Título: BIOGEOCHEMISTRY OF MARINE PHANEROGAMS SOILS

Nombre: Piñeiro Juncal, Nerea

Universidad: Universidad de Santiago de Compostela

Departamento: Escuela de Doctorado Internacional (EDIUS)

Fecha de lectura: 12/02/2021

Programa de doctorado: Programa Oficial de Doctorado en Medio Ambiente y Recursos Naturales

Dirección:

> **Director:** Antonio Martínez Cortizas

> **Codirector:** Miguel Ángel Mateo Mínguez

Tribunal:

> **presidente:** NÚRIA MARBÀ BORDALBA

> **secretario:** María del Mar Sobral Bernal

> **vocal:** STACEY TREVATHAN-TACKETT

Descriptores:

> BIOQUIMICA DE SUELOS

> MICROBIOLOGIA DE SUELOS

> CLASIFICACION DE SUELOS

> GEOQUIMICA ORGANICA

El fichero de tesis ya ha sido incorporado al sistema

> 521451_1303836.pdf

Localización: BIBLIOTECA XERAL USC

Resumen: Marine phanerogams, known as seagrasses, represent a secondary colonization of marine shallow waters by terrestrial plants. Like cetaceans, they returned to the oceans carrying traits of their terrestrial ancestors. Some of the morphological and physiological traits developed to adapt to life inland have allowed seagrasses to overcome some marine life challenges by different mechanisms than algae. For instance, the reinforcement of their tissues with lignin to maintain upright position out of the water may have supported the development of the lacunar system, which allows seagrasses to reduce phytotoxics such as Fe^{2+}, Mn^{2+} or sulfide in their rhizosphere. The development of belowground organs is one of the main characteristics of terrestrial plants and the most outstanding seagrass characteristic from the plant-substrate interaction point of view.

Seagrass meadows are distributed in coastal areas of all continents but the Antarctica. Their canopies promote sediment accretion and canopy structure and belowground organs reduce sediment resuspension, promoting the development of well-structured soils following a chronological sequence in which deeper equals older. Plant derived changes in the substrate are held in the rhizosphere and can vary with meadow maturity. Seagrass

rhizomes and root tips release O2 and organic compounds to the substrate promoting the formation of biogeochemical micro niches in its rhizosphere. The seagrasses are not the sole organism promoting physico-chemical changes in the meadow substrate, macro and micro invertebrates as well as the microbial communities have a role as well in the diagenesis of the substrates. Seagrass from the Posidonaceae genus develop high OM content soils, known as ¿mats¿. The role of mats as long-term carbon reservoirs has boost the interest about the fate of the OM buried in seagrass soils. The OM accumulation in the soil has been linked to (1) particles trapping and deposition favored by the seagrass canopy, (2) the anoxic conditions in the sediments and (3) the refractory character of the seagrass tissues, which can be preserved over millennia.
Traditionally, seagrass substrata have been considered sediments. However, given enough time, the seagrass presence would radically transform it -through additions, removals, transfers and transformations- from a mere accumulation of sediments into a soil. The Unite States Department of Agriculture Soil Taxonomy definition of soil recognizes the possibility of soil formation below seagrasses since its first edition. Nonetheless, subaqueous soils were largely neglected in soil science research. On the other hand, subaqueous soils are only recognized by the World Reference Base for Soil Resources up to 2 m depth at low spring tide. This arbitrary limit implies that the seagrass substrate of the same meadow, with the same age, and same characteristics, could be considered or not a soil on the sole basis of the depth of the water column ¿ approach not consistent with the soil-continuum. The intensity of transformation and the nature of the seagrass soil formed would be determined by several factors like climatic temperature regime, organisms, bathymetry, flow regime, parent material, time, water column attributes, and catastrophic events. Although seagrass soils can be as diverse as their terrestrial counterparts, being permanently water saturated, growing in thickness, and creating favorable conditions to accumulate OM, are traits common to most of them. These characteristics are partially fulfilled as well by subaerial soils from peatlands, tidal or freshwater marshes and mangroves. The full recognition of seagrass substrates as soils would help to clarify the nature of the deposit more efficiently and better reflect the processes through which ecosystem services related to the substrate are provided. Furthermore, it would highlight that even when the plant cover has disappeared (i.e., the meadow has died), ecosystem services will still be provided and the ¿dead¿ substrate will still be a functional ecosystem and not a barren bottom.
This thesis aimed to study the biogeochemistry of seagrass soils and, using the available pedological information, to establish a preliminary soil description and classification. Posidonia oceanica meadows are used as a model for seagrass soils biogeochemistry due to high impact of the plant over the substrate and the large quantity of available information about its interaction with the substrate. The soils are studied using depth as a proxy for time. The seagrass soil sampled are distributed between the Portlligat meadow (Northwest Mediterranean) and along the Andalusian coast (Southwest Mediterranean). Portlligat Bay hosts a well-developed P. oceanica meadow that was established since at least 6000 yr ago. The large quantity of information published about the bay and its meadow and the presence of a well conserved ¿dead¿ section of the meadow, makes of Portlligat a fitting location to study the soil biogeochemistry and its response to cover loss. The Andalusian coast (S Iberian Peninsula, SW Mediterranean Sea) present a large environmental heterogeneity with three main areas, from which two were sampled, the first with the characteristics of the Mediterranean Sea and the other, the Alboran Sea, with intermediate characteristics between the Atlantic Ocean and the Mediterranean Sea. This large environmental variability together with the large area occupied by seagrass meadows (>10,000 ha) represent a wide sampling range, allowing us to look for common biogeochemical processes and the main factors controlling seagrass soils formation and composition.
The first approach used for the study of the biogeochemistry of this soils was a general exploration of their composition through grain size distribution, organic matter (OM) content and elemental composition (X-ray

