (4), 1001-1007.
- 479



481 Fig. 1 supplementary material. Linear functions for the ISE probe calibration matching

482 the millivolt readings of the probe and the S^{2-} concentrations of the Na₂S-9H₂O standard

483 solutions prepared freshly in the laboratory.

GENERAL DISCUSSION



Spartinetea maritimae vegetation class in the less elevated areas of the Marina di Macia saltmarsh, Grado Lagoon.

The progressive reduction of the saltmarsh areas recorded worldwide in the last decades (Waycott et al., 2009) led to an increasing interest around these wetland systems, in particular for its severe consequences on biodiversity and human activities. This interest revealed a very scarce knowledge about saltmarshes and the need to develop suitable strategies to cope with an extremely different system, compared to the well-studied terrestrial ones. In fact, diverse mechanisms are involved, for instance in defining plant zonation and soil evolution; moreover, different procedures are required to analyse such environmental samples, like the anoxic soils subject to quick changes when exposed to air (Le Mer and Roger, 2001; Stephens et al., 2001; Van Breemen, 1982).

The overall aim of this research was therefore to investigate the main variables affecting soil and plant zonation in saltmarshs and to define the relationships between soil and vegetation, in order to elucidate the dynamics involved. This knowledge will be than applied in management programs, for preservation of existing saltmarshes and restoration of new areas. To achieve these aims, soil and vegetation were investigated using a holistic approach, moving from the identification of existing relationships between these two compartments (Chapter 1 and 2) to the evaluation of feedback mechanisms that vegetation drives towards soil (Chapter 3), with additional specific contributions aimed to clarify flooding tolerance in halophytes (Chapter 4) and proposing a new method for the evaluation of sulphides in anoxic soil (Chapter 5).

KEY FINDINGS

This work basically confirmed the central role of flooding in determining both soil features and plant distribution (Chapter 1, 2 and 3). Nevertheless, this finding is unfortunately affected by uncertainties in the evaluation of the hydroperiod, a highly critical point. Flooding has been so far usually evaluated indirectly using the elevation as a proxy (Rasser et al., 2013; Silvestri et al., 2005). This is likely cause of the contradictory results arisen among authors, which either highlighted the presence (Chapter 1 and 2; Marani et al., 2004; Bockelmann et al., 2002) or the lack of correlation (Bockelmann and Neuhaus, 1999; Adam, 1993) between plant zonation and flooding. Elevation data are still today strongly affected by the

inaccuracy of available LiDAR models and by the hard effort that accurate data acquisition requires, especially in saltmarshes where differences of few centimetres of height may result in abrupt changes among plant communities. Considering this, we tried to improve accuracy in the evaluation of the hydroperiod, by using both elevation data and tidal regimes (Chapter 3) and this allowed to obtained more sensitive relationships among variables, testifying the need to improve ways to estimate this parameter. Thus, direct measurements or improved correction factors should be considered for future research.

Plant distribution is also linked to the different degrees of tolerance that halophytes show towards flooding. We noticed that plants with similar ecological niches could use different strategies against flooding: from oxygen diffusion through aerenchyma, to underwater photosynthesis (Chapter 4), or to shift mitochondrial respiration to fermentative processes allowing plant metabolism during anoxia periods (Perata et al., 2011; Visser et al., 2003). Interestingly, some species could translocate oxygen from shoots in contact with air to submerged tissues (*Sarcocornia fruticosa*), while others prefer restoring aerobic conditions in the surroundings of roots (*Limonium narbonense*), an advantage when flooding finishes (Chapter 4).

Flooding is extremely important considering that it drives changes on soil, plants, microbial communities, sulphur or carbon cycles etc. (Reddy and DeLaune, 2008). Soil features change along the flooding gradient, developing severe anoxia and accumulating sulphides with the increasing of flooding, and plant communities count even lower species diversity or monospecific stands (Chapter 1 and 2). Beside this, the present work highlighted the strong feedback mechanisms driven by plants towards soil (Chapter 1, 2 and 3). The conditions determined by flooding could be seriously altered by plants through oxygen released from roots, which could be more or less pronounced, based on plant species. Moreover, the quick leaf renewal rates, exploited by many halophytes against salt accumulation, contribute to soil organic matter content (Chapter 1). The decomposition of litter affects the development of soil, but can also affect the production of sulphides upon anoxia, being carbon a source for microbial metabolism and a driving factor of reduction intensity. The oxygen released from roots leads to the formation of red- mottles along the typical gley coloured horizons (Chapter 1; Richardson and Brinson,

2001), changing locally the redox status of soil (Chapter 3). The consequent improvement of soil conditions around roots enhances in turn plant establishment, highlighting the complexity of soil-plant interactions.

