Universitat de Girona
Escola Politècnica Superior

Incoming Exchange Student - Final Degree Project

Erasmus □ Techno X Other (specify):

Degree course: Grau en Enginyeria Agroalimentària Pla 2010

Title: THE ROLE OF SOIL AS A CARBON SINK IN COASTAL MARSH AND AGRICULTURAL SYSTEMS AT LA PLETERA (GIRONA PROVINCE, SPAIN)

Document: Final Degree Project

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Delivered on : **June, 2016**

The final project entitled “THE ROLE OF SOIL AS A CARBON SINK IN COASTAL MARSH AND AGRICULTURAL SYSTEMS AT LA PLETERA (GIRONA PROVINCE, SPAIN)” has been carried out in the department of Chemical Engineering, Agriculture, and Food Technology, High Polytechnic, University of Girona, Spain, under the supervision of Dr. Maria Gispert, Professor of the University of Girona, Spain and Mr. Kongkruy Chay, Lecturer of Royal University of Agriculture, Cambodia.

ACKNOWLEDGEMENT

This final project entitled THE ROLE OF SOIL AS A CARBON SINK IN COASTAL MARSH AND AGRICULTURAL SYSTEMS AT LA PLETERA (GIRONA PROVINCE, SPAIN) was successfully completed at the University of Girona, under a strong cooperation of lecturers of the Department of Chemical Engineering, Agriculture, and Food Technology, High Polytechnic School (EPS).

This work was financially supported by the European Commission (Erasmus Mundus, Techno II Project) and leaded by Dr. Maria Gispert. I would like to thank to Ministry of Agriculture Forestry and Fisheries, Cambodia giving me the great opportunity to exchange my study in the University of Girona, Spain.

I would like to appreciate and thank Dr. Maria Gispert, my adviser, Head of Soil Science, University of Girona, Spain and Mr. Kongkruy Chay, adviser at Royal University of Agriculture, Cambodia, to facilitate and supervise the development of my final degree thesis. During the field, laboratory and writing steps I had very strong help and assistance from Dr. Maria Gispert. Her valuable comments, explanations and instructions during my study research, and her corrections have been very relevant for the conclusion of the overall study. I also want to give many thanks to all lecturers, staffs, colleagues, and friends in EPS who gave me a very kind help and attention in my research study here.

Finally, I would like to give a gratitude to my parents, sisters and brothers who brought up very well and strongly support me with their love, motivation, till I have got all my achievements and success in study.

SUMMARY

The concentration of atmospheric CO_2 is now one third higher than it was during the eighteenth century, and significantly increased during the last several hundred thousand years. CO_2 concentration is causing substantial warming and other changes in global climate by altering the heat and water balances of Earth's surface and atmosphere. Today, scientists, engineers, and researchers on the carbon cycle are working not only to be sure that the experiment is adequately documented, but also to provide information and tools that can be properly managed. The challenge of controlling atmosphere CO_2 level is a topic of expanding public concern, national policies, and international agreement. The importance of soil dynamics on the organic carbon cycle has been outlined to remark that the soil system may capture and reserve almost 16% of the global CO_2 emissions. It is therefore important to have a wide knowledge of which are the soil compartments having a high potential for carbon sequestration.

The present work is associated to the life project "De-urbanizing and recovering the ecological functioning of the coastal systems of La Pletera" and reports the first preliminary results in order to verify the carbon sequestration potential of different areas either altered by anthropic activities or having a proper and natural ecological development. The main question arisen is the convenience to maintain saltmarsh coastal systems and demonstrate that these areas are strong contributors of soil organic carbon storage, instead of disregard in favour of new land use.

It is known that the saltmarsh ecosystems may retain until 37% of organic carbon due its peculiar dynamics of wet-dry cycles. Similarly, organic agriculture and pastureland may also be consistent in preserving organic carbon in soil if adequately managed. Six soil environments were selected in the whole area of study such as: Ruderal (RU); soil under Elymus elymoides (ELY); soil under Arthrocnemum fruticosum (SAAR); soil under Salicornia patula (SAER); soil under corn cultivation (AGR); soil under pasture (PAS) and soil samples collected at three depths (0-5 cm, 5-20 cm, and 20-40 cm) in order to differentiate the development of soil properties along profiles. Soil characterization included mineralogical and physical analyses such as texture and structural stability of aggregates, determined in order to compare the resistance of the various soils to degradation, together with soil chemical characteristics such as pH, electrical conductivity (EC), Soil organic carbon (SOC), Soil extractable carbon (FA&HA), Total nitrogen (TN), Extraction of easily

extractable glomalin (EE-GRSP), Extraction of total glomalin (GRSP), Dissolve organic carbon (DOC), and soil respiration.

Results clearly showed that SAAR soil was the most adequate to preserve organic carbon as it showed the highest clay content, structural stability, glomalin and soil organic carbon, whilst the carbon loss by respiration was the lowest among the other soils. Similarly, the mineralization coefficient of SAAR soil was 1360%, 1220%, 580%, 360%, 280% lower than AGR, SAER, PAS, RU and ELY respectively. This trend provided a twofold information, on the one end the agricultural soil should be managed differently as it showed a large carbon loss with respect to the carbon content; on the other hand the carbon lost by the SAER soil which should has been apparently a suitable carbon sink was absolutely inadequate to preserve organic carbon. The overall results of this work should be used for eventual soil restoration practices at the Pletera.

LIST OF ABBREVIATIONS

Å	: Angstrom
AM	: Arbuscular mycorrhizae
AMF	: Arbuscular mycorrhizal fungi
C	: Carbon
Cm	: Centimeter
CO_2	: Carbon dioxide
dS	: Decisiemens
EC	: Electrical conductivity
FA	: Fulvic Acid
g	: Gram
Gt	: Gigaton
H	: Hour
HA	: Humic Acid
ha	: Hectare
Mg	: Mega gram
M	: Meter
ml	: Milliliter
mM	: Millimolar
O_2	: Oxygen
pH	: Potential of Hydrogen
SOC	: Soil Organic Carbon
SOM	: Soil Organic Matter
TN	: Total Nitrogen
Tg	: Teragram

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Chapter I. INTRODUCTION

1.1. Terrestrial carbon sinks

Carbon is the element of living things in the world, and about half the dry weight of most living organisms is carbon. It plays an important role in the structure, biochemistry, and nutrition of all living cells. Processes that use carbon must obtain it from somewhere and dispose of it somewhere else. Carbon is introduced in the atmosphere as carbon dioxide, from fossil fuels, by animals through foodstuff, by mineralization of soil organic matter and also in rocks as limestone on the Earth's crust. The natural carbon sinks are terrestrials being however the oceans the biggest carbon sinks. A consistent amount of carbon is grabbed as carbon dioxide from the plants by the process of photosynthesis. Later on some of this carbon is transferred to soil as plants die and decompose. The global potential of soil organic carbon sequestration is estimated at 0.6 to 1.2 Gt C/year, even though may vary according to soil management practices (Lal et al., 2007). Soil carbon sequestration potential represents the first factor to reduce carbon dioxide emission and increase soil quality as, once in the soil, organic carbon is subjected to different biological processes and contribute to the improvement of the majority of soil properties. The influences of soil organic carbon on aggregates formation and stabilization have been generally reported in recent studies (Six et al., 2000), and have shown that once soil aggregate stability has increased it may be an important component for organic carbon storage in soil microsites (Chivenge et al., 2011). In fact, soil aggregates interaction with organic carbon is able to maintain a very resistant and resilient structure thus controlling erodibility and minimizing erosion processes (Bronick and Lal, 2005). The Soil organic carbon content in cropland is mainly dependent upon crop and soil management practices, such as crop species and rotation, tillage methods, manure application, pesticide use, irrigation and drainage, and soil and water conservation (Heenan et al., 2004). For this reason, the soil carbon sequestration potential of cropland is uncertain and efforts are made to modify agricultural management in order to reduce carbon dioxide emission from these soils. By contrast, natural soils (forests, scrubland and rangeland) may be more efficient in soil organic carbon preservation. Despite that, the understanding of the carbon and its distribution along the profile needs further investigation to clarify the carbon dynamics in soil (Wang et al., 2008). A mong the terrestrial ecosystems able to retain organic carbon the value of saltmarshes as effective and efficient carbon sink is becoming increasingly apparent. Due to several characteristics, notably the high salinity of the soils, the potential anaerobic condition created by inundation and input of fertile sediments, saltmarshes have low emission of greenhouse gases, especially CO_2, compared to other terrestrial

ecosystems. Low decay rate may be relevant causing total carbon sequestration rates to be very substantial for these lands and should be included in the carbon trading programmes (Burden and Garbutt, 2013). Despite the high number of studies devoted to clarify the soil carbon sequestration potential there is still insufficient knowledge about the impact of microbial processes on the pathways of carbon within the global carbon cycle. A strong need for understanding additional steps in the global carbon cycle is required (Lal, 2009). Whilst there can be a large unfilled carbon sink capacity of the world soils, there are concerns about the trends towards saturation of terrestrial carbon sink capacity (Canadell et al., 2007), due to increase in soil degradation and desertification (Bai et al., 2008). As highlighted by Lal (2011), the noticeable relationships among soil degradation, food security, and climate change, should be seriously considered in order to stimulate environmental and soil quality restoration thus contrasting the two current main crises of food security and climate change. It is therefore evident that soil carbon sequestration is associated to many aspects of current life and may be absolutely relevant for the wellness of future generations.

1.2. Land use and management effects on soil carbon cycle

According to Falkowski et al. (2000), the carbon exchanges between pools occur as the result of various geological, physical, chemical, and biological processes. However, in the past centuries, anthropogenic activities have seriously transformed the global carbon cycle through land use and cover change, and land abandonment. Although carbon dioxide levels have changed naturally over the past several thousand years, humans seem to push up carbon dioxide concentration in the atmosphere. In nature, flows of carbon between the main compartments (atmosphere, oceans, terrestrial ecosystems, sediments) are fairly balanced, so that carbon levels would be roughly stable without human influence (UNH, 2009). An example of the soil C stocks decline after land use changes is described by Gou et al.(2002), from pasture to plantation (-10%), native forest to plantation (-13%), native forest to crop (-42%), and pasture to crop (-59%). However, the same authors reported also soil C stocks increase after land use changes from native forest to pasture (+8%), crop to pasture (+19%), and crop to secondary forest (+53%), indicating that in some instances soil properties recover may occur under renaturalization processes. Nevertheless, land use and cover change should be correctly managed. Some authors (Pardini, et al., 2003; Gispert, et al. 2013) have reported that according to revegetation practices and used plant species, soil properties impoverishment may occur at a large extent. The worse situation found was under stands of

pine tree plantations which caused organic carbon decline because of drastic pH decreases. Reduction in soil carbon stocks by 12-15% were also found by Gou et al. (2002), when using afforestation with pine plantation.

1.3. Land abandonment in the Mediterranean region

Land abandonment is recognised as a major component of global change in Mediterranean type of climate. However, it is not a new phenomenon and in many cases land abandonment invariably leads to land degradation and desertification. It has been constantly occurring and widely spread in Europe since 1950. It does not mean that land is no longer used; it means a change in land use that may lead to plant cover increase or bare surface. According to Thornes, (2002) the land abandonment is due to external stresses and/or because of its inherently low productive capacity. Land abandonment occurs consequently due to internal changes and external driving forces. Wild fires as well as land use change are currently considered the most serious environmental problems in vulnerable areas. For many years, agriculture carried out in arable land has made the farming systems more stable and, as much soil care has been practised, as quickly the soil may return to previous good conditions. Loumou et al. (2003) and Lasanta (1998) gave a description of land abandonment in the Mediterranean which is marked frequently by the difficulty of accessing to farmland areas especially mountainous and mid-mountainous, where more modern agriculture practices where difficult to apply. The rural abandonment in north-east Spain is an outstanding concern because of the difficulty to complete a natural vegetation succession due to wildfire hazard, plant species restriction, and intensification of erosion processes. Both conditions contribute negatively to soil properties, especially to the reconstitution of organic layers. In fact, the majority of plants growing under frequent fire conditions have a very high carbon/nitrogen ratio, hindering a normal mineralization/humificatoin process. For this reason, large parts of abandoned land in northeast Spain are considered very low contributors to soil organic carbon preservation.

1.4. Wetlands and soil carbon

Wetlands are areas where water is logged, either permanently or seasonally, and own the characteristics of a distinct ecosystem. They differ according to their local and regional differences in topography, hydrology, salinity, vegetation and other factors including human

activities. Wetlands are divided into two main classes: tidal salt-marsh and freshwater marshes (Maltby, 1988). The primary factor that distinguishes wetlands from other land forms or water bodies is the characteristic vegetation of aquatic plants, adapted to unique hydric condition of salty soils. Wetlands may play important roles in the environment, such as water purification, flood control, carbon sink and shoreline stability. Wetlands are also considered the most biologically diverse of other ecosystems, serving as home to a wide range of plant and animal life. As highlighted by Vitt et al. (1990), landscape characteristics control wetlands hydrology and hydrochemistry. Hydrochemistry within wetlands is determined by the pH, salinity, nutrients, soil composition, top soil hardness and the sources of water (both saline and fresh water). Accumulation of salinity in wetland areas have influenced the wetland water and soil chemistry, especially in wetland along the coast (Kiddy, 2010). In wetlands which are not pertaining to river or located by the river, the salinity is accumulated by the interaction between ground and surface water; which could be influenced by human activity. Most nutrients such as carbon, phosphorus, sulphur and nitrogen are found within soils in wetlands. Carbon is the vital nutrient cycled in wetlands and its stability is influenced by the aerobic and anaerobic respiration in the soil. The level of salinity in wetlands can play an important role to mitigate soil carbon dioxide emission through soil respiration (Schlesinger et al., 2013). In fact, wetlands may accomplish to implement two significant functions to climate change, because they could mitigate bad impacts of CO_2 emission through their ability to sink carbon, store and regulate water (U.S. EPA, 2009). More importantly, wetlands may store approximately 44.6 Tg C y^{-1} globally. In salt marshes and mangrove swamps in particular, the average carbon sequestration rate is 201 g CO_2 m^{-2} y^{-1} while peatlands sequester approximately 20-30g CO_2 m^{-2} y^{-1} (Roulet, 2000; Chmura, 2003). According to Hart et al. (2011), constructed wetlands take 10 to 100 years to completely resemble vegetative composition of natural wetlands. The ecological value of natural wetland must therefore be maintained as they play a relevant role in soil carbon sequestration.

1.5. Soil Organic Matter

Soil organic matter (SOM) is the fraction of the soil proceeding from plants and animal residues decomposition metabolised by soil organisms and incorporated to the soil. The role of soil organic matter is to provide critical elements to meet the plant requirements, in terms of macro and micro-nutrients, to sustain life cycle. Under nature conditions nutrients cycling is driven by biological process and climate conditions. These two factors contribute to

the mineralization and humification of soil organic matter producing on the one hand simple elements like magnesium, calcium, potassium, phosphates, sulsphates, water, carbon dioxide, and micro elements which are incorporated to the soil solution for the quick uptake by plants and soil organisms. On the other hand, the most resistant portion of organic matter (mainly containing lignin) is progressively transformed into humus. The humification process is very complex and continuously changing according to site conditions and soil constituents. Irrespective to its complexity the transformation of organic matter into humus may be summarized as following: partial metabolisation of lignin producing glucose, phenol, and aminoacid as shown in the Figure 1.

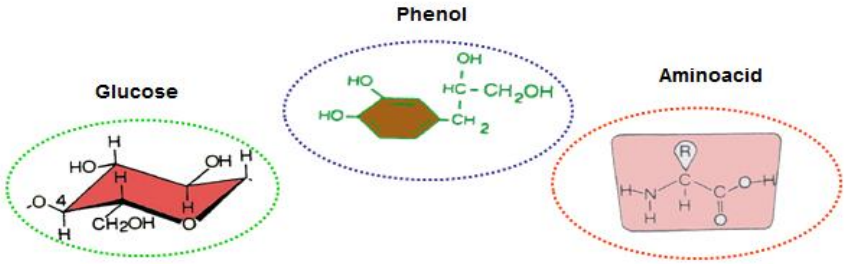


Figure 1. The products of organic material undergoing biochemical metabolic processes.

These products (monomers, low dimension molecules) are the result of biochemical metabolic processes and contribute to the humification processes through polymerisation. At the same time they can also be used by microorganism as energy sources. Going forward with this process, enzymatic activity like poly phenol oxidase transform phenols and link all these substances deriving in always more complex molecules through polymerisation as represented in Figure 2.

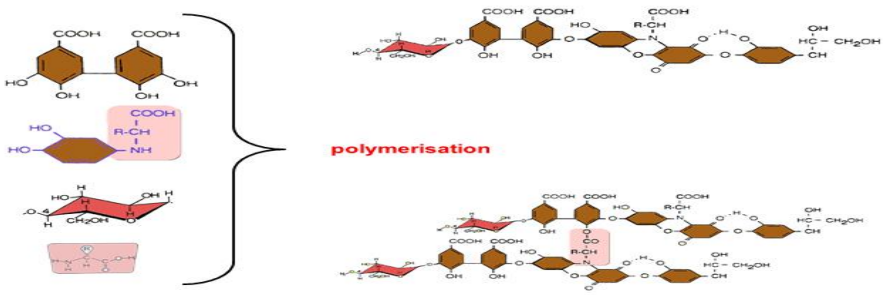


Figure 2. Polyphenol oxidase transform phenols and link all these substances deriving in always more complex molecules through polymerization.

The results of these humus formation paths originate very complex macromolecule with an aromatic nuclei surrounded by oxydhril, carboxyl and phenolic radicals as shown in Figure 3

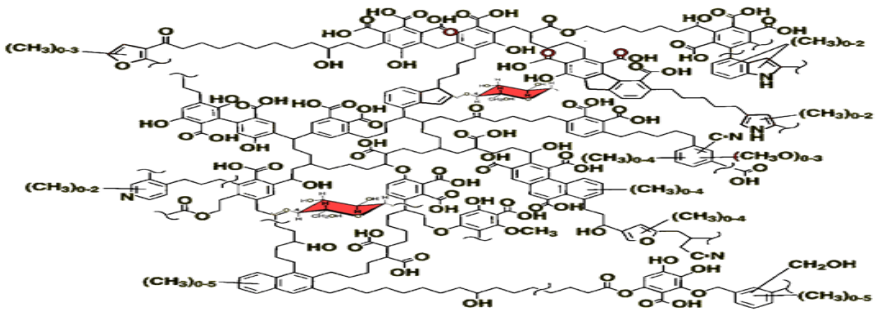


Figure 3. A generic picture of humus macromolecule.

Soil organic matter is typically estimated to contain 58% of carbon, even though the terms 'soil organic carbon' (SOC) and soil organic matter (SOM) are often used interchangeably (Lal, 2004). Soil represents one of the largest carbon sinks on Earth and plays a key role in the global carbon cycle. Although the soil quality cannot be measured directly, it can result from the analysis of a variety of soil properties (maximum dataset for soil quality) in which the soil organic carbon is the most relevant parameter.

As mentioned elsewhere the soil organic material is continuously incorporated to the soil surface as decaying debris or directly into the soil in form of dead roots or soil fauna. This makes that the soil presents different layers in depth being the top layer the darkest one because of the presence of higher amount of fresh and partially humified organic matter. Although it is not possible to appreciate the quality of organic matter or organic carbon visually, in most cases it is possible to observe the maturity of organic matter by the morphology of the soil crumb (Koenig et al, 2010). Soil is split into horizon layers. The A horizon, the surface layer, generally ranges from five to twenty five centimetres, is dark brown in color by the humus substances. According to its properties, it enhances desirable structure, water infiltration and water holding capacity. Other horizons under the A horizon (E, B, and C) form the subsoil (according to the parent material forming the soil), which may consists of higher level of clay than the top soil layer. In general acid rocks as parent material contribute to form quite poor soils, whereas when soil is formed on calcareous rocks usually tends to show better properties and more suitable humic compounds. It is therefore logical that the parent material and the organic horizons are relevant to obtain a better soil and a

higher quality of organic matter. However, independently of the factors of soil formation it is paramount to enhance the capacity of soils to provide the ecosystem service of carbon sequestration through SOM management.

