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**TESI DI LAUREA** 

# Multivariate model-based exploration of ecological niche in plants communities of natural and artificial salt marshes of the Venice Lagoon

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# ABSTRACT

Ecological differences between natural and artificial saltmarshes in the Venice Lagoon were investigated over several months through field sampling, involving the assessment of vegetation coverage, redox potential, sediment granulometry, and altitude.

Indeed, restored ecosystems, exemplified by artificial saltmarshes in the Venice Lagoon, exhibited varying degrees of biological and physical deviation from their natural counterparts.

The gathered data underwent statistical analysis to comprehend the diversities and similarities, as well as the ecological status, of natural and artificial saltmarshes. Building on this novel multivariate dataset, an ecological niche model was constructed to establish a predictive framework for the future restoration of salt marshes.

## **KEYWORDS**

Salt marshes Plants communities Ecological model

# **CHAPTER 1 - INTRODUCTION**

### 1.1 The Venice Lagoon

Coastal lagoons are transitional environments with a significant ecological and socio-economic relevance in the area where they are located. These types of ecosystems are characterized by rich biodiversity, having high natural heterogeneity due to strong gradients of environmental parameters, as well as the diversity of the habitats (Elliott *et al.*, 2007). Unfortunately, however, coastal areas are often involved in negative anthropogenic influences such as pollutant spills, overexploitation, and unregulated maritime traffic (Kjerfve *et al.*, 1996).

The Venice Lagoon, 550 km<sup>2</sup>, is located in the northwest of the northern basin of the Adriatic Sea and has always been a highly dynamic environment. This ecosystem consists of islands, salt marshes, and water bodies and its depth is generally shallow, with only a small portion exceeding 5 meters (Tagliapietra *et al.*, 2018). The Lagoon has been formed because of the action of the rivers, which have deposited sediment over millennia, forming isles and clayey land, and on the other hand, because of the sea pushing the water inland. The action of the sea currents, which, depending on the tides, submerged and resurfaced the sedimented land, designed the dense network of channels that characterize the Lagoon (Fig.1). Nowadays the lagoon is connected to the sea through three entrances, enabling the exchange of over 50% of the whole volume of water inside, in half a day (Gambaro *et al.*, 2009). Consequently, tides play a crucial role in shaping its characteristics and determining various environmental factors (Tagliapietra *et al.*, 2018).



Fig. 1, Image of salt marshes and their internal channels. North Lagoon of Venice, 11-11-2009

(Image source: https://www.mosevenezia.eu/my-product/recupero-morfologico-barene/)

As the Venice Lagoon is subject to a multiplicity of forces, it has undergone several metamorphoses over the centuries, the factors that led to environmental changes over time are both natural, as deposition of river material and erosion and anthropogenic, as river diversion and construction of storm defense works (Solidoro *et al.*, 2010).

As a result it is currently possible to divide the lagoon into three vast extensions: Northern Lagoon, Central Lagoon and Southern Lagoon The northernmost area is the most valuable, retaining characters close to the original ones; the central area has historical and recent artificial features, from land reclamation to other degradation processes; in the southernmost area residual type salt marshes are found (Bonometto 2014).

In the areas with the most evident ecosystem modifications, due to drastic ecological changes, human action at times has played a central role in the maintenance of Lagoon habitats, with interventions aimed at counteracting land silting and coastal erosion (Bonometto, 2014).

As a result of this high variance in habitats and phenomena, the Lagoon of Venice is a well-studied coastal site globally, both ecologically and culturally, and is part of the international Long-Term Ecological Research network (Tagliapietra *et al.*, 2018).

Although the Venice Lagoon is an example of an ecosystem that has been affected by human influence for centuries, it is the most recent interventions that have made the greatest impact on the lagoon habitats, mainly related to activities in the nearby industrial district of Porto Marghera, where some factories are still present (Gambaro *et al.*, 2009), but also to the digging of the Petroli Canal in the central Lagoon and the Philippine clam (*Ruditapes philippinarum*) fishery activity (Bonometto, 2014) (Fig. 2).

Human interventions in the Lagoon have harmed especially salt marshes and seagrass beds, for which environmental conservation and restoration programs have been put in place (Tagliapietra *et al.*, 2018).



Fig. 2, 2010 rate of seabed erosion in the water areas of the lagoon, excluding canals and salt marshes, represented in color scale (D'Alpaos, 2010).

#### 1.2. Salt marshes

Salt marshes, in the Venice Lagoon (Fig. 4), are extensions of tabular land, formed at such an altitude that they are periodically submerged by tides (Bonometto 2014), very few salt marshes are currently above the mean high tide level (Favero *et al.*, 1983). These water fluctuations play a fundamental role in the genesis of these habitats, as when the velocity of water flow decreases, the sediment tends to precipitate to the bottom, favouring the process of sedimentation, which, by facilitating the deposition of sediments such as sand, clay and mud, encourages the formation of salt marshes.

The deposition of different types of sediment changes depending on the type of grain size, in fact this can vary greatly, also affecting the cohesion and permeability of the sediment.

This suggests that the evolution of salt marshes depends on complex interactions between physical, hydrodynamic and geomorphological components (Marani *et al.*, 2006), but also biological components, which will be discussed in more detail later.

The morphology of salt marshes has recurring characteristics: no obvious reliefs, slight basin-like central depressions and slightly raised edges, remarkable tidal flats (Anoè *et al.*, 1984). Tidal flats, mostly mudflats, known locally as 'velme', are marshes parts exposed at low tide and submerged at high tide and are classified as intertidal or subtidal marshes, depending on whether they are above or below the mean spring tide value of 0.50 m (Sarretta *et al.*, 2010). They provide habitat for a variety of marine organisms and play an important role in the coastal ecosystem, they are also significant for coastline protection and for filtering sediments and nutrients (Amos *et al.*, 2004).

Marshes are extremely sensitive and at risk due to climate change. In fact, although the higher parts of salt marshes are better able to withstand the rate of sea level rise (especially due to the presence of vegetation), the tidal flats are in more difficulty. This happens because the amount of sediment supplied to the tidal flats, mainly by the deterioration process of the salt marshes, is insufficient and consequently, their average elevation is decreasing (Carniello *et al*, 2009). Salt marshes boundary erosion is causing losses at large rates, due especially to changes in sediment supply, suffice it to say that in the Venice Lagoon salt-marsh extent has faced a reduction of 76% in the last two centuries (Tommasini *et al.*, 2019).

Considering an important characteristic of this habitat is the continuously changing morphology due to dynamic interactions between erosion and sedimentation processes (Barausse *et al.*, 2015), tracing the main causes responsible for salt marshes erosion is a complex challenge. In environments subject to climate change and anthropogenic pressures, the loss of salt marshes can be triggered by various hydrodynamic forces of different origins. (Tommasini *et al.*, 2019).

Natural causes of salt marshes erosion include tidal currents and wind-induced waves, causing resuspension and transport of sediment to the sea. The disappearance of marsh areas is a cascading and self-reinforcing process, as the

ability of wind to generate waves, inducing the resuspension of sediment, increases with wind speed and the length of the fetch (distance in which the wind blows continuously on a given direction over a water surface), which in turn depends on the marsh areas whose presence limits the fetch itself (Barausse *et al.*, 2015).

Anthropogenic causes of erosion, on the other hand, focus on the absence of freshwater inputs to the lagoon and therefore the lack of riverine sediments, construction of long jetties at the inlets and the excavation of deep lagoon channels (Petroli canal), which have modified the system of lagoon currents (Barausse *et al.*, 2015). Moreover, waves, generated by motor boats, erode bottoms and salt marsh borders, while illegal clam fishing resuspended sediments speeding up the loss of sediments to the sea (Barausse *et al.*, 2015). Also sea level rise, affecting wind-wave climate, is causing a more rapid erosion of the marsh boundaries due to the increase in water depth and consequently in the wave height (Tommasini et al., 2019).

The combined effect of these human and natural actions is a net negative sediment budget for the lagoon: more sediments are lost to the sea with respect to those that enter the lagoon and can rebuild salt marshes (Barausse et al., 2015). The net loss of sediment is evident in the decreasing extent of saltmarsh areas and the increasing depth of the lagoon bottom (Fig. 3) (Tommasini et al., 2019).



Fig. 3, Bathymetries of the Venice Lagoon over the years. Elevations are in meters above mean sea level (Tommasini *et al.*, 2019).

The effect of the loss of areas covered by salt marshes in the Venetian lagoon will have and is having an effect not only at the environmental level, indeed the salt

marshes play a key role within the Lagoon, not only because of their contribution at the ecosystem, but also because of their economic-social importance, which is why we can associate several ecosystem services with this habitat. The concept of ecosystem services encompasses the need to preserve the healthy functioning of these natural regions, as they are responsible for a number of contributions such as habitat provision, nutrient cycling, climate regulation, and cultural services (Costanza *et al.*, 1997).



Fig. 4, The Lagoon of Venice and four sub-basins (A–D), separated by broken lines, showing where the salt marshes are located (Sarretta *et al.*, 2010).

During the past century, unfortunately, the interest in the preservation of these wetlands was relatively minor, in fact, large expanses of tidal flats and open salt marshes were converted to port and industrial complexes, resulting in salt marshes permanent loss and the disappearance of the ecosystem functions and services they once provided (Sarretta *et al.*, 2010). This was as a result of the land reclamation processes carried out between 1927 and 1960, aimed at constructing the industrial area, the Venice airport and facilitating the urban development of the city of Mestre (Sarretta *et al.*, 2010). It was not until 1972, that the first

Special Law for the Venice Lagoon was presented to protect the natural environment (Bonometto 2014).



Fig. 5, Total salt marsh surface (km2) (D'Alpaos, 2010). Saltmarsh areas decreased by more than 50%, from 68 km2 in 1927 to 32 km2 in 2002 (Sarretta *et al.*, 2010)

All these anthropic activities severely damaged the Lagoon (Fig. 5), with losses that are unlikely to be reversible and often permanent. Having reached this point, understanding how the lagoon have responded to past modifications, is necessary in order to prevent further degradation in response to future environmental pressures (Sarretta *et al.*, 2010).

### 1.2.1. Plants communities

The salt marshes are home to salt-loving plants that constantly affect the balance between sediment buildup and erosion (Tagliapietra *et al.*, 2018). This species are mainly halophytic vegetation species, i.e. macrophytes adapted to complete their lifecycle in salty environments. The spatial distribution of halophytic vegetation over salt marshes is not random nor spatially uncorrelated but is, on the contrary, organized in characteristic patches, following a phenomenon called zonation (Fig. 6) (Silvestri *et al.*, 2005).



Fig 6, (Anoè *et al.*, 1984) Schematic profile of lagoon soil and halophytic vegetation in relation to the mean tide level.

Abbreviation	Scientific_Name
Zo	Zostera marina
Sp	Spartina stricta
Li	Limonium serotinum
Ar	Arthrocnemum fruticosum
ghebo	Salt Marsh Internal Channel
Pu	Puccinellia palustris
As	Aster tripolium
Sa	Salicornia veneta
Ju	Juncus maritimus
Art	Artemisia caerulescens
Ph	Phragmites australis

Different types of vegetation can be found in salt marshes depending on their proximity to brackish water and the salinity levels.

First Spartina stricta (Fig. 7) is usually found, it is a resilient grass on the edges in direct contact with brackish water, forming dense patches (Anoè *et al.*, 1984). The perennial saltmarsh grasses of the *Spartina* genus are of particular interest in coastal protection as they are pioneer species (Lo *et al.*, 2017).

Instead in areas that are only occasionally submerged or less salty, *Juncus maritimus* (Fig. 8) is usually present as the prevailing species. (Anoè *et al.*, 1984).



Fig. 7, *Spartina stricta* (Anoè *et al.*, 1984)



Fig. 8, Juncus maritimus

In areas with high salt concentration during high tides, vegetation adapted to extreme saltiness is found, these plants have features like fleshy leaves, downy coverings, or waxy scales to reduce water loss, such as *Halimione portulacoides* (Anoè *et al.*, 1984).

Therefore, the central, slightly depressed parts of the salt marshes where water stagnates and salinity increases due to evaporation are home to succulent halophytes, like *Salicornia veneta* (Fig. 9), *Aster tripolium*, and *Limonium vulgare* (Fig. 11), but it is also possible to see *Puccinellia palustris* (Fig. 10) (Anoè *et al.*, 1984).



Fig.9, Salicornia veneta Fig.10, Puccinellia palustris Fig.11, Limonium vulgare (Anoè et al., 1984).

*Suaeda maritima* thrives in areas where organic matter accumulates, giving the landscape a reddish-purple color in autumn. Furthermore, *Salsola soda* is another plant that prefers well-fertilized soils, rich in nitrogenous substances and so it is found in less salty soils (Anoè *et al.*, 1984).

Also important in Venice salt marshes is *Sarcocornia* spp. (Fig. 12), which is a halophytic succulent plant belonging to the *Amaranthaceae* family, its genus includes 28 species distributed worldwide in saline environments. *Sarcocornia fruticosa* is highly tolerant to salt and dissimilar to *Salicornia*, is perennial (Custodio *et al.*, 2021). *Sarcocornia fruticosa* and *Salicornia*, are fundamental to reinforce salt marshes edges (Bonometto 2014).



