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Dynamics of salt marsh biomes in response to inundation

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Chapter 6

Synthesis and general discussion

Ecosystem engineering in soils is considered of utmost importance for regulating soil communities (Lavelle, 2002). Ecosystem engineers affect the soil physically by moving through the soil, and by mixing soil and/or organic matter, they increase water infiltration and soil aeration, which modify the availability of habitats and sources to other organisms (Jones *et al.*, 1994; Wright & Jones, 2006). Their paramount importance in soil processes justifies studies that attempt to understand how the environment affects ecosystem engineers and, vice versa, how ecosystem engineers influence their surrounding soil (micro)biota. In particular, this thesis focused on understanding (1) whether salt marsh elevation (as a proxy of frequency of inundation) and its associated environmental factors determine the composition of associated bacterial communities of soil ecosystem engineers, and (2) to what extent the composition and traits of the soil bacterial communities are affected by the presence of ecosystem engineers and inundation. I tackle these questions using empirical observations from both field sampling and experimentation in microcosms. The two focal ecosystem engineers in this thesis were the litter-feeding burrowing amphipod *O. gammarellus* and the tall grass *E. atherica*, which both are dominant and key species of the food web of the late-successional stages of European salt marshes (Bakker *et al.*, 1993; Olff *et al.*, 1997; Schrama *et al.*, 2012). This thesis contributes to understanding the ecosystem engineers' interactions -- among them and with the environment-- through a microbial interaction prism.

1. Salt marsh elevation is an important driver of the host's microbiome but its impact differs between host types.

In **Chapter 1**, I detailed why salt marshes are considered ideal for studying changes in intra- and interspecific interactions across physical gradients (Bertness & Leonard, 1997). Differences in elevation lead to differences in the frequency and duration of tidal inundation, which affect many soil properties, such as redox potential, clay content, pH, organic matter content, and salinity (Davy *et al.*, 2011; Janousek & Folger, 2014) (Supplementary material **Chapters 2** and **3**). Thus, (micro)organisms are distributed along the elevation gradient according to their sea-water inundation tolerance (Pennings *et al.*, 2005; Yao *et al.*, 2019). Some species are distributed at both high and low elevations, but most species prefer a certain height above sea level. Dissimilarity in environmental conditions at different elevations can drive an intraspecific phenotypic differentiation (Dunson & Travis, 1994; Hofmann & Todgham, 2009). For example, low-elevation ecotypes have a higher resistance to a high flooding frequency, and ecotypes with higher tolerance to dry conditions have a higher fitness at high elevations (Mendelssohn & Morris, 2002). However, this depends on the life history of the focal species, as this determines the degree of environmental pressure. For instance, plants are sessile organisms that have to adapt to adverse environmental conditions, while soil

animals can move to find shelter or more suitable environments. However, plants and animals are holobionts that comprise a host and associated microbiota (Zilber-Rosenberg & Rosenberg, 2008). The associated microbes might play a role in environmental tolerance (Bulgarelli *et al.*, 2013; McFall-Ngai *et al.*, 2013). Thus, the acclimation of the host to a shift in environmental conditions, such as across the salt marsh elevation gradient, likely co-occurs with shifts in host-associated microbial communities (Carrier & Reitzel, 2018). In this theoretical context, I here discuss the results of the impact of environmental factors on two key ecosystem engineering organisms and their associated microbiome described in the chapters of this thesis.

Low impact of the environment on the associated communities in O. gammarellus may relate with similar life history and tolerance traits at high and low elevation in salt marshes