1.6. Soil glomalin, organic carbon and structural stability

Golmalin, a glycoprotein produced by Arbuscular Mycorrhizal Fungi (AMF) is an indicator of soil carbon storage because mainly formed by recalcitrant humic compounds (Rillig, 2004). Glomalin was discovered by the soil scientist Sara F. Wright (Wright, and Upadhyaya, 1996), and later on many studies have been carried out on this topic. The majority of research works dealt with the capacity of glomalin to increase soil structure stability and favour soil carbon preservation (Rillig et al., 2001; Rillig et al., 2003). It seems that glomalin permeates organic matter after the fungi have deceased, binding silt, sand, and clay particles. Thus, not only does glomalin contain 7 to 40 percent of recalcitrant organic carbon, but it also forms clumps of soil granules improving soil aggregation. As a glycoprotein, glomalin stores carbon in both its protein and carbohydrate (glucose or sugar) subunits. According to this glomalin may be extracted from soil as easily extractable fraction (mainly composed by labile organic compounds i.e. glucose or sugar), and as total glomalin formed by more aromatic humic compounds. As reported by Jonhson et al. (2006) and Borie et al. (2008) glomalin is vital to enhance soil physical properties and facilitate biogeochemical cycling. Moreover, soil having greater amount of glomalin may better resist water and wind erosion and are less prone to forming sealing and crusting processes. A stable structure enhanced by the presence of glomalin may provide a sound pore space and pore size distribution able to manage water and air for beneficial microbial activity. Liefheit et al. (2014) showed the evidence that Arbuscular Mycorrhizal Fungi (AMF) interaction with plant roots constitute the basis for nutrients and carbon interchange at the soil rhizosphere. Fungal hyphae provide nutrients to plant roots while plants ensure carbon supply to hyphae and energy source. As hyphae are subjected to a short lifecycle, as much as their cells go into senescence glomalin is released by the cell walls and dispersed into the soil. This process enables soil organic carbon enrichment in the more stable pool. The following Figure 4 show the conceptual diagram of soil aggregation by the contemporary participation of mineral and organic components.

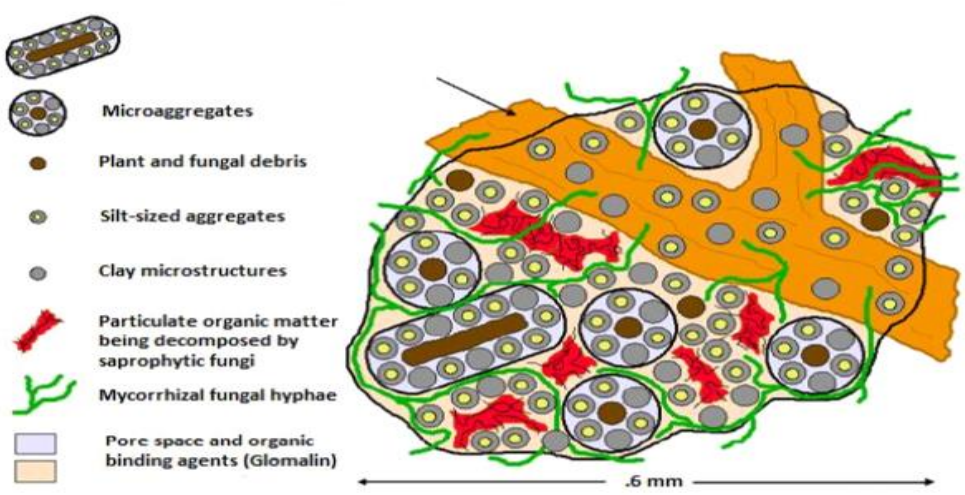


Figure 4. Conceptual diagram of soil aggregation with glomalin implication.

Keeping an abundant amount of glomalin be in native soil is the key to soil stability. Soils that have been damaged by heavy grading, tillage or excessive exploitations are particular in need to restore the quality by increasing the arbuscular mycorrhizae fungi. Conventionally, increasing mycorrhizae in depleted soils will provide the important substances to restore the nutrients cycling and water path to plants (Pal, 2014).

1.7. Objectives

In this chapter, the importance of soil dynamics on the organic carbon cycle has been outlined to remark that the soil system may capture and reserve almost 16% of the global CO_2 emissions. It is therefore important to have a wide knowledge of which are the soil compartments having a high potential for carbon sequestration.

The present work is associated to the life project "De-urbanizing and recovering the ecological functioning of the coastal systems of La Pletera" and reports the first preliminary results in order to verify the carbon sequestration potential of different areas either altered by anthropic activities or having a proper and natural ecological development. The main question arisen is the convenience to maintain saltmarsh coastal systems and demonstrate that these areas are strong contributors of soil organic carbon storage, instead of disregard them in favour of new land use. At this regard, it has been necessary to carry out preliminary soil characterisation of all the areas mentioned in the above project in order to have a first indication of the soil properties, the organic carbon content and the carbon storage capacity.

Chapter II. METHODOLOGY

2.1. Description study area

2.1.1. Characteristics of the study area

The area of this study belongs to the Natural Park of Montgrí-Illes Medes-Baix Ter located in the province of Girona, Catalonia, Spain. The park was created on May, 13th, 2010. It includes two partial natural reserves (Illes Medes and Baix Ter) and a natural integral reserve of the terrestrial part of Illes Medes because of its high ecological value. The Figure 5 showed the setting of the park area (Tourist Office, Estartit).

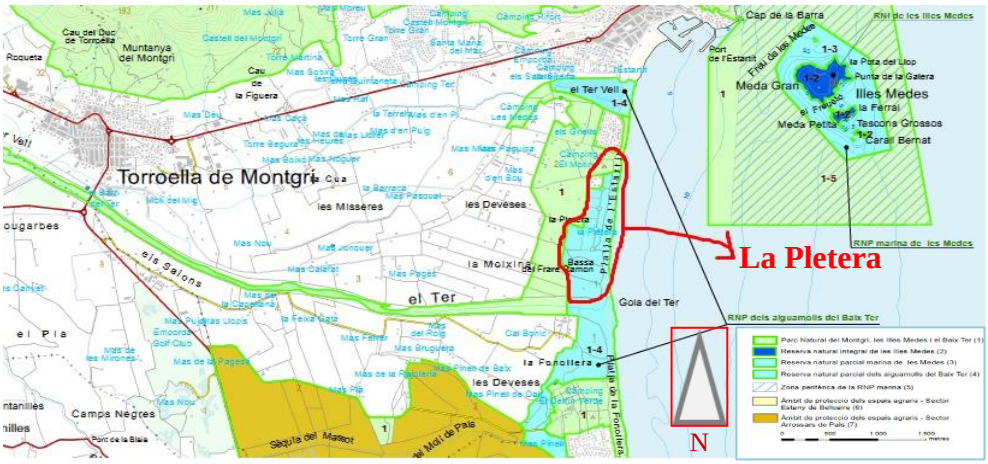


Figure 5. The map of the Pletera area of the National Park, Montgri, Medes Islands and Baix Ter.

2.1.1.1. Geology

The Montgri massif includes Santa Caterina mountain (303m height) and Torre Moratxa and the Roca Maura mountains of 220 and 225 m respectively, close to the Estartit village. The whole massif is situated on the north bank of the Ter river; it stretches from East to West North of the town of Torroella de Montgri. The geological composition is formed by calcareous rocks with a very pronounced tectonics from Jurassic period with inclusions of marls, clayey marls, dolomites, and chalks.

2.1.1.2. Geography

The area of the study is located in the National Park of Montgri- Illes Medes-Baix Ter (42°03103"N, 003°12'31"E), Province of Girona, Catalonia, North East Spain (Figure 6). The Montgri Natural Park, was approved in 2010 with total of 8,192 hectares of land (77%) and

2037 hectares of marine area (23%). The main beach of l'Estartit, is divided into three areas, the main beach, the beach of Els Griells, and the beach of La Pletera. La Pletera saltmarsh is part of the Montgri-Medes Island-Baix Ter Special Protected Area (SPA) and Site of Community Interest (SCI) ES5120016 in Natura 2000 Network. La Pletera has no buildings and neighbourhoods in the surrounding area which is part of a restoration program of coastal dunes and wetlands of the Baix Emporda region.

2.1.1.3. The Pletera project

The Pletera saltmarsh suffered at the beginning of 1980 the uncompleted construction of a touristic urbanization which was abandoned ten years later. This caused an ecological perturbation in an area of about 7 ha. The current result is that this part, which constitutes a space of great natural and ecological interest, is interrupted by different physical barriers such as semiurbanised areas, rubbles accumulation, rocky cliffs, and deteriorated roads which hinder the correct ecological functioning of the lagoon part. The total submerged area of the lagoon occupies 23 hectares. The protecting actions of the area of the Pletera have been conducted to restore the ecological function that where altered by the building works.

Figure 6. Aerial image of the Pletera area.

In Figure 6 it can be observed the two areas filled with rubbles (R) from unknown provenience which cover with almost 2 meters the ancient saltmarsh and that will be excavated along the restoration works. Also the same Figure shows along the paths with three round squares which will be eliminated during the restoration works. As above mentioned the buildings shown in Figure 6 started in 1967 according to the delimitation of the coast line

established by public institutions (red line in Figure 7) which was modified in 2004 enlarging the protected areas (blue line in Figure 7).

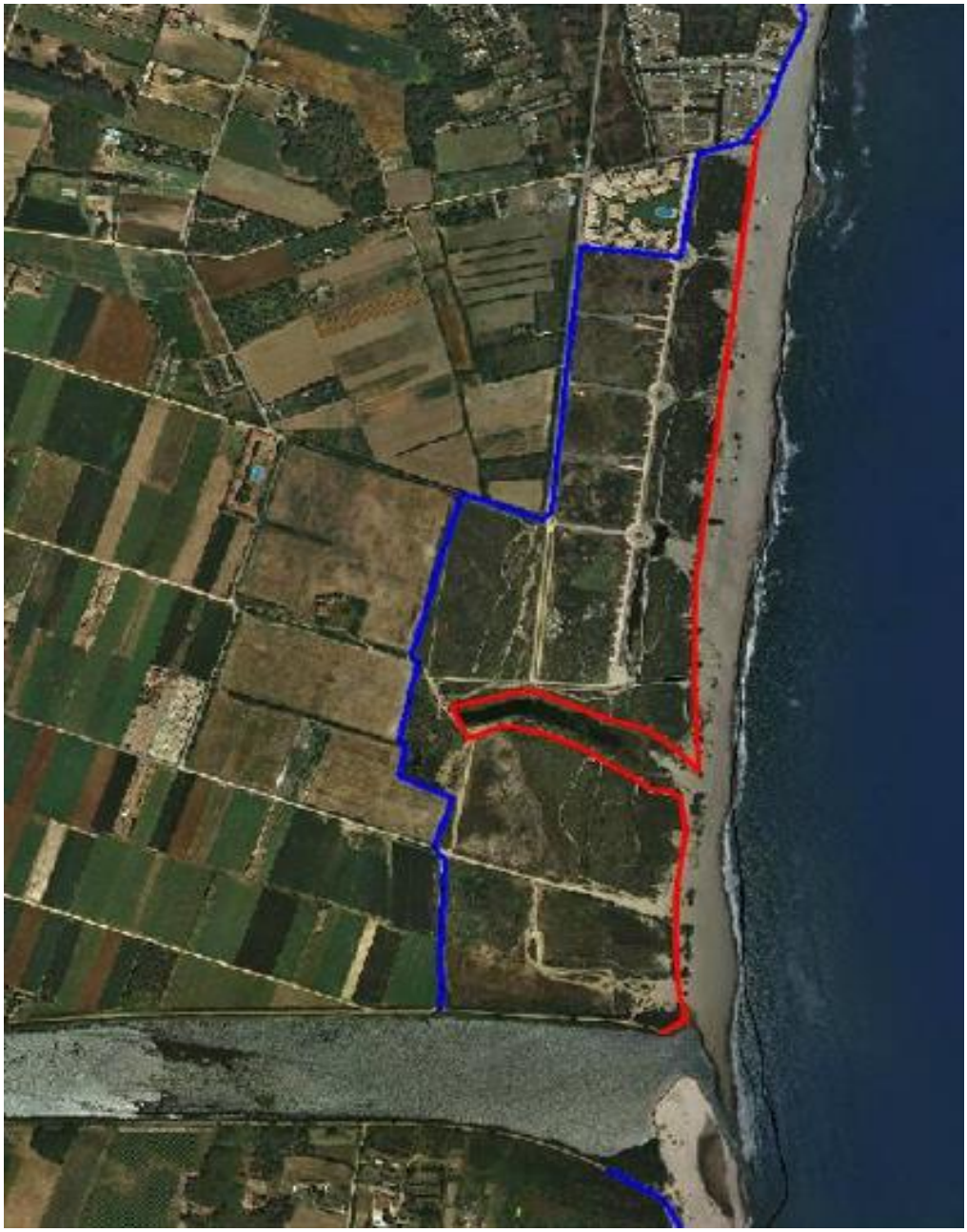


Figure 7. The delimitation of the public domain before 1967 (red) and after 2004 (blue).

The Pletera constitutes a confined lagoon ecosystem of marshes and sparse agriculture soils. The soil texture and the hydrological conditions mainly determine the type of vegetation of the marsh area where 76% of the surface is occupied by salt resistant plants whilst other type of vegetation is present in the perturbed area. The ecological interest of the Pletera lies

in the presence of brackish and hyperhaline coastal lagoons, with well-conserved halophilic and psammophilic plant communities growing in the area as well as established colonies of Spanish toothcarp (Aphanius iberus), a fish endemic to the Iberian Peninsula that is in danger of extinction. One of the aims of the restoration works is to compare the carbon fixation capacity of the perturbed area with respect to saltmarsh ecosystem in order to ascertain the importance of conserving this ecosystem to reduce CO_2 emission into the atmosphere. It is hoped that by replacing the disused urban infrastructures and the mounds of soil covered in Ruderal vegetation by a system of coastal lagoons and adjacent flood belts, the carbon fixation capacity will greatly increase.

2.2. Selection of the environments

According to the characteristics of the area, six environments were selected representing the main soil types and the vegetation cover of the whole area of the study. Replicated soil samples from the perturbed area or Ruderal (RU), and under *Elymus elymoides* community (ELY), under *Arthrocnemum fruticosum* community (SAAR), under *Salicornia Patula* community (SAER), where collected in August-September 2015 for preliminary and comparative study of soil properties and carbon sequestration capacity. Moreover, representative soil samples from an adjacent agricultural soil (AGR), after corn (*Zea mays*) harvest, and a soil under pasture (PAS) covered by *Medicago sativa* were collected. The following Figure 8 shows the selected soil sampling sites at the saltmarsh.

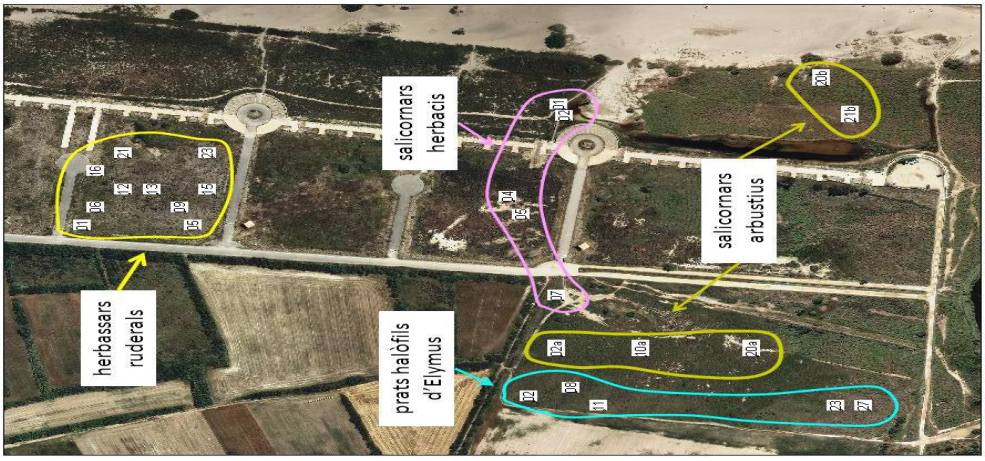


Figure 8. Location of sampling plots at the saltmarsh of the Pletera.

The general view of the selected soil environments with some details of the soil profiles is reported in Figure 9.

Ruderal (RU)

Detail of soil profile in RU

Elymus elymoides (ELY)

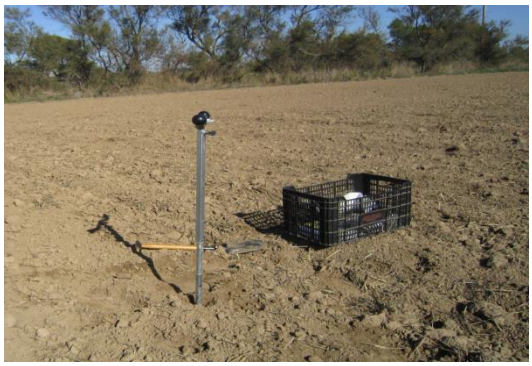
Detail of a plot in ELY

Arthrocnemum fruticosum (SAAR)

Detail of soil profile in SAAR

Salicornia Patula (SAER)

Detail of soil profile in SAER



Agricultural soil (AGR)

Pasture soil (PAS)

Figure 9. The six different selected environments at the area of study.

2.2.1. Soil sampling and classification

The soil sampling strategy has been carried out by establishing five plots of 1 square meter in each selected environment as reported in Figure 8. Soil samples were collected in August-September 2015, at three depths (0-5cm), (5-20cm), and (20-40cm) to assess the mineralogical, physical, chemical and biological properties. A total of 162 samples was finally collected from the selected environments and processed at the laboratory of Soil Science, Polytechnic School, University of Girona.

Ruderal environment (RU)

The soil form Ruderal environment was accumulated at the time of the decision of administration to fill in the marshes area. This soil presents very high amount of coarse material coming from alluvial funs. The plant community of Ruderal is various and it is mainly composed by *Foeniculum vulgare* and *Brachypodium retusum*.

This soil was classified as Xerorthent lithic according to Soil Taxomony (1992).

Figure 10. Detail of *Foeniculum vulgare* in Ruderal environment.

Elymus elymoides environment (ELY)

The soil from *Elymus elymoides* environment was mostly composed by sandy particles. In this area the landscape is mostly covered by *Elymus elymoides* species. This soil has been classified as Xerorthent according to Soil Taxomony (1992).

Figure 11. Detail of *Elymus elymoides* plant in ELY.

Arthrocnemum fruticosum environment (SAAR)

The soil from *Arthrocnemum fruticosum* environment was mostly composed by sand clay. In this area the landscape is mostly covered by *Arthrocnemum fruticosum* species and the soil was classified as Xerorthent even though the periodical presence of sodium in the upper layer may form an Az horinzon with a possible classification of Natriargid (Soil Taxomony, 1992).

Figure 12. Detail of *Arthrocnemum fruticosum* plant in SAAR.

Salicornia Patula environment (SAER)

The soil from *Salicornia Patula* environment was apparently composed by high sand amount. In this area, due to the annual characteristics of this type of plant the surface is moderately covered by Patches of *Salicornia Patula* species with a large part of the land

remaining bare. According to prevalent seasonal salinity this soil does not reach heavy saline conditions and was classified as Xerorthent (Soil Taxomony, 1992).

Figure 13. Detail of sparse *Salicornia Patula* sprouting in SAER.

Corn cultivated environment (AGR)

The soil from corn cultivated environment was apparently homogenous in depth. The surface was covered by corn straws due to the recent harvest. The soil was classified as Xerorthent according to Soil Taxomony (1992).