The ability of plants to modify the chemistry of saltmarsh soils seemed to be strictly linked to the redox status of the soil: sulphides production seemed not to be really limited by plants (Chapter 3), probably due to the very low redox conditions necessary for sulphides production and the limited distance from root surface of oxygen diffusion. On the contrary, zonation can be affected by sulphide production in soils (Gribsholt and Kristensen, 2002), which is proportional to flooding, but also dependent from sulphate availability. Nevertheless, this effect is today still unclear and considered only in few recent works (Borum et al., 2013; Holmer et al., 2005), despite the great amount potentially present in saltmarsh soils. Moreover, the lack of good analytical methods weights to the accuracy of sulphides measurements.

The method proposed in this work (Chapter 5), despite the relative small range of applicability, appeared robust and able to highlight the spatial variability of sulphides in soils. This method will facilitate field studies on the effect of sulphides in zonation processes, and facilitate the mapping of sulphides distribution in saltmarsh soils.

Vegetation can be limited also by the reduced uptake capability of plants upon anoxic conditions. We highlighted that different plant communities are less or more efficient to acquire some nutrients, mostly due to their availability in soil (Chapter 2). The mobility of nutrients like Fe, K and Ca is severely reduced upon anoxia (Julie and Siobhan, 2001) and different species could be able to improve uptake of some elements or tolerate very low amounts (Chapter 2). Flooding is strictly linked to the redox status of soil, defining the availability of an element, but, as previously suggested, plants could alter the redox status and consequently improve the mobility of nutrients present in soil (Chapter 2).

Finally, zonation derived also from plant-plant interactions, due to intra or interspecific competition (Chapter 3; Bockelmann et al., 2002). Previous researches proved that in more elevated areas, competition is even more important, considering that environmental stresses are less severe (Ford et al., 2016; Wang et al., 2006). Plant individuals shape directly changes on plant communities, whereas flooding affect plant zonation only indirectly (Chapter 3). This fact attributes a great role to plant traits that must be considered, together with vegetation, for future restoration programs.

We proved that interactions among abiotic and biotic factors can magnify or quench the original conditions determined by flooding. A pivotal achievement was, therefore, to demonstrate that vegetation and plants could be considered predictor tools of soil conditions, environmental stresses and vegetation dynamics.

The present work highlighted the relevance of a holistic approach when studying saltmarsh system, and the need to move across different levels of detail: from the small scale of plant-soil interface, to the higher level of complexity of vegetation-soils interactions.

FUTURE RESEARCH PERSPECTIVES

Many aspects to investigate in deep and issues to solve arose from the results of the present work. The main hot spots are listed here below.

1) Methods of sampling and analyses have to be improved, especially considering the handling of anoxic soils, subject to quick oxidation when exposed to air. This lack could seriously affect the accuracy of surveys conducted in wetlands.

2) Guidelines regarding conditions for germination (*in situ* or *ex situ*) and growth of halophytes are still missing, despite crucial. For instance, the optimal conditions for water salinity and flooding period required by each species are not known yet.

3) The Soil Taxonomy classification system has to be improved for hydromorphic soils and additional concrete rules are required for a better definition of these soils. This need regards especially submerged soils with mineral iron sulphides phases, not considered yet in the *Sulfiwassent* suborder when the pH is neutralised by carbonates, even if the amount of sulphides is consistent (Chapter 1).

4) Maps of sulphides distribution in saltmarshes are missing, despite they would be a great tool for evaluating the effect of sulphides on plant zonation.

5) Halophytes under submergence could prefer anaerobic metabolism to survive, but this strategy is poorly studied. Plants able to activate fermentative pathways should accumulate reserves during favourable conditions. The amount of reserves and non-structural carbohydrates could explain the great flooding tolerance of species, like *L. narbonense*, able to survive even if completely submerged for long periods (Chapter 4).