Fig. 12, *Sarcocornia fruticosa* (image source: "https://www.freenatureimages.eu/plants/Flora%20S-Z/Sarcocornia%20fruticosa/index.html#Sarcocornia%2520fruticosa%25202%252 C%2520Saxifraga-Jasenka%2520Topic.jpg")

The vegetation of the salt marshes therefore presents a remarkable dynamism, driven by significant variations in chemical, physical, and biotic factors, especially on a small scale -within a few meters (Silvestri *et al.*, 2005). Consider that simple relationships between plant distribution and soil elevation or distance from the creek, are not only applicable across different marshes, but even within the same tidal environment (Silvestri *et al.*, 2005).

A deeper understanding of the spatial and temporal patterns of these species and their interactions with the ecosystem requires accurate studies, particularly on their ecological function within the Venice Lagoon. Biological research of this kind could be useful since environmental alterations of anthropogenic origin have led to a decrease in the extent of salt marshes and a consequent reduction in plant communities.

This loss has a very negative effect on the Lagoon as, according to various studies, marsh vegetation communities can reduce volume loss by 80% in sandy soils and 17% in silty soils, revealing the critical role of salt marshes and their vegetation in coastal defense, playing an important role in these lagoon areas (Lo *et al.*, 2017).

### **1.2.2 Physical parameters**

#### • Soil composition and dynamics

The composition of salt marshes soil is the result of the combined actions of tides, currents, waves and sedimentation, leading to the presence of different grain-sizes such as mud, sand, and clay. The most commonly used scale to describe the granulometry of a soil is the Udden-Wentworth scale, 1922 (Fig. 13).

Millimeters	μm	Phi (ø)	Wentworth size class			
4096 1024		-20 -12 -10	Boulder (-8 to -12)			
256		8	Pebble (-6 to -8			
64		6	Pobble (-2 to -6à)	_		
16		-4	Febble (-2 to -60)	Ve		
3 36		-1 75		ira		
2.83		-1.50	Gravel	פ		
2.38		-1.25	Giaver			
2.00		-1.00-				
1.68		-0.75				
1.41		-0.50	Very coarse sand			
1.19		-0.25				
1.00		-0.00-				
0.84		0.25				
0.71		0.50	Coarse sand			
0.59		0.75				
1/2 - 0.50 -	-500 -	1.00-				
0.42	420	1.25	<u> </u>	p		
0.35	350	1.50	Medium sand	ar		
0.30	300	1.75	ں ۱	מ		
1/4 - 0.25 -	-250 —	2.00-				
0.210	210	2.25				
0.177	177	2.50	Fine sand			
0.149	149	2.75				
1/8 - 0.125 -	-125 -	3.00				
0.105	105	3.25				
0.088	88	3.50	Very fine sand			
0.074	/4	3.75				
1/16 - 0.0625 -	- 63 -	- 4.00-				
0.0550	33	4.25	Coores silt			
0.0440	27	4.50	Coarse silt			
1/22 - 0.0310 -	31 -	4.75				
1/64 0.0156	15.6	6	Medium silt			
1/128 0.0078	7.8	7	Fine silt	_		
1/256 - 0.0039 -	3.9-	- 8 -		n		
0.0020	2.0	9	2	≥		
0.00098	0.98	10				
0.00049	0.49	11	Clay			
0.00024	0.24	12	Ciay			
0.00012	0.12	13				
0.00006	0.06	14				



These sediment patterns are strongly influenced by daily and monthly tidal variations, which determine whether marshes are submerged or exposed; in this way different soil compactness contributes to the unique characteristics of the biotope (Anoè *et al.*, 1984). Compaction and structure of a soil are related to bulk

density (Blake 1965). In a salt marsh context, bulk density can influence various aspects such as soil permeability, water retention capacity and nutrient availability to plants. High bulk density values may indicate soil compaction, which could limit plant growth and influence soil resistance to erosion (Blake 1965). Variations in tidal height contributing especially to the shaping of the marshes, affect also vegetation growth and organic matter accumulation (Tagliapietra *et al.*, 2018).

The accumulation of organic matter in the soil is influenced more specifically by the interplay between plant biomass productivity and decomposition (Roner *et al.*, 2016), for this reason accretion rates of organic and inorganic components vary based on the position within the salt marsh and the distance from channels. If accretion near channels is mainly driven by inorganic sediments, in the innermost part of the salt marsh, on the contrary, the importance of the organic component prevails (Roner *et al.*, 2016).

In general, organic matter is retained and made available to plants and microorganisms in the soil and is therefore very important in salt marshes. The decomposition of organic matter is a key process that influences the availability of nutrients to plants and contributes to soil structure and fertility; for example, very stable organic matter can help maintain good soil structure, retain moisture and provide nutrients to plants over the long term (Schnitzel e Khan 1978). As it is possible to see on the Udden-Wentworth scale (1922) in Fig. 13, silt is a type of sediment consisting of particles (> 63  $\mu$ m) smaller in size than sand. Clayey silts are often rich in organic matter and usually fill the inter-distributary lowlands and back-barrier zones (Da Lio *et al.*, 2018).

The complexity of the balances in these ecosystems underlined till now, is closely linked to their fragility, especially in the context of urbanisation processes, climate change and rising sea levels. For example, the ability of salt marshes to keep pace with sea-level rise is discontinuous: indeed even if, vertically, sediment input contributes to accretion, horizontally, periods of expansion alternate with worrying periods of lateral erosion (Lo *et al.*, 2017).

The sediment properties can influence erosion rates and even in the absence of vegetation, average lateral erosion is lower in silty soils than in sandy soils (Lo *et al.*, 2017).

Also the presence of plants is very important in counteracting erosion phenomena; in fact, their roots play the role of anchoring sediments. Their presence on salt marshes is influenced by many elements, such as salinity and waterlogging. Salinity, for example, gradually increases with soil elevation, probably due to longer evaporation periods at higher altitudes, causing salts in surface soils to become highly concentrated (Silvestri *et al.*, 2005). With regard to soil oxygenation, on the other hand, vegetation plays a key role in increasing aeration, and this can be attributed to variable water uptake among different plant species. This process of water uptake contributes to maintaining a well-ventilated soil layer, which is essential for the survival and persistence of the marsh ecosystem, underscoring the importance of plant-soil interactions in these environments (Boaga *et al.*, 2014).

It's thus understable how studying the interactions in salt marshes between physical processes, such as soil sedimentation and biological processes, e.g. vegetation growth within marshes, is very important for informing action to support their survival (Roner *et al.*, 2016), especially in a historical period when erosion and subsidence have led to a significant reduction in the area of salt marshes in the Venice lagoon, amounting to approximately 35 km2 (Sarretta *et al.*, 2010).

#### • Elevation and oxygen availability

The elevation of salt marshes in the Venice Lagoon shows significant variations, influenced by factors such as geographical position and tidal dynamics (Bonometto 2014).

Significant variations, however, are also found within a single salt marsh. In fact, the strips at the margins, which are locally known as 'strong' salt marshes, are compact, well drained and subject to more pronounced salinity variations and, thanks to the vegetation that retains sediment and detritus, have slightly higher elevations. In the inner core, on the other hand, the salt marsh takes on a concave shape (Bonometto 2014).

The zonation of the elevation of salt marshes, thus described, also has an influence on plant diversity, which is affected by the ground elevation, particularly along the edges of streams as compared to the inner areas of marshes (Silvestri *et al.*, 2005). Many specific species show associations with particular soil elevations (Fig. 14), but these associations may differ among marshes, suggesting that plant distribution is also influenced by local physical characteristics (Silvestri *et al.*, 2005). It was then noted how even slight changes in elevation can alter the marsh flora, making it highly susceptible to shifts in water levels caused by human activities and climate change (Tagliapietra *et al.*, 2018).



Fig. 14, Example of soil profile in salt marshes (Silvestri et al., 2005)

One of the reasons for the correlation between elevation and marsh vegetation could be related to oxygen availability in the soil, measured through the redox potential. In higher areas of salt marshes, where water may be less present and soil aeration may be more effective, the redox potential tends to be higher, indicating better oxygenated conditions. Conversely, in lower areas, the redox potential tends to be lower, suggesting more reducing or anaerobic conditions. Oxygen availability thus emerges as a critical factor for biotope persistence, capable of influencing processes such as plant growth, nitrification, respiration and sulfide oxidation (Lang *et al.*, 2010). Therefore, in salt marshes with small elevation differences, such as natural ones, soil drainage properties have a great impact on oxygen availability (Lang *et al.*, 2010).

The variability of redox potential within the study area follows some recurring patterns, for example, the rate of oxygen consumption is typically similar in streams and areas covered by *Limonium spp.*, but lower at sites with *Halimione portulacoides* and *Juncus maritimus* (Eriksson *et al.*, 2003). This difference could be attributed to variations in hydraulic and nutrient loading among different vegetation types (Eriksson *et al.*, 2003). In general, however, soil oxygen consumption tends to increase during spring and early summer, potentially influenced by factors beyond just temperature (Eriksson *et al.*, 2003). In the end, monitoring changes in oxygenated and anoxic areas can be useful in assessing the conservation status of salt marsh habitats (Lang *et al.*, 2010).

#### 1.2.3 Artificial salt marshes

Artificial salt marshes in the Venice lagoon were created as part of various reclamation and environmental restoration projects in the late '80s, born in response to the challenges posed by erosion and the loss of natural habitats in the Lagoon over the centuries. Over the years indeed, the diminishing presence of natural salt marshes within the lagoon has raised concerns, promoting the creation of artificial salt marshes as a response (Bellafiore *et al.*, 2014). The significance of salt marshes in the Venice area becomes evident through their ecological functions, such as mitigating tidal currents, lessening wave impact, and minimizing the erosive impact of water on the structures within the city. Their restoration was thought to be essential for the protection of the Lagoon ecosystem and the city of Venice. Therefore, it was believed that the creation of artificial salt marshes could contribute to the overall environmental resilience of the Lagoon and provide a habitat for various species of aquatic birds, thus improving the biodiversity of the area (D'Alpaos *et al.*, 2007).

Not always, however, artificial salt marshes has revealed to be as ecological functioning as the natural ones, especially when they are more unstable and subject to rapid changes, which is the case if the vegetation is less structured and subject to rapid species replacement and if the soils lack a defined stratigraphic classification of grain size and porosity (Bonometto 2003). Artificial salt marshes stand out also for their unconventional shapes compared to the typical morphologies of lagoons, designed with compact perimeters e.g. made with wooden poles (Fig.15a). As a result, they have different surface-to-perimeter

ratios (Fig. 15b), compared to natural configurations, which usually exhibit an abundance of concave and jagged forms (Bonometto 2003).



Fig.15a, Poles boundaries during the construction of artificial salt marshes, Venice Lagoon, 19-09-2013 (Image source:<u>https://www.mosevenezia.eu/my-product/recupero-morfologico-barene/</u>)



Fig. 15b, Construction of artificial salt marshes near Valle di Brenta, aerial view, southern Venice lagoon, 26-08-2014 (Image source:<u>https://www.mosevenezia.eu/my-product/recupero-morfologico-barene/</u>)

Often, artificial salt marshes have been created with the primary purpose of reusing dredging or canal excavation sediments (Fig. 16, and Fig. 17), and therefore their locations and shapes are clearly influenced by this purpose and the lack of planning aimed at a real restoration of the Lagoon morphology (L. Bonometto 2003). Dredging sediment from navigation channels and using it to increase the elevation of mudflats (mudflats) and salt marshes (sandbars) has been the most common approach since 2000 (Taramelli *et al.*, 2021). However, the construction of artificial salt marshes has at times improved the morphodynamic functions of some Lagoon areas, increased biodiversity, and strengthened ecosystem resilience, contributing to a more natural and revitalized

environment, proving to be a better approach overall than one more invasive or based on completely artificial materials (Tagliapietra *et al.*, 2018).



Fig. 16, Sediment drainage for construction of artificial salt marshes. Aerial photo, Venice lagoon, 23-10-2012 (Image source: <u>https://www.mosevenezia.eu/my-product/recupero-morfologico-barene/</u>)



Fig. 17, Sediment drainage for construction of artificial salt marshes. Aerial photo, Venice lagoon, 23-10-2012 (Image source: <u>https://www.mosevenezia.eu/my-product/recupero-morfologico-barene/</u>)

Nevertheless, generally, the construction of artificial habitats (Fig. 18) must take into account the morphological and functional specificities of Lagoon areas, not

doing so would mean to simply create 'landfills' covered in vegetation that lack the complex and dynamic functions and peculiarities of the natural habitats (Bonometto 2003). One of the main parameters to consider, during the construction, is the elevation of the artificial salt marsh; in fact, altitudinal balances are critical to the functionality and resilience of salt marshes. A good perimeter/surface ratio, in addition to providing an arrangement that regulates and slows the flow of water, allows better drainage and porosity at greater depths, promoting soil oxygenation. This promotes the development of strong, well-anchored roots of long-lived species. The reinforced soil acts as a natural barrier with high resistance to erosion caused by currents and wind (Bonometto 2014).