Pasture environment (PAS)

This soil was currently covered by alfalfa (*Medicago Saltiva*) and usually used for as pastureland even though sometimes alfalfa is harvested and stored to feed livestock. Apparently the soil is sandy. The soil was classified as Xerorthent lithic according to Soil Taxomony (1992).

2.3. Experimental Layout

Soil samples were transferred at the laboratory of soil and residues of the Department of Chemical Engineering, Agriculture and Food Technology of the University of Girona. All soil samples were air dried, gently crushed and sieved at 0-2 mm for subsequent analyses. Moreover, soil aggregates in the dimensional classes of 0.25-2 mm and 2-5.6 mm were separated for structural stability of aggregates analysis. The soil fraction of 0-2 mm was used for the main soil parameter useful for soil characterization and soil carbon dynamic.

2.3.1. Laboratory mineralogical and physical analyses

2.3.1.1. Total and clay mineralogy

X-Ray Diffraction analysis (XRD) have been carried out for both total and clay fraction mineralogy of the studied soils. The used apparatus is a Bruker, Model D8 Advance Geometry: Bragg-Brentano, mode reflection, Configuration: Theta-2Theta (Figure 14.), using CuKα radiation (λ=1,5406 Å). Dry samples were crushed at <50 µm fraction for total mineralogy by using a RESTCH MM200 mill equipped with silica sphere Ø 5 cm and scanned until 0-60 2θ amplitude reflection. Clay minerals analysis were carried out on <2 µm particles suspension specimens let to settle on glass devices at room temperature (Figure 15.) and scanned at 0-20 2θ amplitude reflection. XRD diagrams will allow defining the different mineral phases of the investigated soils. Figure 14 reminds the general principle of X Ray Diffraction according to the Bragg law.

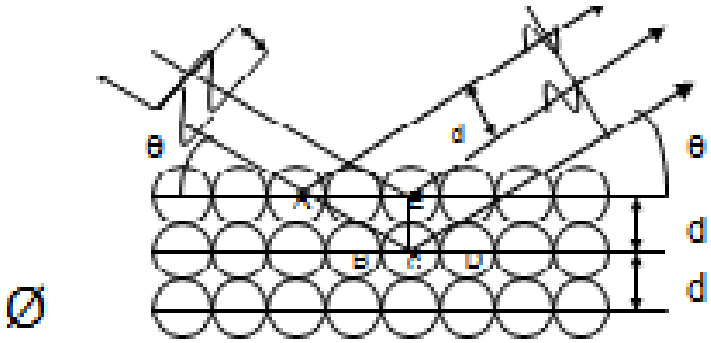


Figure 14. Scheme of the radiation/diffraction over flat mineral d-spacing and the Bragg equation.

Bragg law: $n\lambda = 2\ d \sin \Theta$ (d: crystalline spacing typical of each mineral).

Figure 15. The X-ray diffraction apparatus.

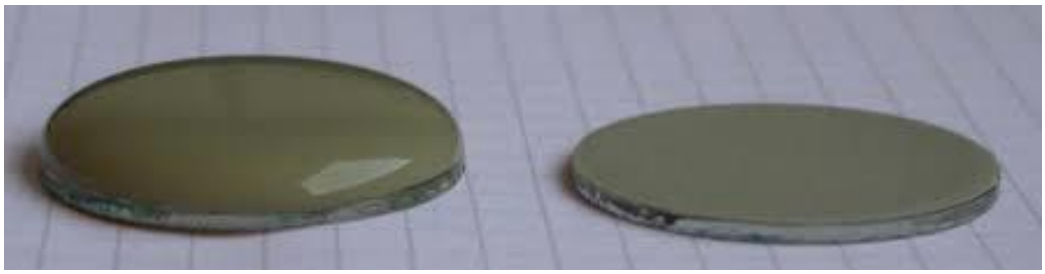


Figure 16. Oriented aggregates for clay minerals analysis by X-ray diffraction.

To identify clay minerals type a subsequent treatment was applied to the clay sample. The first treatment was to expose the clay sample to vapors of Ethylen Glicol Monoetyl Eter (EGME) overnight in a dessicator in order to force the d-spacing expansion in case of presence of expansible clay minerals. After EGME treatment clay samples were subjected to 550ºC treatment in oven during 6 hours. The temperature treatment allows detecting the presence of kaolinite clay mineral which will be destroyed after this treatment.

2.3.1.2. Soil texture

Principle:

The soil textural classes are defined by using Robinson's pipette that is based on the sedimentation of soil particles by gravity. There are three dimensional classes: 2000-200 µm for coarse sand fraction, 200-20 µm for fine sand fraction, 20-2 µm for silt, and <2 µm for clay fraction according to the International Society of Soil Science (ISSS). The recovery of portions at time and given depth makes it possible to collect the specific classes of particles to dry overnight and weighed. The method is based on the Stoke's Law equation (Stoke, 1851).

Used materials and equipment:

- Oven C. R. MARES, S. A. Model: 203.
- Digital balance (GF-1200).
- Rotating shaker.
- Robinson's pipette.

- Beakers 1000 ml.
- Glass cylinders 1000 ml.
- Plastic bottles 1000 ml.
- Sieves of 0.2 mm diameter.

Used chemical and solutions:

- Sodium Polyphosphate 5% [$(NaPO_3)n$].
- Hydrogen Peroxide 35% [H_2O_2].

Procedure:

- 20g of air dried sieved soils were placed in a beaker of 1000 ml and some drops of distillated water.
- 30 ml of hydrogen peroxide was added in order to oxidize the organic matter.
- Samples were heated at 80 ^{0}C for two hours, added 10 ml of H_2O_2 after one hour from the beginning of the heating.
- The suspensions were transferred into a one-liter plastic bottle and 15 ml of sodium polyphosphate was added.
- The plastic bottles were shaken in the rotating shaker overnight.
- After shaking, the suspension was filtered through a 0.2 mm diameter sieve contained in a Buchner funnel to a glass cylinder, then washed and filled with distilled water till 1000 ml.
- Particles >0.2 mm remained in the sieves were carefully washed, dried at 105 ^{0}C for 12 hours, then weighed and the percentage of coarse sand fraction was calculated.
  Coarse sand [CS] % = weight of particles >0.2 mm (g) ×100/initial soil amount (g).

- The suspensions contained particles <0.2 mm were vigorously agitated and put in stable place for 4’48’’ (minutes). Soon after 25 ml of suspension was taken by Robinson’s pipette at 10 cm depth from the surface.

- The volume (25ml) was posed in the small cup, oven dried at 105 ^{0}C and weighed to calculate the silt (S) and clay (C) content corresponding to the fractions 0.02-0.002 and <0.002, which were found at 10 cm after 4’48’’ of sedimentation.

  The S+C fraction was calculated as following:

  [S+C] content = 1st weight (g) of the particles after4’48’’×1000/volume (25ml)

  [S+C]% = [S+C] (g)×100/soil(g)

- The suspension was again taken and sampling of 25 ml suspension at 10 cm depth after 7 hours previous agitation.
- The second 25 ml allowed estimation of the clay content ©, fraction <0.002 mm

  The C fraction was calculated as following:

  C content = 2nd dry weight (g) of particles after 7 h ×1000 / volume (25 ml)

  Clay [C]% = [C] (g) × 100 / soil (g)

  Silt [S]% = [S+C]% – C%

  Finally fine sand was calculated by difference with respect to the previously obtained fractions:

  Fine Sand [SF] % = 100 – (SG%+S%+C%)

  Sand Fraction% = coarse sand% + fine sand%
- The soil texture was determined for each environment from the soil texture triangle as in Figure 17.

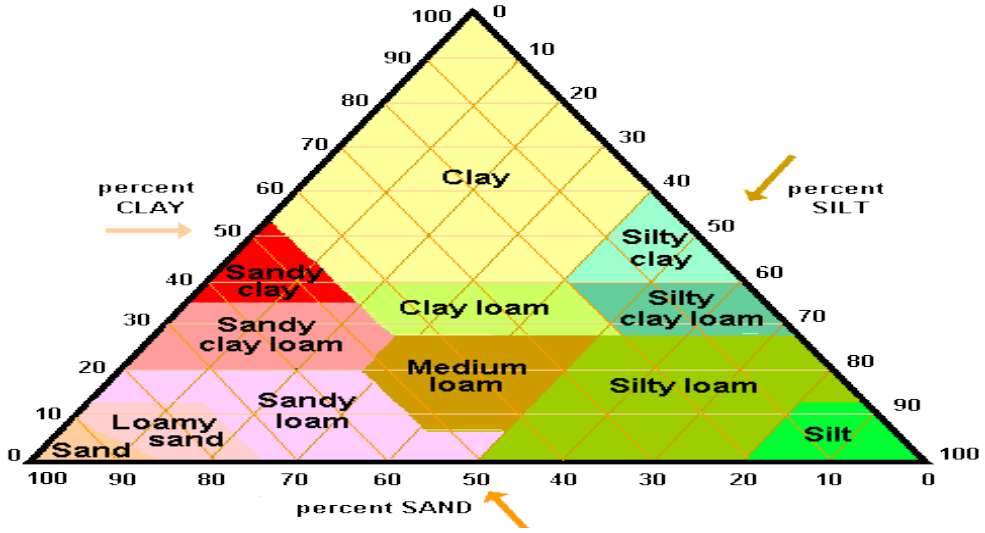


Figure 17. Triangle of soil texture to soil classification is typically made based on the relative proportions of silt, sand and clay.

2.3.1.3. Water stable aggregates (WSA)

Principle

Aggregate stability is a measurement of a resistance of soil structure against mechanical destructive forces of water. Soil structure is defined by the combination or arrangement of primary soil mineral and organic particles. Soil texture, soil structure, and the type of clay mineral, organic matter content and type, cementing agents and cropping history influence the aggregate stability. The mass of aggregated soil remaining after wet sieving as a percentage of the total mass of soil used represented the water stable aggregates. The measurements of WSA were determined by Kemper and Rosenau (1986).

Used materials and Equipment:

- Wet sieving Apparatus model 08.13 by EIjkeamp Inc (Figure 18).
- Sieves of 0.25mm and 2.00 mm diameters.
- Oven C. R. MARES, S. A. model 203 and digital balance (GF-1200).

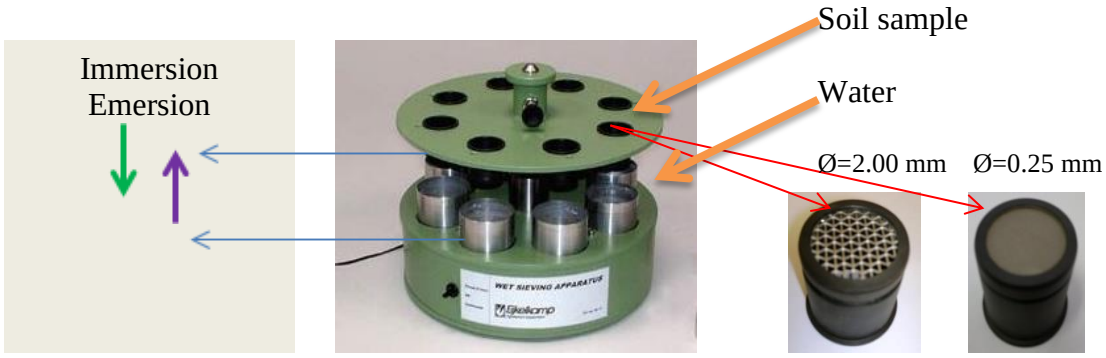


Figure 18. Eijkelkamp wet sieving apparatus and sieves.

Procedure:

- Aliquots of air dried aggregates were placed in a 0.25 mm sieve the 0.25-2.00mm of aggregate fraction, and in a 2.00mm sieve the 2.00-5.6mm of aggregate fraction.
- Soil aggregates were moved repeatedly cycling up and down and sieving in cans full with deionized water along 3 minutes. Aggregates were then subjected to 60 emersion /immersion cycles per minute.
- Soil aggregates surviving disruption and detachment were dried at 105 ^{0}C overnight and weighed.
- The stability of aggregates to water was calculated for each fraction from the following equation taking into account the sand content:

$$\text{WSA}\ (\%) = \frac{M(a+s) - Ms}{Mt - Ms} \times 100$$

Where, M(a+s) is the mass of the resistant aggregates plus sand (g), Ms is the mass of the sand fraction alone (g), and Mt is the total mass of the soil sample (g).

2.3.2. Laboratory chemical analysis

2.3.2.1. Soil pH

Principle:

The acidity or alkalinity of the soil is measured by a pH meter. The meter measures the potential difference between the hydrogen (glass) electrode and the reference (calomel) electrode and converts the reading to pH (Ward, R. C.). The determination of soil pH is done by means of a glass electrode in a soil solution slurry that contains a fivefold volume of water containing 1 M KCl (Forster, 1995).

Used materials and equipment:

- Beaker of 50 ml.
- pH meter (pH/Ion510 PRODUCER).
- Stirrer (AGIMATIC-N).
- Digital balance (Mettler A. E. 100).

Used chemicals and solutions:

- Buffer solution, pH 4.00 and 7.00 for calibration.
- 0.1 N KCl (7.4551 g l^{-1}) and deionized water.

Procedure:

- pH meter should be calibrated by 4.00 and 7.00 pH buffer solutions.
- 10 g of air dried sieved soil (0-2 mm) were added to 25 ml of deionized water in a 50 ml beaker.
- Other 10 g of air dried soil were added to 25 ml of 0.1 N of KCl in another 50 ml beaker.
- Each suspension was stirred for 10 min and let to decant.
- The electrode is immersed in the surface of the soil solution.
- pH values were recorded for H_2O and KCl solution in order to evaluate the ΔpH as follows:

$$\Delta pH = pH_{H_2O} - pH_{KCl}$$

High values of ΔpH may indicate beginning of acidification processes.

2.3.2.2. Soil electrical conductivity (EC)

Principle:

Measurement of soil electrical conductivity (EC) is carried out to estimate the relationship between the amounts of total dissolved salts (TDS) in the soil extraction and their capacity to conduct electricity. EC is generally expressed as dS m^{-1} at 25 ºC (Forster, 1995).

Used materials and equipment:

- Beaker of 100 ml.
- Conductivity Meter (CON510).

- Stirrer (AGIMATIC-N).
- Digital balance, 4 digits (Mettler A. E. 100).

Used chemicals and solutions:

- Buffer KCl solution for calibration (1280 µS cm^{-1}).

Procedure:

- The conductivity meter was calibrated by means of buffer solution of KCl having an EC of 1280 µS cm^{-1}.
- 10 g of air dried soil sieved at 0-2 mm, were added to 50 ml of deionized water in a beaker of 100 ml.
- The solution was stirred for 10 min and let to separate the supernatant.
- The EC electrode was immersed into the solution up to the second ring in the electrode.
- Conductivity value has been taken from the digital screen measuring in mS cm^{-1} and expressed in dS m^{-1} at 25ºC.

2.3.2.3. Soil organic carbon by dichromate oxidation

Principle:

The easily oxidisable components of organic carbon were determined by dichromate oxidation method. This oxidation process is carried out by potassium dichromate as an oxidizing agent and presence of sulphuric acid. The reaction is the following;

$$2K_2Cr_2O_7 + 3C + 8H_2SO_4 \rightarrow 2K_2SO_4 + 2Cr_2(SO_4)_3 + CO_2 + H_2O$$

$$2Cr_2O_7^{2-} + 3C + 16H^+ = 4Cr^{3+} + 3CO_2 + 8H_2O$$

Ferrous ammonium sulphate (Ammonium iron (II) sulfate) solution was titrated with residual amount of dichromate. Ortho-Phosphoric acid 85% was used to improve the reaction under the presence of indicator of diphenylamine. Appearance of green color is the end point of the reaction (Schnitzer, 1991).

Used materials and equipment:

- Erlenmeyer of 250 ml.
- Burette of 25 ml for titration.
- Digital balance, 4 digits (Mettler A. E. 100).

Used chemicals and solutions:

- Concentrated sulphuric acid [H_2SO_4 95% - 98%].
- 1 N $K_2Cr_2O_7$ (49.04 g/l).
- Ortho-Phosphoric acid [H_3PO_4 85%].
- Diphenylamine indicator solution: 0.5 g of diphenylamine dissolved in 20 ml distilled water and then added 100 ml of concentrated H_2SO_4.
- 18 N H_2SO_4: 489.13 ml of concentrated Sulphuric acid diluted in 1000 distilled water.
- Titration solution [0.5 N Mohr's salt]: Ferrous ammonium sulphate [$(NH_4)_2Fe(SO_4)_2.6H_2O$] (196.07 g l^{-1}) dissolved in deionized water, acidified with 10 ml of 18 N H_2SO_4 to prevent oxidation of ferrous iron and then completed with deionized water to 1000 ml.

Procedure:

- 10 ml of 1 N potassium dichromate [$K_2Cr_2O_7$] were added to a suitable amount of air dried soil (0.25mm-2 mm) in Erlenmeyer of 250 ml.
- 20 ml of concentrated sulphuric acid were added to promote the oxidation.
- After 30 min, 100 ml of deionized water and 10 ml of Ortho-phosphoric acid were added.
- Before the titration with Mohr's salt solution, 5 drops of diphenylamine solution were added to facilitate the detection of the end point.
- The residual dichromate was reacted with the titration solution until the green color appears.

  Oxidizable organic carbon can be calculated from the following equation;

$$\text{SOC}\ (\%) = \frac{0.3(\text{A} - \text{B}) \times \text{N} \times \text{CF}}{\text{P}}$$

where, A = milliliters of Mohr's salt solution for blank (ml).

B = milliliters of Mohr's salt solution for soil sample (ml).

N = normality of Mohr's salt solution.

P = weight of soil (g).

CF =correction factor ratio: Theoretical/Real ml of Mohr salt solution consumed for the blank.

- Organic matter (%) was calculated as follow: SOC (%) × $(0.58)^{-1}$

2.3.2.4. Soil extractable carbon, Fulvic and Humic acid

Principle:

Humic substances are considered important in sustainable agriculture because they enhance water-holding capacity, permeability, soil aggregation, buffering capacity, and cation exchange capacity (Cambardella et al., 1992). The role of humic substances in the C cycle is the main source of C reservoir and CO_2 emission that could be changed the CO_2 concentration in the atmosphere and climate change. Humic substances are divided into three fractions: (a) humic acid (HA) which is soluble and precipitated on acidification of alkaline extract; (b) fulvic acid (FA), which is soluble in both dilute alkali and acid; (c) humin. The procedure of extraction is shown in Figure 19 (Schnitzer, 1978).

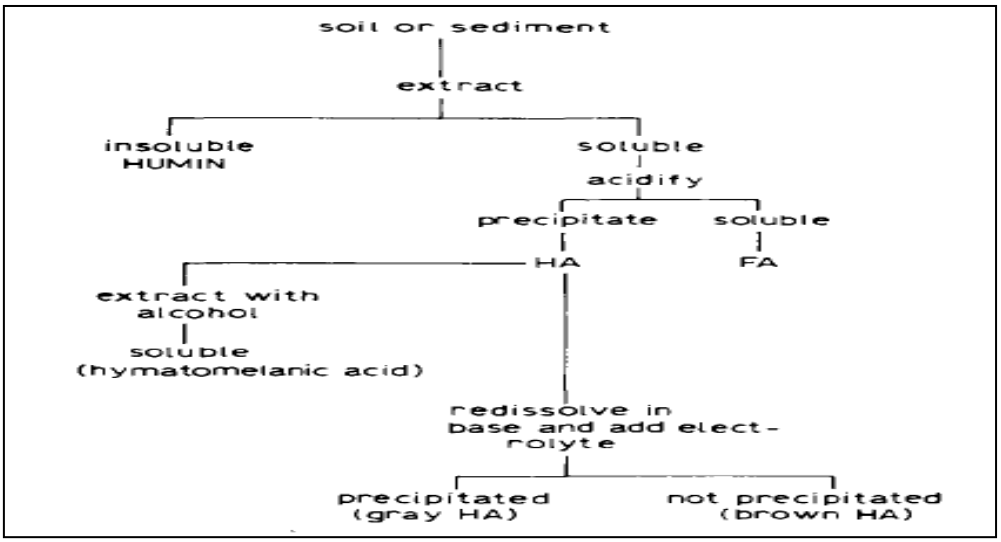


Figure 19. Fractions of Humic substances.