In **chapter 2**, I explored the influence of the environment on the bacterial communities of the digestive tract of *O. gammarellus* (ODT). In general, the composition of bacterial communities from the ODT was not significantly affected by elevation, which may be related to the low impact of the salt marsh environment on host life history and tolerance traits (**Chapter 2**- Appendix B). These results were obtained from the part of the salt marsh not grazed by cattle and deviate from life-history and tolerance traits of *O. gammarellus* populations in salt marsh areas under grazing. In grazed areas, *O. gammarellus* populations show high sensitivity to environmental conditions across elevation (Smallegange & Berg, 2019). I argue that in contrast to the grazed areas, where there is a mosaic of tall and short plant species, there is a dominance of tall vegetation, especially in late successional stages in the absence of grazing (**Chapter 2**, Table 1). In addition, the lack of trampling by cattle favors the movement of the macrodetritivores through the soil and increases water infiltration (Dias & Sprung, 2003a). These two factors, vegetation height, and water infiltration may buffer fluctuations in soil temperature and moisture and, thus, the stress that high-marsh *O. gammarellus*' populations may face during dry months in the absence of cattle grazing. This explanation is supported by the effect of substrate shading by another tall-grass species *Spartina alterniflora*, which reduces high temperatures, dehydration, and soil hardness in high elevation in other salt marshes, promoting higher densities of crabs (*Chasmagnathus granules*) (Bortolus *et al.*, 2002). Therefore, the amelioration of the stress conditions in the high elevation marsh may have prevented intraspecific differences in the physiology of *O. gammarellus*, which together with the ODT selection for certain bacterial genera could have contributed to similar ODT communities among elevations.

A potential symbiont in the digestive tract of *O. gammarellus*

The ODT showed less diversity in bacterial composition than soil and plant litter, and a high proportion of the bacterial abundance (around 60%) was composed of 12 amplicon sequence variants (ASVs), belonging to 5 genera (enlisted in Table 6.1 and **Chapter 2**). The filtering of bacteria from the environment usually observed in (non)-soil animals is based on internal factors, such as the host physiological condition and resource quality, limiting the bacterial diversity and richness compared to soil and litter communities. Interestingly, ODT bacterial communities showed a dominance of *Ca. Bacilloplasma* followed by *Vibrio* species. *Ca. Bacilloplasma* is an unculturable bacterium, and its role in the digestive tract of *O. gammarellus* is still unclear. However, it is considered a non-pathogenic bacterium, not involved in the lignocellulose digestion in isopods (Kostanjek *et al.*, 2007a; Bredon *et al.*, 2018). *Bacilloplasma*-like bacteria and *Mycoplasmas* are prevalent in the gut of other marine and terrestrial organisms, where they are hypothesized to participate in the production of lactic and acetic acids that are sources of carbon for other bacteria (Holben *et al.*, 2002). Recently, the reconstruction of the bacterial genomes from fish gut-associated metagenomes show that *Mycoplasmas* are associated with many host metabolic functions, e.g., riboflavin supplementation, suggesting a mutualistic relationship with their hosts (Rasmussen *et al.*, 2021). However, it would require further meta-transcriptomic and metabolomic studies to understand the role that *Bacilloplasma* plays for its hosts and how it interacts with other bacteria in the gut.

How *Ca. Bacilloplasma* is transmitted between macrodetritivores is unclear (Bouchon *et al.*, 2016). **Chapter 2** is the first to report the presence of *Ca. Bacilloplasma* also in soil and plant litter. As *mycoplasmas* strongly rely on their hosts to survive (Citti & Blanchard, 2013), my findings suggest that *Ca. Bacilloplasma* can be horizontally transferred through the ingestion of feces or remains of dead animals on plant litter and soil particles. Moreover, in the data of **Chapter 2**, I observed that *Ca. Bacilloplasma*-associated reads were more abundant and in higher number of rhizosphere samples compared to bulk soil. This was also observed in **Chapter 4**, where the bacterium was more abundant in rhizosphere than lower layer of bulk soil. These results may indicate that *O. gammarellus* prefer to stay closer to the roots of *E. atherica*.