fluorescence, XRF). Analytical pyrolysis was used as well to study the molecular composition of the OM. The microbial communities structure and activity was explored using Biolog EcoplatesTM and metabarcoding. Finally, a review of the main characteristics of seagrass meadows is presented in an attempt to preliminarily describe and classified them.
The outcome of this approach is reported in five scientific manuscripts, three of which have been published in peer-reviewed journals, one has been submitted and is under revision and the last is being prepared for submission while this thesis is being written.
Manuscript 1 presents the study of a 475 cm-long core from Portlligat bay looking for evidence of horizonation or vertical stratification. This study aimed to better understand the long-term formation processes of a Posidonia oceanica mat, as most of the research performed in those soils are focused in the rhizosphere (short-term processes). To this end, we analyzed the elemental composition, using XRF, the carbonate content and the coarse and fine OM content (above and below 2 mm). The covariation between these soil properties were explored with principal component analysis. Four main physico-chemical signals were found: humification, accumulation of carbonates, texture and OM depletion. The results revealed a highly structured deposit undergoing pedogenetic processes characteristic of soils rather than a mere accumulation of sediments. Organic matter mineralization showed two phases, very likely within and below the rhizosphere. A preliminary classification is attempted classifying this soil as a Fluvaquent Entisol. However, further research is required to properly describe the substratum underneath seagrass meadows.
Manuscript 2 represents the upscaling of the first study. The same approach was used to analyze 15 cores along the Andalusian coast, 13 from Posidonia oceanica meadows and 2 from Cymodocea nodosa meadows, aiming to determine which factors drive the composition of these seagrass soils and looking for common processes. The proxies used were elemental composition (XRF core-scanning), magnetic susceptibility, Corg content and gran size distribution. Furthermore, the cores were dated by 210Pb and AMS14C techniques to estimate soil accretion. A principal component analysis was used to explore the main geochemical processes linked to soil formation. The results showed that terrestrial fluxes play a key role in meadow soil composition. Furthermore, Corg accumulation did not follow any general trend with depth, suggesting that temporal variation in Corg inputs is an important factor in determining carbon depth distribution within the soil. The results suggested that the establishment of well-developed, stable C. nodosa meadows in the Mediterranean may be promoted by adverse environmental conditions to P. oceanica settlement. Metal¿s behavior within the meadow deposit and their interaction with OM and carbonates is unclear. These results highlight the importance of the influence of land-based inputs in the characteristics of seagrass meadow deposits, highly determining their Corg content, as well as the necessity for further studies on metal behavior, to understand their full potential as environmental records.
In Manuscrit 3 Biolog EcoplatesTM were used to assess the patterns of distribution of potential microbial activity within the mat. EcoPlatesTM are a semi-quantitative method developed to characterize microbial functional activity by measuring the respiration of different carbon sources divided into six guilds: carbohydrates, polymers, carboxylic acids, amino acids, amines and miscellaneous compounds. Respiration of the microbial community is revealed by the reduction of a tetrazolium dye that is included with the carbon source. Mat samples from a 130 cm-long core of P. oceanica were incubated, under anaerobic and aerobic conditions. To our knowledge, this represented the first time that soil samples were incubated in Ecoplates under anaerobic condition. The results suggest a pronounced stratification of the microbial community controlled by oxygen availability. Despite the higher aerobic metabolism in the top 40 cm, the anaerobic metabolism was dominant, supporting the high capacity of P. oceanica soils for OM stabilization.
While the previous three manuscripts enabled to observe and explore the composition and change with depth of