Used materials and equipment:

- Beaker of 100 ml.
- Plastic Tubes 50 ml.
- Concentrated HCL[35%].

- Small cups.
- Rotating Shaker.
- Micro pipettes.
- Centrifuge (EBA 21 D-78532 Tuttlingen).
- Oven C. R. MARES, S. A. Model: 203 and digital balance (GF-1200).
- Tetrasodium pyrophosphate [Na4P_2O7].
- Digital balance (Mettler A. E. 100).
- Erlenmeyer of 250 ml.
- Burette of 25 ml for titration.
- Concentrated sulphuric acid [H_2SO_4 95% - 98%].
- 1 N $K_2Cr_2O_7$ (49.04 g/l).
- Ortho-Phosphoric acid [H_3PO_4 85%].
- Diphenylamine indicator solution: 0.5 g of diphenylamine dissolved in 20 ml distilled water and then added 100 ml of concentrated H_2SO_4.
- 18 N H_2SO_4: 489.13 ml of concentrated sulphuric acid diluted in 1000 distilled water.
- Titration solution [0.5 N Mohr's salt]: Ferrous ammonium sulphate [$(NH_4)_2Fe(SO_4)_2.6H_2O$] (196.07 g l^{-1}) dissolved in deionized water, acidified with 10 ml of 18 N H_2SO_4 to prevent oxidation of ferrous iron and then completed with deionized water to 1000 ml.

Principle:

- 4g of air dried soils was placed in plastic tubes of 50ml with 40 ml of Tetrasodium pyroahosphate [$Na_4P_2O_7$].
- Samples were agitated with rotating shaker overnight.
- Centrifuged and the extracted volume placed into a beaker of 100 ml.
- Acidified with concentrated HCL to separate the soluble (FA) and insoluble (HA).
- Both fractions were then transferred into separate small cups, and oven dried at 60^0C to recover the paste.
- 10 ml of 1 N potassium dichromate [$K_2Cr_2O_7$] were added to a suitable amount of the paste of (FA) and (HA) in Erlenmeyer of 250 ml.
- 10 ml of concentrated sulphuric acid were added to promote the oxidation.
- After 30 min, 100 ml of deionized water and 10 ml of Ortho-phosphoric acid were added.

- Before the titration with Mohr's salt solution, 5 drops of diphenylamine solution were added to facilitate the detection of the end point process.
- The residual dichromate was reacted with the titration solution until the green color appears.

Oxidizable organic carbon can be calculated from the following equation;

$$SOC\ (\%) = \frac{0.3(A - B) \times N \times CF}{P}$$

where, A = milliliters of Mohr's salt solution for blank (ml).

B = milliliters of Mohr's salt solution for soil sample (ml).

N = normality of Mohr's salt solution.

P = weight of soil (g).

CF = correction factor ratio: Theoretical/Real ml of Mohr salt solution consumed for the blank.

2.3.2.5. Total nitrogen by Kjeldahl method

Principle:

The total Nitrogen in soil was determined by the Kjeldahl method. The first basic reaction is the conversion of organic nitrogen form into NH_4^+-N by heating the mixture of the soil and sulphuric acid for 1h at 175^0C and 1.5h at 375^0C to digest the nitrogen compounds. The oxidation process is promoted by selenium as alkaline catalyst. The second process is the distillation of NH_4^+-N form previous basification into ammonium (NH_3), addition of Boric acid to avoid NH_3 volatilization and titration with a standard sulphuric acid solution in presence of methylene blue indicator. The end point is reached when the original color of indicator is obtained, indicating the formation of boric acid again by reconversion of NH_3 to NH_4^+ (Kjeldahl, 1983; Forster, 1995).

Used materials and equipment:

- Digestion Unit (J. P. SELECTA Model: 508).
- Distillation Unit (J. P. SELECTA-PRONITRO I).
- Digestion tubes of 100 ml.
- Digital balance, 4 digits (Mettler A. E. 100).


Figure 20. The digestion and distillation units used for the determination of total nitrogen.

Used chemicals and solutions:

- Concentrated sulphuric acid [H_2SO_4 95%-98%].
- Titration solution: Standard sulphuric acid solution [0.01 N H_2SO_4].
- Boric acid [H_3BO_3 4%].
- Indicator [methyl blue + methyl green].
- Sodium hydroxide [NaOH 35%].
- Kjeldahl Catalyst Tablets [99.9% Potassium Sulphate + 0.1% Selenium].

Procedure:

- 5 g of Kjeldahl Catalyst and 20 ml of sulphuric acid were added to1g of soil in a digestion tube to raise the temperature during digestion.
- The digestor was adjusted at 175 ºC for 1 h and then at 375 ºC for 1.5 h.
- After digestion and cooling, 25 ml of distilled water and 4-5 drops of indicator were added to each tube.
- The tube was adjusted in the distillation apparatus and 50 ml of NaOH 35% were automatically introduced in the digestion tube and the distillation was started.
- 150 ml of the distilled solution was collected in a 250 ml Erlenmeyer and 25 ml of Boric acid were added.
- The distilled solution containing NH_3 was titrated with 0.01 NH_2SO_4 standard solution until the original color of indicator reappeared indicating the back formation of Boric acid.

  The milligrams of nitrogen in the soil are calculated as following:

$$\text{mg N} = \text{A ml} \times \text{N} \times 14$$

where, A = ml of the titration solution.

N = normality of sulphuric acid (titration solution).

14 = molecular weight of nitrogen.

The percentage of total nitrogen (%) was calculated with respect to the soil used.

$$\text{TN (\%)} = \frac{\text{N (mg)}}{\text{Soil (mg)}} * 100$$

2.3.2.6. Extraction of Easily Extractable Glomalin (EE-GRSP)

Principle:

Freshly produced glomalin could be extracted from soil by using gentle conditions. This protein is more weakly aggregated with soil particles in the form of soil aggregate. The extraction of easy extractable glomalin could be extracted under high temperature in the presence of a low concentration of sodium citrate. The EE-GRSP pool was extracted by using 20mM citrate, pH 7.0, by one autoclave cycle extraction at 121^0C for 30 minutes (Wright and Upadhyaya, 1998).

Used materials and equipment:

- Polyethylene tubes (50 ml) with screw cup and not tightly closed to relieve pressure during autoclaving.
- Autoclave (PRESOCLAVE 30 L Selecta).
- Centrifuge (EBA 21 D-78532 Tuttlingen).
- Oven C. R. MARES, S. A. Model: 203.
- Vortex (IKA VORTEX GENIUS 3 Model: VG3).
- Graduated cylinder 10 ml.
- 50 ml screw-capped tubes for storing extract.

Used chemicals and solutions:

- 20 mM trisodium citrate dihydrate [$C_6H_5O_7Na_3.2H_2O$] (M.W.: 294.10); 5.882 g of trisodium citrate dihydrate dissolved in one liter of distilled water and pH adjusted at 7.0 with HCl solution.

Procedure:

- An equivalent one gram oven dry soil of air dried sieved (0.25mm-2 mm) soil was added to 8 ml of 20 mM sodium citrate in polyethylene tubes, shacked with vortex to have appropriate soil solution contact, and then autoclaved at 121 ºC for 30 min.
- After autoclave, samples were cooled at room temperature and then immediately centrifuged at 4000 g for 15 min (centrifugation is just to pellet the soil particles and may be conducted at any speed from 3000-10000 g).
- G-Force of 4000 g was converted to 5346 rpm by using a radius of 99.9872208 mm.
- The supernatant that contains the protein was measured by a graduated cylinder and stored in screw capped tubes at 4 ºC.
- The stored solution extraction should be examined for microbial growth contamination and not exceed up to 15 days for their use.

2.3.2.7. Extraction of total glomalin (GRSP)

Principle:

The extraction of total glomalin is consequence of several extraction cycles to make the more stable glomalin forms adhering to particles forming soil aggregates. The total extraction of glomalin is completed under higher citrate concentration. The total glomalin was extracted with 50 mM citrate, pH 8.0, by sequential autoclave extraction cycles at 121 ºC for 30 minutes until the brown color became yellow pale color (Wright and Upadhyaya, 1996).

Used materials and equipment:

- Polyethylene tubes (50 ml).
- Autoclave (PRESOCLAVE 30 L Selecta).
- Centrifuge (EBA 21 D-78532 Tuttlingen).
- Oven C. R. MARES, S. A. Model: 203.
- Vortex (IKA VORTEX GENIUS 3 Model: VG3).
- Graduated cylinder 10 ml.
- 50 ml screw-capped tubes for storing extract.

Used chemicals and solutions:

- 50 mM trisodium citrate dihydrate [$C_6H_5O_7Na_3.2H_2O$] (M.W.: 294.10); 14.705 g of trisodium citrate dihydrate dissolved in one liter of distilled water and pH adjusted at 8.0 with HCl solution. Otherwise, in case of using trisodium citrate 5.5 hydrate [$C_6H_5O_7Na_3.5.5H_2O$] (M.W.: 357.15), 17.8575 g l^{-1} was used.

Procedure:

- An aliquot of 0.25mm-2 mm air dried soil (equivalent to 1 g oven dried soil) was added to 8 ml of 50 mM sodium citrate dihydrate in polypropylene centrifuge tubes.
- The tubes were shacked by vortex to homogenize the soil suspension and then autoclaved at 121 ºC for 30 min. for the first cycle.
- Samples were centrifuged at 4000 g for 15 min. and supernatant decanted and collected in screw caped tubes.
- Soil pellets were re-suspended with 8 ml of 50 mM sodium citrate, shacked, and autoclaved for the second cycle.
- The last two steps were repeated until the supernatant has a yellow pale or limpid color. Extracts were pooled together. A total of seven cycles extractions were carried out in order to reach the yellow pale color in the supernatant.
- Total glomalin extracts were measured with a graduated cylinder and stored at 4 ºC until use.

Quantification of glomalin by Bradford protein assay

Principle:

The determination of protein concentrations in solutions depended on the change in absorbance in an acidic solution of Coomassie Blue G-250, is at 595 nm upon binding of protein (Bradford, 1976). The Bradford protein assay is a quantitative spectroscopic analytical procedure used to measure the concentration of glomalin in soil extraction solution. The current assumption in this method is that all or the vast majority of proteins were destroyed during the harsh extraction procedure except glomalin (Rosier et al., 2006). The significant protein-to-protein variation in absorbance values obtained with the Bradford procedure and give absorbance values close to those for the protein samples. The protein results in a shift of the dye's absorption maximum to 595 nm. As the protein concentration increases, the absorbance of light at 595 nm increases linearly. Although the absorbance of Coomassie blue

dye at 595 nm is proportional to the amount of protein bound, it is necessary to establish a correspondence between absorbance values and known amounts of protein. A series of protein standards dilutions of a protein solution of known concentration should be prepared before measurement. Once measured each standard at 595 nm one will be able to plot the A595 as a function of the known protein content of each standard. After measuring the A595 of unknown sample, the standard curve then can be used to determine the amount of protein corresponding to the absorbance values measured (Wright and Upadhyaya, 1998).

Used materials:

- Visible light spectrophotometer (UV-160A, Shemadzu) calibrated at 595 nm.
- Disposable cuvettes are recommended in order to avoid the coloring with reagent.
- Vortex shaker (IKA VORTEX GENIUS 3 Model: VG3).

Used chemicals and solutions:

- Bradford reagent: Bradford reagent ready-to-use should be used and can be stored at 4 ºC. Alternatively, it can be made by dissolving 100 mg of Coomassie Blue G-250 (available from several sources) in 50 ml of 95% ethanol, adding 100 ml 85% (w/v) phosphoric acid to this solution, and diluting the mixture to 1 liter with water.
- Reagent solution: One volume of Bradford reagent diluted with four volumes of distilled water. The solution should appear brown and pH is 1.1. It is stable for 2 weeks in a dark bottle at 4 ºC.
- Buffer solution: The same buffer concentration used for extraction procedure, i. e. 20 mM trisodium citrate dihydrate for the quantification of EE-GRSP and 50 mM for GRSP.
- Protein standard solution (0.5 mg ml^{-1}): 50 mg bovine serum albumin (BSA) dissolved in 100 ml distilled water.
- Standard concentrations: The standard dilutions should be prepared in the same buffer of the extraction procedure. A convenient standard curve can be made using bovine serum albumin concentration as detailed in Table 1.

Table 1. Preparation of the BSA concentrations to obtain the standard curve.

Conc. of BSA	BSA (ml)	Buffer (ml)	Total volume (ml)
0 µg ml^{-1}	0.00	25.00	25.00
25 µg ml^{-1}	1.25	23.75	25.00
50 µg ml^{-1}	2.50	22.50	25.00
100 µg ml^{-1}	5.00	20.00	25.00
200 µg ml^{-1}	10.00	15.00	25.00

BSA: Bovine serum albumin.

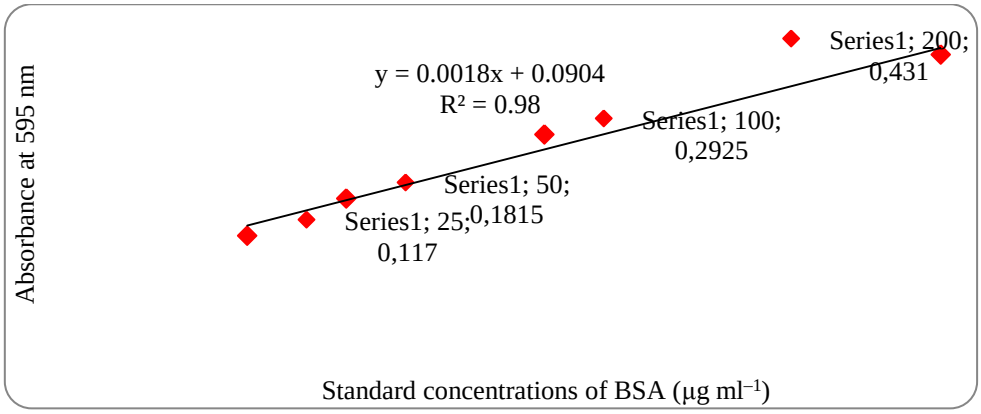


Figure 21. Representative standard curve prepared by the standard concentration of BSA.

Procedure:

- 200 µl of each concentration of BSA were diluted with 4 ml of Bradford reagent solution.
- The assay is useful since the extinction coefficient of a dye-albumin complex solution is constant over a 10-fold concentration range (extinction coefficient of BSA is 0.667).
- Samples are diluted as the standard dilutions, 200 µl of glomalin extracted diluted with 4 ml of Bradford reagent solution.
- Standard and sample dilutions were immediately shacked and incubated for maximum 5 min. before measurements with the spectrophotometer at 595 nm.
- The standard curve of absorbance versus the concentration was prepared and the concentration equation from the curve was determined.
- Absorbance of samples was measured and the concentration (ppm) calculated from the standard concentration equation.
- Total glomalin was calculated with respect to initial soil weight.

2.3.2.8. Dissolved organic carbon (DOC)

Principle:

DOC transport in drained water, from surface of an area of land or building, increases with increasing proportion of wetlands present in the watershed. Especially, with organic soil wetlands or peatlands present. Dissolved organic carbon (DOC) may be estimated by the same oxidation approach (dichromate in the presence of strong acid) although the reagent concentration is different as will be described henceforth (Aitkenhead et al. 1999).

Used materials and equipments:

- Digestor (MULTI-BLOC SYSTEM) provided with Aluminum blocks with holes of 40 mm diameter and 80 mm depth.
- Erlenmeyer of 250 ml.
- Test tubes of 25 ml.
- Burette of 25 ml.

Used chemicals and solutions:

- Concentrated sulphuric acid [H_2SO_4 95%-98%].
- Concentrated ortho-phosphoric acid [H_3PO_4 85%].
- 66.7 mM potassium dichromate [$K_2Cr_2O_7$] (19.6125 g l^{-1}).
- Ferroin solution Indicator [$Fe(C_{12}H_8N_2)_3]SO_4$ M.W.: 692.52 (0.025 M).
- Acid mixture solution: [2 parts of H_2SO_4:1 part of H_3PO_4].
- Titration solution [0.04 N Mohr's Salt]: 15.96 g of ferrous ammonium sulphate [$(NH_4)_2Fe(SO_4)_2.H_2O$] dissolved in liter of deionized water, acidified with 20 ml of concentrated H_2SO_4 to prevent oxidation of ferrous iron then completed by deionized water to 1000 ml.

Procedure:

- 2 ml of 66.7 mM $K_2Cr_2O_7$ was added to 4 ml of water runoff in test tube followed by 7 ml of acid mixture.
- Two blank tubes were prepared by adding 2 ml of 66.7 mM $K_2Cr_2O_7$ and 7 ml of acid mixture in order to control the end point and estimation of water sample.

- The sample tubes and one blank tube were heated at 60 ºC on the digestor for 30 minutes to allow the oxidation of dissolved carbon by dichromate; one blank was left to react at room temperature.
- The contents of each tube was decanted and rinsed with 20-25 ml deionized water in Erlenmeyer of 250 ml.
- 4-5 drops of indicator were added to each Erlenmeyer.
- The excess of non-oxidized dichromate was titrated with the titration solution until the appearance of the end point, the conversion of orange red color to green color.
- Dissolved organic carbon was calculated by the following equation:

$$DOC = \frac{(H - S)(A \times E \times 1000)}{(C \times M \times D) \times 1000}$$

where, DOC = dissolved organic carbon (mg ml^{-1}).

H = volume of titration solution for hot blank (ml).

C = volume of titration solution for cold blank (ml).

S = volume of titration solution for sample (ml).

M = normality of $K_2Cr_2O_7$ (eq l^{-1}).

D = volume of $K_2Cr_2O_7$ (ml).

A = volume of the water runoff sample (ml).

E = conversion factor in the oxidation process that occurs in the Cr^{6+} to Cr^{3+}, the value is equal to 3.

2.3.2.9. Soil respiration

Principle:

Soil respiration is the expression of CO_2 emission from soil. Different methods have been tested in field and laboratory to evaluate this contribution. In the studied soil environments both a static and a dynamic method were used for the estimation of CO_2 flux from surface soil horizon. The static soda lime method was used for both field and laboratory measurements. Soda lime is commonly used as a desiccant because of its ability to absorb water vapor from the air and able to loss the water in a reversible reaction by oven drying. Water is molecularly required to form Sodium and Calcium carbonates, its formation recorded by an increase of soda lime weight. Because of its alkaline properties soda lime removes carbon dioxide very efficiently from the atmosphere according to these reactions:

$$2\ NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$$
$$Ca\ (OH)_2 + CO_2 \rightarrow CaCO_3\downarrow + H_2O$$

Soda lime absorbs carbon dioxide and water resulting from soil respiration but only CO_2 is chemically bounded with soda lime after oven drying. Carbon dioxide respiration can be estimated by the Edwards method (Edwards, 1982), modified by Grogan (1998), and Keith and Wong (2006).

Used materials and equipment:

- Oven C. R. MARES, S. A. Model: 203.
- Glass jars and ceramic cup for soda lime.
- Digital analytical balance, 4 digits (Mettler A. E. 100).

Used solutions and chemicals:

- Soda lime containing: 75% Calcium hydroxide [$Ca\ (OH)_2$], 20% water [H_2O], 3% sodium hydroxide [NaOH], and 1% potassium hydroxide [KOH]. It was used in granular form. It absorbs carbon dioxide in the presence of water vapor.

