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**Interaction of natural organic matter and mobility of
toxic metals in relation to marine water infiltration in
upland soils**

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STATEMENT OF ORIGINAL CONTRIBUTION

The research presented is an original contribution in the field of ecology, focusing on soil chemistry. My supervisors, **Prof. Maria De Nobili**, and co supervisor Prof. **Marco Contin** followed my work constantly and professionally, and their contribution was essential. Lab technician **Aldo Bertoni** and **Andrea Cudini** help me at each step to organize and perform my lab activities. Post Doc fellow **Elisa Pellegrini** assists me in interpretation and presentation of the scientific data.

The thesis entitled “**Interaction of natural organic matter and mobility of toxic metals in relation to marine water infiltration in upland soils**” is organized in three chapters:

Chapter 1. Mazhar S, Pellegrini E, Contin M, Bravo C and De Nobili M (2022), Impacts of salinization caused by sea level rise on the biological processes of coastal soils - A review. *Front. Environ. Sci.* 10:909415. doi: 10.3389/fenvs.2022.909415.

Chapter 2. Flooding and sea water intrusion enhance mobility of airborne Cd, Zn and Pb in coastal soils.

Chapter 3. Seawater flooding impacts on biological activity of organic and conventional farming soils

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And finally, thanks to my family for his enthusiasm and strangeness that I loved, and to all people I met during this work experience for their kindness and help.

SUMMARY

This thesis examines two important issues related to the impact of sea level rise on coastal soils and namely: mobilization of potentially toxic elements (PTE) and biological fertility of cultivated soils.

Worldwide sea level rise (SLR) is amongst the most important consequences of global climate change and a major risk to many countries on Earth. Salinization caused by (SLR) endangers coastal agricultural soils, wetlands and freshwaters which are among the most biodiverse environments on Earth. SLR has important effects on soil biogeochemical cycles through the combined action of submergence and salinity on plant communities, in terms of productivity and type of vegetation.

The deterioration of soil fertility due to soil salt accumulation is a growing concern in coastal areas, but salinity can also make potentially toxic elements more mobile and therefore more bioavailable to soil micro-organisms.

The first part of this thesis draws a brief outline of our present knowledge of the driving factors that determine the diversity of situations that arise from the combined action of the unprecedentedly rapid rise of sea level and the augmented probability of coast inundation events associated with the increased frequency of storms stemming from climatic changes.

A review base study was carried out with the aim to outline the complexity of interactions acting on soil biological processes. The prediction of outcomes is made difficult by the concomitant and sometimes contrasting actions of flooding and seawater intrusion on partly acclimated and non-acclimated environments. Non-salt acclimated plants suffer from osmotic stress, but also from reduced O₂ solubility. Microbial biomass declines with increasing salinity and microbial communities shift in composition. Large concentrations of Cl⁻ inhibit nitrification, but salinity stimulates N₂O fluxes. Impacts on C mineralization rates is variable but enhanced by the larger availability of terminal electron acceptors. The reduction of Fe combined with that of SO₄ could enhance P mobility. Salinization affects methanogenesis which is constrained in favor of SO₄ reduction. The distinction between short- and long-term effects is also important. Saltwater

inundation mobilizes nutrients that add to the loading in adjacent water bodies, reducing water quality.

Less known are risks associated with soil pollution, but salinization may also enhance the solubility and therefore the mobilization of toxic elements, because of cation exchange reactions and formation of complexes with chloride ions, as well as ion couples with sulphate. The second Chapter of the thesis describes some experiments that confirm that metals are made more mobile by saline waters.

Potentially toxic elements (PTEs) are widespread soil pollutants, that are considered toxic, non-degradable, persistent, and bio-accumulative, consequently threatening all living organisms, as well as human health. Salinity changes the chemical behavior of the metals, accumulation of PTEs in soils exposed to salinization by SRL may therefore impose enhanced ecological risks.

To investigate the consequences of increasing salinization and length of flooding events on the mobilization of PTE derived from airborne dusts, we compared the behaviour of Cd, Zn and Pb from two types of sources i.e. mine tailings and smelter exhaust dusts in two soils of similar physico-chemical properties, but subjected to different agricultural management (arable and grassland) when exposed to flooding events of different length with marine and brackish waters. The extent of mobilization depends on several factors, among which the main are the kind of element, the dust source and the salinity of flooding waters, but airborne PTE display a much stronger tendency to be mobilised than metals inherited from the soil parent material. The source of the dust, which determines not only the original speciation of the elements, but also eventually modifies soil conditions, has a strong effect on their release rates. Lower amounts of Pb, Cd and Zn were in fact proportionally released from tailing contaminated soils, than from smelters contaminated soils, probably because of the increase in pH of the water phase. In the presence of easily decomposable organic matter, flooding triggers biochemical redox processes that result in the modification of both the water phase and of solid surfaces.

Soil management also affects the intensity of mobilization as lower amounts of PTE were leached from the grassland soil than from the arable, showing that even

minor modification of soil characteristics such as soil organic matter quality, may significantly affect the extent of PTE mobilization by salinity.

The last part of the thesis (Chapter 3) investigates the effects of submergence with saline waters on soil biological activity and compares two soils that had been managed for more than ten years with conventional farming of organic farming systems.

Conventional farming (CF) necessitates a large amount of chemical fertilizer and use of pesticides to support the yields per hectare, but may impoverish the soil and productivity can be supported only with large energy inputs. Organic farming (OF) systems may provide beneficial solutions to current problems affecting the sustainability of soil biological fertility.

Soil microbial biomass (SMB) and its metabolic quotient (qCO_2) may serve as an indicators of soil environment in both agricultural and natural ecosystems over a long period of time. Chapter 3 describes the impacts of flooding with saline waters on the main biological indicators (microbial biomass, evolution of CO_2 , qCO_2 , ATP) in relation to water potential measured in organically and traditionally cultivated soils, (similar physico-chemical properties, but subjected to different agricultural management).

Soil biomass was found to decrease in both OF and CF during the 5, and 25 days of incubation's periods through gradually increase in salinity. Consequently, CO_2 evolution and metabolic quotient qCO_2 were increased in both OF and CF along decrease in biomass C. Increasing salt levels in submerged soils remarkably decreased soil water potential (ψ), so water was less available to microorganisms.

Soil ATP levels were on average higher in the organic soil and tended to decrease with salinity in both conventional and organic soils, ranging from about 8 to 2 nmol ATP per g soil. Resistance to air-drying was also considered. Ψ of air-dried soils did not reveal clear trends among salt treatments or between soils, while soil ATP results were consistent with soil ATP measured in fresh soils. This result suggests that microbial activity is not only affected by the osmotic stress derived from the saline treatment, therefore by ψ , but also by the possible direct toxic effect of the salts.

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INTRODUCTION

CHAPTER 1

PREFACE

This work was carried out with the aim to outline the driving factors that determine the diversity of situations that arise from the combined action of the unprecedentedly rapid rise of sea level and the augmented probability of inundation events associated with the increased frequency of storms stemming from climatic changes. It examines threats to coastal agricultural land and natural wetlands before focusing on the consequences of salinization on soil biological properties that regulate the contribution of coastal soils to the biogeochemical cycles. The aim is to outline and clarify the complexity of affecting factors and feedbacks that arise from the often-contrasting actions of submergence, availability of electron acceptors and osmotic stress on acclimated (salt marshes) or non-acclimated (coastal freshwater wetlands and agricultural land) ecosystems. A specific discussion is devoted to the consequences on greenhouse gases (GHG) emissions.

This paper, has been published in *Frontiers in Environmental Science*. My contribute referred mostly to developing the structure, contributed to the editing and finalization of the review.



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Impacts of salinization caused by sea level rise on the biological processes of coastal soils - A review

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Soil salinization caused by sea level rise threatens coastal agricultural soils and geochemically important wetlands worldwide. The aim of this review is to outline expected changes in soil biological activity by discussing the combined effects of salt stress and flooding on plants productivity and soil microbial communities, which determine consequences on fluxes of C, N and P. Finally, it outlines the expected repercussions on greenhouse gases emissions. The prediction of outcomes is made difficult by the concomitant and sometimes contrasting actions of flooding and seawater intrusion on partly acclimated and non-acclimated environments. Non-salt acclimated plants suffer from osmotic stress, but also from reduced O₂ solubility. Microbial biomass declines with increasing salinity and microbial communities shift in composition. Large concentrations of Cl⁻ inhibit nitrification, but salinity stimulates N₂O fluxes. Impacts on C mineralisation rates is variable but enhanced by the larger availability of terminal electron acceptors. The reduction of Fe combined with that of SO₄²⁻ could enhance P mobility. Salinization affects methanogenesis which is constrained in favour of SO₄²⁻ reduction. Consequences are largely site specific and difficult to predict because of the complex network of processes occurring simultaneously in different compartments (i.e., soil, microbiome, vegetation). The distinction between short and long term effects is also important. A reliable prediction of outcomes at a planetary scale will only result from more precise inventories and monitoring of areas displaying specific similarities and from the implementation from these well-defined data sets of specifically devised models whose results can be finally combined on a weighted basis.

KEYWORDS

salinization, sea level rise, wetlands, GHG, coastal soils

Introduction

Salinization has occurred throughout the Earth's history via the natural accumulation of salts, released by the weathering of rocks. This process, nowadays intensified by rising temperature, is known as primary salinization. Associated changes in salinity occur over a time scale of approximately 100,000 years, though further variations arise over shorter orbital cycles of 23,000–41,000 years (Neukom et al., 2014). In contrast, much faster increases in salt concentrations caused by anthropogenic actions are known as secondary salinization. Since the onset of civilization, anthropogenic manipulations of the hydrologic cycle have artificially altered the balance between salt accumulation and water inputs, leading to increased salinity in some wetlands, inland aquatic systems and upland soils. Secondary salinization can take place over time scales as short as decades, or even more rapidly (Herbert et al., 2015).

Another threat, associated with climatic changes and the salinization of coastal agricultural soils and wetlands, is due to the worldwide sea level rise (SLR), which also endangers biodiversity, with coastal freshwater wetlands being among the most biodiverse environments on Earth. Wicke et al. (2011) estimated that, globally, 1.1×10^9 ha of land was salt-affected and 14% (1.5×10^8 ha) of this area is classified as forest, wetlands, or other legally protected areas. Blankespoor et al. (2012) projected that, following a 1 m rise in sea level, 64% of freshwater coastal wetlands would be lost and converted to saline systems, with the higher regional loss rates in the Middle East and North Africa (100%), Latin America and the Caribbean (74%), Sub-Saharan Africa (72.5%), East Asia and the Pacific (62.2%). Henman and Poulter (2008) estimated that, worldwide, there are approximately 15×10^6 ha of coastal wetlands below 5 m elevation above the mean sea level (MSL), and thus vulnerable to projected SLR. The Australian and New Zealand Environmental and Conservation Council predicts significantly high salt concentrations in 40,000 km of their waterways and associated wetlands by 2050 (Nielsen et al., 2003).

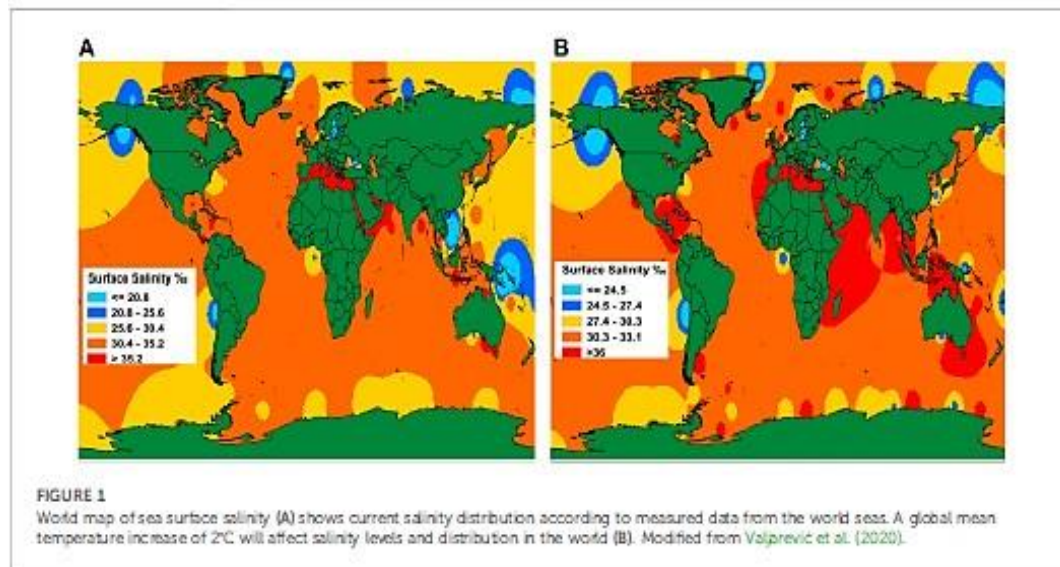
This review starts with a brief outline of the driving factors that determine the diversity of situations that arise from the combined action of the unprecedentedly rapid rise of sea level and the augmented probability of coast inundation events associated with the increased frequency of storms stemming from climatic changes. It then examines threats to coastal agricultural land and natural wetlands before focusing on the consequences of salinization on soil biological properties that regulate the contribution of coastal soils main to the biogeochemical cycles. The aim is to outline and clarify the complexity of affecting factors and feedbacks that arise from the often-contrasting actions of submergence, availability of electron acceptors and osmotic stress on acclimated (salt marshes) or non-acclimated (coastal freshwater wetlands and

agricultural land) ecosystems. A specific discussion is devoted to the consequences on greenhouse gases (GHG) emissions.

Sea level rise and forecasted increases in sea water salinity

The fact that SLR is amongst the most important consequences of global climate change and a major threat to many countries on Earth is widely acknowledged. Habitat and infrastructures of the people living in coastal areas, which host about 10% of the total population of our planet, are likely to face increasingly frequent inundation events and eventually become permanently flooded (Carrasco et al., 2016). The global MSL was calculated to have increased, from 1993 to 2018, at a rate of 3.34 mm per year (based on near real-time satellite altimetry data, Aviso, 2003). As a consequence of global climate change, MSL is projected to increase by 0.26–0.82 m by 2100 (IPCC, 2013; IPCC, 2014), with some models projecting an upsurge of more than 1 m by 2100 (Richardson et al., 2009; Vermeer and Rahmstorf, 2009; Rignot et al., 2011). In addition to the melting of polar caps, the warming of the oceans will also contribute to the global SLR, because of the decrease in density of water with temperature. Vermeer and Rahmstorf (2009) used a semi-empirical method linking temperature changes to SLR, and the resulting projected global SLR by 2100 of 0.75–1.90 m is significantly higher than the IPCC projections (0.26–0.81 m, IPCC, 2013; IPCC, 2014).

Variations caused by gravitational effects resulting from land ice mass changes, thermal expansion and ocean dynamics will be observed at the regional scale. In fact, SLR does not manifest itself as a smooth, linear increase, rather its rates vary over time and between regions, complicating predictions for seawater intrusion. Mediterranean coasts and lands facing the Red Sea, the northeastern part of the Indian Ocean, are potentially more at risk because of the high salinity of marine waters (Figure 1). Valjarević et al. (2020) updated the world maps of sea-surface salinity and calculated the expected increase in salinity (levels and distribution) caused by a 2°C increase in global mean temperature (by the CMIP5 climatological model). According to their projections, salinity levels above 39‰ will be experienced in the area of the Red Sea, the African countries in the Mediterranean, the countries in the Persian Gulf and the Bengal Gulf and Indochina, as well as the Victoria state and the Northern Territory in Australia (Figure 1). In fact, salinization prompted by the SLR varies geographically, because of the spatial variability of regional trends caused by smaller-scale alterations in water temperature, surface winds and geologic activity (IPCC, 2013). Heterogeneity is driven, among other factors, by the salinity of nearby sea surface layers. Climatic factors concur to exacerbate the phenomenon in some regions, e.g. a negative balance between precipitation and soil



evapotranspiration causes salts accumulation in the presence of saline groundwater (Rose et al., 2005).

It is estimated that global soil salinization will continue spreading at a rate of up to 2Mha yr^{-1} (Abbas et al., 2013). Case studies showing the influence of SLR on lagoons and/or estuaries include Lagoa dos Patos, Brazil (Toldo et al., 2000), Lake Illawarra and St. Georges Basin, New South Wales, Australia (Sloss et al., 2006), Venice Lagoon, Italy (Ferla et al., 2007), Pamlico-Albemarle Sound, North Carolina, United States (Pilkey et al., 2009), Wadden Sea, Netherlands/Germany (Dissanayake et al., 2012), Ria Formosa, Portugal (Andrade et al., 2004), Vistula Lagoon, Baltic Sea (Navrotskaya and Chubarenko, 2013), and Manzala Lagoon, Egypt (Frlhy and El-Sayed, 2013). Several aquifers along the densely populated Mediterranean Sea coasts are already suffering seawater intrusion. Speed up of this phenomenon could be particularly intense in this part of the world, because the Mediterranean region, and especially its semi-arid areas, are likely to be seriously affected by a decline in freshwater resources (Kundzewicz and Doll, 2009).

Spatio-temporal drivers of salinization

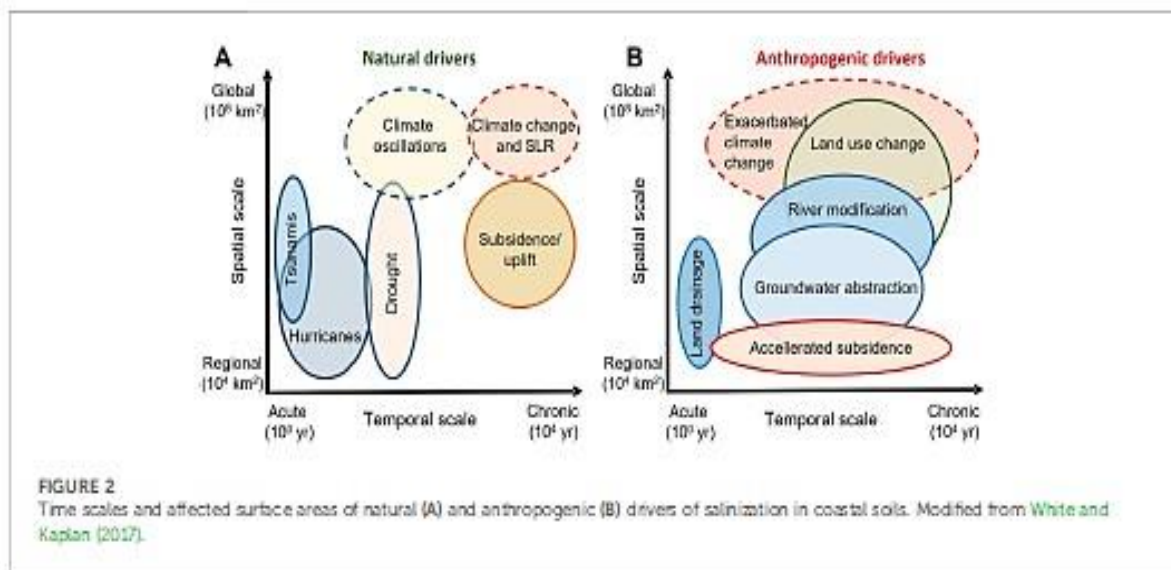
As sea levels rise, seawater intrusion into freshwater ecosystems increases in frequency, duration and spatial extent (Weissman and Tully, 2020). The risk of salinization of coastal soils is related to the frequency of inundation, which increases with proximity to the seashore and decreases with ground height above the MSL. Tides of unusual elevation are becoming more frequent due to a combination of SLR and extreme weather conditions fostered by climate change (IPCC, 2014), and

exacerbate the situation of submerging coastal soils and their salinization.

Strong winds may push the waves of high tides further inland and long drought periods will cause salts to accumulate in surface soil horizons. Once the soils have become submerged, salinity is expected to approach a steady-state equilibrium with brackish waters (Abbas et al., 2013), but even before this happens, evaporation from periodically inundated soils will cause soil salinity to increase at levels much higher than those of the recurrently invading waters. Geographical characteristics contribute to differentiate the effects: within a given tidal range, the frequency of inundation at a particular soil elevation in salt marshes is generally influenced by landscape characteristics, such as length and tortuosity of tidal creeks or distance from the shore. These factors may combine with local climate features, such as the direction of prevailing winds, and lead to locally different inundation patterns (Yang et al., 2015).

The main spatial drivers of variability in salinization intensity are local geomorphology, soil hydrology, textural composition and vegetation cover, which alter pathways and speed of the submarine water discharge, as well as the effects of tidal range and pore water exchange that determine the rates of penetration and drainage of sea water (Guimond and Tamborski, 2021).