Ultimately, the management of salt marshes is functional if a dynamic equilibrium is preserved. Any creation of artificial conditions implies disrupting existing balances or establishing new equilibriums that must align with specific roles in the systemic functionality of the lagoon (Bonometto 2008).



Fig. 18, Artificial salt marshes present in the Venice lagoon in 2009 (in black), to date further ones have been built (Scarton *et al.*, 2009)

#### 1.2.4 Salt marshes restoration

The ability to prevent erosion in the intertidal salt marshes habitat derives from the intricate interaction between vegetation, sediment properties, and elements that collectively contribute to the resilience of these ecosystems. Nevertheless, over the past century, as said before, the Lagoon has experienced significant erosion, redistribution, and export of sediments, resulting in a loss of geomorphological diversity (Tagliapietra *et al.*, 2018). Since the 90s, several dredging and saltmarsh restoration have been carried out. In the Northern part of the Venice Lagoon, saltmarsh restoration took place in the period between 2000 and 2002 (Taramelli *et al.*, 2021).

This erosion phenomenon does not only concern the Venetian lagoon, in fact the gradual loss of coastal habitats across Europe, including intertidal salt marshes, presents a widespread challenge, considering that these habitats are characterized by a constantly changing morphology, shaped in part by the dynamic interactions between erosion and sedimentation processes (Barausse *et al.*, 2015). The focus must therefore be on emphasizing the importance of long-term trends in deposition and erosion for effective coastal ecosystem management (Sarretta *et al.*, 2010).

The threat of soil subsidence, both natural and induced by human activities, along with sea level rise, poses a significant risk. If the combined effect of these processes outpaces the salt marshes' capacity to build up soil, they face the peril of submergence (Barausse *et al.* 2015).

In light of these challenges, the environmental restoration of salt marshes necessitates a multidisciplinary approach. This encompasses not only technical and environmental considerations but also economic and aesthetic aspects. The restoration process integrates engineering solutions for protection, containment, and consolidation, alongside the application of ecological knowledge for biotope restoration (Tagliapietra *et al.*, 2018).

Conservation and restoration are not only critical for flood defense, but also for the overall health and sustainability of coastal ecosystems. These areas have profound differences in values, critical issues, and opportunities for recovery, the study of which is essential to guide actions aimed at rebalancing and restoration (Bonometto, 2014).

An example of salt marsh restoration is the LIFE VIMINE project to counter erosion processes. LIFE VIMINE, funded by the European Commission, focuses on the protection of salt marshes in the northern part of the Venetian lagoon through an integrated soil bioengineering approach, through semi-manual work to reduce environmental impacts (Barausse et al., 2015).



Fig. 19, Fascines produced locally -upper left- and then placed to protect salt marsh edges -upper right and bottom- (Barausse et al., 2015).

The LIFE VIMINE project presents an innovative approach to combating erosion and places strong emphasis on continuous monitoring and maintenance to ensure the long-term effectiveness of protection measures put in place (Barausse *et al.*, 2015).

The integrated approach involves various protection techniques, especially for salt marsh edges: wooden poles screen address intense erosion, gabions (tubular structures) dissipate wave energy, and wooden fascines (Fig.19) to protect against low-energy erosion damage. The project also involves moving sediments to counter erosion and transplanting vegetation to stabilise the soil (Barausse *et al.*, 2015).

By addressing erosion with a combination of innovative techniques, minimizing environmental impact, and incorporating continuous monitoring and stakeholder involvement, the LIFE VIMINE project stands out as a successful example of salt marsh restoration. Unfortunately, not all the restoration actions have been proven as effective and artificial salt marshes, designed to emulate natural environments, sometimes have proved unsuccessful.

From this picture, it can be deduced that the presence of a highly articulated morphology, consisting of time-varying microenvironments with complicated

balances and interdependencies at different levels, challenge artificial proposals. At this point, it is necessary to inquire into the conditions under which these anthropogenic ecosystems function and, more importantly, to understand the reasons for their success or failure.

Nowadays, it also presented the new need to align with the emerging dynamics influenced by climate change, as trying to restore the lagoon to a previous state may not be effective given the changed conditions(Tagliapietra et al., 2018).

## **1.3 Objectives of the study**

The aim of the thesis is to investigate the ecological differences between natural and artificial salt marshes in the Venice Lagoon, analyzing their different degrees of biological and physical deviation.

- The first part of the investigation was carried out in the field, through sampling analyses in five pairs of salt marshes, each pair represented by an artificial and a natural one. The couples have been chosen geographically close to each other, in order to minimize as much as possible factors external to those sampled, which could introduce elements of confusion.
- Data collected on the field was then analyzed to understand the main differences between the plants communities of artificial and natural salt marshes and to highlight which abiotic factors were the most important in determining those differences.
- Finally, to enhance further knowledge on this topic, for future salt marshes restoration projects, it was adapted to the dataset collected, a predictive Generalized Additive Model (GAM) of ecological niche of plants communities of natural and artificial salt marshes.

The generalized additive model (GAM) identifies the points at which the relationship between an independent variable (e.g. altitude) and the dependent variable (e.g. abundance of a species) is optimal. This model may be a functional tool not only to describe the current situation but also to help the design of future restoration works in the Venice lagoon.

In conclusion, this thesis aims to provide, on the basis of the results of the sampling research and GAM modelling, insights that could contribute to help in the sustainable management of these significant environments.

## **CHAPTER 2 - MATERIALS AND METHODS**

#### 2.1 Areas and study period

The sampling project focused on the Central Lagoon of Venice and especially the Southern Lagoon. A coupled design was chosen to be able to have data from artificial and natural salt marshes that were close to each other and were, consequently, affected by similar external conditions. Therefore the comparative analysis of artificial salt marshes provides an insight into the ecological dynamics at play.

Of the five pairs of salt marshes initially selected, one (B3) was excluded from the analysis because the artificial one (B3A) was in such a compromised condition due to erosion and submersion that it was impossible to carry out meaningful samplings.

Hence a total of four pairs was examined, i.e. four natural salt marshes (called B5N, B6N, B8N, B9N) and their artificial counterpart (B5A, B6A, B8A, B9A).

The number salt marshes tags were chosen based on their distance from Chioggia, which is located in the Southern part of the Lagoon (Fig. 20).



Fig 20, Image made with QGis software, using the Google Satellite package. The lines divide the Venice Lagoon in three parts, following the Lagoon's inlets. B8 and B9 are located in the Central Lagoon, instead B6, B5 and B3 are located in the Southern Lagoon.

In the area from Marghera to Chioggia, salt marshes have developed on easily eroded peat and marsh clay soils (Favero *et al.*1983). As shown in Fig. 20, salt marsh B8 is the one closest to the mainland; in this area, salt marshes are residuals of a freshwater environment and generally show a gradual tendency to submergence and erosion, especially in the fronts exposed to the winds (Favero *et al.*1983). On the other hand, salt marsh B6 is the furthest from the mainland, which suggests that it may be affected by tidal fluctuations more directly than other salt marshes.

Salt marshes B6 and B5 are the closest to the Malamocco inlet, which constitutes an important link between the sea and the Lagoon, and it is precisely the entry of sediment from the sea that favours the development of these salt marshes (Favero *et al.*1983). These salt marshes, generated by lagoon processes but influenced by the littoral belt, have an evolution linked to these conditions and show active dynamics (Favero *et al.*1983), linked for example to the ebb and flow of tides and currents from the open sea.

Lastly, the B9 salt marsh is very close to the Porto Marghera area, an important industrial zone in the metropolitan city of Venice. This proximity suggests that it could be subject to the impacts of industrial and port activities. For example, in many areas throughout Porto Marghera, groundwater may come into contact with materials contaminated by pollutants (Master Plan for the remediation of pollutant sites in Porto Marghera, final version - June 2004).

Below are the specifications of each pair, comparing the areas before and after the construction of each the artificial salt marsh:



Pair B5 (BA5 sampled on 04/06/23, BN5, sampled on 01/06/23)

Fig. 21 Image obtained from Google Earth, using "historical imagery" instrument. Each salt marsh has been tagged with the letter B ("Barena") and the adjective N (natural) or A (artificial). The comparison between before the construction of the artificial salt marsh (BA5) and after is visible; 2023 vs 2010.



• Pair B6 (BA sampled on 04/05/23, BN, sampled on 17/04/23)

Fig. 22 Image obtained from Google Earth, using the "historical images" tool. Each salt marsh was tagged with B ("Barena") and the adjective N (natural) or A (artificial). The comparison between before the construction of the artificial salt marsh (BA6) and after, is visible; 2023 vs 2010. This image shows how the BA6 salt marsh incorporates a part of a natural one.



• Pair B8 (BA sampled on 02/10/10, BN, sampled on 10/10/23)

Fig. 23 Image obtained from Google Earth, using "historical imagery" instrument. Each salt marsh was tagged with B ("Barena") and the adjective N (natural) or A (artificial). The comparison between before the construction of the artificial salt marsh (BA8) and after, is visible. This image shows how the BA6 salt marsh includes a part of natural salt marsh; 2023 vs 2008.



• Pair B9 (BA sampled on 17/07/23, BN, sampled on 01/08/23)

Fig. 24 Image obtained from Google Earth, using "historical imagery" instrument. Each salt marsh was tagged with B ("Barena") and the adjective N (natural) or A

(artificial). The comparison between the beginning of the construction of the artificial salt marsh (BA3) and after it was finished, is visible; 2023 vs 2010.



• Pair B3 (BA3 not sampled, BN3, sampled on 03/07/23)

Fig. 25 Image obtained from Google Earth, using "historical imagery" instrument. Each salt marsh was tagged with B ("Barena") and the adjective N (natural) or A (artificial). The comparison between before the construction of the artificial salt marsh (BA8) and after, is visible; 2023 vs 2008.

During the sampling activity which took place from April 2023 to October 2023, a wide variety of climatic conditions were faced. This period covered several seasons, each characterized by a distinctive climate, for example, in the summer of 2023 we recorded temperatures reaching 35°C, highlighting a phase of intense heat.

It is important to note that at certain times, due to the sometimes adverse weather conditions, the collection of samples had to be postponed, for example, in the month of October, exceptional high tides were encountered which made it impossible to carry out some sampling operations at the time of the boat trip.

## 2.2 Preparation of sampling equipment

Preliminary sampling operations were carefully planned before each boat trip, including:

- A consultation on tide conditions during the colder months and weather conditions during the warmer ones. The choice of departure time must be guided by the tides, as some salt marshes may be submerged (especially the natural ones) or some areas may be difficult to reach or inaccessible.
- The definition of three hypothetical transects at different concentric bands (low marsh-mid marsh-high marsh) and a preliminary study on the shape of the salt marsh (Fig. 26). This allowed us to be ready to move within the sampling area with prior knowledge of its characteristics, such as the presence of internal channels.



Fig. 26, Salt marsh B5N. Example of transects hypothesized on the basis of the image provided by Google Earth. Three transects have been marked, the green one identifies the low part, the yellow one for the mid one and the red one for the high one.

- The numbering and placing of specific tags on the bags intended for collecting sediment samples. This measure has proven particularly useful during the warmer months, allowing us to proceed more quickly and avoiding possible confusion or slowdowns during sampling.
- The preparation of two tables to be printed in advance of departure: one to be filled in to note the assigned percentage of each plant species sampled:

Sample	Sarcocornia	Salicornia	Limonium	Spartina marittima	Spartina anglica	Juncus	Green algal mat	Red algal mat	Bare soil	Seagrasses	Aster	Salsola	Halymione	Puccinelia	Inula	Sueda m

Fig. 27, vegetation sampling table, used in the field.

the other to record the redox potential and further observations on the condition of the salt marsh:

Sample	Height	Redox potential	Vegetation Sample	Soil sample	Contermination types	Conterminatio n conditions (Scales)	Missing poles (%)	Damaged fascines (%)	Encrusting organisms

Fig.28, other parameters sampling table, used in the field.

These tools provided us with fundamental support in collecting accurate and detailed data.

### 2.3 Field sampling methodology and instruments

The field sampling methodology and instruments used in this study involved a systematic approach to data collection.

Initially, it was planned to sample a pair of salt marshes per day, but due to time and logistical constraints, sampling one salt marsh at a time was the only option. This decision was also influenced by the sometimes adverse weather conditions.

It was decided to categorize each salt marsh into three concentric bands, namely "low marsh", "middle marsh", and "high marsh", following the classic zoning representation, as explained in the literature by N. Anoè et al., 1984 (see "Introduction") and the transects were then drawn based on their position.

In each area of study, 10 geographical points were sampled: 5 for the putative low marsh, 5 for middle marsh, following transects. In some rare cases (e.g. B8A) and especially in artificial salt marshes, it was also possible to sample the high marsh, in the presence of particularly elevated areas. However, very often, the high marsh was not large enough to carry out a transect, but rather only sampling in a single point and for this reason this level was decided to be excluded from the subsequent analysis.

After arranging the transects, different points along them were chosen at more or less regular distances. Although the selection of transects was planned, the specific choice of points within each transect was influenced by random selection to ensure the randomness of the sampled plant patterns (Fig. 29). Random choosing of the sampling points is particularly useful for obtaining representative and non-subjective data on vegetation cover, avoiding potential biases.