Strong impact of the environment on *E. atherica* may lead to dissimilar bacterial communities at high and low elevation in salt marshes

Plants may show distinct phenotypes in response to abiotic conditions, and these phenotypic responses can be mediated by plant-associated microbes. Microbes can alter a broad range of plant traits by providing novel nutritional and defense

pathways and influencing plant physiological and biochemical pathways (Friesen *et al.*, 2011). For instance, arbuscular mycorrhizal fungi can increase stomatal conductance to improve plants' drought tolerance (Augé, 2000). In salt marshes, plant adaptation to abiotic stress is crucial for their expansion into new habitats due to changes in soil conditions imposed by tidal inundations. However, the contribution of microbes to the local adaptation of plants remains poorly understood. A first step to investigate this relationship is to dissect the patterns of microbiome interactions with salt marsh plants, such as in the sea coach *E. atherica*, which inhabits a broad range of environmental conditions, e.g., across high and low elevations. The genetic and phenotypic adaptation to different elevations has resulted in different ecotypes of *E. atherica* (Bockelmann *et al.*, 2003a; Reents *et al.*, 2021).

Chapter 3 explores the soil biotic factors (root mycorrhiza presence, soil bacterial communities), abiotic factors (flooding frequency, pH, nutrient content, soil texture), and *E. atherica* traits to analyze their effect on the interaction between roots and their associated bacteria. I found that bacterial communities in the rhizosphere of *E. atherica* were significantly different between elevations. These differences in the rhizosphere composition were highly correlated with soil ammonium content. This highlights the overriding effect of the inundation frequency on edaphic factors that in turn shape the soil microbiome and rhizosphere bacterial composition, as the latter is mainly derived from the surrounding community pools (Philippot *et al.*, 2013). This result is consistent with a study on *Limonium vulgare* growing at the same salt marsh when different successional stages are compared, where changes in the soil factors across succession correspond to changes in the rhizosphere community structure (Wang *et al.*, 2016). Besides, as *E. atherica* shows genetic differentiation due to different abiotic conditions across short spatial distances (<100 m) (Bockelmann *et al.*, 2003a; Scheepens *et al.*, 2007), ecotypes may differ in the quantity and quality of root exudates (Pérez-Jaramillo *et al.*, 2017), thus, distinctive rhizosphere bacterial communities. The data on endosphere bacterial communities support this hypothesis (see below).

The endosphere bacterial composition differed significantly from the rhizosphere or soil bacterial composition, showing that ~40% of the endosphere ASVs were exclusive to this compartment. Moreover, the endosphere bacterial composition also differed between *E. atherica* ecotypes. Interestingly, the majority of these inner root bacteria were non-shared between ecotypes. I argue that this difference in bacterial composition at the ecotype level is related at least partially to the dissimilarities in endomycorrhizal colonization, which in the high elevation ecotype was around 4.5 times higher than the low elevation ecotype. There is growing evidence

of bacteria belonging to diverse families living in close association with fungal mycelia (Hoffman & Arnold, 2010). Therefore, fungal-associated bacteria could contribute to the endophytic bacterial communities in roots with high endomycorrhizal colonization. Moreover, the unique ASV sequences found in each ecotype underlie the strong effect of the local environment on inner root bacterial community composition, which may affect *E. atherica* adaptation to local environmental conditions. This is relevant because not all plant species with different ecotypes show differences in their internal microbiome (Singer *et al.*, 2019). However, in *E. atherica*, the ecotypes exerted a strong association with specific bacterial genera and this may even lead to coevolution with ecotype-specific (pathogenic) strains (Laine *et al.*, 2014).

It is interesting to observe that the three dominant and invasive grass species in salt marshes, i.e. *E. atherica*, *Phragmites australis* and *Spartina alterniflora*, all have distinguishable ecotypes adapted to different abiotic conditions (Hanganu *et al.*, 1999; Zogg *et al.*, 2018), which differ significantly in their plant-associated microbiome (Soares *et al.*, 2016; Lin *et al.*, 2019). This observation agrees with a proposed hypothesis on how mycorrhiza can influence plant invasion. This hypothesis states that plant species with facultative symbioses, that is, that easily associate with the microbiome of the new environment and that lack a specific mutualist, are prone to being invasive in the introduced area (Pringle *et al.*, 2009; Coats & Rumpho, 2014). Although more studies are needed to unravel the specific advantages promoted by plant-associated microbes, these examples suggest an essential role of these facultative interactions during the process of plant invasion in salt marshes.