Soil texture determines the thickness of the capillary fringe and the potential maximum flux of solutes to the soil surface (Fiola et al., 2020). The capillary rise is, in fact, lower in sandy layers and can retard the building up of salt accumulation. In the soils of estuarine and former floodplain areas, the succession and textural composition of soil horizons impact the height at which groundwater can rise. Textural discontinuities affect the hydrologic connectivity of the soil and reduce the flow of water and solutes to the surface (Gardner, 2005). Stratigraphic



constraints, such as mud layers, hamper the discharge flow leading to smaller and denser salt fingers where the effect is exacerbated by the eventual presence of buried sand lenses (Wu et al., 2022). These factors are however important only in low-lying soils where the depth of the water table is sufficiently shallow, so the steepness of the banks may zonally reduce the impact of infiltration of salty ground water.

Soil morphology influences the type of vegetation cover that, in turn, affects the flow of water and solutes towards the surface as the suction exerted by plant water uptake and transpiration can greatly accelerate the process (Carmona et al., 2021). Plants affect the distribution of salinity both quantitatively and qualitatively. In fact, Cl^- tends to be excluded from plant nutrient uptake and to accumulate in soil and groundwater as a function of evapotranspiration, with a decreasing gradient of Cl^- concentration with depth (Grimaldi et al., 2009). Presence of roots of neighbour trees exploring the soil, increase the complexity of the spatial variability of Cl^- , enhancing the risk of strong salinization during periods of drought concomitant to the growing season (Humphries et al., 2011). Salt reduces plant evapotranspiration of riparian species but, rising seawaters can lead to the die back of the riparian vegetation in favour of the establishment of halophytes and salt-tolerant woody species (e.g., *Tamarix* spp.). Salt-tolerant plants can take up water at very low soil water potential, thanks to the production of soluble osmolytes stored in the cell vacuoles to regulate the osmotic stress (Flowers et al., 2015). The consequent increase of evapotranspiration further enhances salt accumulation (with the exception of the very dry season where leaf stomata close and transpiration is reduced), exacerbating the process of soil salinization and increasing especially Na^+ levels in the soil. Moreover, vegetation cover is reduced in halophytic compared to riparian communities, entailing an increase in soil temperature that further boosts salt accumulation due to evaporation (Liu

et al., 2019) and causes changes in bacterial community structure (Zhao et al., 2022). Finally, plants can not avoid the passive accumulation of salt in their tissues: this salt is returned to the soil at the end of the growing season, during leaf/root renewal or plant death, supplying salt-enriched litter to the soil and altering OM decomposition.

The natural drivers of coastal soils salinization act through widely differentiated time and spatial spans (Figure 2). They may exacerbate soil salinity for less than a year affecting relatively limited areas or act across much longer periods and at a nearly planetary scale, such as the present climate change and past climate oscillations (White and Kaplan, 2017).

Transient drivers, such as tsunamis or hurricanes, cause direct salinization of groundwater and soils in coastal areas, but previous salt levels may be restored within a year in many geographical regions, as salts can be leached away by intense seasonal rains (Kume et al., 2009). However, in combination with dry climates or low soil permeability, the effects of marine inundation caused by transient exceptional events are expected to last longer. All these natural actions are amplified by anthropogenic drivers such as land drainage (Valipour, 2014), accelerated subsidence (Daltakopoulos et al., 2016), groundwater abstraction (White and Kaplan, 2017), land use changes (Gopalakrishnan et al., 2019), and by all those activities that fuel climatic changes (Figure 2). Anthropogenic drivers may act faster and cause, at the same time, effects that may persist over very long time spans. For instance, the low-lying coastal area near Ravenna (north-eastern Italy) is affected by groundwater salinization from seawater infiltrations fostered by an increase in pumping that will cause threats to freshwater availability (Giambastiani et al., 2020). Gonnee et al. (2013) showed that seasonally enhanced SLR leads to increases in the amount and salt concentrations of submarine ground discharge (in summer). Wood and Harrington (2015) further illustrated, by a two-

dimensional variable density model, that sea level fluctuations push saline water into inland groundwater-fed wetlands through the movement of the subterranean fresh water-saltwater interface.

It is therefore evident that the variety of drivers acting on coastal soils is matched by the large diversity of their spatio-temporal effects. Local factors and their combinations may greatly accelerate the process in specific areas or dampen consequences in others. A holistic approach is therefore necessary to understand the often contrasting outcomes of salinization from SLR on soil biological properties.

Salinization of coastal agricultural lands

Secondary salinization of agricultural soils is one of the most pressing environmental challenges that humankind will face in the current century (UNESCO, 2016). The Food and Agriculture Organization of the United Nations (FAO) estimates that, globally, over 830 Mha of arable land are affected by salinization (Martinez-Antonio and Collado-Vides, 2003). Salinization affects the 17 western states of the United States, up to 3 Mha in Europe, more than 5% of the arable land in Africa, about a fifth in West Asia and 30% in Australia (Chhabra, 1996; Rengasamy, 2006; IPCC, 2007; Ladeira, 2012).

Albeit the salinity of the seas varies, the elemental composition of marine salts is worldwide the same so that constant relative proportions are always maintained among the elements (Míllero et al., 2008). On the contrary the composition of the salts accumulated by inland saline soils depends on the type of rocks from which they formed or that had been in contact with the waters entering the soil, as well as climatic and pedogenetic factors. This large variability of affecting factors is reflected by the variability of the composition of groundwaters, that in general contain a larger proportion of less mobile cations, whereas anions are dominated by the bicarbonate ion and not by chloride (Shvartsev, 2008). As a consequence, salinization of coastal soils, being imposed by sea water, modifies the composition of the soil exchange complex and of the soil solution in relatively similar ways throughout the globe, whereas the salinity affecting inland soils primarily reflects local situations.

Coastal agricultural practices are less resilient than upland agriculture because they need to cope with more frequent changes in ground water salinity, occasional sea inundations, water stresses and waterlogging (Awal, 2014). The sustainability of coastal agriculture is influenced by both climatic and non-climatic factors, of which SLR is the most influential. Apart from SLR, climate change has the potential to affect coastal areas in several ways, such as through increases in temperature and changes in the frequency and intensity of rainfall and storms (Gopalakrishnan et al., 2019).

The deterioration of soil fertility due to soil salt accumulation is a growing concern in coastal areas. Salinity reduces soil quality (by reducing nutrient content and enzymatic activities, Xian et al., 2019), limits the growth of crops, constrains agricultural productivity, and in severe cases, leads to the abandonment of agricultural lands (Diome and Tine, 2015; Sambou et al., 2016). High salt levels in agricultural soil or irrigation waters make water and nutrient uptake difficult for salt-sensitive plants, such as rice, thereby reducing plant growth and crop yields (Kaniewski et al., 2016). Moreover, saltwater inundation mobilizes nutrients that add to the loading in adjacent water bodies, reducing water quality (Herbert et al., 2015). Coastal farming systems result severely affected. In Bangladesh, for example, coastal soil salinity in agricultural lands has been found to dramatically affect crop revenue and the internal migration of farmers (Chen and Mueller, 2018). In Vietnam, more than 30% of the sugarcane plantations have been either destroyed or significantly damaged by the inundation and intrusion of saltwater into the Mekong Delta, resulting in a significant financial loss (Gopalakrishnan et al., 2019).

The productivity of coastal agriculture is increasingly hampered by the biophysical and biochemical constraints imposed by salinity on crop growth (Duarte et al., 2014). Novel perspectives will be, however, offered by studies on biostimulants and plant growth-promoting microorganisms isolated from the rhizosphere of salt tolerant plants (Pereira et al., 2019; Otlewska et al., 2020; Pereira et al., 2021). Literature shows that isolation and inoculation of specific bacterial strains, by soil amendment or foliar spread, can improve nutrient uptake in the plant and decrease plant sensitivity to Na^+ (see, e.g., Etesami and Beattie, 2018).

Adoption of traditional amelioration practices on salt-affected coastal lands not only reduce the direct impact of osmotic stress on crops, but also exerts a positive action on soil biological fertility by reverting the reduction in the biodiversity of the soil microbiome (Sun et al., 2022).

Impacts on freshwater coastal wetlands

The alteration of water chemistry caused by the ions brought in by marine water alters the chemical speciation of elements, their concentration in the soil solution and modifies habitat conditions and substrate availability for soil microorganisms (Shao et al., 2022), shifting the dominant biogeochemical processes within soil (Haywood et al., 2020) and ultimately altering the ability of wetlands to provide key ecosystem services (Wang et al., 2020).

Coastal wetlands are valuable transition environments, sensitive to changes in marine processes and freshwater flows from upstream catchments. Herbert et al. (2015) identified five mechanisms of salinization in coastal wetlands, including: 1)

surface or subsurface seawater intrusion linked to SLR, 2) reduction of riverine freshwater flow, 3) alteration of subsurface freshwater, 4) anthropogenic alteration of coastal geomorphology, and 5) storm surges. The main driver of salinization of coastal freshwater wetlands is undoubtedly surface or subsurface seawater intrusion. Mechanisms 2, 3 and 5 could be probably better considered as climate change-related processes that locally can exacerbate salinization.

The third mechanism responsible for the salinization of coastal freshwater wetlands relates to changes in groundwater recharge and discharge (Galliari et al., 2021; Wu et al., 2022). Aquifers are often exploited so intensively that their natural hydrological regime is strongly disturbed and may be thrown out of balance (Custodio, 2010; Dymond et al., 2019). In particular, changes in seawater intrusion are highly non-linear and exhibit important thresholds, or tipping points, beyond which full seawater intrusion into a coastal aquifer may occur in response to even small sea level and/or groundwater management changes (Elliott et al., 2016). Several aquifers along the densely populated Mediterranean coasts are already suffering seawater intrusion (Mazi et al., 2014).

Anthropogenic manipulations of coastal geomorphology, the fourth mechanism, principally affect coastal floodplain wetlands (Dymond et al., 2019; Mancuso et al., 2020). In the Netherlands, for example, the combination of lowland reclamation in the past centuries and ongoing SLR is expected to lead to strong salinization (Oude Essink, 2001). Similar impacts are expected due to the dredging of deep water channels in the Yangtze and Pearl River Deltas in China (Zhang et al., 2014).

The fifth mechanism, storm surges, can introduce saline water into coastal freshwater wetlands along the estuarine continuum, and in near-shore lagoons and depressional wetlands that have no permanent hydrologic connection to the sea (Guimond and Michael, 2021). The increased frequency of extreme weather events associated with climate change will proportionally increase its contribution to coastal salinization in the near future.

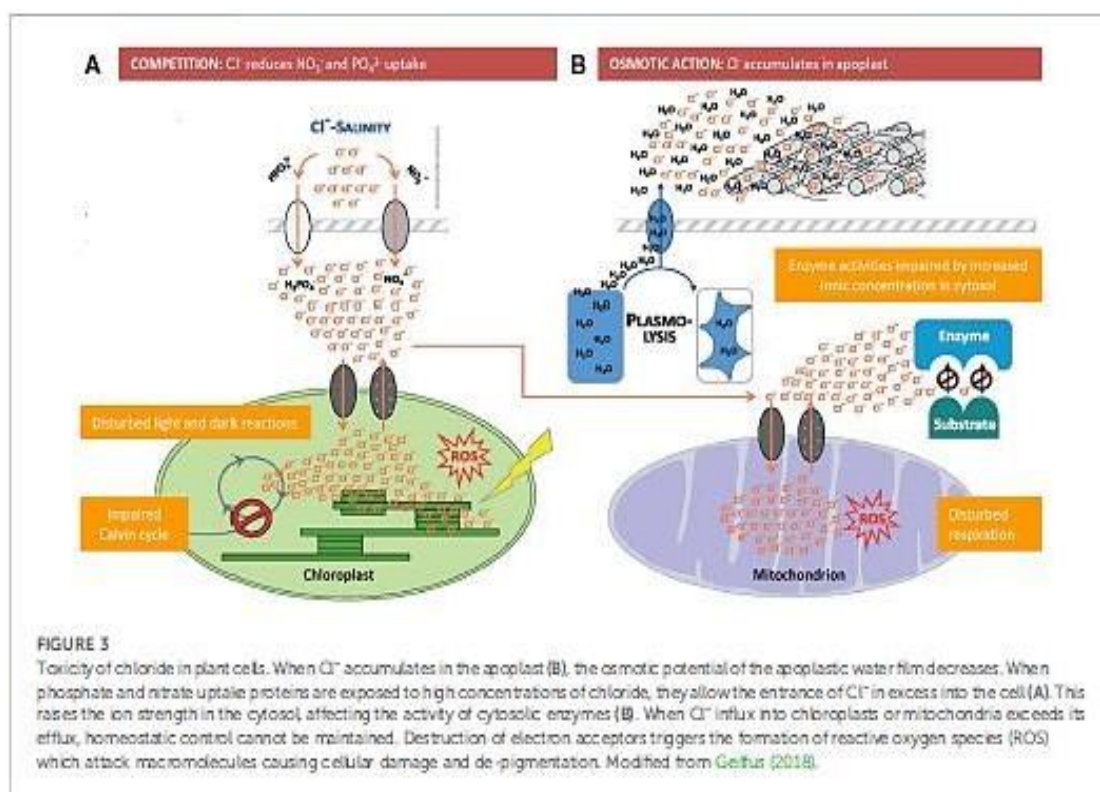
Effects of SLR on the biological activity of wetland soils are linked to O₂ availability, which is not only related to flooding. Elevated ionic concentrations caused by salinity reduce the solubility of gases (Stumm and Morgan, 1996). This affects the diffusion of O₂ in waterlogged soils, resulting in shallower penetration into the soil profile and more negative redox potential. Reduced gas solubility can accelerate GHG emissions by allowing a faster formation of bubbles, and reducing soil microorganisms accessibility to CO₂ and N₂O (McGinnis et al., 2006). In this way, CH₄ oxidation and N₂O reduction might become less efficient when soils are saturated with saline waters than similar soils saturated with fresh waters.

Saline water is denser than fresh water, and seawater intrusion via surface or groundwater movement can result in

the establishment of strong stratification (and the formation of a halocline) in tidal rivers and depressional wetlands (Brock et al., 2005; Revsbech et al., 2005). Stratification is a barrier to the movement of O₂ between the lighter superficial freshwater and the underlying saline strata. Salt loads of merely 2 g L⁻¹ are enough to alter the density of water and produce a degree of stratification similar to the temperature-derived density stratification observed in holomictic freshwater lakes (Findlay and Kelly, 2011).

Ionic displacement has been suggested as a mechanism for desorption of plant nutrients and other chemical species from salinizing wetland soils, particularly inorganic nitrogen (e.g., ammonium) and phosphate (PO₄³⁻), with potential consequences for the fertility of these soils, but also eutrophication of down-stream waters. Therefore, the effects of salinization on wetland biogeochemistry typically include decreased inorganic N availability, decreased C inputs and increased generation of toxic sulfides. All this has negative effects on the biodiversity of wetlands, which include the direct loss of plant species diversity, reduced potential for plant population recruitment, reduction in primary production and subsequent loss of fauna diversity through the loss of habitat and food (Davis and Froend, 1999). Indeed, larger salt and sulfide concentrations induce physiological stress in wetland biota and ultimately can result in large shifts in wetland communities and their associated ecosystem functions (Herbert et al., 2015). Increased salinity is a stress that has been shown to reduce diversity in both terrestrial (Briggs and Taws, 2003; Hobbs et al., 2003) and freshwater aquatic systems (James et al., 2003; Brock et al., 2005). Ecological impacts are influenced by a range of factors including the sensitivity of a species to salt (for both sub-lethal and lethal effects), rates of salinity increase, length of exposure and the life stage at which a species is exposed to salt stress (Cocks, 2003). At soil conductivity above 1.5 dS m⁻¹, reduced growth rates and reduced development of roots and leaves are observed in aquatic plants (James et al., 2003; Nielsen et al., 2003). Smith et al. (2009) found that a considerable loss of freshwater macrophytes may occur after a relatively small increase in salinity and with a severe loss of species already at conductivities of about 1 dS m⁻¹.

Osmotic stress in salt-affected soils challenges the maintenance of turgor pressure in plants (Flowers et al., 2015). Acclimated species are able to accommodate high Na⁺ and Cl⁻ concentrations by intracellular ion compartmentation and the production of osmolytes, i.e., organic solutes that contribute to the osmotic adjustment such as non-structural carbohydrates or amino acids (Gil et al., 2013). Mitochondrial enzymes are sensitive to Na⁺ and especially the cytochrome c, a crucial component of the electron transport chain (Figure 3). In fact, cell respiration was found to be inhibited in mitochondria isolated from salt-stressed plants compared to plants



inhabiting non-saline soils, although the response of overall respiration is very variable (Jacoby et al., 2011).

Chloride is an essential micronutrient in plants and regulates enzyme activities in the cytoplasm; it is a co-factor in photosynthesis, acts as a counter anion to stabilize membrane potential and is involved in turgor and pH regulation (White and Broadley, 2001). However, it can be toxic to plants (Geilfus, 2018) and triggers the production of reactive oxygen species (ROS, Figure 3). Critical concentrations for toxicity are estimated to be 4–7 mg g⁻¹ for Cl⁻-sensitive species and 15–50 mg g⁻¹ for Cl⁻-tolerant species (White and Broadley, 2001). Reduced rhizodeposition of labile organic compounds and an overall decrease in C inputs (due to the alteration of the Calvin cycle) can be expected to have detrimental effects on soil microbial biomass abundance and activity.

Glyphytes, i.e., non-tolerant species to salt, could hardly acclimate to increasing salinity. Therefore, salt stress directly impacts species diversity and composition of plant communities, leading to vegetation shifts in favour of the acclimated halophytic vegetation. Salt-tolerant species are capable to handle the reduced O₂ solubility and mineral N availability (Flowers et al., 2015), osmotic stress and specific ion toxicity, determining the short and long term implications on the net primary productivity and, therefore, on the whole C and N inputs to soil.

Response of microbiota to salt toxicity

Soil contact with sea water is toxic for most soil organisms within 2 h and effects last for at least 48 h (Rath et al., 2016). Exposure to high concentrations of salts affects microorganisms in two ways: by osmotic stress and through specific ion toxicity (Serrano, 1996).

Similarly to plant cells, elevated concentrations of marine salts in the soil water phase cause abnormal Na⁺ and Cl⁻ accumulation in microbial cells and disrupt the uptake of water and essential ions (Figure 3), which may ultimately lead to mortality (Serrano, 1996; Zhang et al., 2019). Toxicity levels inhibiting bacterial growth by about 50% range between 30 and 100 mM Na or between electrical conductivities of 3.0 and 10.7 dS m⁻¹ (Rath et al., 2016).

Increasing salt concentrations in the surrounding medium elevate the osmolarity outside microbial cells and, as external salt concentrations rise, cells lose water. To maintain cell turgor and prevent dehydration, microorganisms produce and accumulate osmolytes in their cytoplasm (Empadinhas and Da Costa, 2008). They have evolved two different osmoadaptation strategies to achieve homeostasis with the surrounding environment, but both are energetically expensive (Gunde-Cimerman et al., 2018).

The first and more widespread strategy, adopted by microorganisms exposed to saline media, is the accumulation

of low molecular weight organic compounds (osmolytes) such as amino acids and carbohydrates within the cell (Oren, 2008). The synthesis of organic osmolytes requires energy in the form of ATP. Oren (1999) calculated that heterotrophic microorganisms need to use between 23 and 79 ATP molecules to produce one molecule of an osmotic solute. Extrusion of Na^+ and uptake of K^+ also consume ATP equivalents.

The second survival strategy, demonstrated in many halophilic prokaryotes, minimizes osmotic potential differences by taking up ions (predominantly potassium) from the medium. This, however, requires the adaptation of intracellular enzymes to elevated ionic concentrations in the cytoplasm, since they should keep their original conformation to preserve enzymatic activity (Oren, 2008). This is not easily achieved because excessive salt concentrations may eventually denature proteins (Frankenberger and Bingham, 1982) and affect enzymes. Even when their solubility is not diminished, the spatial arrangement of amino acids at the active site, which is crucial for maintaining the catalytic efficiency, may be altered at high ionic strengths (Leprince and Quiquampoix, 1996) which favour hydrophobic interactions and de-stabilize electrostatic attraction forces.