6) Finally, species competition appeared even more important than environmental stresses in defining zonation (Chapter 3). Therefore, competition has to be set as an additional relevant variable defining plant distribution and possible changes on soil features, yet unclear.

CONCLUSIONS

This work confirmed the need of a holistic approach to understand saltmarsh systems and the need to move across different levels of detail, from the small scale to the higher level of complexity defined by soil-vegetation interactions. The holistic approach, including different disciplines, provides the most realistic chance to achieve the necessary knowledge and control the increasing loss of saltmarsh areas. The combination of soil features, plant traits and vegetation, tested together, allowed us to define the relevance and the direction of existing relationships (Chapter 3). These findings will allow an easier monitoring of existing saltmarshes and will help to develop better-defined plans for future restoration programs. The monitoring of vegetation indirectly allows to know the degree of environmental stresses involved and the soil features that could allow the presence of a specific vegetation type. On this basis, we can become able to model saltmarsh surface topology and arrange the optimal soil features in order to favour the establishment and growth of specific plants, and indirectly to control vegetation dynamics.

REFERENCES

- Abbiati M., Mistri M., Bartoli M., Ceccherelli V.U., Colangelo M.A., Ferrari C.R., Giordani G., Munari C., Nizzoli D., Ponti M., Rossi R., Viaroli P. (2010) Trade-off between conservation and exploitation of the transitional water ecosystems of the northern Adriatic Sea. *Chemistry and Ecology* 26(S1), 105-119.
- Adam P. (1993) Saltmarsh ecology. Cambridge University Press, Cambridge, UK.
- Adam P. (2002) Saltmarshes in a time of change. *Environmental conservation* 29(1), 39-61.
- Almeida C.M.R., Mucha A.P., Vasconcelos M.T.S. (2004) Influence of the sea rush *Juncus maritimus* on metal concentration and speciation in estuarine sediment colonized by the plant. *Environmental science and technology* 38(11), 3112-3118.
- Anderson E.F., Wilson D.J. (2000) A simple field test for acid volatile sulfide in sediments. *Journal of the Tennessee Academy of Science* 75(3-4), 53-56.
- Armstrong W., Drew M.C. (2002) Root growth and metabolism under oxygen deficiency. *Plant roots: the hidden half* 3, 729-761.
- Barbier E.B., Koch E.W., Silliman B.R., Hacker S.D., Wolanski E., Primavera J., Granek E.F., Polasky S., Aswani S., Cramer L.A., Stoms D.M., Kennedy C.J., Bael D., Kappel C.V., Perillo G.M.E., Reed D.J. (2008) Coastal ecosystem-based management with nonlinear ecological functions and values. *Science* 319(5861), 321-323.
- Batriu E., Pino J., Rovira P., Ninot J.M. (2011) Environmental control of plant species abundance in a microtidal Mediterranean saltmarsh. *Applied Vegetation Science* 14(3), 358-366.
- Bayraktarov E., Saunders M.I., Abdullah S., Mills M., Beher J., Possingham H.P., Mumby P.J., Lovelock C.E. (2015) The cost and feasibility of marine coastal restoration. *Ecological Applications* 26(4), 1055-1074.
- Benayas J.M.R., Newton A.C., Diaz A., Bullock J.M. (2009) Enhancement of biodiversity and ecosystem services by ecological restoration: A meta-analysis. *Science* 325(5944), 1121-1124.
- Bertness M.D., Ellison A.M. (1987) Determinants of pattern in a New England salt marsh plant community. *Ecological Monographs* 57(2), 129-147.