Among bacterial candidates with plant-promoting traits that may contribute to the competitiveness of plants are *Marinomonas* and *Vibrio* (Table 6.1). The former is an abundant colonizer of *E. atherica* (at low elevation marsh) and *Limonium vulgare* in this salt marsh (Wang *et al.*, 2016), as well as in other salt marsh halophytes (Lucena *et al.*, 2016). *Marinomonas* and *Vibrio* have been shown to produce in salt marsh plants the indole-3-acetic acid (Mesa *et al.*, 2015; Lucena *et al.*, 2016), which is a phytohormone that can increase plant growth and abiotic tolerance (Egamberdieva *et al.*, 2019). Interestingly, *Vibrio* was abundant in the endosphere and in the digestive tract of *O. gammarellus* (Chapter 2). The competitive strength of *Vibrio* to colonize eukaryotic hosts strongly relies on its ability to form biofilms, which enhances growth, survival, and protection from predators and antibiotic compounds (Yildiz & Visick, 2009). The observation that *Vibrio* was found in both hosts highlights the great metabolic versatility of this genus that contributes to its host-colonization ability, especially in organisms in areas with frequent tidal flooding.

2. Effects of abiotic factors on the plant-macrodetrivore-microbes interactions

Chapters 2 and 3 provide information about how the environmental conditions affect the microbiome interaction to a different extent in *O. gammarellus* and *E. atherica*. The three components (i.e., microorganisms, plant, amphipod) can directly or indirectly affect each other. However, the environmental conditions may also affect the interaction between *O. gammarellus* and *E. atherica* and with the soil surrounding microbial species. For instance, *E. atherica* is the primary producer of litter material that, when colonized by microbes, is the nutrient source for the amphipod. Moreover, plants contribute to the soil labile carbon, which can be used as an energy source by soil microbes. The ability of *O. gammarellus* to form burrows increases the soil oxygen levels and distribution of organic matter, influencing the mineralization pathways driven by soil microorganisms. This mineralization, in turn, has positive effects on the nutrient uptake of *E. atherica* (Schrama *et al.*, 2015). Even though plant-amphipod interaction is closely linked to soil microbes, little is known about the composition of the microbial communities in the presence of both ecosystem engineers. Thus, to better understand the functioning of salt marshes, it is necessary to analyze the microbial composition from a taxonomical and trait perspective. In chapters 4 and 5, I aimed to take an essential first step to understand plant-amphipod-microbial interactions in a salt marsh ecosystem and assess how these components' interaction is affected by water flooding.

Interspecific interactions- The microbial bridge between plant and bioturbator

- **Ecosystem engineers altered the response of bacterial community over time**
Flooding led to changes in the composition of the soil bacterial communities, but the extent of the changes depended on the soil layer, the frequency of flooding, and the presence of *E. atherica* or *O. gammarellus* alone or in combination. Significant changes in bacterial community composition occurred in the lower soil layer and a frequent flooding frequency. When flooded, the community composition in the lower soil layer in the absence of ecosystem engineers tended to deviate from the non-inundated treatment. This difference in community structure increased over time (Chapter 4). This observation was in line with the highest water content and lowest redox potential at the end of the experiment (33 days). In contrast, either the presence of *E. atherica* or *O. gammarellus* showed less turnover in bacterial composition in the lower soil layer after day 19, suggesting that their presence improved the bacterial community's resistance to inundation. These differences can be explained by the physical effect of the ecosystem engineers on the soil, as the formed burrows and root channels aerate the soil and reduced water retention (Lavelle *et al.*, 2016).

- **Effect of *O. gammarellus* on soil bacteria involved in N-cycling**

The most direct influence of the burrowing invertebrate on the soil was an enhancement of bacteria involved in N-cycling under non-inundation conditions (**Chapter 4 and 5**), which was more evident in treatments without plants. For example, in the *O. gammarellus*-alone treatment enhanced ammonia-oxidizing microbes in the lower soil layer, with *Nitrosomonadaceae* as one of the central taxa in the bacterial networks. I argue that *O. gammarellus* in soils without plants was more active, contributing to soil oxygen content in response to higher burrowing activity. This hypothesis is supported by the higher redox potential observed in the upper soil layer in this treatment compared to the treatment with plants. Thus, their activity promoted microbes able to perform nitrification and ureolysis since both reactions increase in aerobic conditions (McCarty & Bremner, 1991).