Some studies indicate that changes both in microbial community composition and function occur with changes in salinity (Jackson and Valtaire, 2009). The resilience ability of soil fauna seems, however, a function of ecosystem complexity and site specific factors. Salt stress can differently impact the community based on its composition and some organisms might be impacted only marginally (Pereira et al., 2019). Bacteria appear to be more resilient to salinization than fungi, and fungal contribution to microbially derived C is negatively affected by salinity (Shao et al., 2022). Rath et al. (2019) investigated microbial salt tolerance in several saline soils and found that high salt concentrations could inhibit fungal growth by more than 90%. Sardinha et al. (2003) found a strong decrease in the ratio of the fungal biomarker ergosterol to microbial biomass C and that in the least saline site fungi made up 90% of the microbial biomass, but only 17% at the most saline site. The inoculation of salt-tolerant beneficial microorganisms (bacteria in particular) can reduce the salt stress by which the autochthonous microbial community is affected, with consequent positive repercussions to plant or crop growth (Pereira et al., 2016). The variety of microbial responses to salt stress and the existence of beneficial strains could possibly support future strategies to actively face soil salinization, representing a promising perspective in agriculture.

Lower microbial diversity was found in saline soils of the Yellow River delta (Zhao et al., 2020). However, the phyla Proteobacteria, Bacteroidetes, Chloroflexi, Acidobacteria and Planctomycetes, represented more than 70% of the bacterial community in the three different wetlands, indicating the

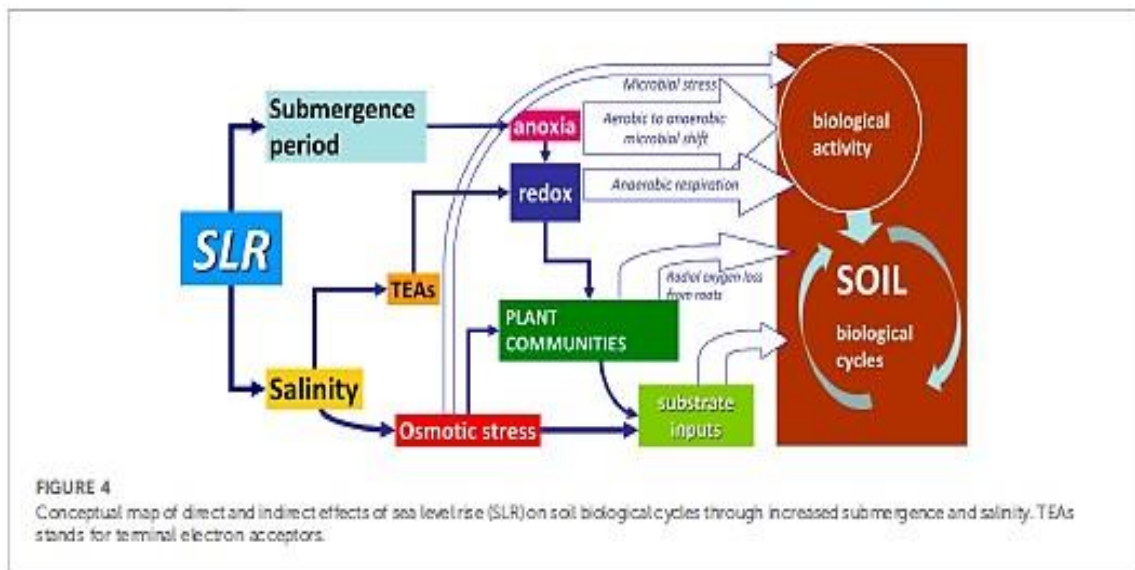
wide adaptation of these phyla to salinity changes. Specifically, Proteobacteria, which were recognized as the most dominant phylum (35–39%), appeared not to be affected by salinity. However, the bacterial composition was different among the wetlands, as revealed by β -diversity, and specific bacterial taxa were suggested to serve as bioindicators of soil salinization (Rath et al., 2019).

The species-specific response of soil microorganisms to increased Cl^- concentration results in modifications of the soil microbial community. However, chlorine is naturally diffused in the environment because saltwater from the oceans is, with an average concentration of 19 g Cl L^{-1} , one of the main sources of atmospheric Cl^- (Stumm and Morgan, 1996). Inorganic chloride is a normal component of the soil solution which is more abundant in coastal regions, where it is carried by marine aerosols (Meira et al., 2006). Setia et al. (2010) suggested that, when chloride is the predominant anion of the soil solution, the nature of the accompanying cations is of little importance and that the osmotic effect plays an important role in reducing rates of organic matter decomposition. However, empirical data from a larger variety of habitats, range of scales and microbial functional groups are still needed to understand how the structure and function of microbial communities will be altered by salinization.

A substantial increase in Cl^- concentration inhibits both nitrification and denitrification, but the associated microbial communities appear to be able to adapt to high Cl^- concentrations over time (Hale and Groffman, 2006). Moreover, Soil anoxia tends to reduce the rate of chlorination of the soil organic matter (SOM) (Bastviken et al., 2007; Bastviken et al., 2009). By affecting the composition of soil microbial cenosis, the increase in chloride availability may even affect the chlorine flux from its inorganic form to organic compounds and vice versa (Gryndler et al., 2008) with consequences affecting the process of chlorination of SOM (Öberg et al., 2005).

The most consistently observed alteration in bacterial communities caused by inundation or infiltration of sea water is a change in the number of methanogenic archaea and a coincident decrease in CH_4 production (Baldwin et al., 2006). Observations suggest that Na^+ and Cl^- alone can inhibit methanogenesis in inland soils but, in coastal systems because of the concomitant increase in SO_4^{2-} , the inhibition is fostered by a less efficient competition with facultative anaerobes (Pattnaik et al., 2000; Mishra et al., 2003; Baldwin et al., 2006; Chambers et al., 2011).

Extracellular enzyme activities are generally larger in non-saline soils compared to naturally saline soils (Rietz and Haynes, 2003; Ghollarata and Raiesi, 2007), even if halophytic microorganisms had the time to adapt and colonize the latter. Enzymatic activity is therefore often measured to assess biological activity in soils affected by



salinization. This provides a convenient way to monitor the consequences of increased salt concentrations on the biological activity of soils (Lemanowicz et al., 2019), however this approach is not without drawbacks. Assays that measure enzyme activities in soil usually test the potential activity at substrate saturation, which is a condition very far from that normally encountered in the substrate starved soil environment (Hobbie and Hobbie, 2013). Moreover, conditions such as temperature or pH can either be optimized or kept close to natural conditions (German et al., 2011; German et al., 2012), which leads to a considerable difference in results (Burns et al., 2013). Current methods which estimate enzyme activities at saturated substrate levels and optimized conditions do not estimate actual *in situ* activities, but rather maximum activities related to the abundance of enzymes, which is largely determined by the size of the soil microbial community (Rath and Rousk, 2015). The observed decrease, therefore, reflects lower microbial biomass contents and only secondarily, a change in functionality of the microbial community.

On the other side, microbial communities faced with unfavourable osmotic potentials can allocate lower energy resources to protein production and therefore release fewer extracellular enzymes. To highlight this, the determination of specific activities (mg substrate transformed per mg of microbial biomass C) would be much a better index of induced functional changes, than activities *per se*. Unfortunately, this approach has scarcely been adopted, although it would also allow direct comparison among different situations and a better generalization of consequences (Moorhead et al., 2013).

Another problem with using enzyme activities as an indicator of overall microbial status is that results can vary considerably between different soils and different enzymes

(Frankenberger and Bingham, 1982; Saviozzi et al., 2011). Therefore, it remains elusive to identify common general patterns from the assessments of extracellular enzyme activities in saline soils. This suggests that other metrics may be preferable to assess microbial functioning in saline soils, e.g., DNA/RNA-based molecular methods (Rath and Rousk, 2015).

Salinity effects on soil biogeochemical cycles

Increasing levels of chloride, sodium, potassium, magnesium and sulphates from sea water intrusion have major effects on biogeochemical processes (Weston et al., 2006; Setia et al., 2010) and the relationships between plants, soil and microbial communities (Nielsen et al., 2003; Munns and Tester, 2008) (Figure 4). SLR has important effects on soil biogeochemical cycles through the combined action of submergence and salinity on plant communities, in terms of productivity and type of vegetation (Hines et al., 2006). In fact, in addition to the anaerobic shift, imposed by the lack of O₂ that accompanies soil submergence, microbial respiration is supported by the larger availability of terminal electron acceptors (TEAs) in sea water with respect to fresh water. TEAs support anaerobic respiration, which partly compensates for the low efficiency of anaerobic transformations. At the same time, changes in plant communities contribute to altering microbial activity due to the different quantity and quality of litter available as a substrate for microorganisms (Barry et al., 2022).

In this paragraph, we will examine the complex interacting factors that drive the modifications in biological activities and govern the outcomes of SLR on the soil biogeochemical cycles of C, N and briefly on those of Fe, S and P.

Carbon cycle

Wetland soils have been estimated to contain from 45 to 70% of the terrestrial C pool (Mitra et al., 2005) and are assumed to play an important role in reducing atmospheric GHG concentrations, and mitigating climate change (McLeod et al., 2011). Coastal wetlands (i.e. salt marshes and mangrove swamps) sequester one order of magnitude more C than an equivalent area of terrestrial forests or peatlands (Chmura et al., 2003; McLeod et al., 2011). Considering that coastal wetland coverage will be reduced by 46–59% by the end of this century, based on current SLR predictions (Spencer et al., 2016), the reduction in C sequestration will have strong impacts on the terrestrial C cycle (Pfeffer et al., 2008; Vermeer and Rahmstorf., 2009), yet the overall effect is controversial and difficult to predict.

SLR will modify C cycling in coastal wetlands in two ways: 1) by increasing the length of submergence periods and, 2) by increasing salinity (Figure 4). The apparently contradictory results found in the literature may respond to the contrasting actions of these two concomitant factors that combine into a complex pattern. If sea rise *per se* will increase the incidence of soil anoxia, which is expected to favour C sequestration, salinity will increase the availability of TEAs and foster SOM mineralization (Stagg et al., 2017). Inundation by brackish waters may result, at least transiently in a negative C balance in freshwater wetlands, but is likely to have opposite effects in salt marsh sites with high elevation above the MSL. Effects of salinity on soil C depend on vegetation shifts (i.e., from non-halophytic to halophytic plant communities) that affect the quantity and quality of organic matter input to soil.

Salinity acts in contrasting ways on salt tolerant rather than salt sensitive plants. In a non-halophytic system inundated by saline waters, C inputs may dramatically diminish within a short period, because of the immediate anoxia and toxicity imposed by flooding and salt water, respectively (Figure 4). On the contrary, the same phenomenon may have a much lower impact on the productivity of the acclimated plant community (Glenn et al., 1992).

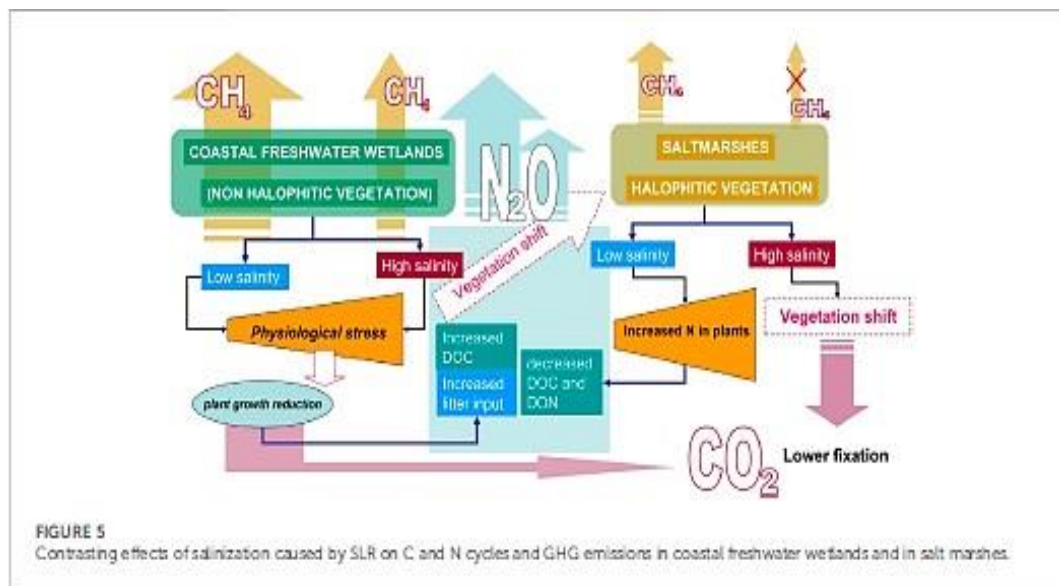
At the same time, in submerged soils, C mineralization efficiency depends on the availability of TEAs. Adverse effects on organic matter decomposition were found after the addition of salts to non-saline soils (Wichem et al., 2006; Wong et al., 2008), but sea salts composition and submergence interact in a complex pattern. Decomposition rates depend on the type of salt: for instance, Na₂SO₄ increased CO₂ emission from submerged soils during laboratory incubation, while NaCl decreased it (Li et al., 2006). The addition of sulphate ions sustains anaerobic respiration and therefore a more efficient decomposition of SOM, compared to strictly anaerobic systems, where microorganisms are forced to derive their energy from fermentation. It also inhibits the reduction of CO₂ to CH₄. So, the increased availability of TEAs which are normal components of seawater is crucial. In tidal freshwater forests, salinity

increased the amount of C mineralized (based on laboratory incubations) suggesting that even relatively low increases in salinity can alter the short-term C dynamics of coastal wetlands (Marton et al., 2012).

The primary consequence of increasing soil salinity on C dynamics of coastal freshwater wetlands is undoubtedly the decrease in plant productivity caused by osmotic stress, and hence, in the long term, the potentially diminished C inputs to the soil (Figures 3, 4). However, in the very short term or following transient pulses in salinity, caused by storms or tsunamis, a transient increase in C inputs to soil, e.g., caused by the more intense shredding of litter from salinity stressed plants, may also be observed. Over longer periods, when salt accumulates, stressed plants become less competitive, fostering changes in abundance and diversity of plant communities that can enforce feedbacks that affect soil formation processes (Ferronato et al., 2018; Pellegrini and Fernández, 2018). Neubauer et al. (2013) showed that the release of CO₂ from soil increased in response to a short-term salinity pulse, but declined over time in response to long-term (3.5 years) exposure. This was attributed to slow indirect feedbacks on soil that affect the composition of SOM (e.g., higher C:N ratios) and are potentially driven by changes in plant productivity and species composition and abundance. In one of the few long-term studies reported in the literature, soils containing larger SOM stocks appear to lose more C in response to salinization, with respect to soils of low C content (Marton et al., 2012). Forecasting the consequences of SLR on organic matter decomposition in wetlands, therefore, requires the integration of biogeochemical dynamics along with vegetation patterns (Figure 5).

Besides governing the release of CO₂ from soils, organic matter mineralization regulates the regeneration and availability of nutrients for plants, also in wetland systems (Reddy and DeLaune, 2020). In this respect, the ensuing decrease in soil microbial biomass and soil enzyme activities may reduce plant productivity in wetland soils, in which extracellular enzymes are crucial for the recycling of nutrients required to sustain plant growth (Pathak and Rao, 1998; Ghollara and Raiesi, 2007).

Perduring submergence combined with salinity cause a decrease in soil microbial diversity: this reduction, besides being driven by a shift to anaerobic or facultative anaerobic microorganisms, is connected with the toxicity of sulfide and it eventually fosters slower SOM decomposition rates (Setia et al., 2013; Qu et al., 2019). Before this happens, any increase in salinity will be transiently accompanied by an increase in concentration in the soil solution of TEAs (such as iron, manganese and sulphate ions), that support anaerobic respiration and therefore stimulate CO₂ production via increased efficiency in the mineralization of organic matter (Chambers et al., 2011; Meiggs and Taillefert, 2011; Weston et al., 2011; Marton et al., 2012; Neubauer et al., 2013). The larger availability of TEAs supports the shift of the dominant pathway of anaerobic metabolism from methanogenesis towards higher



energy yielding pathways (e.g., SO_4^{2-} reduction). This, combined with lower plant productivity, could eventually lead to a reduced carbon sequestration potential (Herbert et al., 2015).

The best studied effect of increased salinity on microbial C cycling in freshwater wetlands is the suppression of methanogenesis caused by the availability of SO_4^{2-} (Weston et al., 2006; Chambers et al., 2011; Poffenbarger et al., 2011; Neubauer et al., 2013). This not only makes CO_2 reduction to CH_4 less advantageous, but even promotes CH_4 oxidation. Recently, tidal freshwater sediments have been shown to support high rates of anaerobic CH_4 oxidation coupled to SO_4^{2-} (Segarra et al., 2015) and Fe (III), Mn (IV), and NO_3^- reduction (Segarra et al., 2013). The interactions between increased SO_4^{2-} and methanogenesis and CH_4 oxidation (aerobic and anaerobic) are likely to vary with site specific factors such as soil and water chemistry, O_2 availability, vegetation type and water level fluctuations.

The balance between C inputs and mineralization rates does not only affect CO_2 emissions and C sequestration potential, but is also crucial in determining the capability of coastal marshes to keep up with SLR. In fact, their resilience depends on the enhanced organic matter accretion and accelerated sediment deposition (Kirwan and Guntenspergen, 2010). The latter may be seriously hampered by anthropic actions that are the main causes of sediment erosion and loss of wetland surfaces (Fontolan et al., 2012).

Nitrogen cycle

Nitrogen often acts as a limiting nutrient for coastal salt marshes and N availability has considerable impacts on the

structure and productivity of plant communities (Mitsch and Gosselink, 2015). Ammonification is the key process that converts organic N into inorganic N, but all the steps of the N cycle are impacted by inundation with marine waters. In tidal zones, increased sea level combined with tides may result in frequent alterations of the hydrologic status of soils, which stimulate decomposition of SOM and loss of N (Djaman et al., 2018). Bai et al. (2005), Bai et al. (2007) showed that flooding duration and frequencies could influence soil N distribution because submergence greatly influences soil properties.

A comprehensive meta-analysis of coastal soil salinization on N pools by Zhou et al. (2017) showed that soil salinization increased plant N content (+18%), soil NH_4^+ (+12%) and soil total N (+210%), although it decreased soil NO_3^- (-2%) and soil microbial biomass N (-74%). Therefore, we can expect the net primary production (NPP) of halophytic plant communities to benefit from this increased N availability, whereas effects on non-acclimated vegetation may be limited or even reversed by the action of osmotic stress.

Nitrate is the most efficient electron acceptor in submerged soils and helps to maintain the soil redox potential at more positive values. Nitrification, which may still go on in aerobic surface water and soil layers, provides a relatively low but continuous supply of this type of TEAs to the denitrifiers, facultative anaerobic bacteria, which produce the energy necessary for their metabolism by reducing NO_3^- to N_2 using electrons derived from the oxidation of substrates. Denitrification has often been observed to decline along salinity gradients (Rysgaard et al., 1999; Craft et al., 2009; Giblin et al., 2010). However, this detrimental effect is limited to ammonium oxidizing bacteria and nitrite oxidizing bacteria:

heterotrophic aerobic denitrifiers can thrive on the nitrate produced by heterotrophic nitrification and may successfully remove most of the N under different salinity conditions (Fu et al., 2019).

Isotopic analysis of N in ocean sediment layers covering the past 300 million years has shown that substantial variations in denitrification rates were associated with planetary climatic changes such as Quaternary glacial and interglacial periods. Enrichments in ^{15}N , indicative of intensified denitrification, regularly occurred during the stages of rapid sea level rise and were followed by a decrease as sea level subsided again (Algeo et al., 2008). We can therefore expect a similar trend to occur during the early stages of inundation of coastal areas by sea water.

Large increases in salinity (16.5 dS m^{-1}) ultimately inhibit soil nitrification (Ardón et al., 2018). In oxic zones, microbial nitrification converts NH_4^+ to nitrite (NO_2^-) and, finally, to nitrate (NO_3^-), but sulfide inhibits nitrifying bacteria (Joye and Hollibaugh, 1995). Therefore, with increased exposure to saltwater, nitrification rates may decrease and affect denitrification, because of the lower availability of substrate (Rysgaard et al., 1999; Noe et al., 2013). Sulfide ions inhibit denitrification directly by inhibiting the reductase enzymes that catalyze the final steps of denitrification, resulting in incomplete denitrification to NO_2^- , NO , or N_2O (Brunet and Garcia-Gil, 1996), or indirectly by reducing NO_3^- availability via the inhibition of nitrification. Increased ionic strength can also interfere with the enzymes associated with denitrification (Glass and Silverstein, 1999). Dissolved salts alter the configuration of the active site of enzymes (non-competitive inhibition), modifying kinetic parameters such as rate and saturation constants (Dinçer and Kargı, 2001).