Procedure:

- The soda lime had been previously prepared in the laboratory as follows: 15 g of pure soda lime were placed in a ceramic cup, oven dried at 105 ºC overnight, cooled in the desiccator, and weighed with the accuracy balance.
- Weighed 30g of soil was placed in glass jar and added with 7 ml of water.
- Cups were immediately closed and stored in the glass jar for 7days and then weighed and oven at 105 ºC for 24h.
- After 24 h the cooled in a desiccator and then weighed with the accuracy balance.
- The weight of carbon dioxide absorbed by soda lime was calculated by multiplying the weight gain after oven drying by 1.69 as a correction factor. As observed each mole of CO_2 chemically bound to soda lime favors the formation of a mole of water, which is then lost by oven drying. Therefore dry mass increase before and after exposure underestimates CO_2 absorbed. The correction factor takes into account that 44 g of CO_2 react with 74 g of $Ca(OH)_2$ to form 100 g $CaCO_3$ and 18 g H_2O. The measured increase in soda lime after oven-drying is 26 (i.e. 100–74). Thus the correction factor of 44/26

(i.e. 1.69) must be applied to the measured mass differences in order to obtain the true value of CO_2 absorbed (Grogan, 1998; Keith and Wong, 2006; Emran et al., 2012a).

2.4. Statistics

Statistical analysis was carried out by using the program Statistics (Version 7.1, 2007, Statsoft Inc., Tulsa, OK, USA). ANOVA was used to investigate the data variations within and between the soil environments along the three sampling depths. ANOVA was run by using the number of replications (3), the mean and the standard deviation of any given parameters at any sampling depth when comparing data variability within each one environment. Likewise, to compare data variability between environments the mean of three values of a given parameter and the standard deviation for the six environments at any sampling depth were run. Factor analysis was performed to provide information on the relationships between the measured variable and the investigated soil environment.

Chapter III. RESULTS AND DISCUSSION

3.1. Soil mineralogy

In order to identify the most abundant minerals in the studied soils, a detailed search of mineralogical parameters was carried out as shown in Table 2.

Table 2. X-Ray diffraction parameters for mineral content identification in the studied soils.

Mineral phase	d_1 (Å)	2θ	d_2(Å)	2θ	d_3(Å)	2θ
Albite $NaAlSi_3O_8$	3.17 (100)	28.07	3.211 (30)	27.76	3.752 (30)	23.69
Aragonite $CaCO_3$	3.396(100)	26.22	1.977 (65)	45.86	3.273 (52)	27.22
Biotite $K(Mg,Fe)_3(AlSi_2O_{10}(OH)_4$	3.37 (100)	26.43	10.1 (100)	8.75	2.66 (80)	33.67
Blodite $Na_2Mg(SO_4)_2{\cdot}4H_2O$	3.25 (100)	27.42	3.29 (95)	27.08	4.56 (95)	19.45
Calcite $CaCO_3$	3.03 (100)	29.40	2.095 (18)	43.14	2.285 (18)	39.40
Chlorite $(Mg,Fe)_5Al(Si_3Al)O_{10}(OH)_8$	7.16 (100)	12.35	4.77 (70)	18.59	3.58 (60)	24.85
Ematite Fe_2O_3	2.69 (100)	33.28	1.69 (60)	54.23	2.51 (50)	35.74
Epsomite $MgSO_4{\cdot}7H_2O$	4.21 (100)	21.08	2.677 (25)	33.45	5.35 (25)	16.56
Gypsum $CaSO_4{\cdot}2(H_2O)$	7.63 (100)	11.59	4.28 (100	20.74	3.07 (80)	29.06
Glauberite $Na_2Ca(SO_4)_2$	3.13 (100)	28.49	2.66 (80)	33.67	6.22 (80)	14.23
Halite NaCl	2.821(100)	31.69	1.994 (55)	45.45	1.628 (15)	56.48
Kaolinite $Al_2Si_2O_5(OH)_4$	7.07 (100)	12.33	1.49 (90)	62.26	3.58 (80)	24.85
Labradorite $(Na,Ca)(Si,Al)_4O_8$	3.18 (100)	28.04	3.21 (70)	27.77	3.76 (70)	23.64
Mirabilite $Na_2SO_4{\cdot}10H_2O$	5.49 (100)	16.13	3.21 (75)	27.77	3.26 (60)	27.33
Muscovite $KAl_2(Si_3Al)O_{10}(OH,F)_2$	3.32 (100)	26.83	9.95 (95)	8.88	2.57 (55)	7.44
Orthoclase $KAlSi_3O_8$	3.18 (100)	28.04	4.02 (90)	22.09	3.80 (80)	23.39
Quartz SiO_2	3.34 (100)	26.65	4.257 (22)	20.85	1.817 (14)	50.14
Sanidine $(K,Na)(Si,Al)_4O_8$	3.26 (100)	27.33	3.22 (90)	27.68	3.27 (75)	27.25
Thenardite Na_2SO_4	2.78 (100)	32.14	4.66 (73)	19.03	3.178 (51)	28.05

d_1, d_2, d_3: Are the three main peaks of maximum intensity to be used for the identification of the mineral phase. Between brackets the XRD intensity value (%); 2θ: XRD angle of a given mineral related to each mineral peak and intensity.

As may be observed this table reports the three principles d-spacing (d_1, d_2, and d_3), the corresponding reflection intensity and the diffraction angle (2θ), and facilitate the identification of not common minerals which may be found in saltmarsh environments. The search of minerals was focused to identify not only the most common phases but also special minerals that may be formed upon salt accumulation. This is because in the area of study we have often noticed saline efflorescence on the soil surface as may be observed by Figure 22.

Figure 22. An example of salt deposition at the Pletera saltmarsh.

X-ray diffraction analysis of soil total mineralogy showed that the main mineral phases present in the samples were quartz, calcite and feldspars amounting to more than 90% of the mineral matter being quartz always the most abundant. Conversely, the Ruderal environment was much richer in calcite corroborating it is allocthonous material. Only in the SAAR environment it was possible to identify the mineral Blodite which is a sodium magnesium sulphate ($Na_2Mg(SO_4)_2 \cdot 4H_2O$) as reported in Table 2 and Figure 23. The possible existence of Mirabilite ($Na_2SO_4 \cdot 10H_2O$) was also noticed though in very few amount.

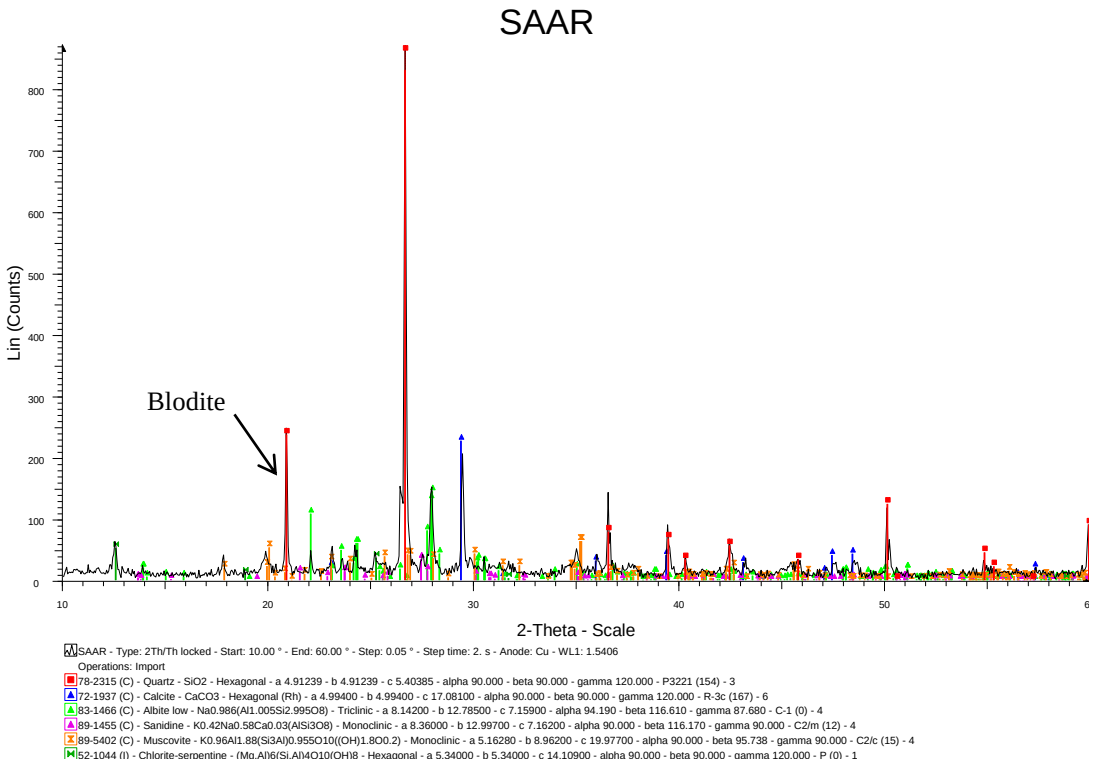


Figure 23.An example of X-ray diffraction diagram of SAAR environment.

The mineralogy of clay showed the presence of muscovite and kaolinite but not the presence of more reactive clays like montmorillonite, probably because of the prevalently abundance of sand and the singularity of the environments. The presence of muscovite and kaolinite was checked by the application of temperature (550Cº) to the clay specimen. This test allows to confirm the presence of the two above clay minerals because kaolinite is destroyed and muscovite resists the temperature treatment (Figure 24). In fact, from Figure 24 it may be observed that the green lines corresponding to kaolinite with peaks at 14 Å and 7Å appearing in the first graph is completely disappeared in the graph reporting the heat treatment.

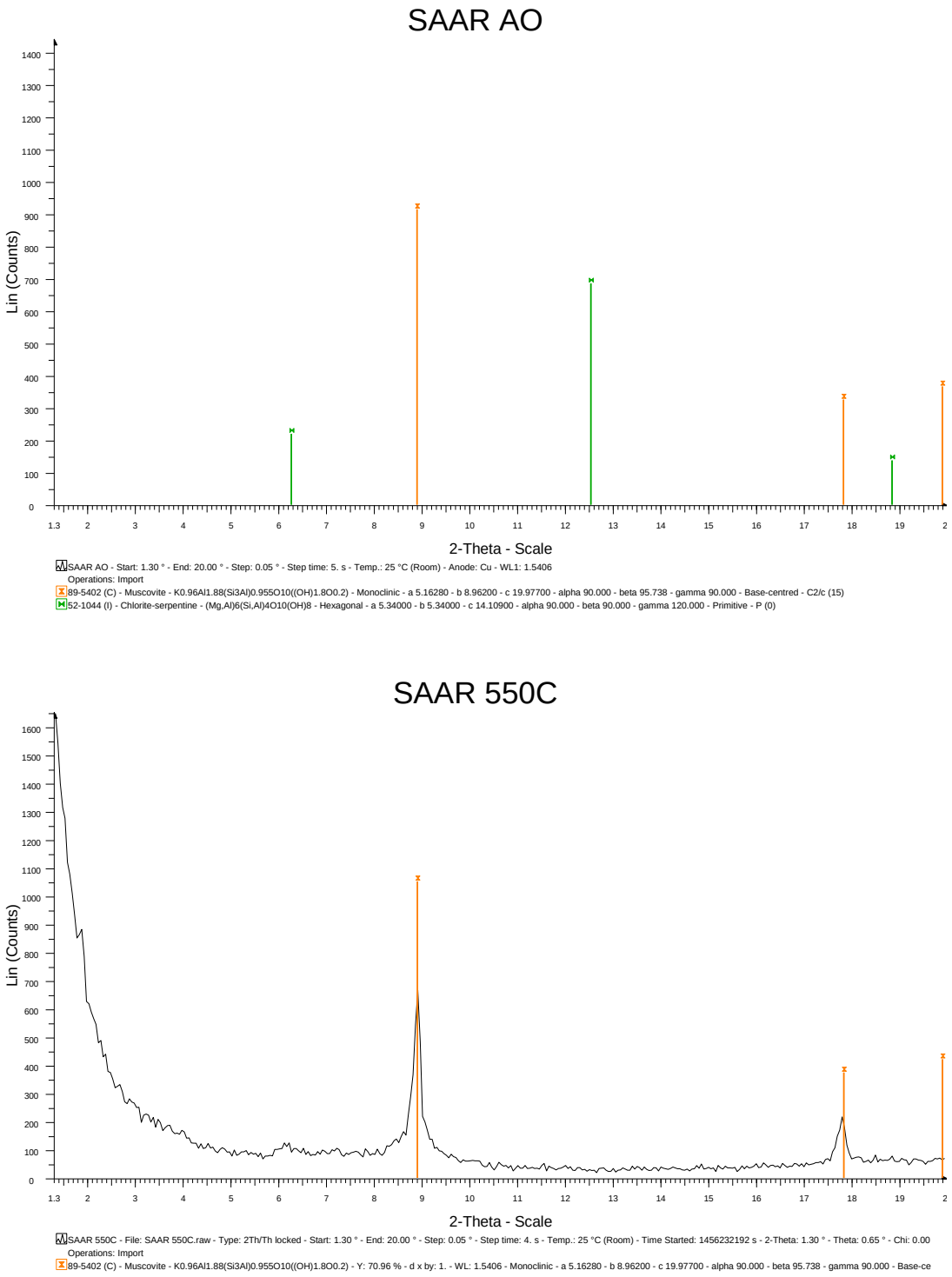


Figure 24. An example of clay mineral diffraction diagram before and after heat treatment.

The results of soil mineralogy have announced the lacking of very reactive clays increasing the importance of soil organic carbon alone in soil properties improvement.

The physiographical characteristics of the studied soils are reported in Table 3. It is worth to outline that each of the six selected soil environment has almost unique plant cover species which may facilitate the relationships between plant and soil properties dynamics.

3.2. Soil physical and chemical characteristics

Table 3. Physiographical characteristics of the studied soils at La Pletera saltmarsh (Estartit, Girona).

Soil	Location	Parent material	Soil use	Main Plant cover	Soil depth (cm)	Horizon development
RU	42°03103"N, 003°12'31"E	Calcareous	Rubbles	*Brachypodium retusum*	75	A/C
ELY	42°03103"N, 003°12'31"E	Calcareous	Meadows	*Foeniculum vulgare* *Elymus elymoides*	75	A/C
SAAR	42°03103"N, 003°12'31"E	Calcareous	Marsh	*Arthrocnemum fruticosum*	75	A/C
SAER	42°03103"N, 003°12'31"E	Calcareous	Marsh		75	A/C
AGR	42°03103"N, 003°12'31"E	Calcareous	Cultivation	*Salicornia Patula*	75	A/C
PAS	42°03103"N, 003°12'31"E	Calcareous	Pasture	*Zea mays* *Medicago sativa*	75	A/C

RU: Ruderal soil environment; ELY: *Elys Elimoides* soil environament; SAAR: *Arthrocnemum fruticosum* soil environament; SAER: *Salicornia patula* soil environament; AGR: Corn (*Zea mays*) cultivated soil environament; PAS: Pasture (*Medicago sativa*) soil environament.

All soil samples were collected in triplicate in each soil environment and analysed for their characterization. According to particle size distribution the soils presented an abundance of sand fractions which is in agreement with their location. Only the SAAR soil showed a sandy clay loam texture for its higher amount of clay (Table 4). As may be observed from this table the fractions coarse and fine sand maintain their percentage along the three examined depths. By contrast, the clay fraction decreased substantially in all the environments indicating that the origin of these soils is prevalently due to the presence of ancient sand dunes. From the descriptive statistic, it is observed a quite high variability of both standard deviation and coefficient of variation which may indicate a high variation of the sampling points. However, the standard deviation and coefficient of variation are very low in Ruderal soil because it represents the added homogenous materials in the altered area.

Table 4. Particle size and textural class at different depths of the studied soils at La Pletera saltmarsh (Estartit, Girona). Descriptive statistics is reported.

0-5 cm depth

		CS(%)			FS(%)			S(%)			C(%)			Textural
Soil	N	×	σ	cv	×	σ	cv	×	σ	cv	×	σ	cv	class
RU	3	**62.71**	6.01	9.59	**18.62**	8.32	44.69	**10.67**	2.31	21.65	**8.00**	4.00	50.00	Loamy sand
ELY	3	**45.55**	12.33	27.07	**46.72**	10.39	22.24	**5.33**	1.51	28.39	**2.40**	1.06	44.09	Sand
SAAR	3	**23.90**	12.94	54.14	**20.11**	3.95	19.66	**27.33**	6.11	22.35	**28.67**	9.45	32.97	Sandy clay loam
SAER	3	**73.67**	9.87	13.40	**5.00**	4.01	80.30	**6.00**	4.00	66.67	**15.33**	2.31	15.06	Loamy sand
AGR	3	**45.27**	0.67	1.47	**21.40**	2.74	12.81	**17.33**	1.15	6.66	**16.00**	2.00	12.50	Sandy loam
PAS	3	**45.18**	2.06	4.57	**23.48**	2.25	9.57	**17.33**	1.15	6.66	**14.00**	2.00	14.29	Sandy loam

5-20 cm depth

		CS(%)			FS(%)			S(%)			C(%)			Textural
Soil	N	×	σ	cv	×	σ	cv	×	σ	cv	×	σ	cv	class
RU	3	**60.18**	5.45	9.05	**17.23**	6.11	35.46	**9.30**	4.88	52.47	**6.76**	3.61	53.40	Loamy sand
ELY	3	**48.88**	19.42	39.73	**43.38**	17.70	40.80	**4.40**	4.51	102.44	**3.33**	1.80	54.11	Sand
SAAR	3	**27.08**	7.53	27.80	**40.25**	4.32	10.73	**20.00**	9.17	45.83	**12.67**	6.11	48.24	Sandy loam
SAER	3	**87.02**	5.86	6.74	**4.98**	2.40	48.25	**2.67**	1.15	43.30	**5.33**	1.15	21.65	Sand
AGR	3	**45.22**	0.67	1.47	**23.45**	2.67	11.37	**15.33**	6.11	39.85	**16.00**	4.00	25.00	Sandy loam
PAS	3	**46.48**	3.09	6.65	**21.52**	1.13	4.78	**22.00**	0.00	0.00	**8.00**	2.00	25.00	Sandy loam

20-40 cm depth

		CS(%)			FS(%)			S(%)			C(%)			Textural
Soil	N	×	σ	cv	×	σ	cv	×	σ	cv	×	σ	cv	class
RU	3	**67.85**	4.19	6.17	**16.15**	2.39	14.81	**9.33**	1.15	12.37	**6.67**	2.31	36.64	Loamy sand
ELY	3	**51.18**	21.54	42.08	**43.48**	20.95	48.18	**2.93**	0.46	15.75	**2.40**	1.06	44.09	Sand
SAAR	3	**29.58**	19.21	64.93	**49.75**	15.46	31.08	**6.67**	4.62	69.28	**14.00**	7.21	51.51	Loamy sand
SAER	3	**48.22**	23.73	49.21	**20.45**	5.99	29.31	**20.67**	14.46	70.00	**10.47**	6.43	60.27	Sandy loam
AGR	3	**51.45**	4.57	8.89	**21.22**	3.93	18.53	**14.00**	0.00	0.00	**13.33**	1.15	8.66	Sandy loam
PAS	3	**46.33**	7.59	16.38	**24.33**	3.94	16.20	**16.00**	4.00	25.00	**13.33**	1.15	8.66	Sandy loam

RU: Ruderal soil environment; ELY: *Elys Elimoides* soil environament; SAAR: *Arthrocnemum fruticosum* soil environament; SAER: *Salicornia patula* soil environament; AGR: Corn (*Zea mays*) cultivated soil environament; PAS: Pasture (*Medicago sativa*) soil environament; N: Number of replications; CS: Coarse sand; FS, Fine sand; S: Silt; C: Clay; ×: Mean of three replicationa; σ: Standard deviation; cv: Coefficient of variation.