- **Effect of *O. gammarellus* on plant traits**

The rise in soil nitrate content in the presence of *O. gammarellus* was also observed in the treatments with plants, which could be explained by the increase of plant aboveground biomass, as well as broader leaves. In agreement, the phenotypic response to higher moisture and nitrates correlated with the data from the field. It was observed that plants inhabiting soils with these soil characteristics showed broader leaves as well (**Chapter 3**). Plant growth in the presence of *O. gammarellus* was correlated with a slight increase in aerenchyma surface area under inundation compared to the plant alone treatment (**Chapter 4**). However, I argue that the increase in plant biomass under inundation was detrimental to the plant as their roots require more oxygen for respiration, which was scarce under inundation conditions. The internal supply of O₂ to the roots may be compensated by more aerenchyma formation, but even so, plants with *O. gammarellus* tended to show higher stress (lower chlorophyll fluorescence) than plants alone.

- **The interactions between *O. gammarellus* and *E. atherica* affect the functional and taxonomic composition of soil microbial communities**

The interactions between *E. atherica* and *O. gammarellus* modified the soil bacterial community composition and stimulated soil microbial processes under inundation and non-inundation conditions (**Chapter 4,5**). Firstly, I posit that this difference in community composition of bacteria in non-inundated soil resulted from root exudates and animal burrowing, affecting the distribution of nutrients and microbes in the soil matrix. The modifications in soil structure by both engineers allowed that the daily surface watering penetrated deeper into the soil through the burrows and root channels (Fischer *et al.*, 2015; Howison *et al.*, 2017). This favored plant growth (but also slightly higher plant stress) as well as bacterial aerobic and anaerobic metabolism (**Chapter 4,5**). These effects on soil moisture

and nutrient distribution may explain the presence of exclusive soils ASVs in the combined engineer treatment (**Chapter 4**). Secondly, it is known that high water content can cause desorption of organic matter from the soil particles, enhancing microbial access to degrade organic matter (Spivak *et al.*, 2019). This effect of water in soils may explain the increase of bacteria able to hydrolyze complex and simple organic compounds in all flooded treatments compared to non-flooded conditions (**Chapter 5**). However, some differences were found in the effects of engineers on soils. For instance, treatments with both engineers in flooded and non-flooded soils, as well as with single *E. atherica* treatment under flooded conditions, differed in the bacterial network topology compared to the *O. gammaarellus* alone or bare soil. The presence of plants increased the network modularity and average path length (**Chapter 4**). These network characteristics suggested that the presence of plants in soils with high moisture content promoted the formation of bacterial subgroups (modules) and bacterial interconnection (Hernandez *et al.*, 2021). This strong effect of roots on the soil bacteria concurs with the positive correlation of the aerenchyma area with the soil bacterial composition. Thus, it is probable that the labile carbon from root exudates improves the cooperation between anaerobic microbes. This reasoning is consistent with enhancing fermentation and microbes that use fermentation-based products for their metabolism when compared to the treatment without plants (**Chapter 5**).

The additional release of nutrients derived from interactions between the two engineers positioned some copiotrophs (r-strategists), such as *Flavobacteriaceae* (Bacteroidetes) *Woeseiaceae* (Gammaproteobacteria), as hubs in community networks (Fierer *et al.*, 2007). That is, bacteria able to use nutrients rapidly are potentially linked to many other species, thus having a central role in the community structure (Aglar *et al.*, 2016). The rhizosphere communities also shifted under inundation, favoring the growth of copiotrophs such as *Burkholderiaceae* (Betaproteobacteria) (**Chapter 4**). As salt marsh soils are characterized by a low soil N content (Tyler *et al.*, 2003), a rhizosphere priming effect strategy is likely to occur, especially under flooded conditions. This represents an important strategy for plants to access organic N when in competition with microbes in the soil. Plants release labile C that often enhances the growth of the rhizosphere community, resulting in an increased microbial demand for N. This demand will then stimulate the microbial community to hydrolyze polymeric organic matter to obtain more N (Moreau *et al.*, 2015). The roots take up an important part of the products of this hydrolysis. The results obtained in **Chapter 5** indicated an increase of bacterial nitrogen assimilation in the presence of plants. However, an increase of soil bacteria that potentially were able to mineralize N organic compounds in the treatments with plants was not found.