Increasing soil salinity stimulated soil N_2O fluxes as well as hydrological NH_4^+ and NO_2^- fluxes more than threefold, although it decreased the hydrological dissolved organic nitrogen (DON) flux (-59%). Soil salinization also increased net N mineralization by 70%, although salinization effects were not observed on the net nitrification, denitrification and dissimilatory nitrate reduction to ammonium (Zhou et al., 2017).

Sulphur, iron and phosphorus cycling

In wetlands, S cycling is tightly coupled to Fe cycling, both of which are driven by biotic and abiotic redox reactions (Smolders et al., 2007; Burgin et al., 2011). In oxic or sub-oxic soils, S and Fe are in their oxidized forms, i.e., SO_4^{2-} and Fe^{3+} , and are reduced to sulfide (S^{2-}) and ferrous (Fe^{2+}) ions during anaerobic microbial respiration. Sulphate reduction results in the formation of sulfide species (H_2S , HS^- , S^{2-}), which are toxic to many organisms (Lamers et al., 2013). In many soils, this toxicity is controlled by the co-precipitation of insoluble forms. Released sulfide and Fe^{2+} ions combine abiotically to form, at first, highly insoluble iron monosulfide (FeS) (Schoepfer et al., 2014), regulating the

concentration of sulfide in the soil solution. Salinization generally favours the formation of FeS_x minerals in wetland soils such as mackinawite and pyrite (FeS_2 , Rickard and Morse, 2005; Tobias and Neubauer, 2019). Nevertheless, the formation of FeS_x minerals retains S in sediments and can have potentially deleterious consequences. Reclamation of anaerobic soils with high concentrations of FeS_x may generate acid sulphate soils. If drained, FeS_x minerals react with O_2 releasing a large amount of H^+ (White et al., 1997), leading to soil acidification and the mobilization of aluminium (Al) and toxic metals (Johnston et al., 2003; Baldwin and Fraser, 2009; Lamers et al., 2013). These processes limit the reclamation of soils that have been submerged by sea water for a long time.

The interaction between S and Fe cycling indirectly also controls P cycling in periodically submerged soils. In fact, the reduction of Fe (III) to the more soluble ferrous Fe (II) form and its reaction in solution with sulfide results in the dissolution of Fe- PO_4 minerals, releasing phosphate ions (Reddy and DeLaune, 2020). The increased availability of P freed from iron-sulphur complexes goes on for weeks to months following saltwater intrusion, and may contribute to the eutrophication of overlying and downstream waters (Lamers et al., 2001; Lamers et al., 2002; Weston et al., 2006). The concomitant increased potential for H_2S build-up and toxicity, and for PO_4^{2-} release, have important implications for overall ecosystem health and downstream eutrophication, though these effects may not be apparent with short-term salinization.

It is obvious that Fe, S, and P dynamics are highly complex and, albeit driven by biological reduction, they are controlled largely by abiotic factors such as water chemistry, SOM, soil type, hydrology and other site-specific factors.

Emission of greenhouse gases

Prediction of trends in the release of greenhouse gases (GHG) from soils is a complex issue where ecosystem type, soil hydrological status and quantity and composition of salts with respect to divalent cations combine in different ways (Figure 4).

Vegetated coastal ecosystems are net C sinks that fix and sequester large amounts of both locally produced and allochthonous organic C. In fact, from 428 to 681 $\text{g C m}^{-2} \text{ y}^{-1}$ are fixed by vascular plants and microalgae in North American salt marshes and rates of salt marsh C accumulation, due to sediment burial, reached on average 218 $\text{g C m}^{-2} \text{ y}^{-1}$ over the last 50–100 years (McLeod et al., 2011). In tidal salt marshes, CO_2 emissions from soils (i.e., soil CO_2 efflux) are highly variable ranging from 240 to 720 $\text{g C m}^{-2} \text{ y}^{-1}$ and this heterogeneity arises from a variety of processes, including autotrophic and heterotrophic respiration and fermentation (Tobias and Neubauer, 2019). Even if the global net C sequestration potential of vegetated coastal ecosystems is difficult to predict, mangrove swamps and salt marshes greatly contribute to the

biological C pool (Chmura et al., 2003) and it would be important to predict changes brought by SLR.

An increase in sea level will obviously increase the length of submergence periods in tidal environments, eventually leading to permanent flooding of the lowest parts of coastland. As a consequence, the time allowed for oxygen diffusion will be restricted and anaerobic decomposition of SOM fostered by anoxia. In general, C mineralization is slower in submerged soils, because of thermodynamic impairments on decomposition, but submergence by sea water implies an increase in salinity, which drives a complex network of consequences.

Weston et al. (2006) observed a shift from methanogenesis to SO_4^{2-} reduction in ocean water-amended sediments, though this biogeochemical shift was not accompanied by changes in microbial community dominance (Edmonds et al., 2009). Poffenberger et al. (2011) performed a meta-analysis on CH_4 fluxes along natural salinity gradients and showed that emissions decreased with increasing salinity. However, the decrease was significant only for salinity regimes above 18 g L^{-1} (polyhaline marshes), whereas for mesohaline marshes, about 52% of the positive GHG balance of the yearly sequestered C was offset by methane emissions that did not significantly differ from those of non-saline systems. This is indirectly confirmed by responses of GHG emissions to storm-surge reductions in salinity: Capocci et al. (2019) used a mixed effects models approach and found that a decrease in salinity can produce increased pulses up to 24% in GHG emissions.

Short-term laboratory incubations simulating saltwater intrusion in tidal forests (Marton et al., 2012) confirmed that CH_4 production was inhibited by salinity, decreasing up to 90% in 5‰ salinity treatment, but variable responses were observed for N_2O production. Tidal forest soils are capable of producing from 0.032 to $1.9 \mu\text{g N}_2\text{O g}^{-1} \text{ soil h}^{-1}$, but most of it is reduced to N_2 , unless the acidity of the freshwater inputs inhibits the N_2O reductase activity. At high levels of seawater intrusion, however, the same enzyme may suffer from inhibition by sulfide ions caused by increased sulphate availability (Sorensen et al., 1980).

The production of GHG is regulated by the availability of TEAs that are preferentially used by microorganisms because of the greater thermodynamic yield compared to methanogenesis (Figure 4). Besides sulphate, the availability of other TEAs can also be influenced by SLR. For instance, humic substances are a widespread component of dissolved organic matter in rivers and can act as TEAs but, because of their poly-electrolytic nature, their solubility is suppressed in sea water. The normal trend can therefore be locally reversed with a lack of TEAs leading to increased methane production.

Conclusions and outlook on future investigation needs

Coastal soils feature some of the most densely inhabited and productive areas of the world and are natural hot spots of

biodiversity. They also harbour biological processes that govern interchange between terrestrial and marine environments and between soils and the atmosphere. Because of the large number of effecting factors acting often in contrasting directions, the consequences of salinization of coastal soils are still difficult to predict on the global scale. In particular, the effects of SLR on C and N cycles and consequently on GHG emissions from coastal soils are highly variable, depending on the contrasting and concomitant effects of flooding and salinity. At present, it is unlikely that this variability can be handled by models aiming to predict consequences at a planetary scale. A reliable evaluation at a planetary scale should result from more precise inventories and monitoring of areas displaying specific similarities and from the implementation from these well-defined data sets, of specifically devised models whose results can be finally combined on a weighted basis.

Besides the collection of data, a deeper knowledge of the mechanisms and effecting factors that act, in both the short and long term on the biological activity of soils affected by SLR is certainly needed. In this way most of the apparently contradictory results that are nowadays reported in literature could be embedded into a holistic insight, allowing scientists and decision makers to devise *ad hoc* strategies and mitigate unavoidable impacts of SLR and salinization at local and regional scales.

Author contributions

MD conceptualized the project and, with SM, developed the structure for the review. MD compiled data and prepared the initial text. EP contributed to the writing of the text. SM, CB, and MC contributed to the editing and finalization of the manuscript. All authors approved the text for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CHAPTER 2

PREFACE

This chapter entitled “**Flooding and sea water intrusion enhance mobility of airborne Cd, Zn and Pb in coastal soils.**” reports the results of a research work was carried out with the aim to investigate the consequences of increased frequency and length of flooding events related to global sea level rise (SRL) on the mobilization of PTE from airborne dusts in coastal soils.

Airborne contamination is a worldwide problem and is expected to intensify in many areas because of increase in severity and length of drought periods combined with extreme weather events.

We compared the behaviour of Cd, Zn and Pb from two types of potential sources of airborne metals, i.e. mine tailings and smelter exhaust dusts in two soils of similar physico-chemical properties, but subjected to different agricultural management (arable and grassland) when exposed to flooding events of different length with marine and brackish waters.

INTRODUCTION

Atmospheric deposition of anthropogenic dusts represents a major pathway of potentially toxic elements (PTE) contamination at the soil surface, because silt size particles can be transported over long distances and reach rural and even remote areas (Ghanavati, 2019; Connan et al. 2013; Tuohy et al., 2015; Jiao et al., 2014). The PTEs released from decommissioning of the mines, waste rock piles and tailings impoundments can act as sources of contamination at the site and could further mobilize by transporting over long distances to marine and terrestrial environment through atmospheric circulation (Trifuoggi et al., 2017; Concas, 2006). The airborne deposits induce some direct or indirect impacts on crop growth by enriching the soil with clay and coarse silt fractions (Luo et al., 2019), and direct accumulation by foliar and root uptake (Günthardt-Goerg et al., 2019; Shahid et al., 2017; Alahabadi et al., 2017; Popek et al., 2017; Schreck et al., 2013).

In the agricultural soils of Europe deposition rates were reported to reach 540 g of Zn and 101 g Pb ha/y, but are lower for Ni and Cd (25 and 2.7 g/ha/y, respectively) and correspond to about 40 to 50% of total Zn inputs, 55 to 70% of total Ni and Pb and 50% of total Cd (Nicholson et al., 2003). It has been estimated that from 50 to 93% of the total input of potentially toxic elements (PTE) to Chinese agricultural soils derives from dry or wet deposition of airborne dusts (Peng et al., 2019).

In the proximity of mining areas, annual deposition rates can reach 42 g/m², therefore surpassing for some elements the quantity that regulations allow to introduce by application of sewage sludge (Sánchez et al., 2017). Following the general implementation of emissions regulations, in many countries the concentrations of many PTE in atmospheric aerosols has decreased in the last years and consequently their inputs to soil from the atmosphere were reduced (Luo et al., 2019; Bai et al., 2005).

The dust particles produced during mining and smelting activities are known sources of long range contamination to both marine and terrestrial environments (Trifuoggi et al., 2017; Concas, 2006). Setttable dust particles produced by mining activities, are mostly deposited within 10 to 70 km away from waste mineral deposits, but it is well known that even coarse mineral particles, the size of small sand grains can actually be carried several hundred kilometres as a consequence of long range atmospheric transport mechanisms (Michèll et al., 2017). The phenomenon is likely to exacerbate because of climatic changes, as dust storms are foreseen to increase in frequency and intensity.

Accumulation PTEs in the salt affected soils may imposed ecological risk (Bartkowiak et al., 2017). Depending on the composition of soil solution concentrations of salts may also change the chemical behaviour of the metals accumulated in soils, causing an increase or decrease in their availability, i.e decrease in salinity could enhance the bioavailability and toxicity of heavy metals (e.g., Cd, Zn, Pb) on marine and estuarine isopods (Riba et al., 2003). Soil salinity intensifies the adverse effects of Cd pollution on plant growth and soil microbial properties (Usman, 2015; Raiesi and Sadeghi, 2019), which lead into the reduction in soil microbial biomass, activity, and functions (Raiesi et al., 2018; Raiesi and Sadeghi, 2019; Wang et al., 2019). Increase in salinity may enhance themobility of Cd, Pb, and Zn (Chu et al., 2015).

In coastal areas PTE contamination impacts could be enforced by another concomitant problem connected with the global climatic change: sea level rise (SLR). The fifth assessment report of the IPCC (AR5) predicts that the terrestrial sea level will continue to rise at higher rates than observed in the past four decades (IPCC, 2013). Together with reduced freshwater inputs from the mainland, caused by water withdrawal for agriculture and longer drought spells, upsurges of saltwater intrusion (Weissman et al., 2020) will impact soil biogeochemical cycles, microbiota diversity and emissions of greenhouse gases incoastal areas (Mahzar et al., 2021), deteriorating the chemical, physical, and biological properties of the soils, altering nutrient cycles and reducing biodiversity. Other negative effects may be expected in contaminated soils, as soilchemical and biological processes

also determine the mobility and redistribution of PTE (Hooda, 2010). It also increases the solubility of Pb and thus its mobility and bioavailability in soils because high ionic strength favours to the formation of Pb complexes with inorganic ligands (Usman et al., 2005; Abbaspour et al., 2008; Acosta et al., 2011; Almaroai et al., 2014; Smolders et al., 2015).

Accumulation of PTEs in soils exposed to salinization by SRL may therefore impose enhanced ecological risks (Bartkowiak et al., 2017), augmenting the mobility of Cd, Pb, and Zn (Chu et al., 2015). The level of mobilization depends on the nature of deposited dusts (such as chemical speciation, particle size distribution) and total amounts of PTEs present, but factors such as soil dissolved organic matter, pH, and the type of salts causing the salinization (Ponting et al., 2021, Correia et al., 2020; Acosta et al., 2011). Also the submergence period is likely to influence mobilization, because the composition of the soil solution is affected by the length of the submergence period (Wang and Wang, 2013), moreover even similar soils may respond differently if kept under different managements (e.g. arable vs. grassland), because of the difference in soil organic matter quality and biological activity. The combined effects related to global sea level rise (SRL) on PTE mobilization from differently managed coastal soils, however, never been investigated.

To investigate the consequences of increasing salinization and length of flooding events on the mobilization of PTE derived from airborne dusts, we compared the behaviour of Cd, Zn and Pb from two types of sources, i.e. mine tailings and smelter exhaust dusts in two soils of similar physico-chemical properties, but subjected to different agricultural management (arable and grassland) when exposed to flooding events of different length with marine and brackish waters.

MATERIALS AND METHODS

Soils

The two soils (Endoskelectric, Cambisols) selected for this study are located (46° 08' 00" N, 13° 13' 40" E) in the upper plain of Friuli-Venezia Giulia (Italy) and belong to two contiguous fields under different land use: arable and grassland. Soils samples were taken with an auger from the surface layer (5-20 cm), sieved (2 mm) and stored moist at 4 °C to preserve biological activity. Their physico-chemical properties and contents of PTE are reported in Table 1. Soil pH was measured at a 1:2.5 soil to water ratio. Total carbon, organic carbon and nitrogen contents were assessed with a CHNS Elemental Analyzer (Vario Microcube, Elementar, Langensfeld, Germany). Carbonates were calculated by difference, subtracting the organic carbon (HCl treated soil) from the total carbon content. Soil texture was measured in a Bouyoucos' cylinder with an ASTM 152H hydrometer.

Cation exchange capacity (CEC) was measured with BaCl₂ buffered at pH 8.2 with triethanolamine (TEA) (Mehlich, 1953). Non-humic (non-phenolic) and humic organic C were extracted by a single extraction with 0.1 M Na₄P₂O₇ plus 0.1 M NaOH and separated by SPE on crosslinked PVP (De Nobili et al., 2008). Soluble organic carbon was extracted following Jiang et al. (2017) and measured by a Shimadzu TOC analyzer.

Table 1. Physico-chemical properties of the arable and grassland soils. PTE concentrations are reported as mean \pm standard deviation.

Soil type	Arable soil	Grassland soil
Clay (%)	17	9
Silt (%)	38	40
Sand (%)	45	51
WHC (ml/100g)	63	71
Carbonates (g kg ⁻¹)	1	2
pH	5.7	6.0
CEC (cmol kg ⁻¹)	21.5	20.5
N (g kg ⁻¹)	1.4	1.8
Corg (g kg ⁻¹)	14.2	16.8
Non-humic to humic extractable C ratio	0.7	0.5
Cd ($\mu\text{g g}^{-1}$)	1.91 \pm 0.02	1.80 \pm 0.23
Pb ($\mu\text{g g}^{-1}$)	34.4 \pm 2.2	43.2 \pm 4.5
Zn ($\mu\text{g g}^{-1}$)	72.2 \pm 4.3	70.7 \pm 5.4

PTE sources

Fine mine tailing dust was collected from abandoned flotation ponds deposits at the site of a blenda mine (Cave del Predil, Friuli Venezia Giulia, Italy - 46° 26' 36" N, 13° 34' 10" E). The dust contained Cd 22 \pm 2 $\mu\text{g g}^{-1}$, Cu 134 \pm 17 $\mu\text{g g}^{-1}$, Pb 5194 \pm 75 $\mu\text{g g}^{-1}$ and Zn 11512 \pm 270 $\mu\text{g g}^{-1}$. Smelter exhaust dust was collected from the stack of a Pb smelter in Zerjav (Meza Valley, Slovenia). The major PTEs were Cd 0.8 $\mu\text{g g}^{-1}$, Cu 21.8 \pm 5 $\mu\text{g g}^{-1}$, Pb 40754 \pm 6903 $\mu\text{g g}^{-1}$ and Zn 17 \pm 6 $\mu\text{g g}^{-1}$.

The crystallographic compositions and particle size distributions of the two dusts are reported in the S1 and S2 sections of the supplementary materials.

Artificial seawater and synthetic rain

Artificial seawater (SW) was prepared according to the standard ISO 10253:2006 and contained all the main salts present in natural seawater: NaCl₂ (26.7 g L⁻¹) MgCl₂ (2.2 g L⁻¹) MgSO₄ (3.2 g L⁻¹) CaCl₂ (1.2 g L⁻¹) KCl (0.7 g L⁻¹) NaHCO₃ (0.2 g L⁻¹) H₃BO₃ (0.05 g L⁻¹). Synthetic rainwater (RW) was prepared dissolving (1.4 g L⁻¹) CaSO₄·2H₂O, 1.50 g L⁻¹ NH₄Cl, 1.92 g L⁻¹ NaNO₃ and 0.32 g L⁻¹ sodium acetate in distilled water (Letardi et al., 2017).

Experimental plan

Contaminated surface soils were prepared by mixing uncontaminated 2 mm sieved moist soils (equivalent dry weight 300 g) with either mine tailings (7 g) or smelter dust (0.95 g). These levels of contamination were chosen in order to achieve with both dusts a comparable contamination level for at least one element (Pb) and are representative of a medium-high deposition level. Soils were then pre-incubated at 25 °C for one week.

Two main experiments were conducted on the artificially contaminated soils and non-contaminated control soils, simulating: a) a short-term inundation (24 h) by sea or brackish waters followed by a rain event, or b) a medium (7 days) and long-term (30 days) inundation. The flooding treatments (short, medium and long) were imposed using artificial sea water at four levels of salinity, i.e. 4.37 g L⁻¹ (S₁), 8.75 g L⁻¹ (S₂), 17.5 g L⁻¹ (S₃) and 34.5 g L⁻¹ (S₄), which corresponded to serial dilutions of 1/8, 1/4, 1/2 and full-strength artificial SW. Mobilization by rain was instead simulated by leaching the soils exposed to short term flooding with artificial rain water.

Short-term flooding was imposed in 50 mL plastic syringes equipped with a valve, on moist soil aliquots corresponding to 20 g dry weight, by slowly pouring 25 mL of salty or distilled water (control, S₀) from the top of the syringe. After 24 hours of equilibration, the valve was opened and the leaching solution was collected from the nozzles by gravitational pull. Soils were left to equilibrate with air for one more day at room T (about 22 °C) and 25 ml of RW were then added and immediately collected after leaching.

Prolonged flooding was imposed on aliquots of moist soil corresponding to 20 g dry weight that were kept submerged under 25 mL of SW in stoppered 50 mL test tubes. The test tubes were incubated at 25 °C for 7 or 30 days maintaining constant layer between 3 to 4 cm water above the soil by replacing weight losses. At the end of the incubation period, tubes were swirled to suspend sedimented soil and centrifuged (4000 rpm) for 20 min to separate the solution for analyses.

All solutions were analysed for dissolved organic and inorganic C (DOC and DIC), conductivity, pH and PTE concentrations.

Analysis of potentially toxic elements

The concentrations of Cd, Pb, and Zn were measured in soils, mine tailings dust, smelter dust and solutions collected after the described treatments. Digestion was carried out according to the USEPA 3052 method (USEPA, 1996). Samples of mine tailings and smelter dust (0.3 g) and soils (0.5 g) were placed in Teflon tubes and treated with 10 mL of 67% HNO₃. Digestion was carried out at 180 °C for 10 min by placing tubes in a microwave irradiation oven. After digestion, all extracts were quantitatively transferred to 20-mL certified flasks, brought to volume with ultrapure water (Millipore Direct-Q System) and then filtered with 0.2 µm Whatman polytetrafluoroethylene (PTFE) membrane filters. Similarly, solutions collected during the experiments were filtered with 0.2 µm Whatman filters. Filtered solutions were diluted with reagent grade water and a scandium solution was added as internal standard (1 mg mL⁻¹).