- Bockelmann A.C., Neuhaus R. (1999) Competitive exclusion of *Elymus athericus* from a high stress habitat in a European salt marsh. *Journal of Ecology* 87, 503-513.
- Bockelmann A.C., Bakker J.P., Neuhaus R., Lage J. (2002) The relation between vegetation zonation, elevation and inundation frequency in a Wadden Sea salt marsh. *Aquatic Botany* 73(3), 211-221.
- Boorman L.A. (2003) Saltmarsh Review. An overview of coastal saltmarshes, their dynamic and sensitivity characteristics for conservation and management. JNCC Report, No. 334
- Borum J., Raun A.L., Hasler-Sheetal H., Pedersen M.Ø., Pedersen O., Holmer M. (2013) Eelgrass fairy rings: sulfide as inhibiting agent. *Marine Biology* 161, 351-358.
- Bradley M.P., Stolt M.H. (2006) Landscape-level seagrass-sediment relations in a coastal lagoon. *Aquatic Botany* 84, 121-128.
- Brouwer H. (1995) Acid Volatile Sulfides (AVS) in sediment: an environmental chemistry experiment. *Jornal of Chemical Education* 72(2), 182.
- Burke D.J., Weis J.S., Weis P. (2000) Release of metals by the leaves of the salt marsh grasses *Spartina alterniflora* and *Phragmites australis*. *Estuarine, Coastal and Shelf Science* 51, 153-159.
- Cadol D., Engelhardt K., Elmore A., Sanders G. (2014) Elevation-dependent surface elevation gain in a tidal freshwater marsh and implications for marsh persistence. *Limnology and Oceanography* 59, 1065-1080.
- Cazzin M., Ghirelli L., Mion D., Scarton F. (2009) Completamento della cartografia della vegetazione e degli habitat della laguna di Venezia: anni 2005-2007. *Lavori Soc. Ven. Sc. Nat.* 34, 81-89.
- Chen D., Li S., Liu H., Chen T., Chen C., Yu C. (2014) Rapid determination of sulfide sulfur in anaerobic system by gas-phase molecular absorption spectrometry. *Analytical Methods* 6(22), 9085-9092.
- Cocco S., Agnelli A., Gobran G.R., Corti G. (2013) Changes induced by the roots of *Erica arborea* L. to create a suitable environment in a soil developed from alkaline and fine-textured marine sediments. *Plant and soil* 368(1-2), 297-313.
- Colmer T.D. (2003) Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant, Cell andEnvironment* 26, 17-36.
- Colmer T.D., Flowers T.J. (2008) Flooding tolerance in halophytes. *New Phytologist* 179, 964-974.
- Costa C.S.B., Marangoni J.C., Azevedo A.M.G. (2003) Plant zonation in irregularly flooded salt marshes: relative importance of stress tolerance and biological interactions. *Journal of Ecology* 91: 951-965.
- Cott G.M., Reidy D.T., Chapman D.V., Jansen M.A.K. (2011) Saltmarshes on peat substrate on the southwest coast of Ireland: edaphic parameters and plant species distribution. *Geo-Eco Marina* 17: 41-49.
- Cronk J.K., Fennessy M.S. (2016) Wetland plants: biology and ecology. CRC press LLC, Washington, DC, 482 pp.
- Crooks S., Pye K. (2000) Sedimentological controls on the erosion and morphology of saltmarshes: implications for flood defence and habitat recreation. *Geological Society*, London, Special Publications, 175(1), 207-222.
- Chytrý M., Otýpková Z. (2003) Plot sizes used for phytosociological sampling of European vegetation. *Journal of Vegetation Science* 14(4), 563-570.
- Curcó A., Ibáňez C., Day J.W., Prat N. (2002) Net primary production and decomposition of salt marshes of the Ebre delta (Catalonia, Spain). *Estuaries* 25: 309–324.
- Cutini M., Agostinelli E., Acosta T.R.A., Molina J.A. (2010) Coastal salt-marsh zonation in Tyrrhenian central Italy and its relationship with other Mediterranean wetlands. *Plant Biosystems* 144(1), 1-11.
- Dunnette D.A., Chynoweth D.P., Mancy K.H. (1985) The source of hydrogen sulfide in anoxic sediment. *Water Research* 19(7), 875-884.
- Elmore A.J., Engelhardt K.A., Cadol D., Palinkas C.M. (2016) Spatial patterns of plant litter in a tidal freshwater marsh and implications for marsh persistence. *Ecological Applications* 26(3), 846-860.