Fig. 29, Image created with QGIS software. Transects were made using salt marsh B8A data acquired by the GNSS altitude instrument (GeoMax zenith GNSS), and the map was created afterwards. Low altitude areas are shown in green, medium altitude areas in red, and high altitude areas in yellow.

The sampling was structured based on the analysis objectives:

#### Study of vegetation cover:

This was done through quadrat sample frames (Fig. 30 and 31) measuring 50 cm x 50 cm, placed randomly, directly on top of the vegetation at specific intervals. Within each quadrat, vegetation information was recorded. Each quadrat has 25 square units within it, which means that it is divided into a grid of 5x5 square units and that one unit (1/25) corresponds to 4% of the total vegetation cover inside the quadrats (Fig. 30).

The assessment of the vegetation cover was carried out by means of the method of squares with multiple assessors (minimum two), where each has the task of assessing the number of units covered for each species within a given sample square.

After both assessors have completed their assessments, the results can be compared and any discrepancies discussed.

The categories sampled included:

- The vegetation typically present in salt marshes, such as *Sarcocornia spp.*, *Salicornia* spp. and *Limonium* spp. (Fig. 27)
- The bare soil, a level that proved to be very effective during the pair comparison analyses
- Presence of stranded seagrasses
- Presence of green algal mat and red algal mat



Fig. 30, Photo taken on the field, showing the quadrat used and the redox potential survey tool EcoTech (on top). One of the square units is highlighted in orange, constituting 4% of the total.


Fig. 31, Photo taken on the field, showing the quadrat used and the soil corer tool (at the bottom).

#### Altitude detection:

We conducted elevation measurements using the GNSS (Global Navigation Satellite System) altitude instrument, GeoMax zenith GNSS, assessing the altitude value from the center of each quadrat sample frame. The instrument is composed of a pole with adjustable height (which was set at 2m for this analysis), for mounting the Zenith GNSS receiver, the GNSS receiver and a tablet that connects to the device via the XPad application.

The GeoMax Zenith GNSS is a GNSS-based position tracking system that utilizes signals from satellites to determine precise locations on Earth. The receiver captures signals from GNSS satellites, containing information about the satellite's position. Using trilateration, it calculates its own position by measuring signal travel times from multiple satellites. Once determined, it provides accurate latitude, longitude, and altitude data for its location.

(Source: Zenith60 model data sheet, <u>https://geomax-positioning.com/it-it/products/gnss/zenith60</u>).

The precision and performance specifications of a GNSS receiver are as follows:

• RTK (Real-Time Kinematic) mode: Hz 8 mm ± 1 ppm (rms) V: 15 mm ± 1 ppm (rms)

• Real-Time Cinematic Compensated with Tilt: additional horizontal uncertainty of 2 cm up to an inclination of 30° (Source: Zenith60 model data sheet, <u>https://geomax-positioning.com/it-it/products/gnss/zenith60</u>).

Subsequently, the geolocalized points obtained, generated via the Xpad application, were downloaded to acquire data regarding specific elevation, longitude, altitude and other information pertinent to each point (Fig. 32).

Name	Lat.WGS84	Long.WGS84	Alt.WGS84	Date	Time
B8AL1	45 23 09.8667	12 11 13.3839	43.790	02/10/23	12:33:20
B8AL2	45 23 09.5709	12 11 12.9224	43.802	02/10/23	12:34:05
B8AL3	45 23 09.2915	12 11 12.2583	43.694	02/10/23	12:35:45
B8AL4	45 23 09.1323	12 11 11.4829	43.659	02/10/23	12:41:36
B8AL5	45 23 09.3118	12 11 10.8550	43.678	02/10/23	12:38:26
B8AM1	45 23 10.1963	12 11 10.3459	43.826	02/10/23	12:00:12
B8AM2	45 23 10.1778	12 11 10.9543	43.880	02/10/23	12:02:21
B8AM3	45 23 10.2060	12 11 11.8072	44.003	02/10/23	12:06:07
B8AM4	45 23 10.2449	12 11 12.5503	43.951	02/10/23	12:11:40
B8AM5	45 23 10.2757	12 11 13.4301	43.844	02/10/23	12:32:28
B8AH1	45 23 10.9262	12 11 12.0913	44.291	02/10/23	12:43:47
B8AH2	45 23 10.9226	12 11 12.4071	44.152	02/10/23	12:44:37
B8AH3	45 23 10.8961	12 11 12.7831	44.140	02/10/23	12:45:08
B8AH4	45 23 10.9350	12 11 13.1666	44.172	02/10/23	12:45:58
B8AH5	45 23 10.9648	12 11 13.5009	44.203	02/10/23	12:46:30

Fig. 32, Table of elevation point sampled, provided by XPad. Artificial salt marsh B8A.

The altitude data were then processed for statistical analysis using R software.

It was necessary to subtract 43 meters and 0.2356 meters from the values of the sampled ellipsoid heights. These values represent respectively the ellipsoid height measured with the GNSS instrument at Chioggia Punta San Felice (considered as the current reference for sea level) and the combined effect of subsidence and eustasy for the Lagoon as a whole (Saretta *et al.*, 2009).

Collection of soil samples:

Adjacent to each quadrat, sediment samples were extracted using a corer boasting a diameter of precisely 9.97 cm and a height of 10 cm. Once gathered, the sediment samples were promptly placed into tagged bags, each bearing a unique label such as L1 (first Low marsh of the transect), L2 (second Low marsh of the transect), and so forth, for accurate identification and record-keeping purposes.

Subsequently, the samples were transported to the laboratories located at the Chioggia Hydrobiological Station, where they underwent analysis and assessment.

#### Redox potential measurement:

The redox potential was measured using the dedicated ecoTech Umwelt-Messsysteme instrument in the quadrats or in the immediate vicinity, recording it in the table (Fig. 28)

The ecoTech redox potential instrument with reference and measuring electrode is used to assess the redox potential of a medium, which indicates its ability to undergo oxidation or reduction reactions.

- The reference electrode (Ag/AgCl reference electrodes) is inserted into the soil so that it is fully immersed and provides a constant voltage as a reference point for measuring the redox potential.
- The measuring electrode (redox electrode acc. to Mansfeldt), partially embedded in the soil, detects changes in the redox potential in the sediment.

These changes are converted into an electrical signal which the ecoTech unit interprets and displays as a numerical reading. The value can be used to assess the oxidation or reduction capacity of the soil, an important element in the management of terrestrial ecosystems (Source: <u>https://www.ecotech.de/</u>).

	Laboratory	Field
Conduction system:	Ag/AgCl	Ag/AgCl
Shaft		
Material:	PVC	PVC
Diameter:	6 mm	12 mm
Length:	80 mm	110 mm
Filling:	3 M KCI+AgCI	3 M KCI+AgCI
Diaphragma:	Ceramic	Ceramic
Salt bridge		
Material:	PVC	PVC
Diameter:	12 mm	25 mm
Length:	130 mm	400 mm (others on request)
Gel-Filling:	3 M KCI+AgCI	3 M KCI+AgCI
Cable		
Length:	3 m, other lengths availables on request	3 m, other lengths availables on request
Fig. 22		

Technical data on the ecoTech reference electrode:

Fig. 33 (Source: ecoTech, https://www.ecotech.de/en/product/agagcl\_reference\_electrodes)

Measuring range:	-1 +1 V
Resolution:	1 mV
Platinum rod	
Material:	99,95 % Pt, hard drawn
Length:	L = 5 mm
Diameter:	1 mm
Shaft	
Material:	Carbon fiber
Length:	30 cm, other lengths available on request
Diameter:	6 mm
Cable	
Length:	3 m, other lengths available on request
Fig. 34	

Technical data on ecoTech measuring electrode:

Fig. 34 (Source: ecoTech, https://www.ecotech.de/en/product/redox electrode acc to mansfeldt)

Overall, we encountered from time to time some difficulties during sampling due to less than ideal conditions of the salt marshes, influenced by tides, degradation and erosion, for example artificial salt marsh B3, which was not sampleable. Summer temperatures slowed down sampling and there were some difficulties in moving inside the salt marsh during the spring period due to the high quantity of mud.

## 2.4 Sediment sieving and granulometry analysis

Sediment samples were collected in the field using the corer and subsequently airdried in the laboratory. They were equally divided between "low marsh" and "mid marsh" (and "high marsh" if present) zones, and each was named according to the sampling point, corresponding to the square (e.g. BA5L1).

As soon as possible the samples were placed in an ARGOLAB TCN50 Plus oven at 105 °C for a period of 72 hours to ensure complete evaporation of the water (Margesin *et al.*, 2005). This step was essential to exclude the weight of water from subsequent measurements, allowing us to accurately determine the sediment content. After a one-day waiting period to allow the samples to cool down to a manageable temperature, the following steps were performed:

- Each dry sample was weighed in its entirety with KERN precision laboratory balance (to two decimal places).
- A quantity of the total sample was taken and weighed with the KERN precision laboratory balance to reach approximately 50 grams.
- These 50 grams were then manually pounded with a ceramic mortar until the sediment was completely disintegrated, thus ensuring the homogeneity of the mixture.
- The sediment was then passed through a sieves stack to separate particles of different sizes in the soil sample. These sieves are typically composed of a series of frames with varying mesh sizes, stacked to allow smaller particles to pass through and retain larger ones. The mesh size of the sieves stack used are:
  - $\cdot > 1 \text{mm}$
  - · 1mm 500 μm
  - · 500 μm 250 μm
  - · 250 µm 63 µm
  - $\cdot < 63 \ \mu m$

This procedure allowed the sediment to be classified according to its particle size; the clay fraction was obtained by sieving less than  $<63 \mu m$ .

For each sieve, the sediment fraction was weighed using the KERN precision laboratory balance, starting with the largest particle size down to a particle size below 63µm.

The individual fractions from the various samples were then added together to obtain a final weight, allowing an assessment of how much sample was lost in the particle size classification process. This was possible due to the comparison with the total weight of the sample, measured at the beginning of the seed analysis.

For each sieving, Excel tables were created to clearly display the percentages of each sediment fraction, allowing the largest and smallest presences within the samples to be identified:

- $\cdot > 1$ mm = very coarse sand
- $\cdot$  1mm 500  $\mu$ m = coarse sand
- $\cdot$  500  $\mu m$  250  $\mu m$  = medium sand
- $\cdot$  250 µm 63 µm = very fine sand
- $\cdot$  < 63  $\mu$ m = clay and silt

According to The Udden-Wentworth 1922 scale of grain size classification (Fig. 13)

Finally, 20 grams of sediment were taken from the initial sample total and analyzed for organic matter content by combustion in a muffle furnace at 550 degrees for 5 hours (followed by a half-day cooling period). The samples were then weighed to determine the organic matter (Om) content in the sediment (Denef et al. 2001).

The weight of organic matter was then obtained by subtracting the final weight of the sample (after combustion) from the initial weight of the sample (before combustion). This difference represents the amount of organic matter present in the sample.

Another important data collected was the Bulk Density (BD), which was determined with the ratio of the dried sample weight to the corer volume (Thorne *et al.* 2014), which was 441.80 cm<sup>2</sup> in our case.

## 2.5 Data analysis

#### Initial analysis

A preliminary exploration of the data was conducted through the use of Excel tables to represent the results of the sampled vegetation coverage. The primary objective of this phase was to obtain an overall view of the distribution of plants communities in the different squares of the study area.

The information gathered during the analyses was entered into Excel tables, organized according to the sampled squares. This structuring of the results allowed a detailed visualization of the levels of plant cover in each square, providing a comprehensive overview of the occupation of the soil by vegetation. The first step was to verify whether each square achieved 100% cover, between vegetation and bare soil.

Subsequently, after the grain-size analysis was performed, the data obtained were used to create bar graphs using Excel. These graphs are fundamental visualization tools in the graphic representation of grain size information. The creation of such bar graphs makes it possible to visually grasp the development of sediment sizes in the various salt marshes.

#### R analysis

At a later stage of data analysis, the statistical programming language R (Version 2021.09.2-382, RStudio Team, 2022) was employed for more advanced processing and an in-depth understanding of the dissimilarities between natural and artificial salt marshes.

Graphs were created using the 'ggplot2' package to explore the relationship between variables. Then, ANOVAs (Analysis of Variance), applied using R software, were used to determine whether the differences observed in the graphs were statistically significant.

#### **Primer analysis**

Using Primer7 & Permanova software the following multivariate analysis were performed.

Initially the dataset was visualized through non metric Multi Dimensional 82 Scaling (nMDS). Abundance data was log transformed (Anderson et al, 2008).

A PERMANOVA analysis was then performed with 'Saltmarsh type' (2 levels: Natural, Artificial) and the "Saltmarsh code" (4 levels: B5, B6, B8, B9) as factors on a Bray-Curtis similarity matrix. Salt marsh type was selected as the fixed factor, while Saltmarsh code as the random factor. A SIMPER analysis (Similarity Percentages) later facilitated the exploration of which species contributed the most to the dissimilarities between groups.