Soil reaction was measured at the three depths both in water and potassium chloride 0.1M in order to see any acidification through ΔpH (Table 5). It is known that potassium has a large affinity for negatively charged soil surfaces and when it acts like that it displaces protons in the soil solution increasing the pH value of soil solution and the ΔpH, advising of an acidification process for a probable presence of protons in the exchange complex. As may be observed in Table 5 this was not the case and the ΔpH was always much lower than 1 pH unit. Effectively, all the soils showed slightly alkaline pH values which was logical for the sampling areas. The pH values did not change markedly along the depths and were rather homogenous as also showed by standard deviation and coefficient variation.

Table 5. Soil reaction and salinity at diferent soil depth at the Platera saltmarsh (Estartit, Girona). Descriptive statistics is reported.

0-5 cm depth

		pH-H_2O			pH-KCl			ΔpH			EC(dS/m)		
Soil	N	×	σ	cv	×	σ	cv	×	σ	cv	×	σ	cv
RU	3	**8.26**	1.33	6.34	**7.67**	1.54	3.87	**0.59**	0.12	20.34	0.15	0.05	6.43
ELY	3	**8.02**	0.34	4.24	**7.46**	0.07	0.91	**0.56**	0.35	62.75	0.25	0.10	38.78
SAAR	3	**7.35**	0.24	3.25	**7.29**	0.29	4.01	**0.09**	0.06	65.47	**12.23**	2.03	16.56
SAER	3	**8.72**	0.13	1.50	**8.72**	0.23	2.59	**0.00**	0.00	0.00	**5.87**	3.43	58.40
AGR	3	**8.25**	0.08	0.92	**7.56**	0.02	0.23	**0.69**	0.08	12.21	0.16	0.02	14.96
PAS	3	**7.71**	0.31	4.01	**7.40**	0.05	0.69	**0.31**	0.10	32.25	1.06	0.78	73.55

5-20 cm depth

		pH-H_2O			pH-KCl			ΔpH			EC(dS/m)		
Soil	N	×	σ	cv	×	σ	cv	×	σ	cv	×	σ	cv
RU	3	**8.36**	0.25	3.04	**7.73**	0.11	1.39	**0.63**	0.17	27.49	0.12	0.03	21.24
ELY	3	**8.40**	0.18	2.16	**7.86**	0.18	2.34	**0.53**	0.28	52.20	0.31	0.14	44.44
SAAR	3	**8.11**	0.16	2.01	**7.94**	0.19	2.38	**0.17**	0.04	23.40	**4.26**	0.48	11.23
SAER	3	**8.71**	0.27	3.15	**8.64**	0.36	4.15	**0.09**	0.04	46.57	**3.14**	0.99	31.45
AGR	3	**8.19**	0.12	1.42	**7.54**	0.04	0.48	**0.65**	0.10	14.86	0.18	0.04	21.35
PAS	3	**7.73**	0.20	2.57	**7.50**	0.02	0.20	**0.25**	0.21	84.66	1.21	0.77	63.89

20-40 cm depth

		pH-H_2O			pH-KCl			ΔpH			EC(dS/m)		
Soil	N	×	σ	cv	×	σ	cv	×	σ	cv	×	σ	cv
RU	3	**8.50**	0.09	1.01	**7.82**	0.14	1.79	**0.68**	0.06	8.14	0.01	0.02	23.04
ELY	3	**8.71**	0.31	3.55	**8.09**	0.29	3.65	**0.62**	0.38	60.53	0.44	0.32	73.10
SAAR	3	**8.51**	0.10	0.01	**8.30**	0.10	1.26	**0.21**	0.02	7.41	**2.87**	0.48	16.77
SAER	3	**8.73**	0.19	2.22	**8.58**	0.31	3.57	**0.18**	0.16	87.75	**2.28**	0.69	30.31
AGR	3	**8.26**	0.06	0.69	**7.55**	0.03	0.43	**0.71**	0.05	7.45	0.18	0.02	9.85
PAS	3	**7.93**	0.11	1.34	**7.60**	0.07	0.92	**0.31**	0.16	51.89	0.85	0.34	39.53

RU: Ruderal soil environment; ELY: *Elys Elimoides* soil environament; SAAR: *Arthrocnemum fruticosum* soil environament; SAER: *Salicornia patula* soil environament; AGR: Corn (*Zea mays*) cultivated soil environament; PAS: Pasture (*Medicago sativa*) soil environament; ΔpH: Value of pH_{H2O}-pH_{KCl}; EC: Electrical conductivity; N: Number of replications; ×: Mean of three replications; σ: Standard deviation; cv: Coefficient of variation.

In Table 5, data of electrical conductivity are also reported. The first point to outline is that soil RU, ELY and AGR showed a very low electrical conductivity (<1 dSm^{-1}). These values are logical considering their position, origin and land use. Conversely, a high electrical conductivity value was found in the soil SAAR and SAER at 0-5 cm depth (Table 5). In this case the EC of SAAR was 108% higher than SAER, probably because of a higher amount of

clay in the former which is able to maintain higher salinity. The EC values of these soils decreased with depth even though SAAR values were still 35% and 24% higher than SAER at 5-20 cm and 20-40 cm respectively.

One way ANOVA was tried in order to explore data variability within and between the studied soil environments at the three sampling depths. This simple statistical treatment allows to compare groups and means of analysed parameters assigning a significance according to standard deviation and number of samples. As may be observed in Table 6 significant data variability was much clearer between soil environments than within each environment. As expected, the highest significant variability was found in SAAR soil for EC values.

The structural stability of aggregates was examined at two different aggregates classes (0.25-2 mm and 2-5.6 mm) with the purpose to establish aggregates classes of resistance at the three different sampling depths. In general, these classes are attributed to the survived aggregates (%) after their immersion/emersion in water for a given time. The classes are the following: 0-20% very low resistance, 20-40% low resistance, 40-60% moderate resistance, 60-80% high resistance, 80-100% very high resistance. By observing Table 7 it can be seen that at 0-5 cm depth soil ELY, SAAR, AGR and PAS belong to high-very high resistant classes, but soil RU and SAER which showed low resistant classes. The highest stability of aggregates was found in SAAR soil which also presented the highest value of EE-GRSP and GRSP (Table 7). However, there was a irregular behavior of WSA value in the two classes of aggregates. This irregular behavior may be probably due to the periodical flooding due to water movement from rainfall and sea storms. However, it may be concluded that higher structural stability of aggregates corresponded to higher glomalin content. Total glomalin (GRSP) was always higher in SAAR (8.88 mgg^{-1}) followed by ELY, RU and PAS soils. The relatively high glomalin value of RU soil may be misleading in the context of the analysed materials. Effectively, RU soil is rubble coming from different places with probably a proper soil history interrupted at the time of its removal to fill the saltmarsh area.

Table 6. One-way ANOVA results for exploring significant data variability for selected soil parameters within and between soil environments along different sampling depths.

	CS		FS		S		C		pH_{H2O}		pH_{KCl}		EC		TN	
Soil	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Within soil environments along three depths																
RU	1.65	0.269NS	0.12	0.887NS	0.18	0.839NS	0.15	0.868NS	0.75	0.513NS	0.47	0.644NS	0.95	0.439NS	2.17	0.195NS
ELY	0.07	0.931NS	0.04	0.963NS	0.56	0.590NS	0.47	0.645NS	4.40	0.067NS	7.54	0.023*	0.64	0.558NS	15.24	0.004**
SAAR	0.12	0.886NS	7.55	0.023*	6.93	0.028*	3.97	0.080NS	33.53	0.001**	18.11	0.003**	50.11	0.000***	9.95	0.012*
SAER	5.03	0.052NS	12.40	0.007**	3.64	0.092NS	4.69	0.059NS	0.01	0.993NS	0.16	0.856NS	2.39	0.172NS	0.73	0.520NS
AGR	5.07	0.051NS	0.46	0.652NS	0.65	0.553NS	1.01	0.421NS	0.53	0.615NS	0.31	0.744NS	0.50	0.630NS	1.45	0.307NS
PAS	0.06	0.939NS	0.10	0.911NS	5.16	0.050NS	10.44	0.011*	0.90	0.456NS	11.54	0.009**	0.22	0.806NS	9.49	0.014*
Between soil environments along three depths																
0-5	11.53	0.000***	14.89	0.000***	19.82	0.000***	11.77	0.000***	1.99	0.153NS	1.82	0.144NS	26.27	0.000***	9.07	0.001**
5-20	14.17	0.000***	8.78	0.001**	7.77	0.002**	5.08	0.010*	7.84	0.002**	14.65	0.000***	30.46	0.000***	2.42	0.098NS
20-40	1.81	0.185NS	4.61	0.014*	3.08	0.051NS	3.78	0.028*	9.93	0.001**	13.84	0.000***	26.72	0.000***	5.19	0.009**

RU: Ruderal soil environment; ELY: *Elys Elimoides* soil environament; SAAR: *Arthrocnemum fruticosum* soil environament; SAER: *Salicornia patula* soil environament; AGR: Corn (*Zea mays*) cultivated soil environament; PAS: Pasture (*Medicago sativa*) soil environament; CS: Coarse sand; FS, Fine sand; S: Silt; C: Clay; EC: Electrical conductivity; TN: Total nitrogen.
NS: Not significant; *: p-level <0.05; **: p-level <0.01; ***: p-level <0.001.

Table 7. Structural stability of aggregates and glomalin related soil protein at diferent soil depth at the Platera saltmarsh (Estartit, Girona). Descriptive statistics is reported.

0-5 cm depth

		EE-GRSP (mg/g)			GRSP (mg/g)			WSA(0.25-2) (%)			WSA(2-2.56) (%)		
Soil	N	×	σ	cv	×	σ	cv	×	σ	cv	×	σ	cv
RU	3	**0.86**	0.18	20.93	**2.66**	1.55	41.13	**27.21**	10.44	38.36	**20.25**	9.18	45.33
ELY	3	**1.02**	0.12	11.57	**5.31**	2.09	39.28	**87.63**	9.01	10.28	**88.87**	8.52	9.59
SAAR	3	**1.14**	0.19	16.51	**8.88**	2.93	32.99	**98.78**	2.84	3.60	**86.04**	1.15	1.33
SAER	3	**0.26**	0.05	18.91	**0.46**	0.08	16.71	**73.75**	9.02	12.23	**47.04**	36.11	76.76
AGR	3	**0.43**	0.05	10.99	**1.17**	0.11	9.18	**77.81**	1.59	2.05	**63.26**	25.84	40.85
PAS	3	**0.80**	0.20	25.25	**2.47**	0.55	0.22	**79.92**	12.17	15.23	**68.81**	38.14	55.42

5-20 cm depth

		EE-GRSP (mg/g)			GRSP (mg/g)			WSA(0.25-2) (%)			WSA(2-2.56) (%)		
Soil	N	×	σ	cv	×	σ	cv	×	σ	cv	×	σ	cv
RU	3	**0.78**	0.04	5.17	**2.33**	0.10	4.32	**21.20**	7.67	36.18	**19.56**	9.33	47.69
ELY	3	**0.42**	0.13	32.09	**2.02**	0.58	28.72	**83.20**	8.49	10.20	**81.72**	0.73	0.89
SAAR	3	**0.69**	0.15	20.79	**2.89**	0.79	27.64	**86.04**	1.15	1.33	**42.70**	13.06	30.58
SAER	3	**0.17**	0.19	115.22	**0.82**	1.09	132.65	**34.50**	11.85	15.90	**39.26**	34.96	89.06
AGR	3	**0.48**	0.05	11.05	**2.29**	0.04	1.91	**66.67**	2.94	4.41	**30.53**	6.95	22.78
PAS	3	**0.65**	0.11	16.93	**3.01**	0.29	9.66	**66.97**	0.67	1.00	**25.18**	13.61	54.06

20-40 cm depth

		EE-GRSP (mg/g)			GRSP (mg/g)			WSA(0.25-2) (%)			WSA(2-2.56) (%)		
Soil	N	×	σ	cv	×	σ	cv	×	σ	cv	×	σ	cv
RU	3	**0.83**	0.10	12.26	**2.13**	0.54	22.27	**20.12**	5.78	28.72	**18.68**	6.22	33.29
ELY	3	**0.15**	0.092	62.08	**0.89**	0.37	41.61	**14.78**	12.36	83.61	**75.34**	16.62	22.06
SAAR	3	**0.25**	0.063	25.53	**1.03**	0.51	48.99	**33.06**	17.71	53.56	**8.35**	6.63	79.47
SAER	3	**0.30**	0.25	84.62	**0.74**	0.44	58.95	**28.30**	17.99	29.32	**2.92**	3.22	110.17
AGR	3	**0.57**	0.07	11.95	**1.63**	0.38	22.99	**52.99**	12.54	23.67	**9.62**	6.50	67.60
PAS	3	**0.53**	0.04	6.82	**1.69**	0.28	16.39	**40.56**	8.50	20.95	**18.04**	11.64	64.51

RU: Ruderal soil environment; ELY: *Elys Elimoides* soil environament; SAAR: *Arthrocnemum fruticosum* soil environament; SAER: *Salicornia patula* soil environament; AGR: Corn (*Zea mays*) cultivated soil environament; PAS: Pasture (*Medicago sativa*) soil environament; EE-GRSP: Easily extractable glomalin related soil protein; GRSP: Glomalin related soil protein; WSA(0.25-2): Aggregates in the class 0.25-2 mm; WSA(2-5.6): Aggregates in the class 2-5.6 mm; N: Number of replications; ×: Mean of three replications; σ: Standard deviation; cv: Coefficient of variation.

By contrast, the glomalin value of SAAR, ELY, PAS, AGR and SAER were coherent with the in-situ soil dynamics (Table 7). The values of EE-GRSP and GRSP of SAER soil were consistently the lowest at any depth with respect to other soils. It is worth to remark that GRSP value in SAAR soil was 20 times higher than SAER value which means an increase of 1830%. However, at 5-20 cm depth GRSP in SAAR soil increased by 250% and at 20-40 cm depth the increase was 39% with respect to SAER, indicating that the very active layer of glomalin is at 0-5 cm depth. GRSP in ELY soil decreased at the same rate of SAAR soil (Table 7) whilst EE-GRSP and GRSP values in AGR and PAS soils were quite similar at the three sampling depths. No relationship was found between GRSP and WSA in the two dimensional classes because of the contrasting values of GRSP and WSA in RU at any depth, due to particular origin of this material. However, when these values were neglected, a very high correlation was found between GRSP and $WSA_{(0.25\text{-}2)}$ ($y=2.84x+73.16$, $r= 0.996$, $P<0.01$), and $WSA_{(2\text{-}5.6)}$ ($y=4.32x+55.00$, $r= 0.868$, $P<0.01$) at 0-5 cm depth (Hontoria et al., 2009).

A very similar trend was found between the soil environments when analyzing the soil organic carbon content which values were in the following order SAAR> ELY> RU> PAS>AGR>SAER (Table 8).

Table 8. Soil organic carbon, total nitrogen content and carbon/nitrogen ratio at different soil depths at the Platera saltmarsh (Estartit, Girona). Descriptive statistics is reported.

0-5 cm depth									
		SOC (mg/g)				TN (mg/g)			C/N
Soil	N	×	σ	cv	N	×	σ	cv	
RU	3	**16.18**	6.21	38.38	3	**0.34**	0.003	0.86	**47.35**
ELY	3	**23.63**	7.40	31.32	3	**0.74**	0.02	2.72	**31.93**
SAAR	3	**40.08**	2.52	5.30	3	**1.88**	0.08	4.39	**21.31**
SAER	3	**4.40**	0.99	22.43	3	**0.22**	0.01	2.72	**20.00**
AGR	3	**5.77**	0.83	14.48	3	**0.29**	0.003	1.21	**19.80**
PAS	3	**11.45**	0.61	5.36	3	**0.59**	0.02	2.59	**19.41**
5-20 cm depth									
		SOC (mg/g)				NT (mg/g)			C/N
Soil	N	×	σ	cv	N	×	σ	cv	
RU	3	**15.29**	4.43	28.96	3	**0.30**	0.01	3.62	**50.96**
ELY	3	**18.58**	3.63	19.54	3	**0.39**	0.01	2.47	**47.64**
SAAR	3	**14.94**	0.73	4.89	3	**0.46**	0.02	4.04	**32.47**
SAER	3	**3.69**	2.81	75.96	3	**0.17**	0.01	7.88	**21.71**
AGR	3	**5.17**	1.03	17.87	3	**0.25**	0.01	3.80	**20.68**
PAS	3	**9.57**	1.69	44.52	3	**0.37**	0.003	0.74	**25.86**
20-40 cm depth									
		SOC (mg/g)				SOC (mg/g)			C/N
Soil	N	×	σ	cv	N	×	σ	cv	
RU	3	**9.32**	3.47	37.26	3	**0.28**	0.01	1.81	**33.28**
ELY	3	**7.04**	0.44	6.30	3	**0.13**	0.01	5.88	**54.15**
SAAR	3	**6.02**	1.62	8.11	3	**0.24**	0.004	1.76	**25.08**
SAER	3	**1.96**	1.44	73.13	3	**0.11**	0.01	8.52	**17.81**
AGR	3	**4.22**	0.62	14.72	3	**0.36**	0.01	2.76	**11.72**
PAS	3	**6.59**	2.91	44.53	3	**0.27**	0.003	1.08	**24.41**

RU: Ruderal soil environment; ELY: *Elys Elimoides* soil environament; SAAR: *Arthrocnemum fruticosum* soil environament; SAER: *Salicornia patula* soil environament; AGR: Corn (*Zea mays*) cultivated soil environament; PAS: Pasture (*Medicago sativa*) soil environament;SOC: Soil organic carbon; NT: Total nitrogen; C/N: Carbon/Nitrogen ratio; N: Number of replications; ×: Mean of three replicationa; σ: Standard deviation; cv: Coefficient of variation.

As expected very good relationship was found between organic carbon and nitrogen ($y= 0.044x-0.6063$, $r= 0.965$, $P<0.01$). C/N ratio values of ELY, SAAR, SAER, AGR, and PAS were relatively lower (≈50%) than RU soil, once more indicating that this soil may have proper mineralization process.

At the first glance it may be inferred that the soil SAAR is able to retain much more soil organic carbon (SOC) because almost 10% is formed by total glomalin (GRSP) which means more recalcitrant humic compounds even though this property is rather quickly decreased at increasing soil depth. Effectively, GRSP and SOC decreased in a very similar way along depth: from 0-5 cm to 5-20 and 20-40 cm GRSP decreased by 67% and 88% respectively; and from 0-5 cm to 5-20 and 20-40 cm SOC decreased by 62% and 85%

respectively, corroborating certain relationships and appointing SAAR soil as the most suitable for organic carbon storage.

3.3. Carbon dioxide production and storage

This assumption was also confirmed by laboratory trials of carbon respiration potential. As may be observed in Table 9 the carbon dioxide production of SAAR soil resulted the lowest among the examined soils which increased by 370%, 170%, 79%, 38% and 35% in RU, ELY, PAS, AGR, SAER respectively. The Ruderal soil showed the highest CO_2 production and consequently the highest C-CO_2 loss. However, its GRSP-C and coefficient of mineralization values may not be considered reliable because of the artifact caused by its provenience.

Table 9. Carbon dioxide emission potential, organic carbon emitted with CO_2, organic carbon from glomalin related soil protein and mineralization quotient. Descriptive statistics is reported.