- Erich E., Drohan P.J. (2012) Genesis of freshwater subaqueous soils following flooding of a subaerial landscape. *Geoderma* 179, 53-62.
- Fiedler S., Sommer M. (2004) Water and redox conditions in wetland soils-their influence on pedogenic oxides and morphology. *Soil Science Society of America Journal* 68(1), 326-335.
- Flowers T.J., Hajibagheri M.A., Clipson N.J.W. (1986) Halophytes. *The Quarterly Review of Biology* 61: 313–337.
- Flowers T.J., Colmer T.D. (2008) Salinity tolerance in halophytes. *New Phytologist* 179, 945-963.
- Ford H., Garbutt A., Ladd C., Malarkey J., Skov M.W. (2016) Soil stabilization linked to plant diversity and environmental context in coastal wetlands. *Journal of Vegetation Science* 27(2), 259-268.
- Frederiksen M.S.,Holmer M., Borum J., Kennedy H. (2006) Temporal and spatial variation of sulphide invasion in eelgrass (*Zostera marina*) as reflected by its sulfur isotopic composition. *Limnology and Oceanography* 51, 2308-2318.
- French J.R., Stoddart D.R. (1992) Hydrodynamics of salt marsh creek systems: Implications for marsh morphological development and material exchange. *Earth surface processes and landforms* 17(3), 235-252.
- Gribsholt B., Kristensen E. (2002) Effects of bioturbation and plant roots on salt marsh biogeochemistry: a mesocosm study. *Marine Ecology Progress Series* 241, 71-87.
- Hasegawa P.M., Bressan R.A., Zhu J.K., Bohnert H.J. (2000) Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* 51: 463-499.
- He Q., Bertness M.D., Bruno J.F., Li B., Chen G.Q., Coverdale T.C., Altieri H.A., Bai J., Sun T., Pennings S.C., Liu J., Ehrlich P.R., Cui B. (2014) Economic development and coastal ecosystem change in China. *Scientific reports* 4(5995), 1-9.
- Herzog M., Pedersen O. (2014) Partial versus complete submergence snorkeling aids root aeration in *Rumex palustris* but not in *R. acetosa. Plant, Cell and Environment* 37, 2381-2390.

- Hinsinger P., Plassard C., Tang C., Jaillard B. (2003) Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. *Plant and Soil* 248(1-2), 43-59.
- Holmer M., Frederiksen M.S., Møllegaard H. (2005) Sulfur accumulation in eelgrass
 (*Zostera marina*) and effect of sulfur on eelgrass growth. *Aquatic Botany* 81(4), 367-379.
- Holmer M., Pedersen O., Krause-Jensen D., Olesen B., Petersen M.H., Schopmeyer S., Koch M., Lomstein B.A., Jensen H.S. (2009) Sulfide intrusion in the tropical seagrasses *Thalassia testudinum* and *Syringodium filiforme*. *Estuarine, Coastal and Shelf Science* 85, 319-326.
- Holmer M., Wirachwong P., Thomsen M.S. (2011) Negative effects of stressresistant drift algae and high temperature on a small ephemeral seagrasss pecies. *Marine Biology* 158, 297-309.
- Holmer M., Hasler-Sheetal H. (2014) Sulfide intrusion in seagrasses assessed by stable sulfur isotopes-a synthesis of current results. *Frontiers in Marine Science* 1, 64.
- IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Stocker, T.F., Qin D., Plattner G.-K., Tignor M., Allen S.K., Boschung J., Nauels A., Xia Y., Bex V., Midgley P.M. (eds.) Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.
- Jenačković D.D., Zlatković I.D., Lakušić D.V., Ranđelović V.N. (2016) Macrophytes as bioindicators of the physicochemical characteristics of wetlands in lowland and mountain regions of the central Balkan Peninsula. *Aquatic Botany* 134, 1-9.
- Jeppesen E., Søndergaard M., Pedersen A.R., Jürgens K., Strzelczak A., Lauridsen T.L., Johansson L.S. (2007) Salinity induced regime shift in shallow brackish lagoons. *Ecosystems* 10(1), 48-58.
- Jiang J., De Angelis D.L., Smith III T.J., Teh S. Y., Koh H. L. (2012) Spatial pattern formation of coastal vegetation in response to external gradients and positive feedbacks affecting soil porewater salinity: a model study. *Landscape Ecology* 27(1), 109-119.

Julie K.C., Siobhan F.M. (2001) Wetland plants: biology and ecology. CRC Press.