Environmental Data was normalized and the differences between the sites were explored through a PERMANOVA analysis performed on a Euclidean distance matrix. A SIMPER analysis was also performed to understand which abiotic variables determine the most dissimilarities (Anderson et al, 2008).

To explore the correlation of the variability in plants communities and in environmental variables we operated a DistLM analysis (Distance based linear models).

The DistLM (Distance-based Linear Models) analysis includes two distinct worksheets: Resemblance and Predictor Variables. The stepwise method was selected for the best model to identify the most significant predictors influencing the measured similarities between sampling units. This type of analysis evaluates how predictor variables influence measured similarities, providing a detailed understanding of relationships between variables and similarities/distances between sampling units.

## 2.6 GAM model methodology

An ecosystem is a biotic and functional system or unit capable of supporting life and includes all the biological and non-biological variables present in that unit; modelling and computer simulation is a common approach to study of ecosystems.

Main advantages of using models are related to study complex systems, reveal system priorities and weaknesses in our knowledge, but also to test scientific hypotheses by simulating ecosystem reactions (Jrargensen and Bendoricchio, 2001).

Models can be used to study an ecosystem and select the most suitable environmental technology to solve the ecological problem under investigation, to study the complexity of an ecosystem and also they are useful to use as a synthesis tool (Jrargensen and Bendoricchio, 2001).

In its mathematical formulation, a model in environmental science, has the following components:

• <u>Forcing functions or external variables</u>: functions or variables of an external nature that affect the state of the ecosystem; if these forcing

functions are varied, how will it affect the state of the ecosystem? The model wants to predict what will happen if.

In the case of this study the external variables are considered salt marsh elevation, soil redox potential, soil different grain-size, soil organic matter and calculated bulk density.

• <u>State variables</u>: a quantity representing the state or condition of a system at a given time. In the case of this study, the state variables is the percentage of vegetation coverage of each species sampled.

Modelling with the R programme was chosen to represent the sampled research data.

In the course of the research, R programming language was used in conjunction with the "gam" package to create a Generalized Additive Model (GAM) and the "mgcv 1.9-1" package. GAMs are semi-parametric extensions of generalized linear models, useful for fitting nonlinear relationships without prior assumptions on the shape of the response, and has been shown to perform favorably compared to other novel methods (Lou *et al.*, 2018).

First, an Excel table containing the information required for the analysis was used. Within the table, the following variables are identified:

- Continuous response/dependent variable: percentage of vegetation coverage of each species sampled (e.g. *Sarcocornia spp., Salicornia spp., Limonium spp.*).
- Continuous predictive/independent variables: Altitude (m), Redox (mV), Grain-size (g), Organic matter (%) and Bulk density (g/cm<sup>3</sup>).
- Categorical predictive/independent variable: 'Saltmarsh type' (indicated as N or A)
- Categorical random variable: 'Saltmarsh code' (B5, B6, B8, B9)

The process of creating the model was divided into several steps:

• Creation of the design matrix: Initially, a design matrix was created using the variable 'Saltmarsh type', which is a categorical variable, including the designation N for natural and A for artificial. To interpret the coefficients of the categorical variable as fixed effects, the intercept was removed from the model. This was done by using the -1 option in the design matrix creation function.

> dummy <- model.matrix(~ Saltmarsh\_type - 1, data = my\_dataset)</pre>

• Combination of the design matrix with the dataset: Next, the 'dummy' design matrix was combined with the original 'mydataset' dataset using the 'cbind' (column bind) function. This allowed the columns of the 'dummy' matrix to be added to the dataset.

> data\_with\_dummy <- cbind(my\_dataset, dummy)</pre>

- Specify Saltmarsh\_code as a categorical random variable: In order to indicate to the model that 'Saltmarsh code' is a categorical random variable, which encapsulates the distinction for each salt marsh (B5,B6,B8,B9), the term bs ="re" was used with a 'random effect' basis to model this variable
- Creation of the GAM model: Finally, the GAM model was created using the 'gam' function of the 'gam' package. In the model code below, 'Sarcocornia' was defined as the response variable, while all other variables preceded by 's()' were considered as predictor variables.

```
> Modello <- gam(Sarcocornia ~ s(Altitude) + s(Redox) + s(a) + s(b) + s(c) + s(d)
+ s(e) + s(BD) + s(Omc) + s(Saltmarsh_code, bs="re") + Saltmarsh_type, data =
data with dummy, family = gaussian(), method = "REML")
```

```
The terms "a, b, c, d, e" represent the different grain sizes obtained by sieving during laboratory analysis, then in order:
```

- $\cdot > 1$ mm = a
- $\cdot 1$ mm 500  $\mu$ m = b
- $\cdot$  500  $\mu m$  250  $\mu m$  = c
- $\cdot 250 \ \mu m 63 \ \mu m = d$
- $\cdot < 63 \ \mu m = e$

Moreover, "Omc" represents the Organic material content detected in the samples and "BD" the measured Bulk density.

Within the model code, there are also two key parameters: 'family' and 'method'. The parameter 'family' was set to 'gaussian()', indicating that it is a generalized linear model with a normal error distribution.

The 'method' parameter was set to 'REML' (Restricted Maximum Likelihood), which was used to estimate the model parameters.

These parameters are crucial for determining the error distribution and the method of estimating the model parameters, which are essential for correctly interpreting the results obtained.

Finally, summary() and draw() from the "gratia" package were used to display the results.

With this GAM model, the sampling results were interpreted to obtain a better understanding of the relationships between the variables and the species of interest. A second model, i.e. a mixed model (GLMM), was also created to broaden the understanding of the data in its complexity. However, during the analysis, the presence of some outliers was noted that could have negatively affected the accuracy of the mixed model. These outliers could have compromised the mixed model's ability to provide accurate predictions.

In fact, the Generalized Additive Model (GAM) is known to be more robust when it comes to handling data with non-linear patterns or with the presence of outliers. In addition, the variables in the dataset produced showed smoother patterns, which suggested that GAM might be a more suitable option.

After a careful evaluation, GAM model was chosen for the analysis. This choice proved to be the best as GAM was able to more accurately and flexibly capture the structure of the data, providing more reliable results than the mixed model, which had difficulty handling the outliers in my dataset.

## **CHAPTER 3 - RESULTS**

#### 3.1 Displaying sample data

Comparative analysis between plants communities in Artificial and Natural salt marsh ecosystems:



Fig. 35, The boxplot, created using R software, shows the data distribution of percentage of vegetation coverage for each species in the sampled natural and artificial salt marshes.

The distribution of the data collected, through the boxplot (Fig. 35), highlights several interesting aspects. First of all, in the comparison between bare soil and vegetation cover, bare soil exhibits a higher median (Q2) in artificial salt marshes. This presents a difference in the quantity of unvegetated soil between artificial and natural environments.

Furthermore, *Salicornia* spp. has a Q2 higher in artificial salt marshes. On the other hand, *Limonium* and *Sarcocornia* genus show higher average medians in natural marshes. Another important note is that the presence of *Puccinellia* spp. is generally higher in natural marshes.

In general, outliers are observed in the dataset.



Ν

## Comparative analysis between bare soil coverage in Artificial and Natural salt

Fig 36, Distribution of data regarding bare soils in artificial and natural salt marshes.

Saltmarsh type

After the graphic rendering of the overall picture (Fig 35), in Fig 36 it was investigated the distribution of data between the bare soil sampled in the natural and artificial salt marshes.

Interesting is the higher Q2 in artificial salt marshes.

Δ

0.25

0.00

An Anova analysis was then performed to assess statistical significance to determine whether the differences between the averages are statistically significant. It is useful to understand whether the observed differences are random or not.

ANOVA BARE	SOIL:				
Calture analy taxes	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Saltmarsn_type	1	0.3104	0.3104	12.78	0.000000
Residuals	78	3.1160	0.0399		

A significant (p-value = 0.000606) difference can be observed between these two habitats. The box plot (Fig. 36) suggests that in artificial salt marshes, we have a significantly higher percentage coverage of bare soil compared to natural ones.

E A 苗 N

# Comparative analysis between percentage of soil parameters in Natural and Artificial salt marsh ecosystems:



Fig. 37a, The boxplot, created using R software, shows the data distribution of soil parameters sampled for natural and artificial salt marshes.

Fig. 37a shows that the soil grain size above 1 mm (very coarse sand) has the highest median between all the grain sizes in the natural habitat, but is between lowests in the artificial ones, after the silt (sediment <  $63 \mu$ m). I

n addition, artificial salt marshes are composed of a large proportion of 500  $\mu m$  - 250  $\mu m$  sediment, classified as medium sand.

They also have a higher bulk density and a lower percentage of organic material content, than their natural counterparts.



Comparative analysis between redox potential in Artificial and Natural salt marsh ecosystems:

Fig. 37b, Distribution of data regarding soil redox potential in artificial and natural salt marshes.

In Fig 37b the distribution of data between the redox potential sampled in the natural and artificial salt marshes was investigated, created with R software. It's noticeable the difference between redox potential distribution in natural and artificial environments, with a higher median in artifical ones. An Anova analysis was performed to assess statistical significance to determine whether the differences between the averages are statistically significant.

ANOVA REDOX POTENTIAL:

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Saltmarsh_type	1	703875	703875	36.64	4.67e-08 ***
Residuals	78	1498523	19212		

A significant (p-value = 4.67e-08) difference can be observed between the two habitats. The box plot (Fig.37b) suggests that in artificial salt marshes there are significantly higher levels of redox potential, both overall and on average, compared to natural salt marshes.

## 3.2 Multivariate analyses

## 3.2.1 Abundance data

Source	df	SS	MS	Pseudo- F	P(perm)	Unique perms	P(MC)
Saltmarsh_code	3	18790	6263,4	3,1822	<u>0,001</u>	998	0,001
Saltmarsh_type	1	17347	17347	4,3322	<u>0,051</u>	362	0,021
Saltmarsh_code x Saltmarsh_type	3	12013	4004,3	2,0344	<u>0,017</u>	999	0,019
Res	72	4172E5	1968,3				
Total	79	1,8987E5					

Table 1- PERMANOVA table of results of abundance data:

There is a significant interaction (p value= 0,017) between the location (salt marsh code) and the type of salt marsh (N or A) in determining the differences in the communities.

Consequently, a pair-wise PERMANOVA tests were carried out to assess the differences between the pairs of salt marshes.

In the analysis 'N' and 'A' refer to natural and artificial groups within a specific salt marsh. PERMANOVA is used to determine whether significant differences in multivariate vegetation compositions exist between 'A' and 'N'. The average similarity between/within groups is calculated, within the level, in percentage.

Table 2 - Pairwise PERMANOVA results, within level 'B5' of factor 'Saltmarsh code':

Groups	t	P(perm)	Unique perms	P(MC)
N, A	2,2471	<u>0,006</u>	993	0,005

Table 5 - Average similarity between/within gloups within level b5	Tabl	e 3 - Avera	age similarity	y between/within	groups within	level 'B5':
--	------	-------------	----------------	------------------	---------------	-------------

	Ν	A
Ν	49,986	
A	<u>41,936</u>	59,224

There is a significant difference between artificial and natural B5 with an average similarity of 42%

Table 4 - Pairwise PERMANOVA results, within level 'B6' of factor 'Saltmarsh code':

Groups	t	P(perm)	Unique perms	P(MC)
N, A	2,1348	<u>0,001</u>	992	0,003

Table 5 - Average similarity between/within groups within level 'B6':

	Ν	Α
Ν	37,489	
A	26,229	39,44

There is a significant difference between artificial and natural B6 with an average similarity of 26%

Table 6 - Pairwise PERMANOVA results, within level 'B8' of factor 'Saltmarsh code':

Groups	t	P(perm)	Unique perms	P(MC)
N, A	1,3687	0,117	991	0,149

Table 7 - Average similarity between/within groups within level 'B8':

	Ν	Α
Ν	29,789	
A	<u>31,505</u>	41,738

There is not a significant difference between artificial and natural B8 with an average similarity of 31%

Table 8 - Pairwise PERMANOVA results, within level 'B9' of factor 'Saltmarsh code':

Groups	t	P(perm)	Unique perms	P(MC)
N, A	2,0818	<u>0,01</u>	994	0,014

Table 9 - Average similarity between/within groups within level 'B9':

	Ν	Α
N	39,683	
A	32,372	43,868

There is a significant difference between artificial and natural B9 with an average similarity of 32%

## MDS DISPLAYING

MDS analysis depicts the arrangement of objects in a two-dimensional space, reflecting the relationships of similarity or difference between them. Through these graphs (Fig. 38 and Fig. 39), it is possible to better visualize the distance between each natural and artificial salt marsh sampled, but also the differences observed in the overall picture.



Fig 38, Abundance MDS displaying, taking into consideration the factor Saltmarsh type.



Fig. 39, Abundance MDS displaying, taking into consideration the factors Saltmarsh type and Saltmarsh code.

In MDS displaying (Fig. 38 and Fig. 39) the distances between vegetation abundance in natural (N) and artificial (A) salt marshes are present, but not so defined.