Interestingly, the bacterial communities that increased in the presence of plants were the N_2 fixers. Likely, the ammonium produced by N_2 fixation was shared between ANAMMOX, fast-growing bacteria and plants, since all of them showed an increase in abundance or growth in flooded soils. As some of the bacteria that increased in the presence of *O. gammarellus* and plants were fast-growers (e.g., *Flavobacteriaceae*) and the plants presented higher biomass, it is likely the competition for N may be higher in the combined treatment compared to plants alone. After 33 days of the experiment, the stimulation of SOM decomposers of lignin may have started in the combined engineer treatment since the abundance of laccase-producing microbes was higher than in the plant alone treatment. This result could indicate a faster positive priming effect in the presence of *O. gammarellus* compared to the plant alone.

It is worth noting that the factors driving the rhizosphere priming effect are still largely unknown, but there are (a)biotic factors reported to affect this priming effect. For instance, soil waterlogging can cause a negative priming effect in rice plants, in other words, less SOM decomposition. This negative effect was hypothesized to result from the bacterial community shift from the use of humified SOM as their main energy source to the plant-derived inputs (Ge *et al.*, 2012). In addition, microfauna, such as the nematodes predate on microbes in the rhizosphere, regulating microbial turnover and composition, subsequently affecting the root priming effect (Kou *et al.*, 2018). I posit that the closely associated microbes to the micro and macrofauna can also affect the rhizosphere bacterial community and likely the rhizosphere priming effect. For instance, *Ca. Bacilloplasma*, which was found in high abundance in the digestive tract of *O. gammarellus* (Chapter 2), was found in the rhizosphere of *E. atherica* even in higher abundance compared to the lower soil layer (Chapter 3 and 4), suggesting changes in rhizosphere composition due to the presence of another soil engineer. This finding substantiates recent reports in which the interaction of two ecosystem engineers resulted in a different assembly of rhizosphere bacteria compared to single engineers (Jacquioid *et al.*, 2020). To sum up, the results indicated that both engineer and inundation potentially modulate the level of plant rhizosphere priming effect on the composition of soil microbial communities.

Do the interactions of both ecosystem engineers increase methanogens or denitrifying bacteria?

Regarding the ecosystem level effects of the interaction among engineers in a soil inundation context, I will focus here on the potential increase in potent greenhouse gases, i.e., methane and nitrous oxide. Methane production in salt marsh sediments is considered negligible due to high soil sulfate content and the competition with

sulfate-reducing bacteria (Bartlett *et al.*, 1987; Poffenbarger *et al.*, 2011). However, intermittent freshwater inundation can also cause brackish soil conditions in salt marshes (Bakker, 2014), reducing soil sulfate content and thus, allowing methanogens to emerge. Moreover, methanogens can more efficiently use some electron donors, such as methanol or trimethylamine, than their competitors (Magonigal *et al.*, 2004; Yuan *et al.*, 2013). In **Chapter 5**, methanogen abundance was increased under inundation (anaerobiosis) in all the engineer treatments. In addition, they were more abundant in the combined treatment compared to the *O. gammarellus* alone treatment. This observation supports our previously discussed effect of releasing labile carbon by the roots into the soil, which increased diverse anaerobic microbes. However, since there was no difference in the methanogen abundance when compared to the plant alone treatment, *O. gammarellus* did not promote their abundance and, potentially, the methane emission. On the contrary, it increased aerobicity, reducing the anaerobic environment needed for methanogenesis. A higher average (albeit not significant) of methane oxidizers and aerobic respiration in *O. gammarellus* presence was observed. Thus, possibly a higher burrowing activity could have decreased the methanogens abundance significantly. Other reports of larger burrowing invertebrates such as crabs, e.g., *Neohelice granulata*, highlight their potential importance in limiting the anaerobic carbon degradation and increasing the CO₂ release (Martinetto *et al.*, 2016; Xiao *et al.*, 2021).