- Kiehl K., Esselink P., Bakker J.P. (1997) Nutrient limitation and plant species composition in temperate salt marshes. *Oecologia* 111(3), 325-330.
- Koch M.S., Mendelssohn I.A., McKee K.L. (1990) Mechanism for the hydrogen sulfide-induced growth limitation in wetland macrophytes. *Limnology and Oceanography* 35, 399-408.
- Koretsky C.M., Haveman M., Cuellar A., Beuving L., Shattuck T., Wagner M. (2008) Influence of *Spartina* and *Juncus* on saltmarsh sediments. I. Pore water geochemistry. *Chemical Geology* 255(1), 87-99.
- Kroon H., Visser E.J.W. (2012) Plasticity as a plastic response: how submergenceinduced leaf elongation in *Rumex palustris* depends on light and nutrient availability in its early life stage. *New Phytologist* 194, 572-582.
- Kristensen E., Rabenhorst M.C. (2015) Do marine rooted plants grow in sediment or soil? A critical appraisal on definitions, methodology and communication. *Earth-Science Reviews* 145, 1-8.
- Laanbroek H.J. (2010) Methane emission from natural wetlands: interplay between emergent macrophytes and soil microbial processes. A mini-review. *Annals of Botany* 105(1), 141-153.
- Lamers L.P.M., Govers L.L., Janssen, I.C.J.M., Geurts J.G.M., Van der Welle M.E.W., Van Katwijk M.M., Van der Heide T., Roelofs J.G.M., Smolders A.J.P. (2013) Sulfide as a soil phytotoxin - a review. *Frontiers in Plant Science* 4, 268.
- Lang F., von der Lippe M., Schimpel S., Scozzafava-Jaeger T., Straub W. (2010) Topsoil morphology indicates bio-effective redox conditions in Venice salt marshes. *Estuarine, Coastal and Shelf Science* 87, 11-20.
- Le Mer J., Roger P. (2001) Production, oxidation, emission and consumption of methane by soils: a review. *European Journal of Soil Biology* 37(1), 25-50.
- Lee R.W. (1999) Oxidation of sulfide by *Spartina alterniflora* roots. *Limnology and Oceanography* 44, 1155–1159.
- Liu Z., Cui B., He Q. (2016) Shifting paradigms in coastal restoration: Six decades' lessons from China. *Science of The Total Environment* 566, 205-214.

- Marani M., Lanzoni S., Silvestri S., Rinaldo A. (2004) Tidal landforms, patterns of halophytic vegetation and the fate of the lagoon of Venice. *Journal of Marine Systems* 51, 191-210.
- Marschner P. (1995) Mineral Nutrition of Higher Plants. London: Academic Press.
- McAlpine K.G., Wotton D.M. (2009) Conservation and the delivery of ecosystem services. *Science for the Environment* 295. Department of Conservation, Wellington.
- McGrath J.A., Paquin P.R., Di Toro D.M. (2002) Use of the SEM and AVS approach in predicting metal toxicity in sediments. *Fact sheet on environmental risk assessment* 10, 1-7.
- Millero F.J. (1986) The thermody-namics and kinetics of the hydrogen sulfide system in natural waters. *Marine Chemistry* 18, 121-147.
- Möller I. (2006) Quantifying saltmarsh vegetation and its effect on wave height dissipation: Results from a UK East coast saltmarsh. *Estuarine, Coastal and Shelf Science* 69: 337-351.
- Mommer L., Wolters-Arts M., Andersen C., Visser E.J.W., Pedersen O. (2007) Submergence induced leaf acclimation in terrestrial species varying in flooding tolerance. *New Phytologist* 176, 337-345.
- Mucha A.P., Almeida C.M., Bordalo A.A., Vasconcelos M.T. (2005) Exudation of organic acids by a marsh plant and implications on trace metal availability in the rhizosphere of estuarine sediments. *Estuarine, Coastal and Shelf Science* 65, 191-198.
- Newton A., Carruthers T., Icely J. (2012) The coastal syndromes and hotspots on the coast. *Estuarine, Coastal and Shelf Science* 96, 39-47.
- Newton A., Icely J., Cristina S., Brito A., Cardoso A.C., Colijn F., Dalla Riva S., Gertz F., Hansen J.W., Holmer M., Ivanova K., Leppäkoski E., Canu D.M., Mocennim C., Mudge S., Murray N., Pejrup M., Razinkovas A., Reizopoulou S., Pérez-Ruzafa A., Schernewski G., Schubert H., Carr L., Solidoro C., Viaroli P., Zaldívar J.-M. (2014) An overview of ecological status, vulnerability and future perspectives of European large shallow, semi-enclosed coastal systems, lagoons and transitional waters. *Estuarine, Coastal and Shelf Science* 140, 95-122.