#### SIMPER ANALYSIS

The SIMPER analysis shows which biotic variables contributed the most to the dissimilarities between groups:

• Groups B5N & B5A Average dissimilarity = 58,06

Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bare soil	0,04	0,23	11,86	1,40	20,42	20,42
Sarcocornia	0,29	0,38	10,66	1,36	18,36	38,79
Limonium	0,18	0,03	10,02	1,22	17,25	56,04
Puccinellia	0,13	0,03	6,57	1,42	11,32	67,36
Salicornia	0,08	0,06	6,31	0,85	10,87	78,23
Green algal mat	0,03	0,08	4,73	1,56	8,14	86,37
Juncus	0,07	0,00	4,05	0,60	6,97	93,34

With an average dissimilarity of 58% salt marshes B5N and B5A differ, at a biomass level, in different amounts of bare soil, *Sarcocornia* spp. and *Limonium spp*.

• Groups B6N & B6A Average dissimilarity = 73,77

Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bare soil	0,04	0,29	16,62	0,99	22,53	22,53
Limonium	0,19	0,00	11,32	1,11	15,34	37,87
Sarcocornia	0,23	0,13	10,83	1,33	14,68	52,55
Salicornia	0,04	0,18	10,25	0,97	13,90	66,45
Green algal mat	0,11	0,15	8,97	1,24	12,17	78,62
Puccinellia	0,08	0,05	5,03	1,13	6,81	85,43
Juncus	0,07	0,00	3,99	0,48	5,40	90,84

With an average dissimilarity of 74% salt marshes B6N and B6A differ, at a vegetation cover level, in different amounts of bare soil, *Limonium* spp.and *Sarcocornia* spp.

• Groups B8A & B8N Average dissimilarity = 68,79

Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Sarcocornia	0,23	0,32	19,25	1,15	28,10	28,10
Limonium	0,18	0,15	14,09	1,13	20,58	48,68
Bare soil	0,16	0,04	8,80	1,59	12,85	61,52
Puccinellia	0,08	0,13	8,74	0,96	12,77	74,29
Juncus	0,00	0,09	5,40	0,54	7,88	82,17
Salicornia	0,09	0,00	5,24	1,18	7,66	89,83
Aster	0,05	0,01	3,55	0,39	5,19	95,01

With an average dissimilarity of 69% salt marshes B8N and B8A differ, at a vegetation cover level, in different amounts of *Sarcocornia spp., Limonium spp.* and bare soil

#### • Groups B9A & B9N Average dissimilarity = 68,25

Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Salicornia	0,26	0,10	13,88	1,29	20,52	20,52
Limonium	0,14	0,29	12,24	1,58	18,10	38,62
Sarcocornia	0,12	0,13	9,86	1,13	14,58	53,20
Green algal mat	0,16	0,00	9,20	1,40	13,60	66,80
Puccinellia	0,11	0,07	5,91	1,13	8,74	75,53
Bare soil	0,03	0,10	5,71	1,06	8,45	83,98
Juncus	0,00	0,06	3,82	0,49	5,65	89,63
Spartina anglica	0,04	0,00	2,31	0,35	3,42	93,05

With an average dissimilarity of 68% salt marshes B9N and B9A differ, at a vegetation cover level, in different amounts of *Salicornia spp., Limonium spp.* and *Sarcocornia spp.* 

Overall *Sarcocornia spp.*, bare soil, *Limonium* spp., and *Salicornia* spp. are the variables that contribute the most to the dissimilarities between groups.

## 3.2.2 Environmental data

Source	df	SS	MS	Pseudo- F	P(perm)	Unique perms
Saltmarsh_code	3	130,83	43,609	9,4581	<u>0,001</u>	996
Saltmarsh_type	1	159,44	159,44	5,3889	<u>0,021</u>	369
Saltmarsh_code x Saltmarsh_type	3	88,757	29,586	6,4166	<u>0,001</u>	996
Res	72	331,98	4,6108			
Total	79	711				

Table 10- PERMANOVA table of results of environmental data:

There are significant differences in the groups defined by 'Saltmarsh code' and 'Saltmarsh type', this means that there are significant environmental differences both within the groups (B5, B6, B8, B9) and between natural and artificial salt marshes.

Table 11 - Pairwise PERMANOVA results, within level 'B5' of factor 'Saltmarsh code':

Groups	t	P(perm)	Unique perms	P(MC)
N, A	3,6968	<u>0,001</u>	988	0,001

Table 12 - Average similarity between/within groups within level 'B5':

	Ν	Α
Ν	2,3297	
A	<u>4,8038</u>	3,3976

There is a significant difference between artificial and natural B5 with an average similarity of 5% Table 13 - Pairwise PERMANOVA results, within level 'B6' of factor 'Saltmarsh code':

Groups	t	P(perm)	Unique perms	P(MC)
N, A	3,1968	<u>0,001</u>	994	0,001

Table 14 - Average similarity between/within groups within level 'B6':

	Ν	Α
N	2,1148	
A	<u>4,8169</u>	4,2885

There is a significant difference between artificial and natural B6 with an average similarity of 5%

Table 15 - Pairwise PERMANOVA results, within level 'B8' of factor 'Saltmarsh code':

Groups	t	P(perm)	Unique perms	P(MC)
N, A	3,8443	<u>0,002</u>	990	0,001

Table 16 - Average similarity between/within groups within level 'B8':

	Ν	Α
N	2,1934	
A	<u>4,0456</u>	2,8129

There is a significant difference between artificial and natural B8 with an average similarity of 4%

Table 17 - Pairwise PERMANOVA results, within level 'B9' of factor 'Saltmarsh code':

Groups	t	P(perm)	Unique perms	P(MC)
N, A	4,2591	<u>0,001</u>	990	0,001

Table 18 - Average similarity between/within groups within level 'B9':

	Ν	Α
Ν	1,6588	
A	<u>3,9846</u>	2,8747

There is a significant difference between artificial and natural B9 with an average similarity of 4%

#### MDS DISPLAYING



Fig. 40 Environmental MDS displaying, taking into consideration the factor Saltmarsh type.  $_{6_{T}}$ Saltmarsh type



Fig. 41 Environmental MDS displaying, taking into consideration the factors Saltmarsh type and abiotics variables.

In MDS displaying (Fig. 40) the distances of environmental levels are explicitly outlined, more evident here, than in the biomass case (Fig. 38). It can be noted, indeed, a pattern has formed to the right for natural salt marshes and one to the left for artificial ones, highlighting a linear distance between all components of the environmental dataset.

#### SIMPER ANALYSIS

The SIMPER analysis shows which abiotic variables contributed the most to the dissimilarities between groups:

#### • Groups B5N & B5A Average squared distance = 24,45

Variable	Av.Value	Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
Redox	-1,18	1,3	6,58	2,33	26,92	26,92
B density	-0,333	1,71	4,49	1,87	18,37	45,29
< 63 µm	0,538	0,315	3,63	0,87	14,84	60,13
> 1mm	0,845	-0,503	3,03	1,06	12,39	72,51
250 μm-63 μr	n -0,643	0,115	2,47	0,71	10,10	82,61
1mm - 500 μm	n 0,267	0,635	1,6	1,00	6,53	89,14
Organic m. c.	0,28	-0,804	1,28	1,84	5,23	94,37

The variables that contributed the most to the dissimilarities between groups are redox potential, bulk density and sediment as clay and silt (< 63  $\mu$ m).

## • Groups B6N & B6A Average squared distance = 24,53

Variable	Av.Value	Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
B density	-1,15	1,17	5,99	1,53	24,43	24,43
Altitude	1,71	0,356	3,92	2,29	15,97	40,40
500 μm - 250 μm	-0,141	1,16	3,78	0,75	15,39	55,78
< 63 µm	-0,259	0,378	2,48	0,67	10,09	65,87
Organic m. c.	1,86E-2	-1,33	2,08	1,40	8,46	74,34
1mm - 500 μm	-2,42E-2	-1,08	1,95	1,16	7,93	82,27
250 μm - 63 μm	0,568	0,301	1,89	0,96	7,68	89,95
Redox	0,585	1,14	1,66	0,63	6,76	96,71

The variables that contributed the most to the dissimilarities between groups are bulk density, altitude and medium sand (500  $\mu$ m - 250  $\mu$ m).

## • Groups B8A & B8N Average squared distance = 16,77

Variable	Av.Value	Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
500 μm - 250 μm	0,954	-0,801	4,9	0,98	29,22	29,22
> 1mm	-0,416	1,55	4,74	1,25	28,24	57,46
Organic m. c.	-0,695	0,703	2,26	1,79	13,49	70,95
250 μm - 63 μm	0,33	-0,607	2,01	0,78	12,01	82,96
1mm - 500 μm	-0,983	-0,351	0,863	1,02	5,15	88,11
Redox	-3,9E-2	-0,719	0,682	1,15	4,07	92,18

The variables that contributed the most to the dissimilarities between groups are medium sand (500  $\mu$ m - 250  $\mu$ m) coarse sand (1 mm) and organic material material.

• Groups B9A & B9N Average squared distance = 17,23

Variable	Av.Value	Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
Organic m. c.	-0,682	1,2	4	1,61	23,20	23,20
> 1mm	-0,921	0,819	3,45	1,60	20,02	43,23
250 μm - 63 μm	1,04	-0,537	3,3	1,03	19,15	62,38
< 63 µm	0,322	-0,747	1,94	0,62	11,27	73,65
1mm - 500 µm	0,388	-7,36E-2	1,62	0,70	9,40	83,04
B density	0,453	-0,445	1,49	0,74	8,67	91,71

The variables that contributed the most to the dissimilarities between groups are organic material material, coarse sand (1 mm) and very fine sand (250  $\mu$ m - 63  $\mu$ m).

There are different abiotic parameters that contribute to the dissimilarities between the natural and artificial groups, the most frequent being bulk density, medium sand, and organic material content, represented moreover in Fig. 41.

#### **DistLM**

The Distance-based linear model (DistLM) was used to assess relationships between predictor variables (abiotic factors) and a response variable (biotic factors).

Variable	SS(trace)	Pseudo- F	Р	Prop.
Altitude	2422,7	1,0082	<u>0,417</u>	1,276E-2
Redox	11348	4,9584	<u>0,001</u>	5,977E-2
1mm	7034,9	3,0013	<u>0,012</u>	3,7052E-2
63 µm	2736,8	1,1407	<u>0,346</u>	1,4414E-2
Organic material content	21580	10,002	<u>0,001</u>	0,11366
Bulk density	10947	4,7722	<u>5,7655E-2</u>	5,7655E-2

Table 19 - Marginal test:

The marginal test is able to assess the individual influences of each predictor variable on the response variable.

Variable	AICc	SS(trace)	Pseudo- F	Р	Prop.	Cumul.	res.df
Organic material content	616,27	21580	10,002	<u>0,001</u>	0,11366	0,11366	78

Table 20 - Sequential test:

The sequential test uses the adjusted Akaike information criterion (AICc), the model with the lowest AICc value is considered the best.

AICc	R^2	RSS	No.Vars	Selections
616,27	0,11366	1,6829E5	1	5

Table 21 - Best solution:

Since organic material content has the lowest AICc value (616.27), it was indicated as the best predictor variable, explaining approximately 11.37% of the variance in the response variable.

#### 3.3 GAM model results

Some of the most representative vegetation coverage sampled data, according to the multivariate analysis performed and according to the literature, were chosen as dependent variables in the GAM model created.

#### SARCOCORNIA

Parametric coefficients:

	Estimate	Std.Error	t value	Pr(> t )
(Intercept)	1.02909	0.25118	4.097	0.000129 ***
Saltmarsh_typeN	0.09454	0.12036	0.785	0.435305

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value
s(Altitude)	4.3665	5.453	3.438	0.007523 **
s(Redox)	1.8217	2.281	1.282	0.264205
s(a)	3.7484	4.635	3.145	0.019298 *
s(b)	1.3655	1.637	0.302	0.599508
s(c)	1.0000	1.000	0.813	0.370809
s(d)	1.7251	2.158	0.973	0.423384
s(e)	1.8485	2.262	1.435	0.247633
s(Om)	1.0000	1.000	0.359	0.551192
s(BS)	1.0000	1.000	4.554	0.037008 *
s(Saltmarsh_code)	0.9149	1.000	10.799	0.000265***

R-sq.(adj) = 0.418 [The model explains 42% of the data] Deviance explained = 56.3% REML = 20.491



Fig. 44 GAM model's results for *Sarcocornia spp.*, significant predictors circled in red.

As the altitude increases, there is an initial increase in *Sarcocornia* spp. until reaching an optimal elevation, consequently, beyond that level, its units decrease. This is what GAM models are designed for: to show optimal points in the

relationship investigated.

It is also significant the slight negative relationship between coarse sediment (a = 1mm) and the genus *Sarcocornia*.

It is possible then, to identify a linear relationship between *Sarcocornia* spp. and Bulk density, outlining a positive relationship.