The frequent flooding disturbance in soil increased the bacteria able to reduce nitric oxide to nitrous oxide. However, the abundance of microbes performing this denitrification step was similar between single or combined engineer treatments, suggesting that *nosZ* communities are not sensitive to the presence of another engineer. Besides, even though the denitrifiers were more abundant (NO reducers gene *norB*, 19.4 ± 2.2 mean CPM ± SD) compared to ANAMMOX (*nrfH* gene, 6.7 ± 0.66 CPM ± SD) in the soil, the latter increased with plant presence. This result suggested that the plants may increase the microbial N₂ release without the potential leaking of the greenhouse potent gas. Moreover, bacteria with the *nosZ* gene may scavenge a large part of the N₂O, reducing the gas emission to the atmosphere. Similar to *norB* communities, there were no abundance differences in the presence of single or combined engineers' treatments. This scavenging of N₂O is also favored in soils with high moisture content since the diffusion of the gases is slower than in aerated soils (Elzenga & van Veen, 2010). Finally, other considerations remain to be explored such that this composition of denitrifying community may differ to the internal part of *O. gammarellus* burrows or in the rhizoplane soil (An *et al.*, 2021). Also, whether the difference of denitrification rate (and likely potential release of N₂O) between elevations observed in Chapter 3 correspond to differences in denitrifying community composition (**Chapter 3**, Angell *et al.*, 2018).

Table 6.1. Abundant bacterial families or genera associated with salt marsh hosts.

Phylum or Class	Bacterial family or genus	Host or source	Study	Putative role	Reference
Bacteroidetes	<i>Algitalea</i>	ODT core Soil Plant litter	Chapter 2	Core microbiome ODT. Digestion of algae.	(Yoon <i>et al.</i> , 2015)
Tenericutes	<i>Ca. Bacilloplasma</i>	ODT core Rhizosphere Soil Plant litter	Chapter 2, 4	Commensal in the digestive tract of <i>O. gammarellus</i> and other detritivores	(Kostanjšek <i>et al.</i> , 2007a)
Gammaproteobacteria	<i>Leucothrix</i>	ODT core Soil Plant litter	Chapter 2	Associated to seaweed and the gill of crabs	(Zhang <i>et al.</i> , 2016a; Xie <i>et al.</i> , 2017)
Bacteroidetes	<i>Mariibacter</i>	ODT core Soil Plant litter	Chapter 2	It is associated with seaweed, intertidal soil, and seawater.	(Xie <i>et al.</i> , 2017)
Gammaproteobacteria	<i>Vibrio</i>	ODT core Soil Plant litter Endosphere core, more abundant in low elevation	Chapter 2 Chapter 3	Found in fresh, brackish, and seawater, often associated with aquatic animals and plants. Some species are pathogens to humans, and others promote plant growth.	(Imhoff, 2005)
Gammaproteobacteria	<i>Marinomonas</i>	Endosphere core, more abundant in low elevation	Chapter 3	Marine bacteria associated with plants and fish; capable of degrading lignin-derived components.	(Lu <i>et al.</i> , 2020)
Gammaproteobacteria	<i>Burkholderiaceae</i>	Rhizosphere and soil	Chapter 4	Increase in rhizosphere due to inundation	This study. (Estrada-De Los Santos <i>et al.</i> , 2001)
Bacteroidetes	<i>Flavobacteriaceae</i>	Rhizosphere and soil	Chapter 4	Interaction <i>O. gammarellus</i> and <i>E. atherica</i> .	This study.