- Nielsen L.B., Finster K., Welsh D.T., Donelly A., Herbert R.A., De Wit R., Lomstein B.A. (2001) Sulphate reduction and nitrogen fixation rates associated with roots, rhizomes and sediments from *Zostera noltii* and *Spartina maritima* meadows. *Environmental Microbiology* 3(1), 63-71.
- Pandža M., Franjić J., Škvorc Ž. (2007) The salt marsh vegetation on the East Adriatic coast. *Biologia* 62(1), 24-31.
- Pedersen O., Binzer T., Borum J. (2004) Sulphide intrusion in eelgrass (*Zostera marina* L.). *Plant, Cell and Environment* 27(5), 595-602.
- Pedersen O., Colmer T.D., Sand-Jensen K. (2013) Underwater photosynthesis of submerged plants - recent advances and methods. *Frontiers in Plant Science* 4(140), 1-19.
- Pennings S.C., Callaway R.M. (1992) Salt marsh plant zonation: the relative importance of competition and physical factors. *Ecology* 73(2), 681-690.
- Perata P., Armstrong W., Voesenek L.A. (2011) Plants and flooding stress. *New Phytologist* 190(2), 269-273.
- Pezeshki S.R. (2001) Wetland plant responses to soil flooding. *Environmental and Experimental Botany* 46(3), 299-312.
- Ponnamperuma F.N. (1984) Effects of flooding on soils. *Flooding and plant growth* 9-45.
- Rabenhorst M.C., Megonigal J.P., Keller J. (2010) Synthetic iron oxides for documenting sulfide in marsh pore water. *Soil Science Society of America Journal* 74(4), 1383-1388.
- Rasser M.K., Fowler N.L., Dunton K.H. (2013) Elevation and plant community distribution in a microtidal salt marsh of the western Gulf of Mexico. *Wetlands* 33: 575-583.
- Reddy K.R., DeLaune R.D. (2008) Biogeochemistry of wetlands: science and applications. CRC press, Washington, DC, 800 pp.
- Reed D.J. (1990) The Impact of Sea-level Rise on Coastal Salt Marshes. *Progress in Physical Geography* 14(4), 465-481.

- Rhodes D., Nadolska-Orczyk A., Rich P.J. (2002) Salinity, osmolytes and compatible solutes. In: Läuchli A, Lüttge U, eds. Salinity: environment–plant–molecules. Dordrecht, the Netherlands: Kluwer, 181–204.
- Rickard D., Morse J.W. (2005) Acid volatile sulfide (AVS). *Marine Chemistry* 97(3), 141-197.
- Richardson J.L., Brinson M.M. (2001) Wetland soils and hydrogeomorphic classification of wetlands. *Wetland Soils: Genesis, Hydrology, Landscapes, and Classification* 209-227.
- Riley D., Barber S. (1971) Effect of ammonium and nitrate fertilization on phosphorus uptake as related to root-induced pH changes at the root-soil interface. *Soil Science Society of America Journal* 35(2), 301-306.
- Rinaldo A., Belluco E., D'Alpaos A., Feola A., Lanzoni S., Marani M. (2004) Tidal networks: form and function. In: Fagherazzi S., Marani M., Blum L.K. (eds.) The ecogeomorphology of tidal marshes. American Geophysical Union, Washington, DC, 75-91.
- Rivoal J., Hanson A.D. (1993) Evidence for a large and sustained glycolytic flux to lactate in anoxic roots of some members of the halophytic genus *Limonium*. *Plant physiology* 101(2), 553-560.
- Sanchez J. M., Otero X.L., Izco J. (1998) Relationships between vegetation and environmental characteristics in a salt-marsh system on the coast of Northwest Spain. *Plant Ecology* 136(1), 1-8.
- Silvestri S., Defina A., Marani M. (2005) Tidal regime, salinity and salt marsh plant zonation. *Estuarine, Coastal and Shelf Science* 62(1), 119-130.
- Simas T., Nunes J.P., Ferreira J.G. (2001) Effects of global climate change on coastal salt marshes. *Ecological Modelling* 139(1), 1-15.
- Soil Survey Staff (1999) Soil Taxonomy. 2nd edition. Agricultural Handbook 436, United States Department of Agriculture. Washington D.C., U.S.A.
- Soil Survey Staff (2014) Keys to Soil Taxonomy. 12th ed. United States Department of Agriculture, Natural Resources Conservation Service.