#### SALICORNIA

Parametric coefficients:

	Estimate	Std.Error	t value	$Pr(\geq  t )$
(Intercept)	0.08817	0.10385	0.849	0.399
Saltmarsh_typeN	-0.08972	0.07831	-1.146	0.256

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Altitude)	1.0000	1.000	3.036	0.0860	
s(Redox)	1.0000	1.000	1.658	0.2023	
s(a)	1.7855	2.251	2.481	0.0794	
s(b)	1.0000	1.000	5.379	0.0234 *	×
s(c)	1.4953	1.827	3.575	0.0591	
s(d)	1.0000	1.000	5.819	0.0186 *	×
s(e)	1.0000	1.000	4.759	0.0327 *	:
s(Om)	1.0000	1.000	0.077	0.7829	
s(BS)	11.0000	1.000	1.834	0.1802	
s(Saltmarsh_code)	0.4747	1.000	0.904	0.1618	

R-sq.(adj) = 0.15 [The model explains 15% of the data] Deviance explained = 27.7% REML = -2.9292



Fig. 45 GAM model's results for *Salicornia spp.*, significant predictors circled in red.

In the Fig. 45 all the grain size have a positive relationship with the genus *Salicornia*, but the model shows the most significant are coarse sand (b = 1mm - 500 µm), very fine sand (d = 250 µm - 63 µm) and clay and silt (e = < 63 µm). Altitude, although partially signifying, has a negative relationship with *Salicornia* spp.

#### LIMONIUM

Parametric coefficients:

	Estimate	Std. Error	t value	$\Pr(> t )$
(Intercept)	0.18045	0.04176	4.321	6e-05 ***
Saltmarsh_typeN	-0.01240	0.07669	-0.162	0.872

Approximate significance of smooth terms:

	edf H	Ref.df	F	p-value	
s(Altitude)	2.217e+00	2.795	1.023	0.291970	
s(Redox)	2.544e+00	3.170	3.195	0.029120	*
s(a)	5.322e+00	6.412	4.724	0.000539	***
s(b)	1.000e+00	1.000	0.221	0.640282	
s(c)	1.475e+00	1.784	0.384	0.569396	
s(d)	1.752e+00	2.196	0.824	0.516420	
s(e)	2.288e+00	2.810	1.067	0.269451	
s(Om)	1.000e+00	1.000	13.530	0.000510	***
s(BS)	1.000e+00	1.000	0.109	0.743001	
s(Saltmarsh_	code) 2.949e-07	1.000	0.000	0.395488	

R-sq.(adj) = 0.559 [The model explains 56% of the data] Deviance explained = 66.8% REML = -5.7962



Fig. 46 GAM model's results for *Limonium spp.*, significant predictors circled in red.

In this graph (Fig.46), it can be observed that the variation in *Limonium* spp. is positively linked to coarse sediment (a = 1mm), pointing out that further increases in the amount of coarse sediment cause an increase in the presence of *Limonium* genus.

More complex is the non-linear relationship with redox potential, as the predictor variable (Redox) increases, there is an increase in the *Limonium* spp. response. However, after a certain point, the response begins to decrease, indicating a decrease in the presence of *Limonium* spp. even as the predictive variable continues to increase.

In the end, it is possible to observe the positive linear relationship with organic material content. The presence of *Limonium spp*. in areas with a high percentage of organic material may be dictated by the fact that *Limonium* spp. is found in depressed areas where organic matter accumulates.

#### PUCCINELLIA

Parametric coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.07243	0.02682	2.701	0.00872 **
$Saltmarsh\_typeN$	0.04713	0.04734	0.996	0.32294

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Altitude)	1.000e+00	1.000	3.142	0.08078	
s(Redox)	1.000e+00	1.000	6.238	0.01493	*
s(a)	1.132e+00	1.250	4.972	0.01630	*
s(b)	1.000e+00	1.000	6.785	0.01128	*
s(c)	1.000e+00	1.000	7.121	0.00952	**
s(d)	1.641e+00	2.059	3.399	0.03653	*
s(e)	1.173e+00	1.322	6.515	0.01114	*
s(Om)	1.000e+00	1.000	2.556	0.11453	
s(BS)	1.000e+00	1.000	0.001	0.98164	
s(Saltmarsh_code)	3.060e-07	1.000	0.000	0.46535	

R-sq.(adj) = 0.209 [The model explains 21% of the data] Deviance explained = 31.9% -REML = -33.885



Fig. 47 GAM model's results for *Puccinellia spp.*, significant predictors circled in red.

First of all, it's possible to underscore how Altitude and Redox negatively affect the presence of *Puccinellia* genus, considering that redox potential has the most significant effect (Fig. 47).

Although, the significance of all sediment grain sizes in this case could underscore the idea that this genus varies due to the interaction of other factors not included in the model. Alternatively, another explanation could be that the GAM model fails to capture the real trend in the relationship between grain-size and the presence of *Puccinellia spp*.

#### **BARE SOIL**

Parametric coefficients:

	Estimate	Std. Error	t value	$\Pr(> t )$
(Intercept)	0.25861	0.04519	5.723	3.95e-07 ***
Saltmarsh_typeN	-0.23247	0.08571	-2.712	0.00879 **

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Altitude)	3.503e+00	4.408	15.380	4.72e-07	***
s(Redox)	3.645e+00	4.494	2.991	0.0274	*
s(a)	1.000e+00	1.000	0.634	0.4291	
s(b)	1.000e+00	1.000	0.860	0.3575	
s(c)	3.581e+00	4.423	2.296	0.0695	
s(d)	1.000e+00	1.000	0.849	0.3607	
s(e)	1.761e+00	2.149	0.943	0.4215	
s(Om)	3.818e+00	4.619	2.035	0.0812	
s(BS)	1.000e+00	1.000	0.303	0.5842	
s(Saltmarsh_code)	1.526e-07	1.000	0.000	0.8134	

R-sq.(adj) = 0.641 [The model explains 64% of the data] Deviance explained = 73.8% REML = -13.453





The model explains how the relationship between Bare soil and Altitude is strongly positive after a certain elevation, in lower areas instead this association is very negative (Fig. 48).

Regarding the Redox potential, it contributes to the presence of bare soil in a more complex manner, initially observable an increase in response as a predictor variable changes and then a subsequent decrease. This type of pattern may indicate some sort of reversal point in the relationship between the variables involved.

Bare soil then presented also a negative relationship with medium sand (c = 500  $\mu$ m - 250  $\mu$ m).

Regarding the interaction between bare soil and organic material the relationship is complex and nonlinear, with a decrease in the response as a predictor variable changes, followed by a subsequent increase.

#### **GREEN ALGAL MAT**

Parametric coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.05078	0.08095	0.627	0.533
$Saltmarsh\_typeN$	-0.05874	0.06497	-0.904	0.370

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Altitude)	5.7133	6.877	2.838	0.01373	*
s(Redox)	1.0000	1.000	0.266	0.60800	
s(a)	1.0000	1.000	7.594	0.00785	**
s(b)	1.0000	1.000	7.275	0.00918	**
s(c)	4.8013	5.855	3.774	0.00335	**
s(d)	2.9903	3.706	3.651	0.01161	*
s(e)	1.0000	1.000	7.389	0.00868	**
s(Om)	1.5983	1.963	2.956	0.06876	
s(BS)	1.0000	1.000	2.596	0.11263	
s(Saltmarsh_code)	0.4139	1.000	0.707	0.17020	

R-sq.(adj) = 0.434 [The model explains 43% of the data] Deviance explained = 58.8% REML = -26.959



Fig.49, GAM model's results for green algal material sampled, significant predictors circled in red.

It is possible to identify in Fig. 49 a particular relationship between green algal mat and Altitude, showing two optimal points.

The significance of all sediment grain sizes in this case, could underscore the idea that green algal mat varies due to the interaction of other factors not included in the model. Alternatively, another explanation could be that the GAM model fails to capture the real trend in the relationship with the grain-sizes.

Finally, the relationship with organic material content is negative.

## 3.3.1 Validation of the model: Grado-Marano Lagoon dataset

To validate the model, it was decided to adapt it to the dataset presented in Pellegrini et al., (2018), in which a similar analysis conducted, however, in the Grado-Marano Lagoon is presented. Only the predictor variables Altitude, Redox and Saltmarsh code were retained, for consistency with the Grado-Marano data.

## **VENEZIA SARCOCORNIA**

Parametric c	oefficients:			
	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.9337	0.2191	4.261	5.97e-05 ***

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Altitude)	3.8488	4.847	2.277	0.053048 .	
s(Redox)	1.0000	1.000	1.499	0.224699	
s(code)	0.8994	1.000	8.941	0.000827 ***	

R-sq.(adj) = 0.158 [The model explains 16% of the data] Deviance explained = 21.9% REML = 16.419



Fig.50, GAM model's results for *Sarcocornia* spp. sampled in Venice Lagoon, significant predictors circled in red.

#### **GRADO-MARANO SARCOCORNIA**

#### Parametric coefficients:

	Estimate	Std. Error	t value	$Pr(\geq  t )$
(Intercept)	30.40	3.53	8.611	9.72e-12 ***

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Altitude)	1.9714	2.446	0.518	0.5348	
s(Redox)	1.8905	2.328	2.661	0.0804	
s(code)	0.7374	1.000	2.807	0.0533	

R-sq.(adj) = 0.199 [The model explains 20% of the data] Deviance explained = 26.1% REML = 260.32



Fig.51, GAM model's results for *Sarcocornia* spp. sampled in Marano-Grado Lagoon, significant predictors circled in red.

Regarding *Sarcocornia* genus, in both Venice and Grado-Marano lagoons, it is presented by the model as a different significant interaction with the environmental parameter, but the curves of the graph (Fig. 50 and Fig. 51) show a similar trend for altitude factor.

#### **VENEZIA BARE SOIL**

#### Parametric coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.14237	0.01782	7.99	1.73e-11 ***

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Altitude)	4.418e+00	5.471	7.737	5.28e-06	***
s(Redox)	3.493e+00	4.344	3.187	0.0136	*
s(code)	1.055e-05	1.000	0.000	0.9154	

 $\begin{array}{ll} R\text{-sq.(adj)} = & 0.447 \ [The model explains 45\% of the data] \\ Deviance explained = 50.2\% \\ REML = & -19.07 \end{array}$ 



Fig.52, GAM model's results for Bare soil sampled in Venice Lagoon, significant predictors circled in red.

#### **GRADO-MARANO BARE SOIL**

Parametric coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	8.3328	0.7387	11.28	8.31e-16 ***

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Altitude)	2.9717388	3.698	10.658	5.29e-06	***
s(Redox)	2.1275243	2.614	2.385	0.102	
s(code)	0.0002061	1.000	0.000	0.651	

R-sq.(adj) = 0.5 [The model explains 50% of the data] Deviance explained = 54.3% REML = 189.1



Fig.53, GAM model's results for Bare soil sampled in Grado-Marano Lagoon, significant predictors circled in red.

Regarding bare soil sampled in Venice and Grado-Marano salt marshes, in Fig. 52 e and Fig. 53 the trend of the relationship between bare soil and altitude is similar, being negative at low elevation.
## **VENEZIA SALICORNIA**

	Estimate	Std. Error	t value	$Pr(\geq  t )$
(Intercept)	-0.06226	0.08737	-0.713	0.478

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value
s(Altitude)	1.000	1	6.143	0.0154 *
s(Redox)	1.000	1	6.384	0.0136 *
s(code)	0.823	1	4.651	0.0200 *

R-sq.(adj) = 0.109 [The model explains 11% of the data] Deviance explained = 14.1% REML = -15.661



Fig.54, GAM model's results for *Salicornia* spp. sampled in Venice Lagoon, significant predictors circled in red.

## **GRADO-MARANO SALICORNIA**

Parametric coefficients:

	Estimate	Std.Error	t value	$Pr(\geq  t )$
(Intercept)	2.0167	0.6637	3.038	0.00366 **

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Altitude)	2.50854	59 3.121	4.680	0.00509	**
s(Redox)	2.544667	5 3.124	2.192	0.09413	
s(code)	0.000206	57 1.000	0.000	0.92388	

R-sq.(adj) = 0.289 [The model explains 29% of the data] Deviance explained = 35%% REML = 182.95



Fig.55, GAM model's results for *Salicornia* spp. sampled in Grado-Marano Lagoon, significant predictors circled in red.

Even if the model is better adapted to Marano-Grado dataset, note how Altitude graphs (Fig. 54 and Fig 55) present overall a negative relationship with Salicornia spp. instead redox potential a partially positive one.

## **VENEZIA LIMONIUM**

Parametric	coefficien	its:		
	Estimate	Std. Error	t value	$Pr(\geq  t )$
(Intercept)	0.06751	0.10958	0.616	0.54

#### Approximate significance of smooth terms:

	edf	Ref.df	F	p-value
s(Altitude)	1.6384	2.037	1.123	0.3203
s(Redox)	2.5862	3.232	2.940	0.0453 *
s(code)	0.4983	1.000	0.993	0.1149

R-sq.(adj) = 0.136 [The model explains 14% of the data] Deviance explained = 18.8% REML = -3.2066



Fig.56, GAM model's results for *Limonium* spp. sampled in Venice Lagoon, significant predictors circled in red.