Metals were detected and quantified by plasma optical emission spectrometry (ICP-OES) (ICP-OES 5800 Agilent technologies). The calibration was performed using 0.2 to 20 µg mL⁻¹ standard solutions prepared in 0.67 % nitric acid from a 1000 ppm multielement certified solution (Merck solution IV). The method detection limit (MDL) was calculated for each element as 3 times the standard deviation of a blank solution.

Speciation modelling

In order to rationalize the speciation of metals in the analysed solutions at in equilibrium with the expected solid phases and CO₂ dissolved at atmospheric pressure, was computed with Visual Minteq 3.1. (Gustafsson, 2014), using measured PTE concentrations, pH, DOC and DIC values. Interactions with DOM were computed by the Nica-Donnan model. Input files are reported in section 2 of the supplementary materials. Ionic strengths values were also computed with Visual Minteq 3.1.

RESULTS

After only 24h of contact with synthetic brackish waters of increasing salinity, the mobility of all the examined PTE, increased for both soil management types in the uncontaminated control soils (Fig.1) and for both types of dusts in the contaminated soils (Fig. 2 and 3).

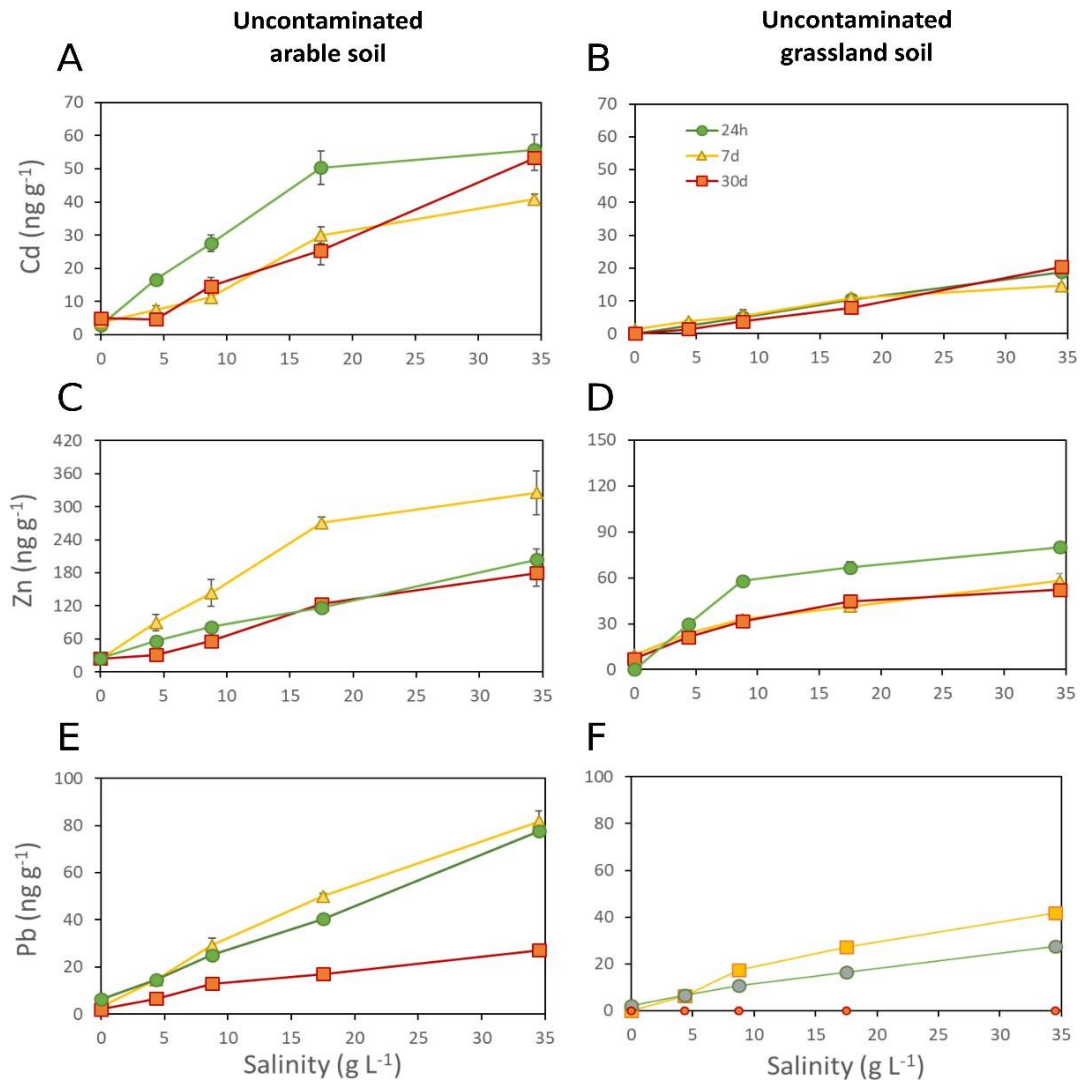


Figure 1. Amounts of Cd, Zn and Pb mobilized per gram of soil from the uncontaminated arable (left) and grassland (right) soils by waters of increasing salinity. Data points are averages (n=3) +/- standard deviation.

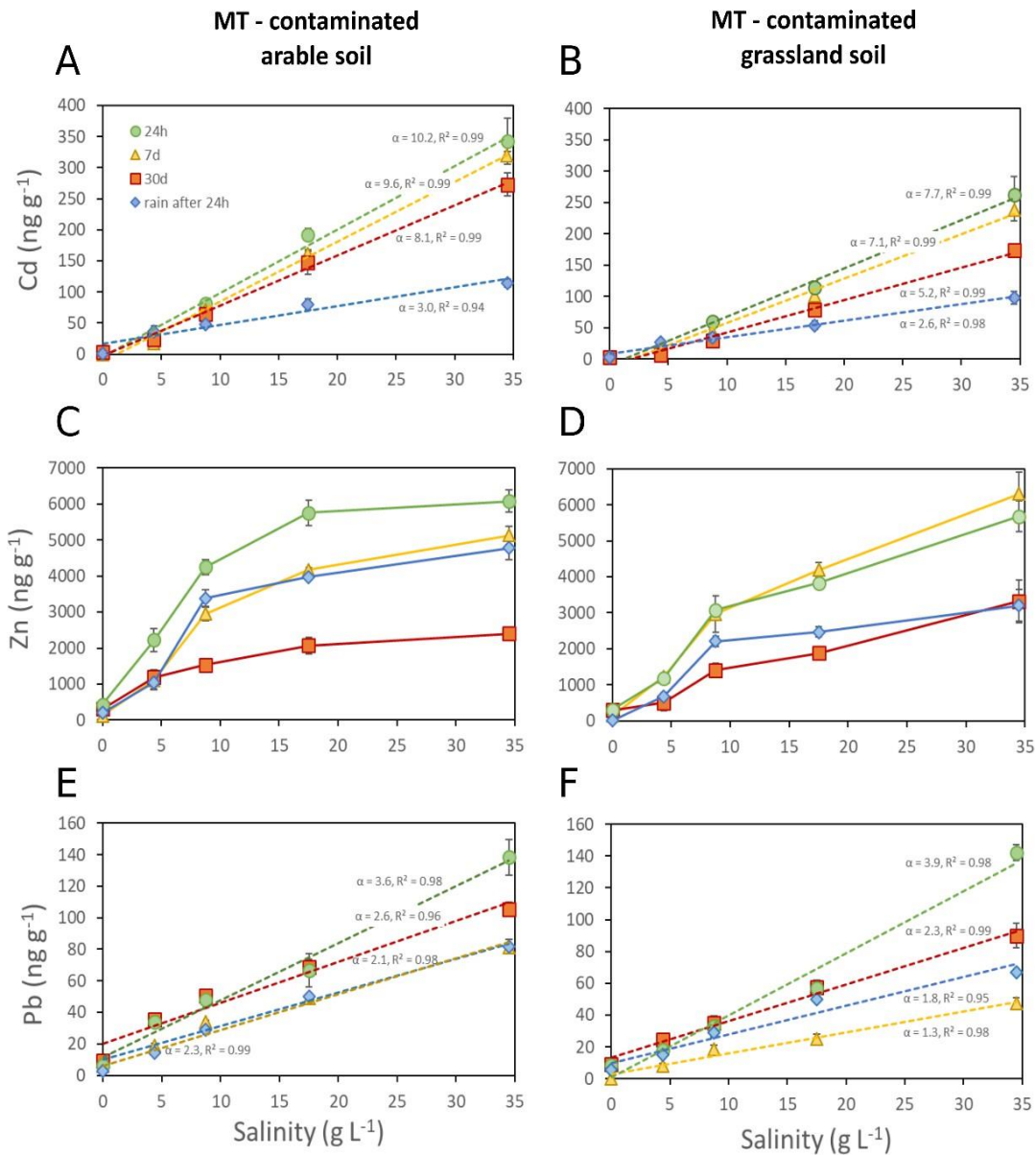


Figure 2. Amounts of PTE mobilized from the mine tailing (MT) contaminated arable (left) and grassland (right) soils by waters of increasing salinity. Graphs show trends of Cd, Zn and Pb leached after 24 hours contact (green) and from a subsequent rain event (blue), and after 7 (yellow) or 30 days of submergence (red). Data points represent average (n=3) +/- standard deviation. Dashed lines are linear models applied on data points for which slope (α) and R^2 is reported. Solid lines refer to non-linear trends.

Even artificial rain water (RW), when applied to soil that had been drained after being submerged for 24h with SW of increasing salinity and equilibrated for further 24h (Fig. 2 and 3), was able to mobilize in most cases, amounts of PTE larger than those from the simple displacement of the metals dissolved in the volume of liquid phase retained within soil columns by the soils' water holding capacity (metals retained from the previous short term flooding treatment) . This was particularly true at the low salt concentrations ($<10 \text{ g L}^{-1}$) where amounts of metal leached from either the mine tailing (MT)- and smelter exhaust (SE)-contaminated grassland soil were in some cases comparable or even larger (e.g. Pb from SE in grassland soil, (Fig. 1E) than those mobilized by marine waters ($>17.5 \text{ g L}^{-1}$). This points out to the possibility that equilibrium is not reached after 48h. Therefore, in order to investigate the kinetics of dissolution of the two sources, we highlighted the concomitant action of different affecting factors based on incubations of flooded soils for 7 and 30 days.

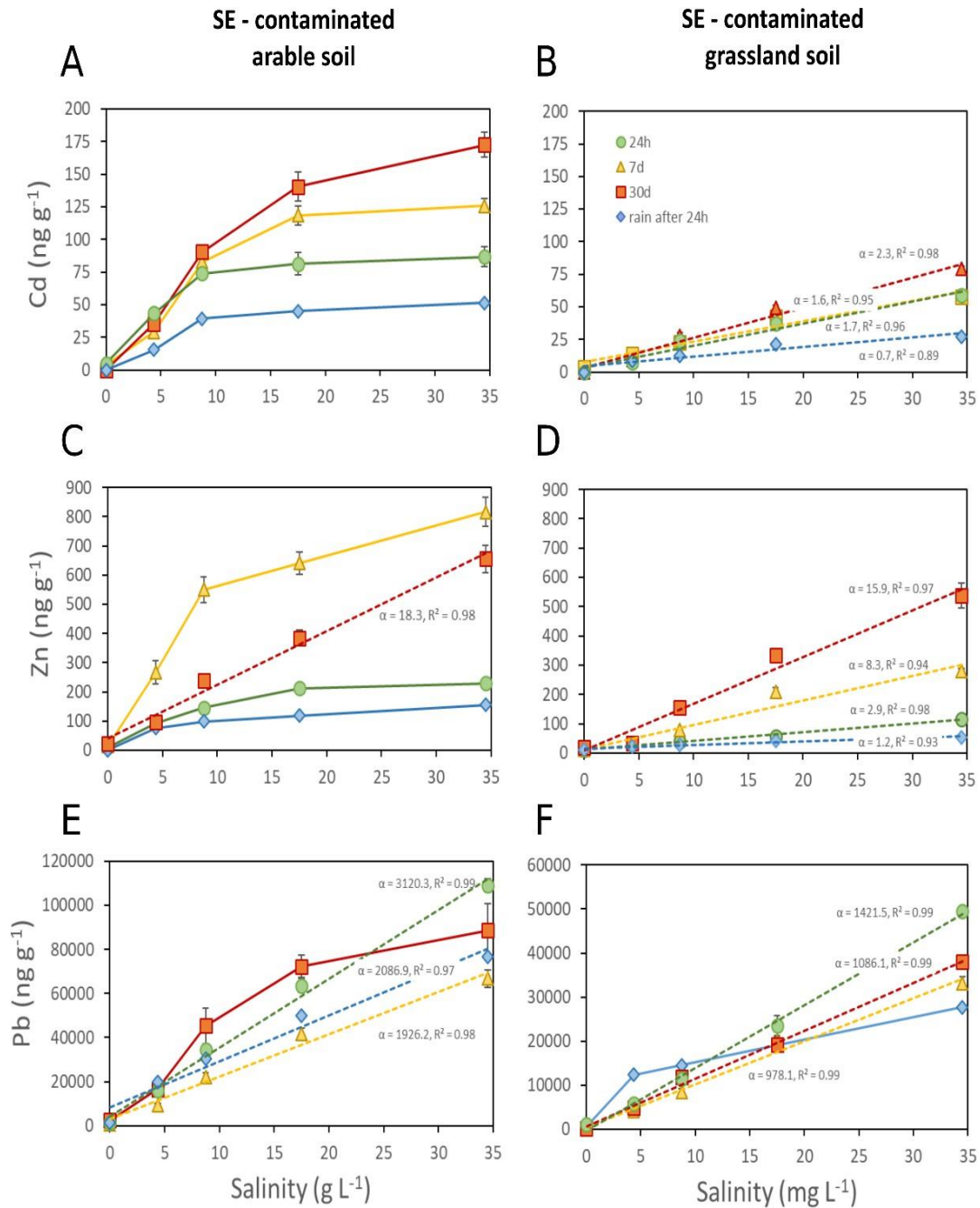


Figure 3. Amounts of PTE mobilized from the smelter exhausted SE contaminated arable (left) and grassland (right) soils by waters of increasing salinity. Graphs refer to PTE leached after 24 hours contact (green) and subsequent rain event (blue), and after 7 (yellow) or 30 days submergence (red). Data points are averages ($n=3$) +/- standard deviation. Dashed lines are linear models applied on data points for which slope (α) and R^2 is reported. Solid lines refer to non-linear trends.

Effect of contamination source

The two dust samples employed to contaminate soils, had very different size distributions. The MT dust was mostly composed of very fine particles (62% of which were $<2 \phi$, with $\phi = \log_2(\text{particle size})$), whereas the SE dust contained only 14% of particles below 2ϕ and was mostly composed ($>69\%$) of particles between 6 and 11 ϕ . Size distribution probably affected the kinetics of dissolution from SE, as shown by the levelling off of mobilization of Cd, Zn and Pb at larger salt concentrations, which is evident in the trends of SE contaminated soils flooded only for 24h. In fact, Cd displayed a nearly perfect linear increase in mobility with increasing salinity in all treatments and in both soils when contaminated with MT dust (Fig. 2A, B), but displayed a more or less pronounced tendency to level off in leachates collected after 24h at the two highest salt concentrations, in both the arable and grassland soils contaminated with SE (Fig. 3A, B and 4A, B), in spite of the lower level of Cd contamination (1.95 versus $11.96 \mu\text{g Cd g}^{-1}$) reached for this metal with the addition of SE (Fig. 5A, B) and of the lower concentration measured in the leached solution (Fig. 3A, B).

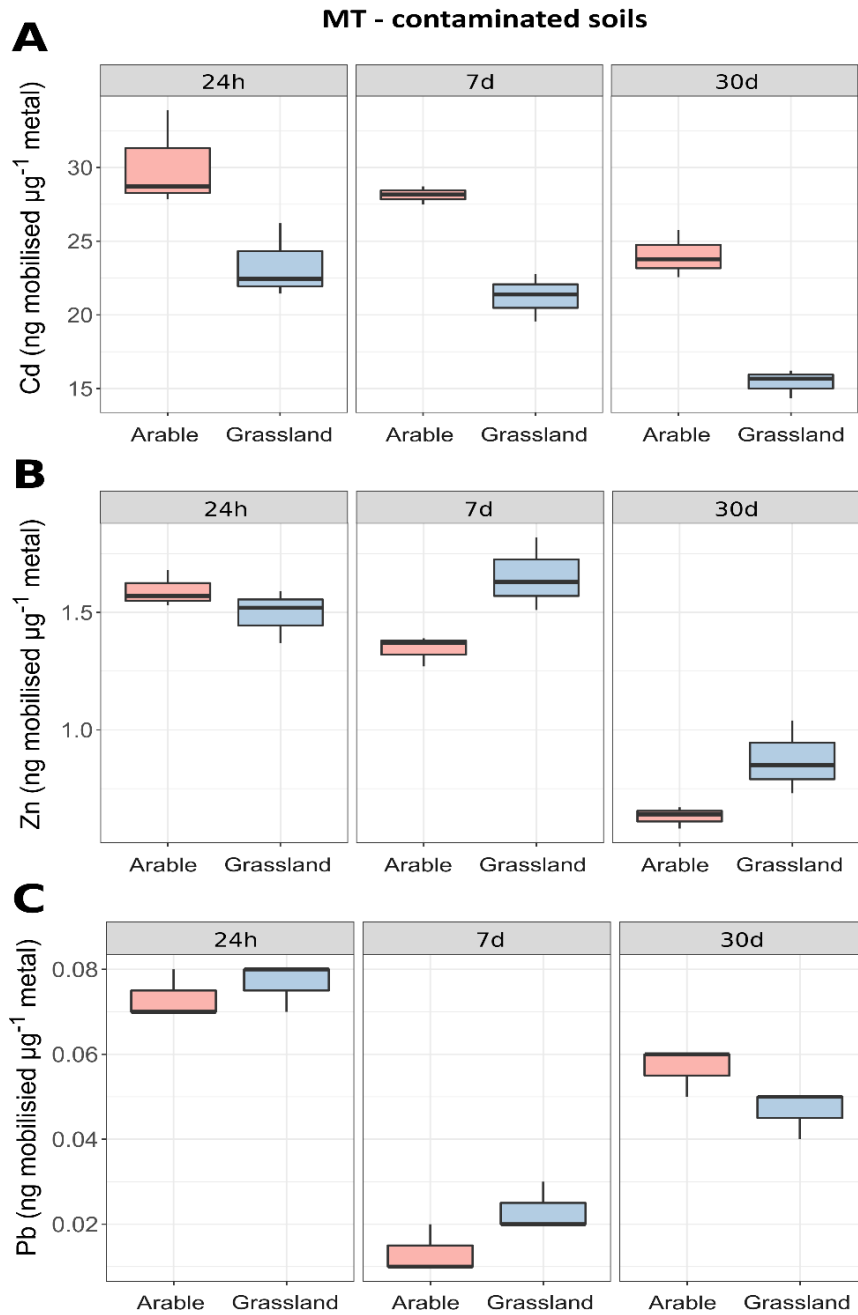


Figure 4 Metal mobilization normalized per metal total content in arable or grassland soils contaminated with mine tailings (MT), after 24h and 7 or 30 days of exposure to high salinity (34.5 g L^{-1}).

The effect is also evident in the case of Pb (Fig. 4A, B, and 5A, B), considering that the contaminated soils were added about the same amount of this element (range 1852 to 1970 $\mu\text{g g}^{-1}$). In fact, much more Pb was mobilized from SE than from MT contaminated soils and in all treatments, amounts of PTE leached and leaching trends with salinity strongly depended on the source of contamination.

Besides the difference in particle size distribution, dissolution kinetics are certainly influenced also by the speciation of the elements: MT dust, being derived from processing of a blend ore contains mineral sulphides, whereas SE that form during the smelting treatment contain oxides (see supplementary materials).

For Pb from both airborne dust sources the trends with increasing salinity were well described by linear models, but these displayed significantly different slopes (Fig. 2, 3).

At the maximum salt concentration, corresponding to that of sea water, about 300 ng of Cd were leached per gram of the MT contaminated arable and grassland soil after 24h of contact, but these amounts did decrease slightly when flooding with saline water was prolonged for 7 days and even more after 30 days of flooding (Fig. 2). On the contrary, the amount of Cd mobilized from SE contaminated soils after 7 and 30 days was proportionally much larger compared to the 24h treatment (Fig. 3).