- Stephens S.R., Alloway B.J., Parker A., Carter J.E., Hodson M.E. (2001) Changes in the leachability of metals from dredged canal sediments during drying and oxidation. *Environmental Pollution* 114(3), 407-413.
- Studer C., Braendle R. (1987) Ethanol, acetaldehyde, ethylene release and ACC concentration of rhizomes from marsh plants under normoxia, hypoxia and anoxia. In: Crawford RMM, ed. Plant life in aquatic and amphibious habitats, Special publication 5, British Ecological Society. Oxford: Blackwell Scientific Publications, 293-301.
- Sun Z.G., Sun W.G., Tong C., Zeng C.S., Yu X., Mou X.J. (2015) China's coastal wetlands: conservation history, implementation efforts, existing issues and strategies for future improvement. *Environment International* 79, 25–41.
- Tessier M., Gloaguen J.C., Bouchard V. (2002) The role of spatio-temporal heterogeneity in the establishment and maintenance of *Suaeda maritima* in salt marshes. *Journal of Vegetation Science* 13(1), 115-122.
- Townend I., Fletcher C., Knappen M., Rossington K. (2011) A review of salt marsh dynamics. *Water and Environment Journal* 25(4), 477-488.
- Underwood G.J.C. (1997) Microalgal colonization in a saltmarsh restoration scheme. *Estuarine, Coastal and Shelf Science* 44, 471-481.
- Van Breemen N.V. (1982) Genesis, morphology, and classification of acid sulfate soils in coastal plains. In: Kittrick, J.A., Fanning, D.S., Hossner, L.R., (eds) Acid Sulfate Weathering. Soil. Sci. Soc. Am. Spec. Publ. 10, 95-108.
- Van de Koppel J., Altieri A.H., Silliman B.R., Bruno J.F., Bertness M.D. (2006) Scaledependent interactions and community structure on cobble beaches. *Ecology Letters* 9:45-50.
- Visser J.W., Voesenek L.A.C.J., Vartapetian B.B., Jackson M.B. (2003) Flooding and Plant Growth. *Annals of Botany* 91(2), 107-109.
- Voesenek L., Colmer T.D., Pierik R., Millenaar F.F., Peeters A.J.M. (2006) How plants cope with complete submergence. *New Phytologist* 170: 213–226.
- Wang Q., Wang C.H., Zhao B., Ma Z.J., Luo Y.Q., Chen J.K., Li B. (2006) Effects of growing conditions on the growth of and interactions between salt marsh plants: implications for invasibility of habitats. *Biological Invasions* 8(7): 1547-1560.

- Waycott M., Duarte C.M., Carruthers T.J., Orth R.J., Dennison W.C., Olyarnik S., Calladine A., Fourqurean J.W., Heck K.L., Hughes A.R., Kendrick G.A., Kenworthyj W.J., Short F.T., Williams S.L. (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences USA* 106(30), 12377-12381.
- Windham L., Weis J.S., Weis P. (2003) Uptake and distribution of metals in two dominant salt marsh macrophytes, *Spartina alterniflora* (cordgrass) and *Phragmites australis* (common reed). *Estuarine, Coastal and Shelf Science* 56(1), 63-72.
- Wright A.J., Kroon H., Visser E.J., Buchmann T., Ebeling A., Eisenhauer N., Fisher C.,
 Hildebrandt A., Ravenek J., Rosher C., Weigelt, A., Weisser W., Voesenek L.A.C.J.,
 Mommer L. (2017) Plants are less negatively affected by flooding when growing
 in species-rich plant communities. *New Phytologist* 213(2), 645-656.
- Wyn Jones G., Gorham J. (2002) Intra- and inter-cellular compartments of ions. In: Läuchli A, Lüttge U, eds. Salinity: environment–plant–molecules, Dordrecht, the Netherlands: Kluwer, 159-180.
- Zedler J.B., Morzaria-Luna H., Ward K. (2003) The challenge of restoring vegetation on tidal, hypersaline substrates. *Plant and Soil* 253(1), 259-273.