0-5 cm depth

Soil		CO_2 (mg/g)			C-CO_2 (mg/g d)			GRSP-C (mg/g)			q		
	N	×	σ	cv	×	σ	cv	×	σ	cv	×	σ	cv
RU	3	**10.20**	4.67	45.78	**3.78**	0.55	14.55	**2.56**	0.97	37.89	**0.23**	0.10	43.47
ELY	3	**7.36**	0.44	5.94	**2.01**	0.12	5.94	**2.04**	0.62	30.39	**0.19**	0.07	36.45
SAAR	3	**2.73**	0.29	10.53	**0.74**	0.08	10.53	**2.71**	1.11	41.08	**0.05**	0.04	31.47
SAER	3	**3.68**	0.87	23.59	**1.33**	0.24	17.73	**0.32**	0.02	6.25	**0.66**	0.11	17.22
AGR	3	**4.33**	0.73	16.79	**1.97**	0.20	10.10	**0.46**	0.11	24.43	**0.73**	0.07	10.00
PAS	3	**4.89**	0.79	16.24	**1.83**	0.22	11.82	**1.00**	0.12	11.79	**0.34**	0.06	17.64

RU: Ruderal soil environment; ELY: *Elys Elimoides* soil environament; SAAR: *Arthrocnemum fruticosum* soil environament; SAER: *Salicornia patula* soil environament; AGR: Corn (*Zea mays*) cultivated soil environament; PAS: Pasture (*Medicago sativa*) soil environament; C-CO_2: Organic carbon los by CO_2 emission in mg/g day: GRSP-C: Organic carbon from total glomalin related soil protein; q: Coefficient of mineralization; :N: Number of replications; ×: Mean of three replications; σ: Standard deviation; cv: Coefficient of variation.

Not only the SAAR soil showed the lowest CO_2 production at 0-5cm depth, but consequently the glomalin carbon (GRSP-C) and coefficient of mineralization (q) were also the lowest value (Table 9) indicating that SAAR soil may accomplish the main function of organic carbon storage in the saltmarsh area of the Pletera.

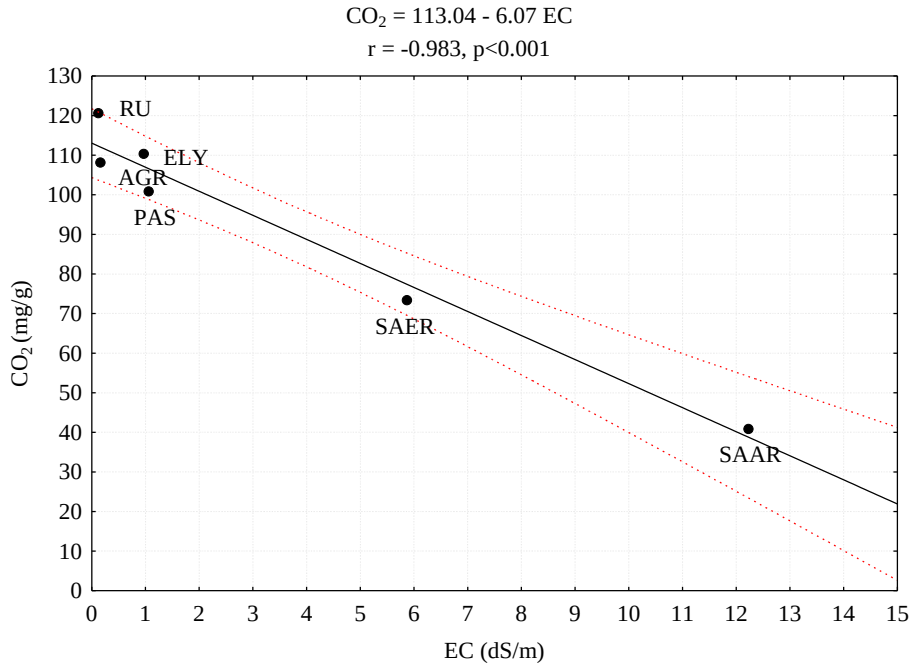


Figure 25. Linear regression equation of CO_2 as a function of EC.

A very interesting and significant relationship was fond between CO_2 emission and EC indicating that saline conditions may hinder mineralization processes of organic compounds favoring carbon retention in soil. From the Figure 25 it is clear how SAAR soil is a suitable candidate for constituting a carbon sink.

Table 10. One-way ANOVA results for exploring significant data variability for selected soil parameters within and between soil environments along different sampling depths.

	$WSA_{(0.25\text{-}2.00)}$		$WSA_{(2.00\text{-}5.60)}$		SOC		EE-GRSP		GRSP	
Soil	F	p	F	p	F	p	F	p	F	p
Within soil environments along three depths										
RU	0.653	0.554NS	0.03	0.974NS	1.78	0.247NS	0.33	0.729NS	0.24	0.795NS
ELY	53.18	0.000***	1.18	0.370NS	9.55	0.014*	44.88	0.000***	9.80	0.013*
SAAR	22.99	0.002**	63.20	0.000***	295.29	0.000***	28.49	0.001**	15.99	0.004**
SAER	10.02	0.012*	1.97	0.220NS	1.30	0.341NS	0.40	0.690NS	0.23	0.800NS
AGR	8.26	0.019*	8.68	0.017*	2.58	0.156NS	4.58	0.062NS	18.04	0.030*
PAS	16.40	0.004**	3.83	0.085NS	4.62	0.061NS	3.07	0.121NS	8.52	0.018*
Between soil environments along three depths (cm)										
0-5	19.98	0.000***	3.33	0.041*	31.60	0.000***	16.38	0.000***	11.17	0.000***
5-20	44.27	0.000***	5.20	0.009**	14.40	0.000***	9.68	0.001**	4.96	0.011*
20-40	3.31	0.042*	23.53	0.000***	4.44	0.016*	12.48	0.000***	4.85	0.012*

RU: Ruderal soil environment; ELY: *Elys Elimoides* soil environament; SAAR: *Arthrocnemum fruticosum* soil environament; SAER: *Salicornia patula* soil environament; AGR: Corn (*Zea mays*) cultivated soil environament; PAS: Pasture (*Medicago sativa*) soil environament; WSA: Water stable aggregates in 0.25-2.00 mm dimensional class; WSA: Water stable aggregates in 2.00-5.60 mm dimensional class; SOC: Soil organic carbon; EE_GRSP: Easily extractable glomalin related soil protein; GRSP: Glomalin related soil protein.
NS: Not significant; *: p-level <0.05; **: p-level <0.01; ***: p-level <0.001.

Data variability significance of WSA, SOC and GRSP within and between soil environments was also checked with one way ANOVA. As expected significant variation of data was found between soil environments along the three sampling depths for the two aggregates classes, SOC, EE-GRSP and GRSP (Table 10). Surprisingly, significant data variability was found in the aggregate class 0.25-2 mm within ELY, SAAR, SAER, AGR, PAS, but RU. Also SOC, EE-GRSP and GRSP data varied significantly within SAAR and ELY soils at the three sampling depth.

The bulk density of the soils was 1.36 gm^{-3}, 1.28 gm^{-3}, 1.17 gm^{-3}, 1.30 gm^{-3}, 1.39 gm^{-3}, 1.37 gm^{-3} for RU, ELY, SAAR, SAER, AGR, PAS respectively. These values allowed to calculate the weight of the different soils at 0-5 cm depth. According to this, the corresponding values of SOC, GRSP, GRSP-C and C-CO_2 were also calculated in $Mgha^{-1}$(Table 11). As may be observed the SAAR soil maintains the highest SOC and the SAER soil the lowest. According to the values reported in Table 10, it is possible to calculate an approximate gain or loss of soil organic carbon by using the ratio GRSP-C/SOC and C-CO_2/SOC. Results indicate that AGR, SAER, RU, and PAS soils, may have a soil organic carbon loss by CO_2 emission of 26.16, 22.72, 7.54, 7.14 $Mgha^{-1}$ respectively, according to the soil samples analysed. By contrast, SAAR and ELY soils may have a soil organic carbon gain of 4.9 and 0.13 $Mgha^{-1}$ respectively, according to the soil samples analysed.

Table 11. Mean values of soil organic carbon (SOC), glomalin related soil protein (GRSP), organic carbon content in GRSP (GRSP-C), carbon-carbon dioxide (C-CO_2), and the proportion of GRSP-C and C-CO_2 to SOC for the studied soils at La Pletera (Estartit, Girona). Results refer to 0-5 cm depth.

Soil	SOC ($Mgha^{-1}$)	GRSP ($Mgha^{-1}$)	GRSP-C ($Mgha^{-1}$)	GRSP-C/SOC (%)	C-CO_2 ($Mgha^{-1}$)	C-CO_2/SOC (%)
RU	11.02	1.81	1.74	15.78	2.57	23.32
ELY	15.12	3.40	1.31	8.66	1.29	8.53
SAAR	23.45	5.19	1.58	6.73	0.43	1.83
SAER	2.86	0.30	0.21	7.34	0.86	30.06
AGR	4.01	0.81	0.32	7.98	1.37	34.14
PAS	7.84	1.69	0.69	8.80	1.25	15.94

RU: Ruderal soil environment; ELY: *Elys Elimoides* soil environament; SAAR: *Arthrocnemum fruticosum* soil environament; SAER: *Salicornia patula* soil environament; AGR: Corn (*Zea mays*) cultivated soil environament; PAS: Pasture (*Medicago sativa*) soil environament.

As may be observed from the Tables 9 and 11 some soil parameters like CO_2 emission and GRSP-C among others have been analysed only in the 0-5 cm depth soil layer because previous data appointed to this layer as the most appropriate the retain soil organic carbon and minimize soil organic carbon loss by CO_2.

According to different works reported in the literature the data of GRSP-C/SOC were of the same order of what reported by Rillig et al. (2001) who calculated that GRSP may represent from 7% to 42% of soil organic carbon.

A correlation matrix was obtained analyzing simultaneously all the analysed parameters of all soil environments at 0-5 cm depth. The correlation coefficient and the degree of significance are showed in Table 12, marked in red color. There are interesting correlations that may be related to the soil dynamics. For instance, the relationship between the coefficient of mineralization (q) and the SOC content (Figure 26).

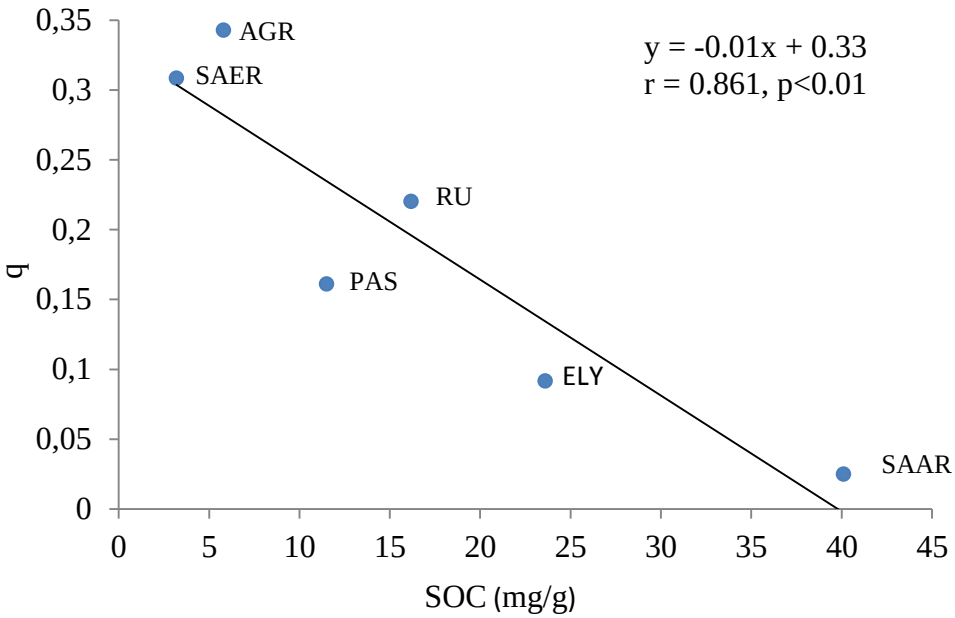


Figure 26. Linear regression equation between q and SOC.

3.4. Correlation matrix between analysed parameters

Many other examples of relationship reported in Table 12 are worth to be outlined as the following: negative correlation of coarse sand with all the organic compounds; Positive correlation between WSA and the majority of organic compounds; Negative correlation between clay and CO_2 indicating that a higher amount of clay may form a better structure and favor the preservation of organic carbon in soil microsites.

Table 12. Correlation matrix among the analysed parameters for the 0-5 cm depth.

0-5 cm depth	CS	FS	S	C	WSA_1	WSA_2	pH_{H2O}	pH_{KCl}	EC	SOC	EC-SOC	HA	FA	GRSP	EE-GRSP	GRSP-C	CO_2	$C\text{-}CO_2$	q
FS	-0.437																		
S	-0.799	-0.159																	
C	-0.508	-0.532	0.843*																
WSA_1	-0.534	0.735	0.020	-0.111															
WSA_2	-0.833*	0.799	0.360	0.051	0.722														
pH_{H2O}	0.932**	-0.384	-0.778	-0.482	-0.461	-0.798													
pH_{KCl}	0.855*	-0.645	-0.596	-0.101	-0.422	-0.797	0.830*												
EC	-0.426	-0.331	0.551	0.814*	0.046	0.254	-0.449	0.040											
SOC	-0.809*	0.405	0.562	0.419	0.396	0.854*	-0.819*	-0.615	0.665										
E-SOC	-0.933**	0.440	0.716	0.455	0.628	0.819*	-0.974***	-0.787	0.432	0.777									
HA	-0.821*	0.355	0.635	0.441	0.734	0.605	-0.742	-0.635	0.241	0.433	0.850*								
FA	-0.840*	0.258	0.678	0.592	0.664	0.691	-0.870*	-0.544	0.604	0.712	0.940***	0.870*							
GRSP	-0.845*	0.433	0.564	0.442	0.525	0.880*	-0.838*	-0.606	0.678	0.985***	0.832*	0.552	0.803*						
EE-GRSP	-0.845*	0.657	0.461	0.198	0.666	0.959***	-0.894*	-0.756	0.405	0.902**	0.903**	0.624	0.805*	0.927**					
GRSP-C	-0.821*	0.530	0.469	0.337	0.632	0.917**	-0.821*	-0.603	0.609	0.959***	0.838**	0.575	0.812*	0.988***	0.955***				
CO_2	0.413	0.380	-0.563	-0.853*	-0.101	-0.195	0.432	-0.081	-0.983***	-0.579	-0.447	-.0332	-0.654	-0.617	-0.358	-0.557			
$C\text{-}CO_2$	0.318	0.477	-0.556	-0.827*	0.176	-0.108	0.372	-0.104	-0.973***	-0.586	-0.315	-.0838*	-0.466	-0.566	-0.279	-0.476	0.943***		
q	0.711	-0.598	-0.418	-0.070	-0.318	-0.853*	0.815*	0.786	-0.268	-0.861*	-0.729	-0.305	-0.524	-0.808*	-0.892*	-0.806*	0.161	0.231	
N_T	-0.796	0.181	0.707	0.599	0.209	0.718	-0.832*	-0.568	0.759	0.965***	0.754	0.404	0.709	0.936**	0.812*	0.878*	-0.680	-0.723	-0.806*

CS: Coarse sand; FS: Fine sand; S: Silt; C: Clay; WSA_1: Water stable aggregates in 0.25-2 mm dimensional class; WSA_2: Water stable aggregates in 2-5.6 mm dimensional class; EC: Electrical conductivity; SOC: Soil organic carbon.; e-soc: Extractable SOC; HA: Humic acids; FA: Fulvic acids; GRSP: Glomalin related soil protein; EE-GRSP: Easily extractable glomalin related soil protein ; GRSP-C: Organic carbon in glomalin extraction; CO_2: Carbon dioxide emission from soil; C- CO_2: Organic carbon loss from carbon dioxide emission; q: Coefficient of mineralization.
*: p-level <0.05; **: p-level <0.01; ***: P-level <0.001.

As examples of correlations betwenn the analysed parameters, it may be observed that sand has a negative and significant correlation with SOC (Figure 27).

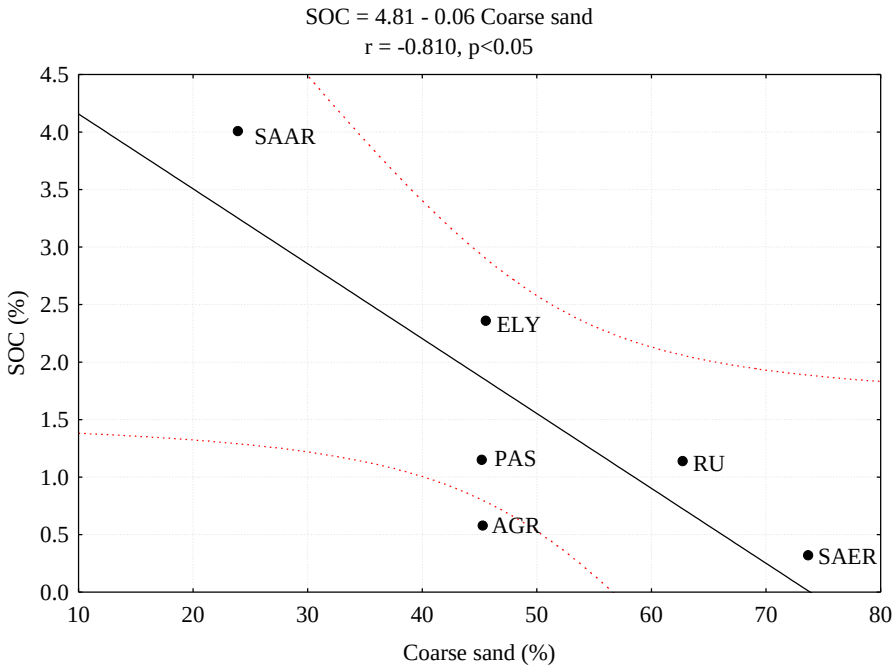


Figure 27.Linear regression equation between SOC and coarse sand.

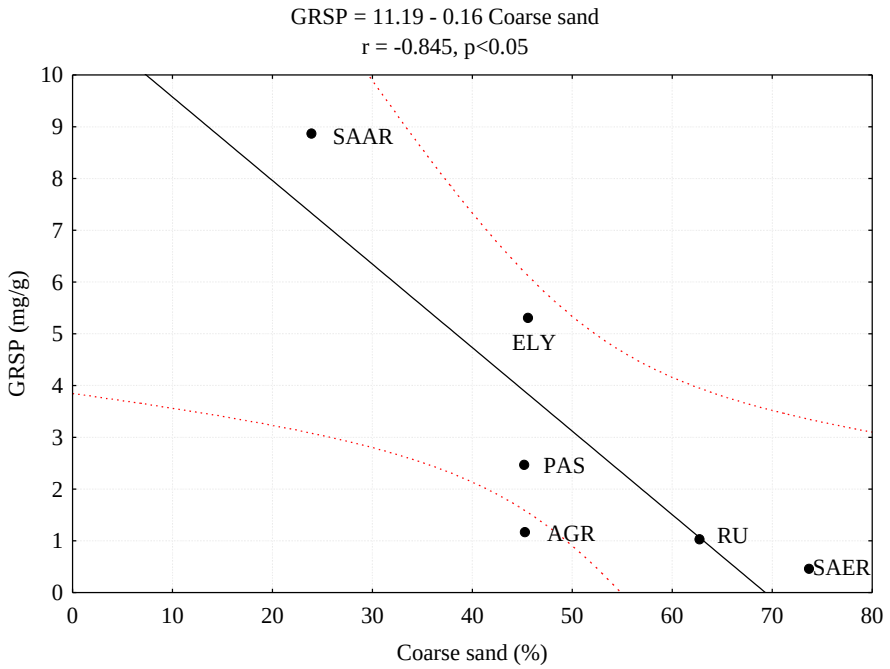


Figure 28. Linear regression equation between GRSP and coarse sand.

Similarly, GRSP was negatively correlated with the coarse sand (Figure 28) showing approximately the same scatter of point and indicating the same carbon source in each environment.