Zn mobility displayed in all cases a tendency to level off at higher salinity, even if in the SE contaminated soils, the amount of Zn added was much lower, resulting in lower concentrations of the element in the leached solutions. The type of contamination source also influenced the amounts of metals solubilized after longer submergence periods: much larger amounts of Zn were, for instance, mobilized from SE in both arable and grassland soils after 7d and 30d that after 24h (Fig. 3C, D).

At the highest salinity level (34.5 g l^{-1}), however, larger normalized amounts of PTE were dissolved by marine water from the SE than from the MT contaminated soils (Fig. 4 and 5).

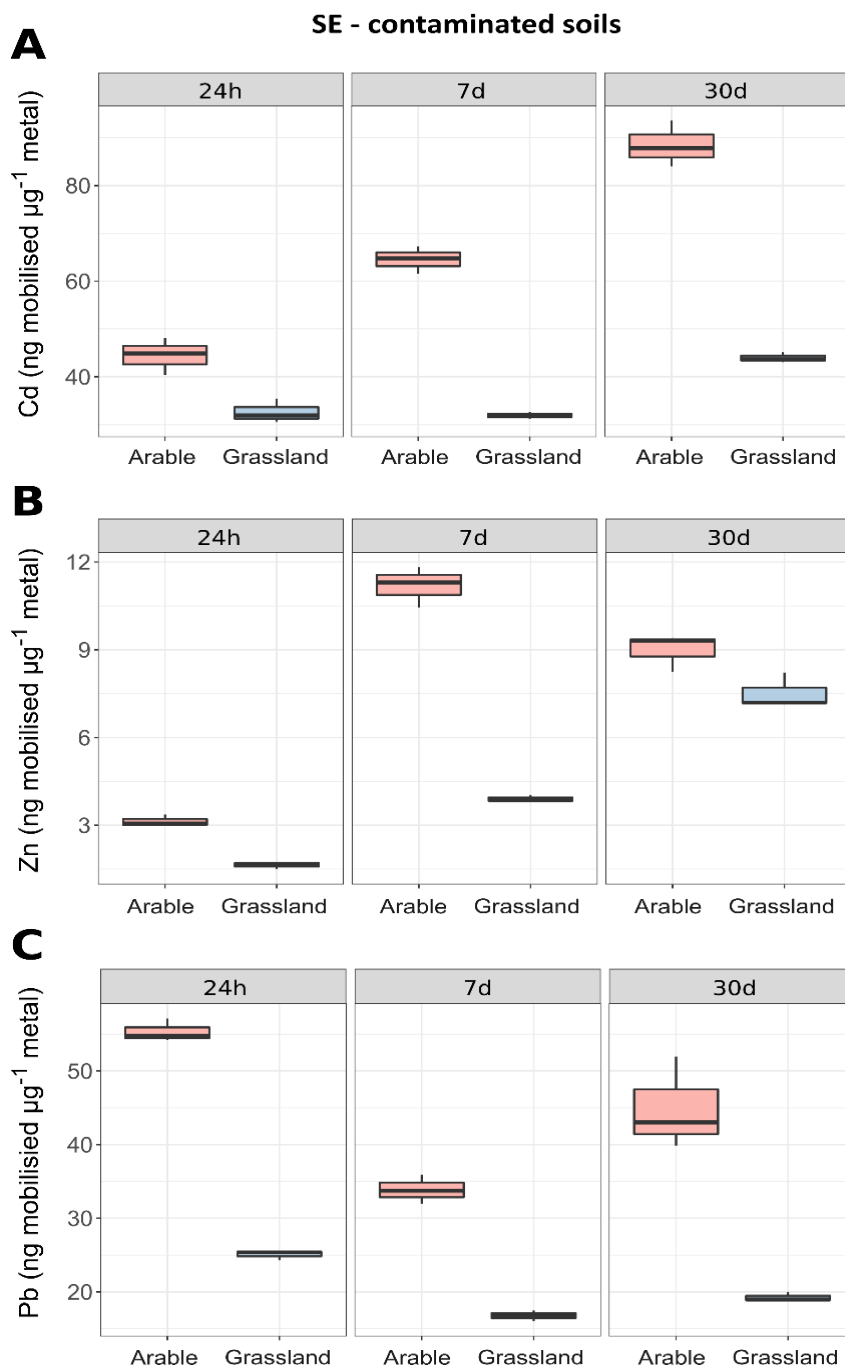


Figure 5 Metal mobilization normalized per metal total content in arable or grassland soils contaminated with smelter exhausted (SE) dust, after 24h and 7 or 30 days of exposure to high salinity (34.5 g L^{-1}).

Effect of soil management

The amount of PTE released from uncontaminated soils, always increased with the salinity of flooding waters, but significantly larger amounts of Cd, Pb and particularly Zn were leached from the arable soil (Fig. 1), in spite of the fact that the two soils had about the same content of PTE (Table 1). Also from the contaminated soils, saline waters released lower amounts of PTE from the grassland than from the arable soil, irrespectively of the source of contamination. This remains true considering the normalized amounts (ng mobilized per total μg of metal) of PTE leached from contaminated soils at the largest salt concentration (Fig. 4 and 5).

The DOC of the solutions extracted from soils not exposed to flooding increased in waters of increasing salinity and, although the two soils are very close in composition, and in particular have a similar organic C content, the grassland soil released significantly more water soluble C than the arable soil at all levels of salinity (see Fig.1 in supplementary materials).

Flooding time affected not only the DOC but also the pH and DIC of the water phase (Table 1 in supplementary materials). The combined effects of pH, DOC and DIC changes on PTE speciation in solution were modelled with Visual Minteq 3.1 (see supplementary materials). Modelling showed that these parameters however had only a marginal effect on solubilization and metal speciation of Cd and Zn at low salinity levels, but that Pb was affected by the level of DOC. However, at high salt concentrations and in soils contaminated with MT at the higher Zn concentration, computed solubility indexes show that Zn solubility might have been limited by concentration of DIC, as in some solutions appeared to be oversaturated with respect to Zn carbonates.

DISCUSSION

The soils used in this experiment are located within a scarcely polluted area and PTE (Tab. 1) are in great part inherited from the parent material. As such they may be occluded in sparingly soluble or larger size mineral particles, or have undergone some type of aging process. Albeit more PTE were leached from all soil treatments at increasing salinities, much lower amounts of PTE were proportionally leached from the control soils than from the soils contaminated with the two dusts, showing that airborne PTE are potentially much more easily mobilized by salinity than that the corresponding elements inherited through weathering of parent materials. Incubation conditions were chosen in order to mimic the situation occurring at the soil surface, where fallout of airborne particles accumulates.

Flooding with marine or brackish water rapidly modifies the composition of the soil solution and of the exchange complex, shifting the existing chemical equilibria and affecting the solubility of toxic metals (Ciszewski et al., 2016). The mobilization observed for Cd, Zn and Pb is driven by cation exchange, but also by changes in the speciation of these metal cations which are able to establish new bonds with the inorganic ligands that dominate the composition of sea salts. The speciation of trace metals in synthetic sea water was calculated by Turner (1981) and shows that solubility is principally governed by hydrolysis and complexation with chloride and carbonate ions.

In soil, however, other factors contribute to the observed mobilization: the strong increase in ionic strength weakens electrostatic attractions with solid surfaces and the hydrolysis of the divalent cations with formation of $\text{Me}(\text{OH})^+$ species further reduces attraction by negatively charged surfaces, decreasing the possibility that these elements compete with the much more abundant cations from seawater for sorption within the double layer of solid surfaces. Hydrolysis reactions are driven by the electronic configuration of the element and by pH, so marine or brackish waters which display relatively constant pH ranges, would not induce changes in the hydrolysis behaviour of PTE, but significant changes may occur within the soil, depending on the source of contamination and length of the submergence period and were indeed observed in our experiment (Fig. S3).

Flooding caused the pH to drop slightly in all soils, probably because of the trapping of CO₂ in the water phase, but, under the experimental conditions chosen, the low depth of the water layer did not cause soils to become fully anaerobic, so the pH changed only slightly with time. These conditions reflect those expected to occur in the upper part of the surface layer (0-2 cm) of coastal flooded soils, unless the overlaying waters become anaerobic, and therefore mimic the situation expected under field conditions. The pH was strongly affected by the source of contamination, which eventually, as in the case of MT significantly increased the pH of the leached water already after 24h and maintained a higher pH, compared to the other treatments, even after much longer flooding periods.

The effect of pH on PTE mobilization is highly significant, but also depends on the length of flooding (Fig. 6, 7). In the case of Cd and Zn the effect decreases after 30 days of submergence, whereas for Pb the decrease starts already after 7 days as shown by the slope of the regression lines.

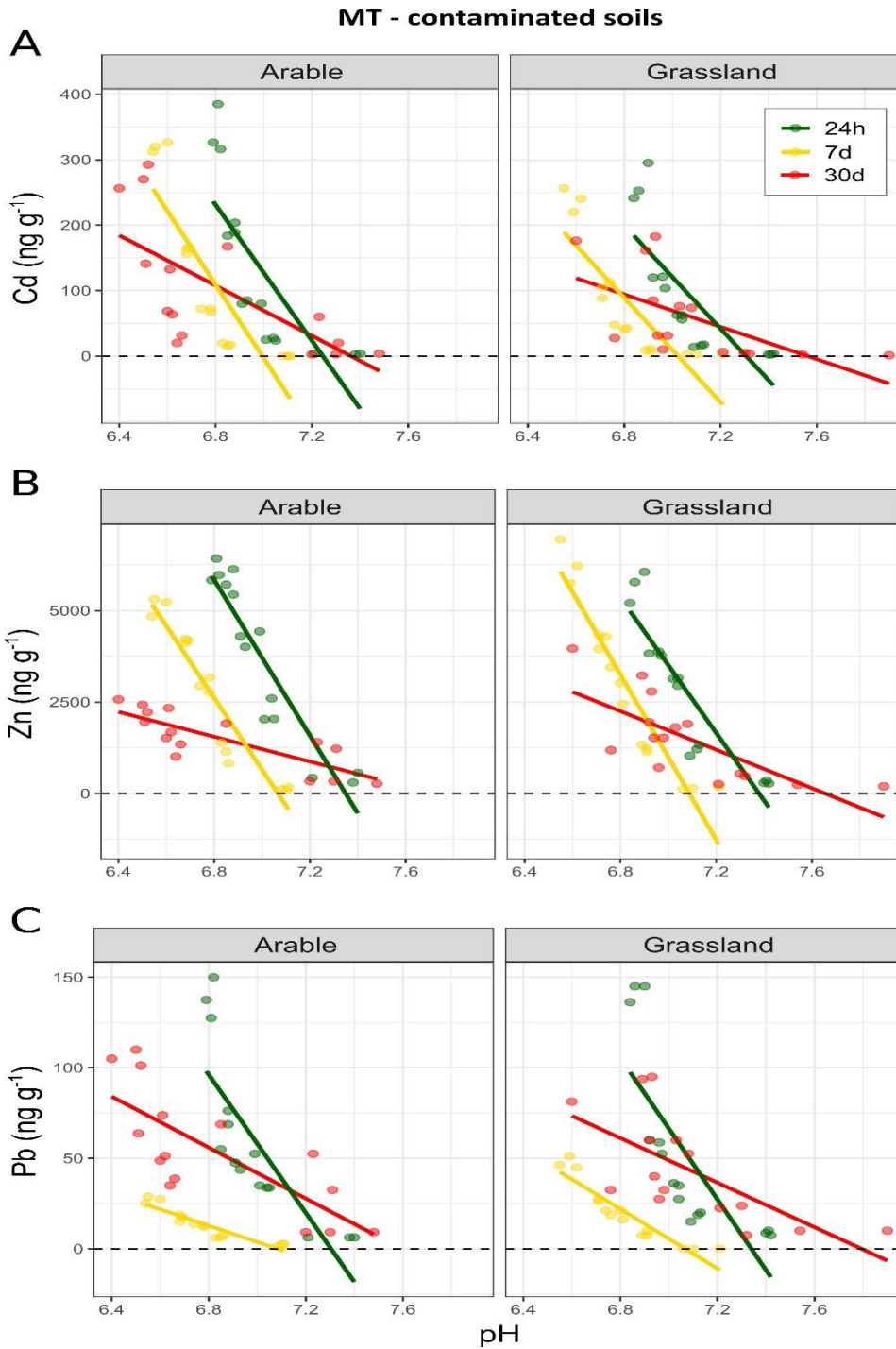


Figure 6 pH effect on metal mobilization in arable or grassland soils contaminated with mine tailings (MT), after 24h and 7 or 30 days of exposure to water of increasing salinity (from 0 to 34.5 g L⁻¹).

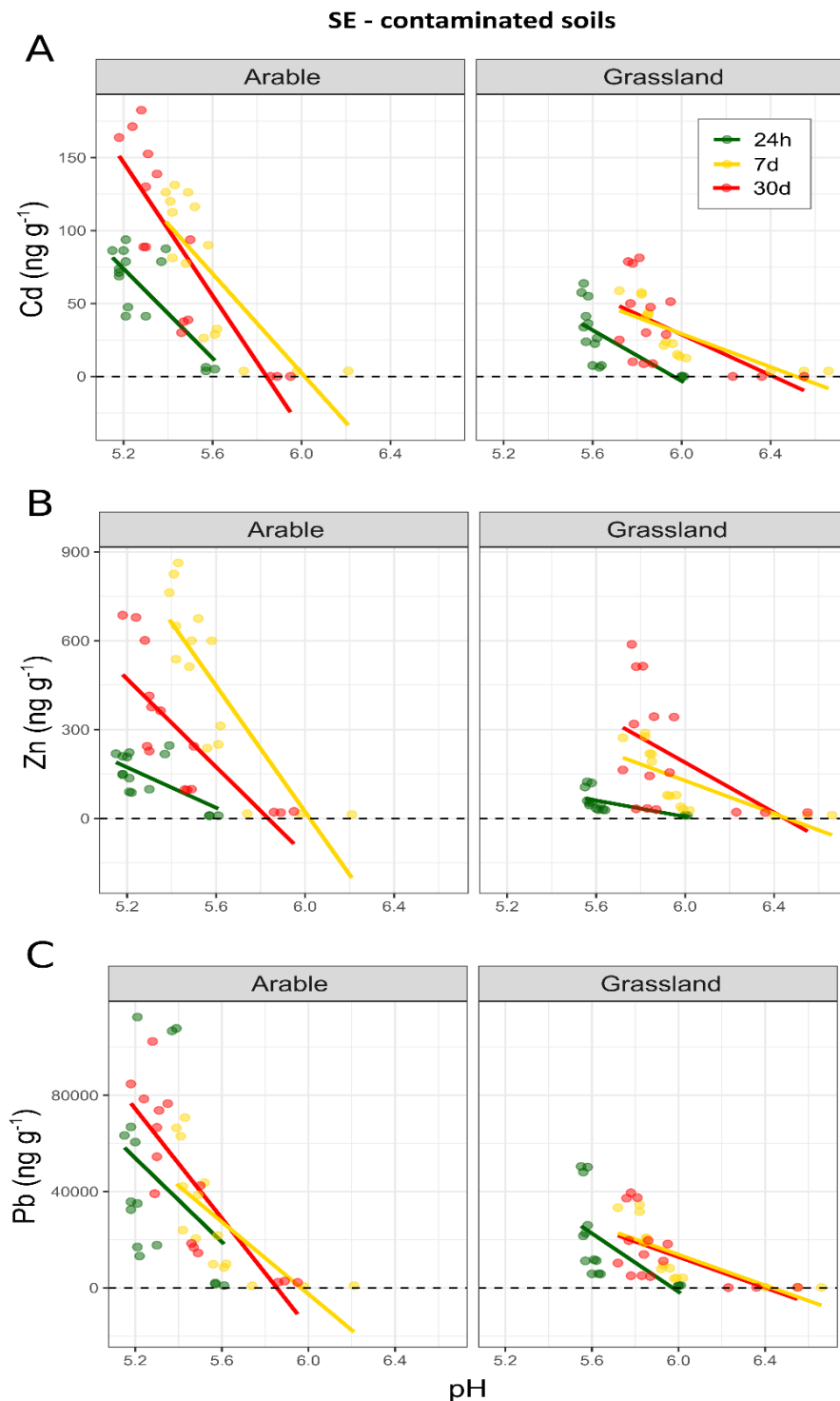


Figure 7 pH effect on metal mobilization in arable or grassland soils contaminated with smelter exhausted (SE) dust, after 24h and 7 or 30 days of exposure to water of increasing salinity (from 0 to 34.5 g L⁻¹).

The combination of chemico-physical modifications induced by the salts dissolved in marine or brackish waters with kinetic dissolution effects that become evident comparing trends of short and longer submergence periods enhances, however, the complexity of the system (Liu et al., 2019). The significant difference in the amount of DOC solubilized by marine and brackish waters from the two uncontaminated soils at all salinity levels (Fig. S3) may have affected the behaviour of Pb, but not that of Cd and Zn. In fact, speciation modelling indicated that a substantial fraction of this metal was associated with fulvic acids in solution. However, this did not result in larger amounts of Pb being solubilized from the grassland soil. It is possible that some of the potentially mobilized Pb could have been retained by interaction (complexation) with the non-soluble humified fraction of organic C, which was larger in the grassland soil compared to the arable (supplementary). Qualitative differences, generated by soil cultivation, may have therefore overcome the small difference in SOM between the two soils.

CONCLUSIONS

The rise of the sea level driven by global climate changes will increase the frequency of flooding events in coastal areas. This work shows that flooding by brackish or marine waters will enhance the mobility of PTE and in particular will favour the dissolution of the toxic elements contained in airborne dusts from mining and smelting activities.

The extent of mobilization depends on several factors, among which the main are the kind of element, the dust source and the salinity of flooding waters, but airborne PTE display a much stronger tendency to be mobilised than metals inherited from the soil parent material.

The source of the dust, which determines not only the original speciation of the elements, but also eventually modifies soil conditions, has a strong effect on their release rates. Lower amounts of Pb, Cd and Zn were in fact proportionally released

from MT contaminated soils, than from SE contaminated soils, probably as a consequence of the increase in pH of the water phase.

In the presence of easily decomposable organic matter, flooding triggers biochemical redox processes that result in the modification of both the water phase and of solid surfaces, that are not the simple result of the blockage of gaseous exchange with the atmosphere and act in a dynamic way. Although this is not likely to occur in the upper 2 cm of soil, this may eventually occur in the field affecting not only the dissolution of PTE, but also the nature of the soil surfaces and of the soil solution: these factors will need to be investigated with further experiments.

Soil management also affects the intensity of mobilization as lower amounts of PTE were leached from the grassland soil than from the arable, showing that even minor modification of soil characteristics such as soil organic matter quality, may significantly affect the extent of PTE mobilization by salinity.

CHAPTER 3

PREFACE

This chapter entitled “**Seawater flooding impacts on biological activity of organic and conventional farming soils**” reports the results of a research work carried out to investigate the consequences of increased frequency and length of flooding events related to global sea level rise (SRL) on the biological activities of the organic and conventional soils.

The following study was carried out to determine the saline flooding impacts on osmotic stress and key biological indicators, i.e. microbial biomass, soil respiration and ATP, in relation to organic and traditional management systems of cultivated soils.

INTRODUCTION

A broad set of intensive soil cultivation by conventional farming (CF) negatively effects on ecosystem services provided by soil microbiome by changing good soil structure, cation exchange capacity and leaching of essential nutrients (Reganold and Wachter, 2016; United Nations Environment Programme, 2016; Powers and Jetz, 2019). Organic farming (OF) systems could provide valuable solutions to existing problems affecting the soil microbiome (Pandey et al., 2018; Peltoniemi et al., 2021). OF is central to the foodmaking process, as well as quality of life, which cautiously uses natural resources and conditions for sustainable development (Cristache et al., 2018). It also helps to maintain adequate levels of soil organic carbon (SOC), which is essential for soil biological properties, good physical structure and enhancing of cation exchange capacity, with positive impacts on nutrient and water cycling (Papadopoulos et al., 2014; Fernandez, 2016). Several studies indicate that regular organic inputs from OF can be more than carbon lost during tillage, such that SOC concentrations increase (Fernandez et al., 2016; Messmer et al., 2012), which leads to decrease the rate of nutrient loss by leaching or erosion (Bender et al., 2015) and can also increase nutrient use efficiency (Fernandez et al., 2016; Snapp et al., 2010). A broad set of sustainability indicators compare organic based against conventional systems across many environmental and socioeconomic metrics (Shennan et al., 2017). Nevertheless, in comparison with CF under experimental and field conditions, yields (per hectare) from OF may be reduced up to 50% compared to CF (Seufert and Ramankutty, 2017; Meemken and Qaim, 2018). Therefore, yield production may be one of the main limitations of OF.