seagrass soils, to infer its biogeochemical process, in Manuscript 4 we compared three cores of the Portlligat meadow with different status: the first one is the same core studied in Manuscript 1 and Manuscript 3; a second core was taken in an area of death meadow (were the seagrass cover has been lost) and the third core was sampled in a living meadow next to the death meadow area. In this study we aimed to understand how seagrass cover losses affect the carbon cycle in the soil by analyzing (1) total, inorganic and organic carbon and nitrogen contents; (2) using analytical pyrolysis (Py-GC¿MS) to assess the molecular composition of the geochemically reactive organic matter (<2mm fraction) and its relation to depth from the soil surface, as a proxy for time; and (3) metabarcoding to infer bacterial and archaeal diversity changes. The results showed the preferential mineralization of polysaccharides and guaiacyl and syringyl lignin, and a selective preservation of p-hydroxybenzoic acid moieties and methylene chain compounds. Soil depth and plant cover showed significant effects on microbial richness and diversity. Spatial variations in soil organic matter inputs and microbial differences in community composition between cores highlighted the importance of intra-meadow variability. Cover loss resulted in a release of soil organic carbon and the promotion of archaeal communities in the rhizosphere, whereas no effect was found in deeper layers. This study highlights the importance of soil stabilization against erosion to avoid the release of millenary organic matter stocks.
Manuscript 5 presents a review of the large effort invested in seagrass substrates research to contribute to the amendment of the lack of pedological studies in subaquatic marine environments, by summarizing the data typically used for soil description and classification. A literature review of papers published between 1980 and 2020 yielded more than 3,800 references from which the available information about bulk density, grain size, redox, pH, carbonate content, organic carbon content and porosity was gathered. Large knowledge gaps were found geographically and in seagrass diversity, being the temperate meadows, especially those from Posidonia and Zostera genus, the ones more studied. Nonetheless, some conclusions could be inferred. (1) Most seagrass substrates have sandy textures, being the Posidonia genus not able to grow in muddy substrates. (2) Although they promoted OM accumulation, the seagrasses are unlikely to form organic soils. The formation of Histosols seems to be a rare situation linked to the Posidonia genus. (3) The most likely classification of seagrass derived soil is as Entisols according to the ST-USDA, and as Fluvisols or Gleysols according to the WRB-FAO, however, further research is needed to properly classify those soils.
The result from the five manuscripts allow the discussion of the stratification of the biogeochemical processes in these soil between the rhizosphere and the subsoil. The rhizosphere holds both plant and microbial deriver processes, with a predominant aerobic potential microbial activity, and it is enriched in easily degradable compounds compared to the subsoil. In the subsoil the predominant potential activity is anaerobic and p-hydroxybenzoic acid moieties and methylene chain compounds are selectively preserved. The biogeochemical processes are led by the microbial communities and changes in plant physiology do not seem to affect the subsoil.
The degradation of organic matter with time within the soil is discussed using the core from the manuscript 1 as a reference due to its geophysical setting and the low change of continental sediment arrivals, that provide a steady input of both organic and inorganic materials. This degradation seems to follow three phases: a fast degradation phase, a low degradation phase and a stabilization phase. The death of the meadow, i.e., plant over loss, promotes an acceleration of this degradation due to a priming effect but only in the rhizosphere, not affecting deeper layers of the soil.
The use of P. oceanica as a model for seagrass soil biogeochemistry may have led to the over estimation of processes linked to organic matter degradation due to the high organic matter content found in its soil compared to other seagrass soils. Furthermore, Posidonia oceanica forms enduring meadows assuring a steady

transformation of the substrate, while other seagrass may form transitory meadows. The impact over the substrate of transitory meadows far lower. Those processes linked to plant physiology may be overestimated in P. oceanica soils as well. Last, the range of substrates colonized by P. oceanica is a fraction of the substrates that can be colonized by seagrasses.

Summarizing, the major findings of this thesis are (1) that there are two distinct biogeochemical compartments in seagrass soils, the rhizosphere and the subsoil. (2) That the main processes found were somewhat related to OM accumulation and mineralization; (3) changes in plant physiology, or death, affect the rhizosphere biogeochemistry, but not the subsoil; and that (4) not all substrates below seagrass meadows meet the characteristic of a soil, but given time, all substrates below seagrass meadows are likely to be transformed into soils. The use of P. oceanica soils as a model may have overestimated the importance of OM geochemistry in the soil, as them show higher OM concentrations than other seagrasses soils. As found in the Portlligat meadow, it is likely that seagrass meadows would have a high spatial diversity and the examination of their spatial distribution is recommended to select the area more suitable for an experiment or for samples retrieval. It would be particularly interesting to compare the inorganic chemistry of meadows with contrasting land basin composition. Metal behavior in the soil is unclear. Further research on the biogeochemistry of metallic elements in the soil would contribute to the efforts devoted to fully explore the potential of these soils as environmental archives of past human activities, as metal content is a key proxy for mining, metallurgy and industrial development.