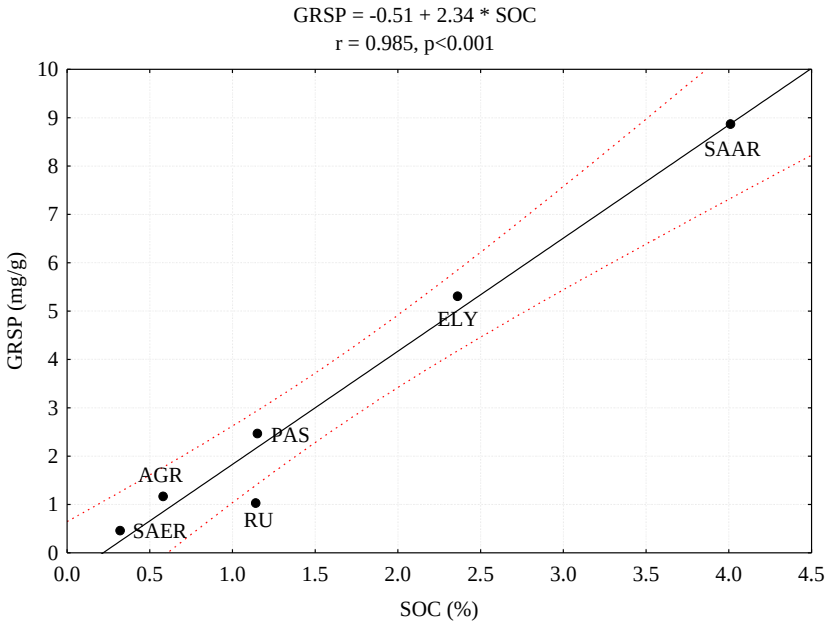


Figure 29. Linear regression equation between GRSP and SOC.

Effectively, Figure 29 demonstrates the extremely high and significant positive correlation between GRSP and SOC, inferring that GRSP is part of SOC.

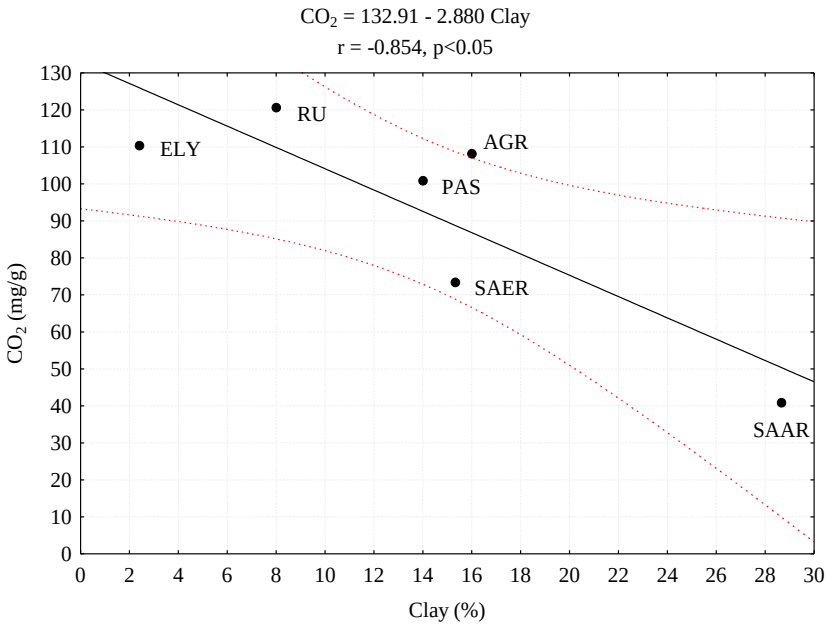


Figure 30.Linear regression equation between CO2 and clay.

As previously mentioned, it is also possible to postulate that the different amount of clay in the examined soils is able to contrast CO_2 emission (Figure 30).

3.5. Multivariate factor analysis

Factor analysis was performed to provide information on the relationships between the measured variables and soil environments at 0-5 cm depth. Factors were selected by using the eigenvalue which measures the variance in all the variables accounted for by one factor. Factors were named according to variable's loadings and the correlation coefficients between the original variables and each factor. Scores for factors assisted in determining the relevance of each soil environments for contributing to soil properties improvement and facilitating an approach to establish the most suitable soil environment able to delay organic carbon mineralization.

Table 13. Variables loadings (correlation <0.700) on the extracted three factor structure. Data were analysed simultaneously for the six studied environments to assess the general dynamics of soil parameters.

Variables	Organic carbon reserve	Organic carbon loss	Aggregation
Coarse sand	-0.763		
Fine sand			
Silt			
Clay		**0.909**	
WSA $_{0.25\text{-}2.00}$			**0.955**
WSA $_{2.00\text{-}5.60}$			**0.825**
pH H_2O	-0.813		
pH KCl	-0.837		
EC		**0.929**	
SOC	**0.871**		
E-SOC	**0.735**		
HA			**0.891**
FA			**0.718**
GRSP	**0.823**		
EE-GRSP	**0.896**		
GRSP-C	**0.819**		
CO_2		-0.933	
C-CO_2		-0.968	
Q	-0.988		
N_T	0.802		
Absolute variance	61.84	19.71	10.29
Cumulative variance	61.84	81.56	91.85

EC: Electrical conductivity; SOC: Soil organic carbon.; e-soc: Extractable SOC; HA: Humic acids; FA: Fulvic acids; GRSP: Glomalin related soil protein; EE-GRSP: Easily extractable glomalin related soil protein ; GRSP-C: Organic carbon in glomalin extraction; CO_2: Carbon dioxide emission from soil; C- CO_2: Organic carbon loss from carbon dioxide emission; q: Coefficient of mineralization.

From the factor analysis a three factor structure was obtained, the first factor explaining 61.84% of total variance into variables, the second factor explaining 19.71% of total variance and the third factor explaining 10.29% of total variance. According to the weight of each factor, they were named "organic carbon reserve", "organic carbon loss" and "aggregation" (Table 13). It may be observed that the factor of organic carbon reserve is related with the soil organic parameters whilst the second factor with the organic carbon loss and the third factor with the soil aggregate stability.

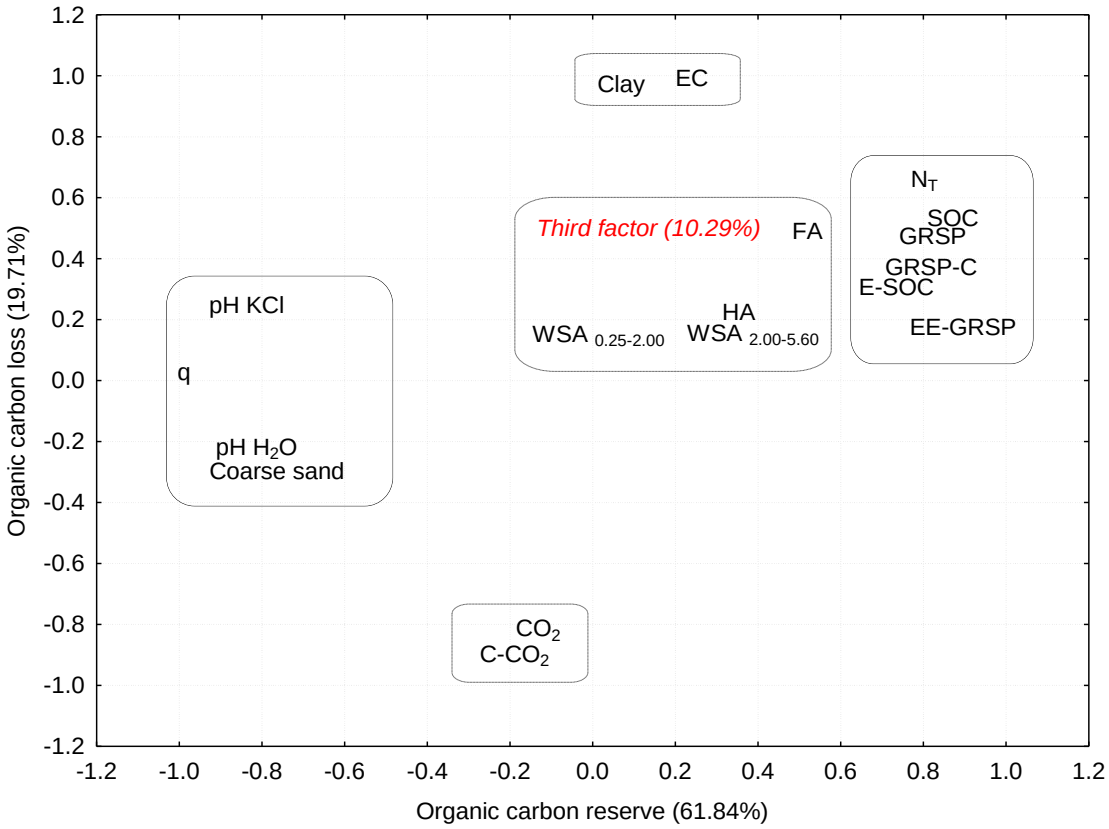


Figure 31. Ordination plots representing the first two factors and related soil variables.

What above mentioned is represented graphically in Figure 31. It can be seen that all the parameters contributing to organic reserve are positioned in the positive site of factor 1 (Organic carbon reserve) whilst contrasting parameters like coarse sand or mineralization coefficient are positioned in the negative site. The factor 2 (organic carbon loss) oppose clay and EC to CO_2 and C-CO_2 indicating that Saline conditions together with clay may contrast carbon dioxide production.

Table 14. Factor score enabling to establish the contribution of each soil environment to the variables in the factor structure.

Soil environment	Organic carbon reserve	Organic carbon loss	Aggregation
Ruderal plants	0.230	-0.405	-1.927
Elymus Elymoides	0.797	-1.183	0.435
Arthrocnemum fruticosum	1.166	0.530	0.234
Salicornia patula	-1.573	0.705	-0.166
Zea mays	-0.686	-0.390	0.705
Medicago sativa	0.064	-0.356	0.717

As expected the SAAR (*Arthrocnemum fruticosum*) soil environment was statistically proved to be the best environment for carbon preservation and storage showed also by the highest score in Table 14.

4. Conclusions

The present work was done in order to have a preliminary view of the soil dynamics among the different environments at the saltmarsh Pletera, Estartit (Girona). Even though results represent the picture of one sampling campaign, they may be sufficiently informative of the soil dynamics occurring in this area. In particular, the work enabled to differentiate the soil carbon, glomalin, aggregation and carbon dioxide behavior among the soils examined.

This first approach of carbon storage capacity may help stakeholders and decision makers to orientate future restoration works in the Ruderal area taking into account that the vegetation community which is more adequate for carbon storage is *Arthrocnemum fruticosum*.

5. Acknowledgements

I am particularly grateful to the project Techno II and the University of Girona who gave me the possibility to spend a fruitful experimental period in Spain.

6. References

Aitkenhead J.A., Hope, D., Billett, M.F. 1999. The relationship between dissolved organic carbon in stream water and soil organic matter pools at different spatial scales. Hydrologic Processes 13, 1289-1302.

Bai, J.G., Dent, D.L., Olsspn, L., Schaepman, M.E. 2008. Proxy global assessment of land degradation. Soil Use and Management 24, 223–234.

Borie, F., Rubio, R., Morales, A. 2008. Arbuscular mycorrhizal fungi and soil aggregation. Soil Science and Plant Nutrient 8(2), 9-18.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemstry* 72, 248-254.

Burdent, A., Garbutt, R.A. 2013. Carbon sequestration and biogeochemical cycling in a saltmarsh subject to costal mananged. Estuarine, Coastal, and shelf Science 120, 12-20.

Cambardella, C. A., Elliott, E.A. 1992. Particulate soil organic-matter change across a grassland caltivation sequence. Soil Science56, 777-783.

Canadell, J.G., Pataki, D.E., Gifford, P., et al. 2007. Saturation of the terrestrial carbon sink. In: Canadell et al. (eds.), Terrestrial Ecosystems in a Changing World. Germany: Springer. pp. 59–78.

Chivenge P., Vanlauwe B., Gentile R., Six, J. 2011. Organic resource quality influences short-term aggregate dynamics and soil organic carbon and nitrogen accumulation. Soil Biology and Biochemistry 43, 657–666.

Chmura, G. L. 2003. Global carbon sequestration in tidal, saline wetland soils. Global Biogeochemical Cycles, 17(4).

Edwards, N.T. 1982. The use of soda lime for measuring respiration rates in terrestrial systems. Pedobiologia, 23, 321-330.

Falkowski, P., Scholes, R.J., Boyle, E., Canadell, J., Canfield, D., Elser, J., Gruber, N., Hibbard, K., Högberg, P., Linder, S., MacKenzie, F.T., Moore, B.3., Pedersen, T., Rosenthal, Y., Seitzinger, S., Smetacek, V., Steffen, W. 2000. The Global Carbon Cycle: A Test of Our Knowledge of Earth as a System. Science 290 (5490), 291–296.

Forster, J. 1995. Soil sampling, handling, storage and analysis. Chapter 3. In: Alef, K. and Nannipieri, P. (Eds.), Methods in Soil Microbiology and Biochemistry. London: Academic Press. pp. 49-122.

Gispert, M. 2013. The impact of land management and abandonment on soil enzymatic activity, glomalin content and aggregate stablilty. Geodema 202-203, 51-61.

Gou, L.B. and Gifford, R.M. 2002. Soil carbon stocks and land use change: a meta analysis. Global change Biology 8, 345-360.

Grogan, P. 1998. CO_2 flux measurement using soda lime: The appropriate correction for water formed during CO_2 adsorption. Ecology 79, 1467-1468.

Hart, T.M., Davis, S.E. 2011. Wetland development in a previously mined landscape of east Texas, USA: Wetlands Ecol Manage 19, 317-329.

Heenan D.P., Chan K.Y., Knight P.G. 2004. Long-term impact of rotation, tillage and stubble management on the loss of soil organic carbon and nitrogen from a Chromic Luvisol. Soil Till. Res 76, 59–68.

Johnson, N.C., Hoeksema, J.D., Bever, J.D., Chaudhary, V.B., Gehring, C., Klironomos, J., Koide, R., Miller, R.M., Moore, J., Moutoglis, P., Schwartz, M., Simard, S., Swenson, W., Umbanhowar, J., Wilson, G., & Zabinski, C. 2006. From Lilliput to Brobdingnag: Extending Models of Mycorrhizal Function across Scales. BioScience 56(11), 889-899.

Keith, H., and Wong, S.C. 2006. Measurement of soil CO_2 efflux using soda lime absorption: both quantitative and reliable. *Soil Biology and Biochemistry* 38, 1121-1131.

Kemper, W.D., and Rosenau, R.C. 1986. Aggregate stability and size distribution. In Klute, A. (Ed) Method of soil analysis. Agron. Monogr. 9. Part 1,2nd ed. ASA and SSSA, Madison, Wisconsin, U.S.A.: SSSA, pp. 425-442.

Kiddy, P.A. 2010. Wetland ecology principles and conservation 2nd edition. New York: Cambridge University Press. pp 391-397.

Kjeldahl, J.Z. 1983. A new method for the determination of nitrógeno in organic matter. Analytical Chemistry 22, 366.

Koenig, R., Isaman, V. 2010. Topsoil quality guidelines for landscaping. A review: Utah state university. Published document (1914). Retrieved from: http://extension.usu.edu/files/publications/publication/AG-SO-02.pdf. Accessed on: 18/May/2016.

Lal, R. 2004. A reviewpoint: Soil carbon sequestration impacts on the global climate change and food security. Science 304, 1623-1626.

Lal, R., Follett, R.F., Stewart, B.A., Kimble, J.M. 2007. Soil carbon sequestration to mitigate climate change and advance food security. Soil Science 172(12), 943-956.

Lal, R. 2009. Challenges and opportunities in soil organic matter research. European Journal of Soil Science 60, 158–169.

Lal, R. 2011. Sequestering carbon in soils of agro-ecosystems. Food Policy 36, 33-39.

Lasanta, T. 1988. The process of desertion of cultivated areas in the central Spainish Pyreness. Pirineos 132, 15-36.

Leifheit, E.F., Veresoglou, S.D., Lehmann, A., Morris, E.K., Rillig, M.C. 2014. Multiple factors influence the role of arbuscular mycorrhizal fungi in soil aggregation: a meta-analysis. Plant Soil 374, 523-537.

Loumou, A., Giourga, Ch. 2003. Olive groves:" The life and identity of the Mediterranean". Agriculture and Human Values 20, 87-95.

Maltby, E. 1988. Global wetlands- History, Current status and future. In: Hook, D.D., McKee, W.H., Smith, Jr, H.K., Gregory, J., Burrell, jr, V.G., DeVoe, M.R., Sojka, R.E., Gilbert, S., Banks, R., Stolzy, L.H., Brooks, C., Matthews, T.D, and Shear, T.H. (eds). The ecology and management of wetlands. Volume1: Ecology of wetlands. United States: Timber Press. pp 3-13.

Pal, A. 2014. Role of glomalin in improving soil fertility: A review. International journal of plant and soil science 3(9), 1112-1129.

Pardini, G. 2003. Relative influence of wildfire on soil properties and erosion processes in different Mediterranean environment in NE Spain. The Science of Total Environment 328, 237-246.

Rillig, M.C., Wright, S.F., Nichols, K.A., Schmidt, W.F., Torn, M.S., 2001. Large contribution of abuscular mycorrhizal fungi to soil carbon pools in tropical forest soil plant and soil 233, 167-177.

Rillig, M.C., Rasmey, P.W., Morris, S., Paul, E.A., 2003. Glomalin an abuscular-mychorrhizal fungi soil protein responds to land use change. Plant and Soil 253, 293-299.

Rillig, M.C., 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. Canadian Journal of Soil Science 84, 355-363.

Rosier, C.L., Hoye, A.T., and Rillig, M.C. 2006. Glomalin-related soil protein: Assessment of current detection and quantification tools. Soil Biology and Biochemistry 38, 2205-2211.

Roulet, N. T. 2000. Peatlands, Carbon Storage, Greenhouse Gases, And The Kyoto Protocol: Prospects And Significance For Canada. Wetlands 20(4), 605–615.

Schlesinger, W.H. and Bernhardt, E.S. 2013. Biochemistry an analysis of global change 3rd edition. Oxford: Elsevier inc. pp 233-273.

Schnitzer, M., 1978. Humic substances: chemistry and reactions. In: Schnitzer, M. and Khan, S. U. (eds), *Soil Organic Matter* Developments in soil science 8. Elsevier Science Publishers B.V. 01-64.

Schnitzer, M. 1991. Soil organic matter. The next 75 years. *Soil Science,* 151, 41-58.

Six J., Elliott E.T., Paustian K. 2000. Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry 32, 2099–2103.

Soil Survey Staff. 1992. Keys to Soil Taxonomy. In: SMSS Technical Monograph Nº. 19. 5th Ed. Blacksburg, Virginia: Pocahontas Press, Inc. 541. pp.71-79.

Thorne, J.B. 2002. The evolving context of Mediterranean desertification. In: Geeson, N.A., Brandt, C.J., Thornes, J.B. (eds). Mediterranean desertification. A Mosaic of processes and responses. West Sussex: John Wiley and Sons. pp 5-15.

Tourist Office Estartit, and Pasqual, J. and Quintana, X. (eds.) Tourist Information Dossier: *Torroella de Montgri i l'Estartit*.(s.n).

University of New Hampshire(UNH). 2009. An Introduction to the Global Carbon Cycle. Retrieved from: http://globecarboncycle.unh.edu/CarbonCycleBackground.pdf. Accessed on 20-May-2016.

U.S. EPA. 2009. Synthesis of Adaptation Options for Coastal Areas, Washington D.C., U.S., Environmental Protection Agency, Climate Ready Estuaries Program, EPA 430-F-08-024.

Vitt, D.H., Chee, W. 1990. The relationships of vegetation to surface water chemistry and peat chemistry in fens of Alberta, Canada. Plant Ecology 89(2), 87–106.

Wang, Z.M., Zhang, B., Song, K.S., Liu, D.W., Li, F., Guo, Z.X., Zhang, S.M. 2008. Soil organic carbon under different landscape attributes in croplands of Northeast China. Plant, Soil and Environment 54, 420–427.

Wright, S.F., and Upadhyaya, A. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Science 161, 575-586.

Wright, S.F., and Upadhyaya, A. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant and Soil 198, 97-107.