Under changing climate conditions, it will be essential to understand the long-term benefits and limitations of OF systems, particularly on soil fertility and biodiversity (Azarbad, 2022). As sea levels rise, low-lying coastal areas are increasingly inundated with saltwater, gradually increasing soil accumulation of soluble salts (Werner et al., 2013). In areas where precipitation is scarce or occurs

only within defined short periods during the year, salts can remain in the soil for a long time before being leached away. Soil salinization is the accumulation of salts in soils at a level that negatively impacts agricultural productivity, environmental health, and economic welfare (Rengasamy, 2006).

To evaluate the state of the soil environment, the indicators are usually physical, chemical, and biochemical, e.g., the content of organic carbon, total nitrogen, C biomass, the level of FDA (Fluorescein diacetate hydrolysis) (Piotrowska-Długosz and Charzyński, 2012) and the content of ATP (Wen et al., 2005). Biological parameters or bioindicators included in the monitoring program are also used to assess the ecological risk relevant to soil health and quality (Suter et al., 2000). Biological factors are often more difficult to interpret than physical or chemical factors due to their complexity in monitoring (Kennedy and Papendick, 1995). For these purposes, the use of microbial parameters seems to be very promising given their importance for soil quality (Tate, 2000).

High solubility of organic matter in saline soils represents a major pathway, by which C and N can be lost (Peinemann et al., 2005; Wong et al., 2008). Increasing salt levels in soil solution limit the availability of the water to plants and microorganisms, due to severe osmotic stress (Brady and Weil, 2002). Salinity causes the soil microbial biomass and activity to decrease (Rietz and Haynes, 2003; Tripathi et al., 2006; Yuan et al., 2007; Chowdhury et al., 2011; Mavi and Marschner, 2012). Soil respiration, i.e., the amount of CO₂ that is released from a soil sample per hour used as a proxy of microbial activity (Chowdhury et al., 2011; Yan and Marschner, 2013), increases as a response to the stress (Mamilov and Dilly, 2002). The metabolic quotient (qCO₂), i.e. an index derived from CO₂ data, provides a measure for the microbial C-use efficiency. The qCO₂ is calculated as the ratio between respiration and biomass C, represents the specific respiration of the living microbial biomass. High values of metabolic quotient qCO₂ are taken as a sign of low microbial efficiency and are assumed as indicators of stress (Anderson and Domsch, 1993; Wardle and Ghani, 1995).

Water potential is a fundamental concept now widely accepted for quantifying the energy state of water in soil, organic materials, plants, seeds, and microorganisms

(Papendick & Campbell, 1981). In soil, it is affected by salt content and soil characteristics (e.g. texture) and can help to understand how differences in soil
Moreover, soil adenosine triphosphate (ATP) is another useful indicator of the microbial activity (Contin et al., 2001; Dyckmans et al., 2003), overcoming the simple estimate of the total biomass C and N of the soil community and focusing on the actual microbial active pool.

The following study was carried out to determine the saline flooding impacts on the main biological indicators (microbial biomass, evolution of CO₂, qCO₂, ATP) in relation to water potential measured in organically and traditionally cultivated soils.

MATERIAL AND METHODS

Soil properties

Conventionally and organically cultivated soils were collected (0–20 cm depth and sieved to < 2 mm) from cultivated areas of Basiliano, (municipality) Friuli- Venezia Giulia region, at 12 km southwest of Udine, Italy.

Table 1. Physio-chemical characteristics of organic and conventional farming soils

Soil type	Organic	Conventional
pH	7.67	7.92
EC (dS m ⁻¹)	0.64	0.36
CaCO ₃ (g kg ⁻¹)	61	71
C (%)	2.04	2.03
Clay (%)	23	21
Silt (%)	14	14
Sand (%)	63	65

Physiochemical properties of CF and OF are reported in Table 1. Soil pH was measured at a 1:2.5 soil to water ratio. Electrical conductivity EC (dS m⁻¹) was measured with 1:2 of soil to water ratio by using a Crison (CM 35) conductivity meter. Total organic carbon (C %) and nitrogen (N %) were measured with a CHNS Elemental Analyzer (Vario Microcube, Elementar, Langenselbold, Germany). Carbonates were calculated by difference, subtracting the organic carbon (HCl treated soil) from the total carbon content. Soil texture was measured in a Bouyoucos' cylinder with an ASTM 152H hydrometer.

Experimental Setup

About 200 mg of organically and traditionally cultivated dry soils were submerged for 6 h with fresh (control) or saline waters (treatments) at increasing concentrations (Fig 1); i.e. T₁ (4.37 g L⁻¹) corresponding to a dilution from the stock saline solution of 1:8, T₂ (8.75 g L⁻¹, 1:4), T₃ (17.5 g L⁻¹ 1:2) and T₄ (stock saline solution of 34.5 g L⁻¹). To determine the microbial biomass C ($\mu\text{g g}^{-1}$) and microbial respiration (CO₂), soils were further incubated at 25 °C for 5 and 25 days in closed containers with 25 ml NaOH (to trap CO₂).



Figure 1. Fresh soils (OF and CF) immersed with fresh or saline waters for 6 h.

Microbial Biomass C

Microbial biomass carbon (B_c) was measured by the Fumigation-Extraction method (Vance et al., 1987). Moist soil portions, equivalent to 25 g dry soil, were fumigated with chloroform (CHCl₃) for 24 h. The soil samples (both fumigated and non-fumigated) were then transferred to 80-ml plastic tubes and extracted with 60 ml (0.5 M) K₂SO₄ solution on an orbital shaker at 170-rev min⁻¹ for 30 minutes and after, solutions were filtered by 150 mm Whatman filter paper. Total organic carbon (TOC) were measured in a TOC analyzer (SHIMADZU TOC-5000A). The difference in TOC between the fumigated and non-fumigated samples corresponds to the microbial biomass C in the soil (Jørgensen, 1996).

Microbial respiration CO₂ and metabolic quotient

Carbon dioxide evolution was measured by pH auto titration (METTLER TOLEDO DL 50) of 5 ml NaOH along 0.5 mol HCl solution. The metabolic quotient was calculated according to Anderson and Domsh (1978) $q\text{CO}_2 = \text{mg g}^{-1} \text{CO}_2\text{-C} \times \text{mg Bc}^{-1} \times \text{h}^{-1}$.

Soil water potential

Soil water potential (ψ) was measured using a dew point psychrometer (WP4C, METER Group, Inc. USA) after 5 days of incubation, and on fresh or air-dried soil samples (n=3). Water retention curves were obtained for the two soils (organic and conventional) submerged in fresh and saline water (34.5 g L⁻¹). Soils were dried at 25 °C and several sampling points were collected measuring, in parallel, ψ and soil humidity.

Soil ATP

Soil ATP was determined using the acid extraction method (D.S. Jenkinson, J.M. Oades A method for measuring adenosine triphosphate in soil *Soil Biol. Biochem.*, 11 (1979), pp. 193-199), with minor modifications. Briefly, soil ATP was extracted from fresh samples with a solution composed by 0.6 M imidazole, 1.10 M TCA and 0.25 M sodium hydrogen phosphate. A second extraction was performed for all samples with the same solution but containing 0.5 nmol ATP L⁻¹, in order to determine possible loss of ATP during extraction. 25 mL of each extractant was added to about 2 g of fresh soil, immediately sonicated for 2 min using a SONOPLUS instrument (Bandelin, Berlin, Germany) equipped with a 12.5 mm diameter probe at 60 % power. After 10 min ice cooling, samples were filtered using Whatman 42 filter paper. ATP contents were determined in supernatants using a Multi label Counter microplate reader (PerkinElmer, USA) in luminescence mode after addition of 50 μ l luciferin-luciferase, 131 μ l TRIZMA buffer and 9 μ l NaOH 1M solution, to 10 μ l ATP extract (BioThema AB, Sweden). Measurements were taken after 4-5 minutes of substrate-enzyme addition (n=3). The ATP calibration curve was assessed using standard ranging from 2.5 to 50 nM ATP. Soil ATP was then expressed as nmol ATP per g of soil or of biomass C.

Statistical analyses

ANOVA was conducted on soil water potential (ψ) and ATP data in order to assess significant differences among salt treatments and soil incubation periods (P<0.05). Tukey's test was applied as post hoc test. Normality and homoscedasticity of data were assessed before conducting the analysis via Shapiro-Wilk's (P>0.05) and Bartlett's (P>0.05) tests, respectively.

RESULTS

Effect of salts on microbial biomass and respiration

After 5 days of incubation, soil biomass C (mg g^{-1}) (Fig 2A) decreased along the salinity gradient, with the higher biomass C reduction at intermediate salinities corresponding to 1:4 artificial seawater dilution (17.5 mg L^{-1}), while slightly increase at 1:8 (34.5 mg L^{-1}) in both soils (CF, OF).

CO_2 evolution ($\text{mg g}^{-1} \text{ h}^{-1}$) (Fig 2B) in both OF ($R^2 0.83$) and CF ($R^2 0.92$) increased along salinity. The metabolic quotient $q\text{CO}_2$ ($\text{mg g}^{-1} \text{ h}^{-1}$) (Fig 2, C) increased in both OF ($R^2 0.96$) and CF ($R^2 0.87$) along the decrease in biomass C.

During the 25 days of incubation period, soil biomass C (mg g^{-1}) (Fig 3A) found to decrease in both OF and CF during the 25 day incubation's periods through gradually increase in salt contents, following the order T_1 (4.37 mg L^{-1}) < T_2 1:2 (8.75 mg/l), < T_3 , 1:4 (17.5 mg/l) < T_4 , 1:8 (34.5 mg/l) and in both OF and CF. The percentage decrease of biomass C in CF (1.5%) was slightly larger than in OF (1.2%). CO_2 evolution ($\text{mg g}^{-1} \text{ h}^{-1}$) (Fig 3B) and metabolic quotient $q\text{CO}_2$ ($\text{mg g}^{-1} \text{ h}^{-1}$) (Fig 3C) increased in both OF and CF along decrease in biomass C.

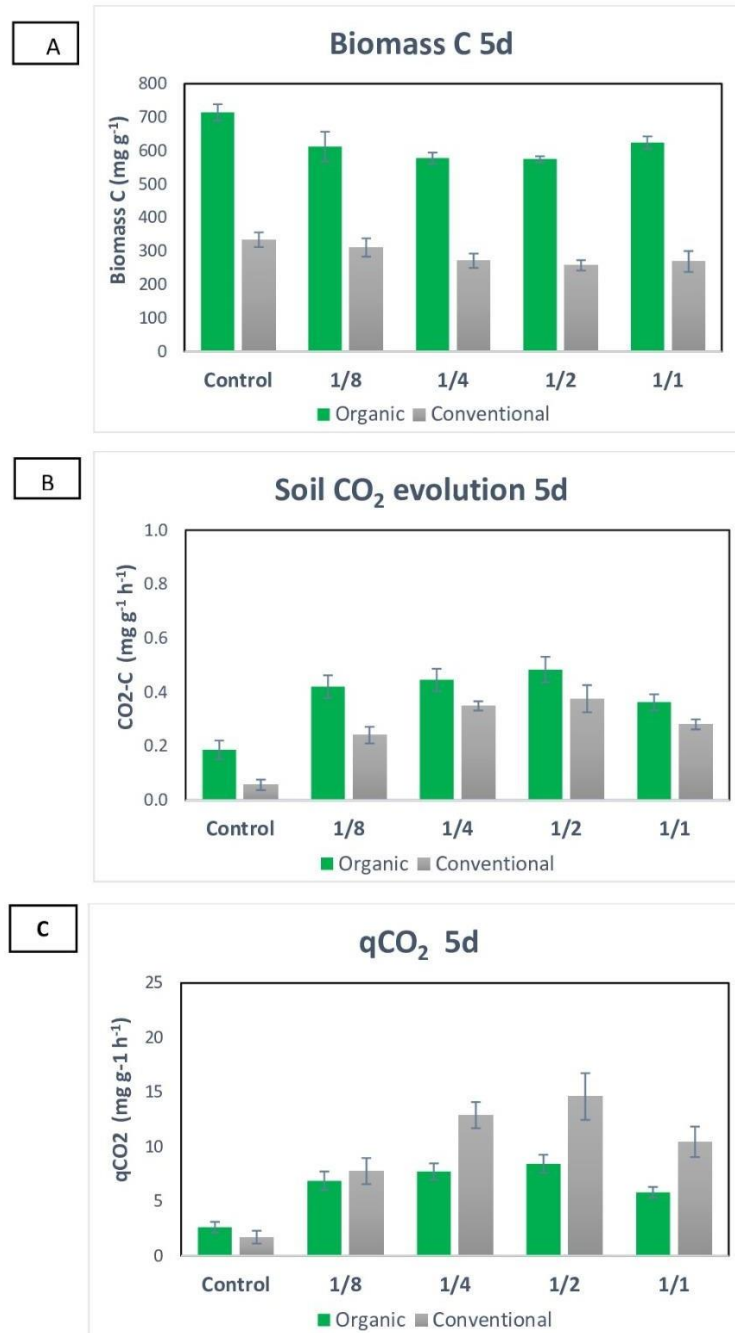


Figure 2. Biomass C (mg g⁻¹), CO₂ evolution (mg g⁻¹ h⁻¹) and qCO₂ in organic and conventional soils along salinity gradient at 5 days of incubation period.

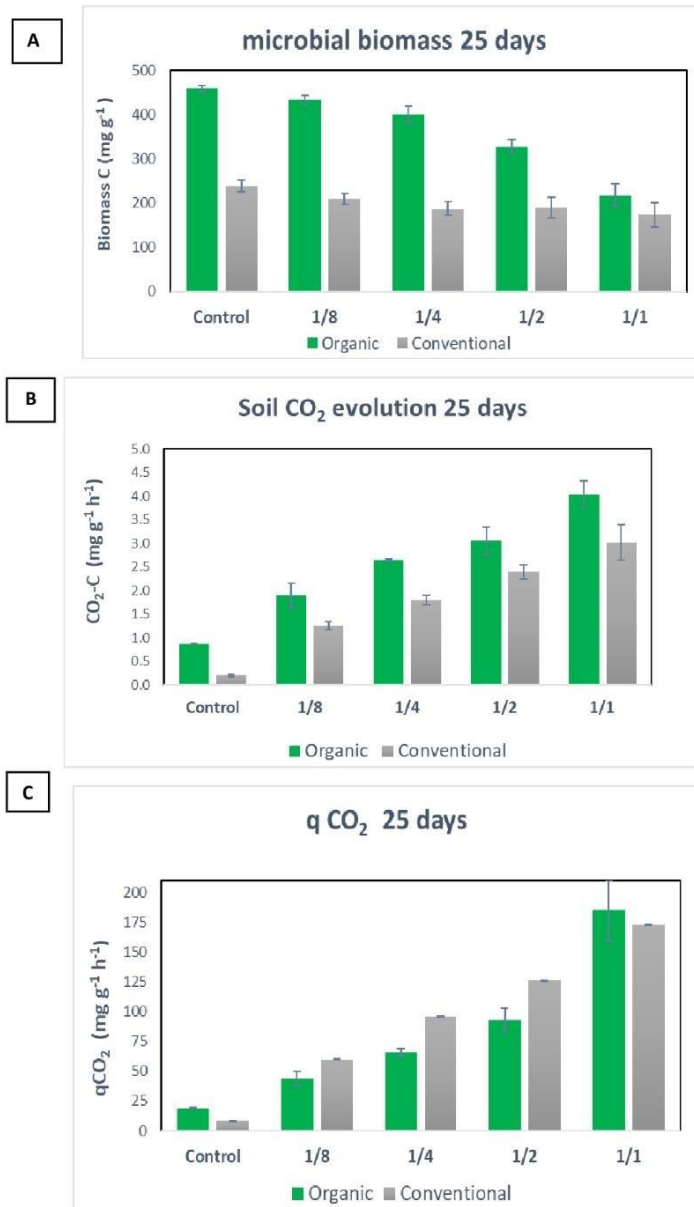


Figure 3. Biomass C (mg g⁻¹), CO₂ evolution (mg g⁻¹ h⁻¹) and qCO₂ in organic and conventional soils along salinity gradient at 25 days of incubation period.

Salt stress effect on soil water availability and soil ATP

Increasing salt levels in submerged soils remarkably decreased soil water potential (ψ) that ranged from about 0 MPa (low salt level, 4.4 g L⁻¹) to -2.5 MPa at the largest salinity (34.5 g L⁻¹) (Fig 4).

The osmotic effect derived from the presence of salt was also clear from the soil water retention curves (Fig 5) for both conventional and organic soils. In fact, at same soil water content, the presence of salt determined a more negative ψ that represent a larger fraction of unavailable water for plants and microorganisms.

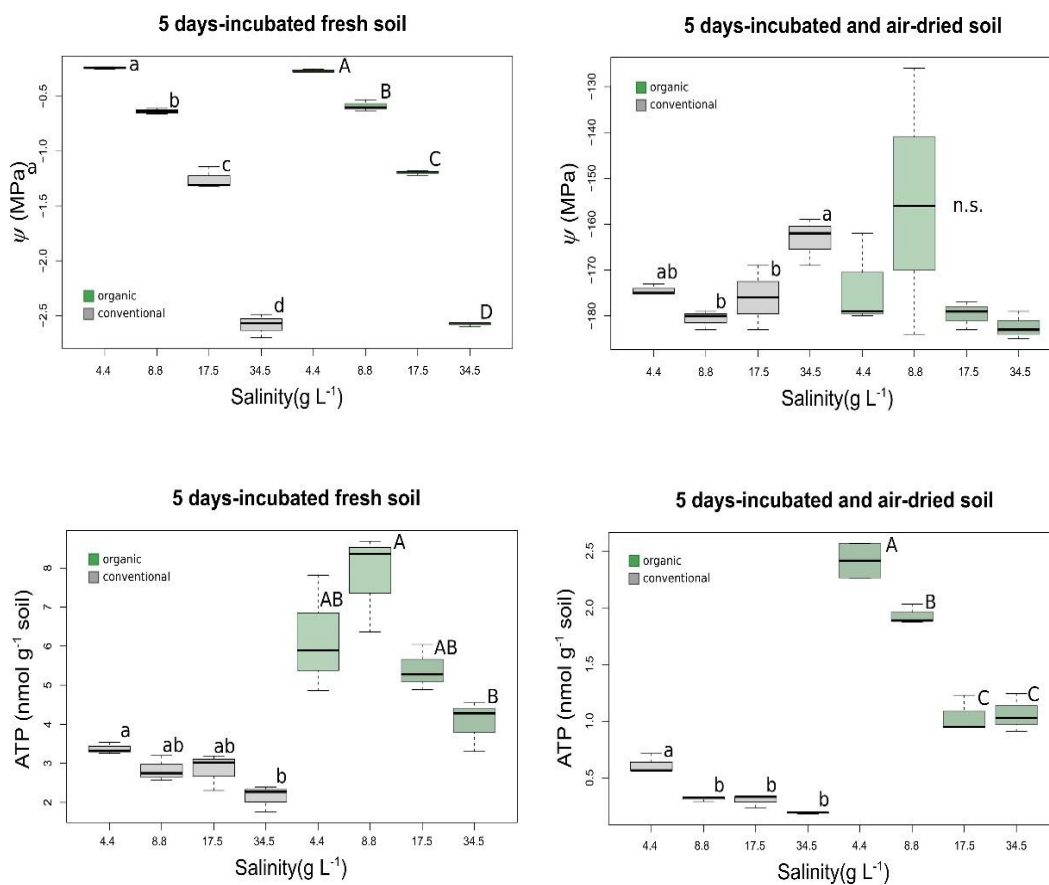


Figure 4. Soil water potential (ψ) and soil ATP measured in organic and conventional soils after 6 h submersion in fresh or saline water of increasing salt concentration. Soils were drained and incubated for 5 days at 25 °C. ψ and ATP were measured on fresh or air-dried samples. Letters refer to significant statistical differences based on Tukey's post hoc test.

Soil ATP was on average higher in the organic soil and tended to decrease with salinity in both conventional and organic soils, ranging from about 8 to 2 nmol ATP per g soil (Fig 4). Soils were then air-dried and ψ and ATP again determined. Ψ of air-dried soils did not reveal clear trends among salt treatments or between soils, while soil ATP results were consistent with soil ATP measured in fresh soils. This result suggests that microbial activity is not only affected by the osmotic stress derived from the saline treatment, therefore by ψ , but also by the possible toxic effect of the salts.