#### **GRADO-MARANO LIMONIUM**

Parametric coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	39.057	3.743	10.43	1.66e-14 ***

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Altitude)	2.5768	3.201	3.624	0.0173	*
s(Redox)	2.1325	2.618	4.022	0.0161	*
s(code)	0.7608	1.000	3.181	0.0416	*

R-sq.(adj) = 0.284 [The model explains 28% of the data] Deviance explained = 35% REML = 263.83



Fig. 57, GAM model's results for *Limonium* spp. sampled in Grado-Marano Lagoon, significant predictors circled in red.

Regarding *Limonium* genus sampled in Venice and Grado-Marano salt marshes, in Fig. 56 and Fig. 57 it's possible to underscore that redox potential has a significant relationship with this genus, in both case studies.

## **VENEZIA PUCCINELLIA**

## Parametric coefficients:

	Estimate	Std. Error	t value	$Pr(\geq  t )$
(Intercept)	0.09600	0.01333	7.201	3.45e-10 ***

#### Approximate significance of smooth terms:

	edf	Ref.df	F	p-value
s(Altitude)	1.00e+00	1	0.632	0.42918
s(Redox)	1.00e+00	1	10.226	0.00201 **
s(code)	2.89e-07	1	0.000	0.64255

R-sq.(adj) = 0.117 [The model explains 12% of the data] Deviance explained = 13.9% REML = -.47.939



Fig. 58, GAM model's results for *Puccinellia* spp. sampled in Venice Lagoon, significant predictors circled in red.

## **GRADO-MARANO PUCCINELLIA**

Parametric coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	) 3.6500	0.7876	4.634	2.47e-05 ***

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Altitude)	5.0191544	6.102	2.943	0.0157	*
s(Redox)	2.4365363	2.982	3.942	0.0127	*
s(code)	0.0002161	1.000 0.000	0.8415		

R-sq.(adj) = 0.322 [The model explains 32% of the data] Deviance explained = 40.7% REML = 196.44



Fig. 59, GAM model's results for *Puccinellia* spp. sampled in Grado-Marano Lagoon, significant predictors circled in red.

In the case of *Puccinellia* spp. both for Venice and Grado-Marano salt marshes, the same redox potential levels have a negative relationship with this genus (Fig. 58 and Fig 59).

Overall it is possible to verify how the GAM model adapts well to both datasets and sometimes presents similar results, agreeing with what was obtained from the data sampled in the Venice lagoon. Note that sometimes the values do not fit the model as one might expect, but this is probably dictated by the fact that many variables were not taken into consideration for reasons of mirroring between the two dataset.

# **CHAPTER 4 - DISCUSSION**

Our results brought to light an overall picture that highlights some differences between natural and artificial salt marshes in the Venice Lagoon. Significant differences emerged both in the plant communities and the abiotic factors such as granulometry and redox potential.

One of the differences that particularly stands out is the variation in bare soil in natural and artificial habitats (Fig. 36), and since the latter is higher in the sampled artificial salt marshes, it is an indication of the uncertain ecological successional state and instability of these environments. Indeed, the higher percentage of vegetation cover present within natural salt marshes delineates these habitats as more resistant to phenomena such as erosion, as in general fine root density has been identified as the best predictor of erosion resistance in marshes (Marin-Diaz et al., 2022). Therefore, a major disadvantage of artificial salt marsh is identified in the lower vegetation cover.

A second important annotation on bare soil, is its positive relationship with the elevation, in fact in the GAM model (Fig. 48) it is possible to see that as elevation increases, bare soil is more present on sampled areas. This means that salt marshes, with particularly high altitude, often have a lower percentage of vegetation cover. For this reason, artificial salt marshes construction should definitely take into account elevation levels, being different soil elevations characteristic of each halophilic species (Silvestri et al., 2005), and areas that are too high are not colonized.

A second element of disparity that is relevant is related to the artificial and natural salt marshes grain size investigation.

While in natural environments, material greater than 1 mm is the most common, medium sand (500  $\mu$ m - 250  $\mu$ m) on the other hand constitutes the predominant grain size in artificial salt marshes (Fig. 37). This finding is probably related to the method of construction of these artificial ecosystems, which involved pumping sediments dredged as a result of excavations in the Venice lagoon. The deposited dredged material was exposed to severe erosion in the initial stages of salt marsh construction (Widdows *et al.*, 2006), and therefore underwent a reduction in sediment grain heterogeneity. In these environments, variation of physical aspects of the sediment, such as grain sizes and soil density, are related to erosion phenomena that can be higher in artificial ecosystems, because the sediment was not naturally deposited (Widdows *et al.*, 2006).

Plus, the high presence of medium sand in artificial salt marshes contributes to soil erodibility, because the extent of subsidence detected on sandy sediments shows more pronounced subsidence than other soil types (Da Lio *et al.*, 2018).

In general however, the multivariate analysis (pag. 50) showed how the distance between these two groups (N and A) is clearer in abiotic variables (Fig. 40). In fact although the plant community may occur in different amounts depending on whether sampled in a natural or artificial area, the average similarity (pag. 53) turns out to be far less between natural and artificial salt marshes when just abiotic variables, such as elevation, redox potential and soil properties, are taken into account. By studying salt marshes vegetation coverage, it was noticed through SIMPER analysis (pag. 57) how some vegetation variables affect the differences between the groups taken into analysis (N and A) more, therefore probably only some of the sampled genus are better adapted to artificially created marshes. These differences between natural and artificial salt marshes are mainly contributed by the genus *Sarcocornia*, *Salicornia* and *Limonium*.

In light of this result and taking into consideration the comparative analysis between plant communities (Fig. 35), in the sampled areas, *Sarcocornia* spp. and *Limonium* spp. have a higher median in natural salt marshes, while *Salicornia* spp. has a higher median value in artificial salt marshes. At this point, the GAM model is a useful tool to explore the variables that influence the distribution and understand its type of relationship with plant communities.

Investigating the GAM model's predictions on the vegetation cover sampled, it emerged that *Sarcocornia* spp. has a negative relationship with coarse sediment (< 1 mm) (Fig.44). This could mean that *Sarcocornia* spp. prefers finer sediment, that usually are present where water velocity decreases, allows the sediments to be deposited more easily (Roner *et al.*, 2016).

The model also identified a positive relationship between *Sarcocornia* spp. and elevation, in fact the plant association that includes *Sarcocornia* is associated with a moderately high elevation range within the Venice Lagoon wetland habitat (Scarton *et al.*, 2000), as proposed in plants zonation by Silvestri *et. al* (2005). The relation, though, is non-linear, in fact the GAM model graphs (Fig. 44) shows an optimal point, beyond which *Sarcocornia* spp. units decrease. Changes in salt marshes elevation, caused by erosion, therefore can affect the growth and the proliferation of this plant genus, because it, as many others, shows an association with a particular soil elevation (Silvestri *et al.*, 2005), and even slight changes in altitude can alter it (Tagliapietra *et al.*, 2018).

Anyway, it would be interesting to investigate, in the future, what is the optimal interaction between *Sarcocornia* spp. and coarse sediment, considering the quite different distribution of this specific grain size in natural and artificial environments (Fig. 37).

Finally, the model shows a linear relationship between *Sarcocornia* spp. and bulk density, delineating a positive correlation. The reason for this positive relationship (Fig. 44) could probably be related to the fact that *Sarcocornia* spp. is better anchored when the soil is compact (Scarton et al., 2002). However, the high level of bulk density (Fig. 37a) in artificial salt marshes may be related to a too compact soil, where Sarcocornia spp. struggle more to proliferate, considering its higher diffusion in the natural environment (Fig. 36).

Ultimately, the relationship between *Sarcocornia* spp. and bulk density highlights the importance of considering the relation between vegetation and soil parameters in restoration projects, as the root action of *Sarcocornia* spp. plays a key role in reinforcing edges (Bonometto 2014).

Another fundamental vegetation element in this kind of environment is *Salicornia spp.*, which in the model was positively correlated to very fine sand (250  $\mu$ m - 63  $\mu$ m) and clay/silt (>63  $\mu$ m). This suggests that it is more likely to find these plants in areas where the soil composition especially consists of these grain sizes,

which is actually higher in sampled artificial salt marshes, than natural ones (Fig. 37a). What the model shows is probably related to the fact that finer sediments are especially found where water stagnates, in these areas salinity increases and succulent halophytes, such as *Salicornia spp.*, are more present (Anoè et al., 1984).

However, sediment distribution in salt marshes is quite dynamic, because it is closely related to soil drainage, which promotes access to water and oxygen and controls sediment exchanges throughout the area (Marani *et al.*, 2004). Investigating soil composition thus proves to be very important in restoration actions, considering its role in the ecological stability of these areas (Bonometto *et. al*, 2019) and its link with the plant species that characterise them.

Very interesting is the case of *Limonium spp*., more largely present in the natural salt marshes sampled than in artificial ones (Fig. 35), which it presents itself as an important indicator in the comparison between these two groups, for several reasons.

Firstly, the GAM model indicates a positive relationship between *Limonium* spp. and the presence of coarse sediments (<1 mm) in the soil (Fig.46). This suggests that *Limonium* spp. is more present in soils with a certain amount of this type of sediment. Indeed, coarse sediment has a higher median value (Fig. 37a) in natural marshes, and *Limonium* spp. has a higher distribution in this environment (Fig. 36).

The distribution of *Limonium* spp. is usually correlated with the morphology and the soil properties of the marshes (Silvestri et al, 2005), probably that is the reason why the model shows a significant relationship between *Limonium* spp. and redox potential (Figure 46). This correlation is negative so in areas with a high redox potential, *Limonium* spp. is less present. A low redox potential is usually recorded in the low areas of salt marshes, where in fact *Limonium* spp. is usually present (Silvestri et al., 2005), which means that these plants are probably adapted to low areas of salt marshes more effectively than other species.

Considering Figure 37b, the medium-lower values of the redox potential can be attributed to natural salt marshes, increasing the probability of finding *Limonium* spp., compared to their artificial counterparts.

Finally, the model indicates a positive linear relationship between *Limonium* spp. and the organic material content in the soil (Fig. 46), which is probably related to the fact that these plants are often found in depressed areas where organic matter can accumulate (Ronet *et al.*, 2016). Fig. 37a shows that in natural marshes the organic matter content is higher on average, compared to artificial counterparts, and therefore there will be a higher probability of finding *Limonium* spp. All these abiotic variables can therefore be considered as factors influencing the occurrence of the genus *Limonium*, on the one hand, and boosters of deviation degrees between natural and artificial salt marshes, on the other.

In conclusion, the research carried out during this project has demonstrated significant ecological differences between natural and artificial salt marshes in the Venice Lagoon, analysing their different degree of biological and physical deviation. On the other hand, the model was a functional tool to investigate the type of relationship between the biotic and abiotic parts.

It can therefore be seen that, as much as ecological engineering related to artificial salt marshes can contribute to limiting the decline of marine species and habitat degradation (Dafforn *et al.*, 2015), studying and understanding the vital services of natural intertidal ecosystems must remain the main priority in order to proceed with effective restoration.

Future research should also investigate what interventions can be made on the artificial counterparts in the lagoon to increase plant colonization and the resilience of these ecosystems to natural and anthropogenic phenomena such as the high erosion rate found. In the future, however, it will be necessary to coordinate ecological engineering efforts with other management strategies for coastal environments, to make restoration more durable (Dafforn *et al.*, 2015).

# **CHAPTER 5 - CONCLUSION**

In conclusion, the detailed analysis of the data reveals significant differences between natural and artificial salt marshes. The percentage of vegetation cover is significantly higher in natural salt marshes, while in artificial ones it is lower, a relevant factor in relation to erosion resistance.

Multivariate analyses show a significant divergence in vegetation between the two ecosystems, with an average dissimilarity of 67% between the groups natural-artificial.

With regard to abiotic factors, multivariates showed an average dissimilarity of 95% between the groups natural-artificial.

Focusing on the variation in the abiotic variables sampled, is important in order to fully understand the dynamics behind such pronounced differences, given their contribution in differentiating between the two groups (N and A). On the other hand, vegetation and abiotic factors interact through feedbacks, so a holistic view of salt marsh ecosystems remains necessary, this means considering the ecosystem as a whole.

The particle size distribution between the two types of sampled areas shows significant distinctions, with medium sand predominating in artificial marshes. Additionally, the redox potential is on average higher in artificial salt marshes. Bulk density and organic matter emerge as key factors in the differentiation between natural and artificial ecosystems, influencing soil properties, erosion rate and ultimately plant cover.

Finally, plant components such as *Sarcocornia* spp., *Salicornia* spp., and *Limonium* spp. emerge as important indicators in the differentiation between natural and artificial habitats, with their distribution influenced by altitude, redox potential, and soil grain size.

*Sarcocornia* spp. showed a positive association with bulk density, as soil compactness may allow better anchoring of its roots. *Salicornia spp.*, on the other hand, has a positive relationship with fine sediments and *Limoniums* spp. with coarse sediments and organic material, these findings are probably related to their location within the salt marshes, in relation to the altitude level preferred by these plants.

In summary, the analysis emphasises the complexity of salt marshes ecosystems and the challenge of replicating these balances through human intervention. Grain size emerges as an important factor affecting restoration efforts, while the GAM model provides valuable insights into the optimal maxima of variables, such as redox potential and elevation, to support the growth of key species.

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