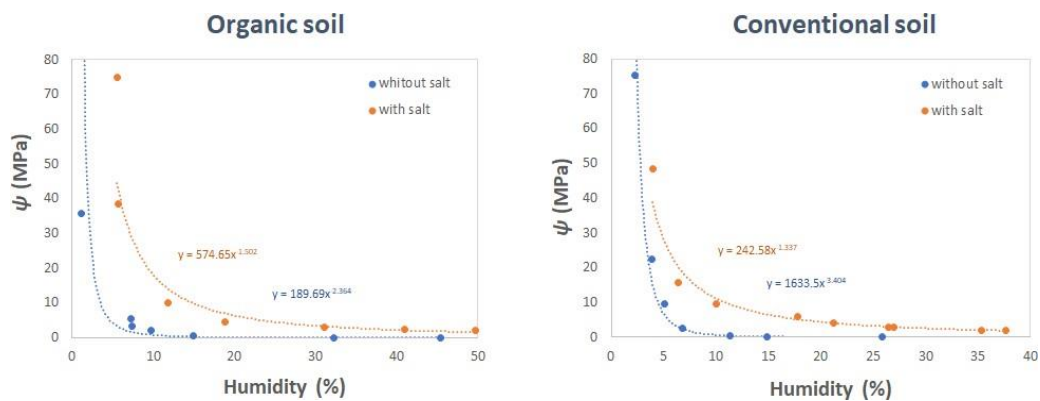


Figure 5. Soil water retention curves of organic and conventional soils submerged with fresh or saline water (34.5 g L^{-1}).

Microbial community recovery from salt stress

Fresh soils samples were further recover for 20 additional days (25 days of incubation in total) in order to assess the recovery ability of the microbial community towards the stress after longer recovery period (25 instead of 5 days) (Fig 6). Apparently, the organic soil did not show the ability to recover. Soil ATP was on average lower after 25-day compared to 5-day incubation period (about half). Some recovery occurred instead for the low salt treatments (4.4 and 8.8 g L^{-1}) of the conventional soil.

In order to assess the possible influence of the microbial pool on soil ATP, data

were normalized and expressed as nmol ATP per g of biomass C (Fig 6). Normalized data allowed to detect the recovery ability of the organic soil for most of the salt treatments applied (with the exception for 8.8 g L⁻¹). Moreover, the recovery of the conventional soil was even more sharp.

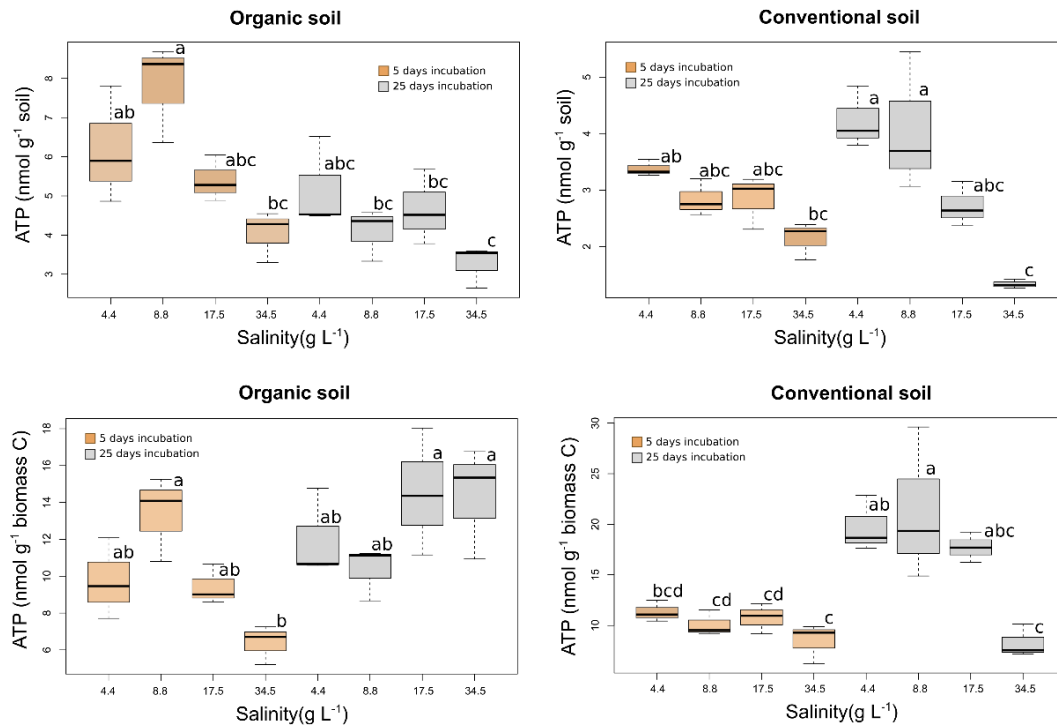


Figure 6. Soil ATP recovery in organic and conventional soils after 5- or 25-day incubation. Soils were submerged in fresh or saline water of increasing salt concentration. Soils were drained and incubated for 5 or 25 days at 25 °C. Letters refer to significant statistical differences based on Tukey's post hoc test.

DISCUSSION

Soil salinity is well known to decrease the microbial biomass carbon and nitrogen in soils (Zhang et al., 2019; Bourahla et al., 2016; Pankhurst et al., 2001; Rietz and Haynes, 2003 and Yuan et al., 2007), which is also confirmed by our data (Fig 2A, 3A). Soil salinity also increased soil respiration and the effect was more pronounced after 25 days of incubation (after the imposed stress). The toxicity of ions, in particular Na^+ and Cl^- , lead to nutrient imbalances in microbial growth and enzyme synthesis and activity (Marinari et al., 2012). Microbial community is less competitive in saline soils, because only specialized and adapted species survive. Salt accumulation in the soil increased the extracellular osmotic pressure (Rath et al., 2015) and microorganisms failing to acclimate to the osmotic stress might have died or become inactive, thereby decreasing microbial diversity and abundance.

Rapid increase in salinity resulted in greater respiration (Fig 2A, 2B). Abiotic stress is known to affect soil respiration, but in a way that is dependent on soil conditions. In fact, Wang et al. (2014) reported that soil respiration decreases in mesic and xeric ecosystems but increases in hydric systems, after a drought event. Salt stress can be assimilated to drought stress, because of the lack of water for microbial activity (due to the osmotic effect). Moreover, to treat soils with increasing salt concentrations, we exposed soils to 6 hours flooding.

Saline soils were found to enhance the $q\text{CO}_2$ (Fig 2C, 3C), therefore, to enhance the respiration rate per biomass C unit. Nevertheless, the $q\text{CO}_2$ could be affected by a shift in the composition of the microbial population (Hattori 1992; Wardle and Ghani 1995).

The salt stress effect was similar in organic and conventional soils (Fig 2, 3), regardless the higher initial biomass C in the organic one. The absence of differences between soils with different organic matter content is probably to attribute to the very similar properties of the investigated soils (Table 1). Higher microbial diversity and the presence of certain beneficial microbes in the soil environment under organic farming would ensure better soil resilience. Moreover, organic matter and the water holding capacity of organic soils, contribute greatly

to soil resilience to stresses (Azarbad, 2022).

Increasing salt levels in submerged soils remarkably decreased soil water potential (ψ) (Fig 4, 5). Salt accumulation in the soil increased the extracellular osmotic pressure. Low osmotic potential at high salt concentration reduces water availability to microbes and plants. Moreover, it may draw water out from the cells due to the osmotic effect (Rath et al., 2015).

Soil ATP content decreased with salinity (Fig 6). Soil ATP is a widely used index of microbial activity and, despite uncertainties in its determination (Blagodatskaya and Kuzyakov, 2013), it nicely reflects changes in total microbial biomass. Contin et al. (2001) found a strong linear relationship ($R^2 = 0.94$) between ATP and biomass C with a stable ratio of about $12 \mu\text{mol ATP g}^{-1}$ biomass C, confirmed by our data (Fig. 6).

The effect of drying and rewetting on soil microbial processes have been studied in the literature (Griffiths et al., 2003; Herron et al., 2009; Ilstedt et al., 2000; Schimel et al., 2007; Xiang et al., 2008). In this case, the microbial activity peaks in the first 24 h after rewetting (Fierer & Schimel, 2003). This is because, upon rewetting, cells of sensitive microbes lyse, whilst other microbial genotypes release the organic solutes they accumulated during the dry phase that act as substrate (Halverson et al., 2000). Furthermore, soil aggregates break down and their previously protected organic matter is exposed and can then be more easily decomposed (Jin et al., 2013). Differences after rewetting may be due to soil moisture and soil type, aggregation and concentration of potentially bioavailable soil organic matter (Anderson and Ingram, 1993). However, drying and rewetting can also kill some microbes and change microbial community structure which, in turn, could influence nutrient cycling (Fierer et al., 2003; Schimel et al., 2007). Moreover, the response pattern of bacteria and respiration upon rewetting dry soil will depend on both the duration of drought and the duration of the incubation of moist soil before drying and rewetting (Meisner et al., 2015).

Based on ATP normalized by biomass C (Fig 6) soils recovered fast after the stress, and already after 5 days of incubation. However, the effect of salt stress is still visible and testify that microbial community in a soil hardly recover after an

abiotic stress, regardless the hypothesized advantage of a larger amount of soil organic matter.

CONCLUSION

Salt affects soil properties but also soil microbial activity. Despite recovery seem possible, it takes place only after a while, which is a medium-long term of absence of the salt stress. Under field conditions, soil salinity is a constantly changing factor, providing an experimental challenge for short- and long-term studies. To date, there is a shortage of growth-based assessments of microbial responses to salinization. There is limited evidence for a connection between the salinity level in soil and the microbial tolerance to salt which is highly dependent of microbial community composition. However, there are some indications that communities autochthonous of saline soils could be better adapt to high salt concentrations than non-acclimated communities.

GENERAL CONCLUSION

Because of the large number of effecting factors acting often in contrasting directions, the consequences of salinization of coastal soils are still difficult to predict on the global scale. In particular, the effects of SLR on C and N cycles and consequently on GHG emissions from coastal soils are highly variable, depending on the contrasting and concomitant effects of flooding and salinity. At present, it is unlikely that this variability can be handled by models aiming to predict consequences at a planetary scale (Chapter 1). This work shows that flooding by brackish or marine waters will enhance the mobility of PTE and in particular will favour the dissolution of the toxic elements contained in airborne dusts from mining and smelting activities. Soil management also affects the intensity of mobilization as lower amounts of PTE were leached from the grassland soil than from the arable, showing that even minor modification of soil characteristics such as soil organic matter quality, may significantly affect the extent of PTE mobilization by salinity (Chapter 2). Salt remarkably affects microbial biomass and respiration, with an apparent inability of both soils to recover. Salinity tend to affects soil properties but also soil microbial activity. Despite recovery seem possible, it takes place only after a while, which is a medium-long term of absence of the salt stress.

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SUPPLEMENTRY MATERIAL CH 2

[Sottotitolo del documento]

Arable soil												
Uncontaminated					Tailings					Smelter		
24 hours												
Salinity (g L ⁻¹)	pH	Conductivity (μS cm ⁻¹)	DOC (mg L ⁻¹)	DIC (mg L ⁻¹)	Salinity (g L ⁻¹)	pH	Conductivity (μS cm ⁻¹)	DOC (mg L ⁻¹)	DIC (mg L ⁻¹)	Salinity (g L ⁻¹)	pH	Conductivity (μS cm ⁻¹)
0	5.89 ± 0.04	19.4 ± 0.21	6.89 ± 1.02	3.41 ± 0.34	0	7.33 ± 0.1	148 ± 56.3	15.97 ± 2.51	12.99 ± 1.2	0	5.58 ± 0.02	48.8 ± 3.7
4.37	4.78 ± 0.05	4416 ± 5.7	5.43 ± 1.94	1.42 ± 0.28	4.37	7.03 ± 0.02	4503 ± 70.9	14.36 ± 1.76	11.25 ± 1.1	4.37	5.24 ± 0.05	4216 ± 12
8.75	4.73 ± 0.06	8436 ± 35	6.82 ± 0.37	1.82 ± 0.1	8.75	6.94 ± 0.04	7970 ± 697	15.88 ± 2.02	13.09 ± 1.67	8.75	5.19 ± 0.02	7900 ± 19
17.5	4.78 ± 0.03	15196 ± 245	9.64 ± 0.81	1.46 ± 0.67	17.5	6.87 ± 0.02	15405 ± 784	16.25 ± 2.89	14.37 ± 3.06	17.5	5.18 ± 0.03	13446 ± 4
34.5	4.85 ± 0.05	28100 ± 565	13.93 ± 2.59	0.49 ± 0.56	34.5	6.81 ± 0.02	25366 ± 115	19.06 ± 0.22	18.09 ± 3.72	34.5	5.32 ± 0.1	27633 ± 5
7 days												
0	5.51 ± 0.05	28.7 ± 15.9	11.05 ± 2.49	1.75 ± 0.61	0.00	7.1 ± 0.02	142 ± 8.6	14.22 ± 1.58	23.67 ± 2.71	0	5.98 ± 0.24	80.6 ± 42
4.37	4.76 ± 0.01	4520 ± 105	7.08 ± 2.13	0.81 ± 0.34	4.37	6.85 ± 0.02	4313 ± 97.1	13.25 ± 1.35	14.08 ± 1.6	4.37	5.6 ± 0.03	4350 ± 28
8.75	4.71 ± 0.04	8293 ± 179	7.3 ± 0.53	0.89 ± 0.67	8.75	6.77 ± 0.02	7873 ± 257	14.92 ± 1.22	16.34 ± 1.49	8.75	5.49 ± 0.08	7900 ± 34
17.5	4.71 ± 0.03	15416 ± 46	9.19 ± 1.64	1.05 ± 0.9	17.5	6.68 ± 0.01	14953 ± 395	17.62 ± 2.31	17.3 ± 1.1	17.5	5.48 ± 0.05	14426 ± 1
34.5	4.69 ± 0.04	27700 ± 818	12.89 ± 0.71	1.14 ± 0.33	34.5	6.56 ± 0.03	27766 ± 208	26.46 ± 2.81	23.42 ± 0.62	34.5	5.41 ± 0.02	26900 ± 2
30 days												
0	5.7 ± 0.09	30.6 ± 2.4	10.48 ± 1.97	1.12 ± 0.53	0	7.33 ± 0.14	137 ± 10.4	19.19 ± 1.3	38.59 ± 7.36	0	5.9 ± 0.05	63 ± 6.7
4.37	4.86 ± 0	4246 ± 49.3	4.53 ± 0.66	0.33 ± 0.39	4.37	6.87 ± 0.38	4520 ± 36.1	13.07 ± 1.18	21.75 ± 4.22	4.37	5.47 ± 0.02	4510 ± 95
8.75	4.8 ± 0.02	8130 ± 52.9	4.65 ± 1.05	0.34 ± 0.33	8.75	6.82 ± 0.36	8356 ± 35.1	15.73 ± 5.65	21.36 ± 8.21	8.75	5.36 ± 0.12	8356 ± 40
17.5	4.76 ± 0.03	14850 ± 62.4	7.27 ± 0.54	0.94 ± 0.33	17.5	6.66 ± 0.17	15610 ± 265	22.87 ± 3.58	28.04 ± 5.09	17.5	5.32 ± 0.03	15690 ± 1
34.5	4.82 ± 0.05	27366 ± 152	12.71 ± 0.05	1.84 ± 0.41	34.5	6.47 ± 0.06	28633 ± 611	29.62 ± 4.19	30.61 ± 2.73	34.5	5.23 ± 0.05	28866 ± 2

Table S1. ...

Grassland soil													
Uncontaminated					Tailings					Smelter			
24 hours													
Salinity (g L ⁻¹)	pH	Conductivity (μS cm ⁻¹)	DOC (mg L ⁻¹)	DIC (mg L ⁻¹)	Salinity (g L ⁻¹)	pH	Conductivity (μS cm ⁻¹)	DOC (mg L ⁻¹)	DIC (mg L ⁻¹)	Salinity (g L ⁻¹)	pH	Conductivity (μS cm ⁻¹)	DOC (mg L ⁻¹)
0	5.79 ± 0.01	22.4 ± 8.08	13.79 ± 1.3	5.52 ± 1.51	0	7.41 ± 0.01	99.5 ± 8.92	9.83 ± 0.39	11.2 ± 1.95	0	6 ± 0.01	46.6 ± 2.9	17.5 ± 0.01
4.37	5.23 ± 0.01	4396 ± 15.2	12.05 ± 0.45	3.04 ± 0.51	4.37	7.11 ± 0.02	4376 ± 135	11.17 ± 1.46	10.65 ± 1.57	4.37	5.62 ± 0.02	4236 ± 17	17.5 ± 0.01
8.75	5.08 ± 0.01	8423 ± 66.5	15.55 ± 2.42	3.98 ± 0.55	8.75	7.03 ± 0.01	8266 ± 65.1	12.31 ± 2.76	11.99 ± 2.62	8.75	5.6 ± 0.03	8036 ± 98	17.5 ± 0.01
17.5	5.29 ± 0.02	15263 ± 142	18.17 ± 1.13	4.25 ± 0.75	17.5	6.95 ± 0.03	15150 ± 210	14.24 ± 2.83	15.71 ± 3.33	17.5	5.57 ± 0.01	15013 ± 5	17.5 ± 0.01
34.5	5.11 ± 0.01	27300 ± 1253	25.01 ± 2.01	4.97 ± 0.1	34.5	6.87 ± 0.03	27566 ± 351	7.82 ± 6.53	8.71 ± 9.13	34.5	5.56 ± 0.02	27433 ± 3	17.5 ± 0.01
7 days													
0	6.01 ± 0.01	21.1 ± 7.68	18.93 ± 6.66	6.96 ± 0.78	0	7.12 ± 0.08	1383 ± 7.8	16.85 ± 2.3	27.74 ± 1.18	0	6.54 ± 0.13	60.9 ± 9.1	17.5 ± 0.01
4.37	5.34 ± 0.01	4303 ± 119	6.52 ± 0.41	2.37 ± 0.23	4.37	6.9 ± 0.01	4153 ± 447	11.22 ± 3.74	10.62 ± 2.02	4.37	6 ± 0.02	4413 ± 25	17.5 ± 0.01
8.75	5.34 ± 0.02	8263 ± 128	7.96 ± 0.2	2.5 ± 0.14	8.75	6.79 ± 0.03	5573 ± 622	14.81 ± 1.66	13.92 ± 2.77	8.75	5.94 ± 0.02	8230 ± 26	17.5 ± 0.01
17.5	5.23 ± 0.07	15276 ± 198	7.03 ± 2.24	1.95 ± 0.3	17.5	6.72 ± 0.02	13066 ± 1126	24.66 ± 2.3	22.59 ± 1.16	17.5	5.85 ± 0.01	15480 ± 1	17.5 ± 0.01
34.5	5.23 ± 0.03	28600 ± 360	16.59 ± 5.29	4.05 ± 0.72	34.5	6.59 ± 0.04	26600 ± 424	24.92 ± 6.57	22.58 ± 7.33	34.5	5.79 ± 0.06	28333 ± 2	17.5 ± 0.01
30 days													
0	6.75 ± 0.06	75.3 ± 13.2	12.35 ± 1.31	18.97 ± 5.09	0	7.59 ± 0.29	297 ± 91.5	27.92 ± 1.58	69.26 ± 0.87	0	6.38 ± 0.16	101 ± 36.	17.5 ± 0.01
4.37	5.54 ± 0.15	4256 ± 20.8	8.62 ± 0.28	1.31 ± 0.22	4.37	7.16 ± 0.18	4610 ± 52.9	21.87 ± 2.16	26.39 ± 3.44	4.37	5.83 ± 0.05	4496 ± 28	17.5 ± 0.01
8.75	5.4 ± 0.14	8170 ± 30	9.69 ± 1.43	2.47 ± 0.59	8.75	6.89 ± 0.12	8520 ± 50	25.92 ± 1.13	31.7 ± 3.44	8.75	5.83 ± 0.11	8460 ± 72	17.5 ± 0.01
17.5	5.47 ± 0.02	14946 ± 100	12.86 ± 1.46	3.17 ± 0.29	17.5	7.01 ± 0.08	15753 ± 83.8	29.15 ± 1.42	30.56 ± 2.46	17.5	5.86 ± 0.09	15623 ± 1	17.5 ± 0.01
34.5	5.57 ± 0.04	27800 ± 100	23.83 ± 2.46	6.34 ± 0.87	34.5	6.81 ± 0.18	29150 ± 70.7	41.39 ± 2.15	35.79 ± 3.26	34.5	5.78 ± 0.03	28866 ± 3	17.5 ± 0.01

Table S2. ...