Future perspectives

Below is a list of prospective study areas for which this thesis could be used as a basis:

- Co-evolutionary relationship between *Ca. Bacilloplasma* and *O. gammarellus* and other detritivores

I find *Ca. Bacilloplasma* useful as a species indicator of co-evolutionary dynamics with *O. gammarellus* and other detritivores. Mycoplasmas have minimal genetic information and therefore acquire many nutrients from their hosts (Razin *et al.*, 1998). This high dependency on their host favors the rapid adaptation to (new) hosts (Delaney *et al.*, 2012). Moreover, the high abundance of *Bacilloplasma* in the digestive tract of *O. gammarellus* (**Chapter 2**) and its presence in other macrodetritivores suggest a host dependency on the bacterium. To disentangle the role of this bacterium in the digestive tract, I recommend first reconstructing the genome of *Ca. Bacilloplasma* to uncover its genetic potential. After that, the genome can be correlated to the microbial genes that are constantly expressed in the digestive tract of macrodetritivores through metatranscriptomic approaches. A positive correlation between the genes of the reconstructed genome and the expressed genes, with those genes that complement the metabolism of the invertebrate, could indicate a symbiosis of *Ca. Bacilloplasma* with its host. This hypothesis could be verified by performing manipulative experiments measuring host fitness at different levels of *Bacilloplasma* colonization in the gut while tracking nutrient fluxes. The results of those experiments together could disentangle the role of this bacterium in the host ecology. Besides, I suggest studying further the different strains of *Ca. Bacilloplasma* in diverse *O. gammarellus* populations that are locally adapted to specific salt marsh conditions. Since both organisms may need to adapt to differences in, for example, soil salinity, they may require physiological traits that may be under selection. It would be interesting to know how the invertebrate and its symbiont support each other's adaptation to environmental changes.

- Plant intraspecific differences in microbial community composition

Chapter 3 and other studies have provided examples of the influence of the environmental (soil) factors on root microbiome of a few salt marsh plant species (Dollhopf *et al.*, 2005; Wang *et al.*, 2016; Soares *et al.*, 2016). However, the role of the plant intraspecific differences driving the plant microbial composition is still unclear. For example, *E. atherica* adapted to low elevation shows a distinct phenotype compared to *E. atherica* inhabiting high elevation. Furthermore, there is a genetic differentiation between high- and low-elevation plants (Bockelmann *et al.*, 2003b). However, a high diversity of genets is also present at the same elevation

and at short distances < 5 m, indicating a large genetic intraspecific variability with potential differences in traits (Scheepens *et al.*, 2007). Therefore, it is challenging to unravel the contribution of intraspecific adaptations to the associated plant microbiome. A recent study shows that each *E. atherica* ecotype mediates the effect of flooding on soil exo-enzymes activities and belowground litter breakdown (Tang *et al.*, 2021). Thus, intraspecific adaptations of *E. atherica* may have important implications for soil carbon accumulation in salt marshes. However, it is still unclear which specific plant genes and functional traits drive the differences in the associated microbiome and plant surrounding bulk soil. Therefore, manipulative experiments coupled with field observations could help to disentangle which root and plant traits e.g., root exudates composition, drive the microbial communities (Friesen *et al.*, 2011). This information would be relevant not only for wetland plants but also for a broad understanding of plant trait effects on the plant microbiome.

- Soil community resilience after flooding

Community stability is defined as the tendency of a community to return to a mean composition after a disturbance (Pimm, 1984; Shade *et al.*, 2012). Stability includes community resistance and resilience. In **chapter 4**, I observed that the presence of the ecosystem engineers increases the resistance of the bacterial community after the flooding events compared to bare soil. However, I did not measure whether the soil community recovers after the last flooding event (resilience). I posit that after some weeks, plants will become more vigorous, and the young *O. gammarellus* will increase in size. Thus, the soil aeration will increase significantly. However, the soil community may not have had the same composition before the flooding events. Therefore, the soil communities may undergo a post-disturbance reorganization to find an alternative stable composition (Scheffer *et al.*, 2001; Jurburg *et al.*, 2018). Further experiments may include a follow-up of the short- and long-term impact on soil communities after the last flooding.

Additionally, the effect of flooding on all organisms that compose the soil food web remains unexplored. Soil harbors dense communities of organisms not studied here, such as viruses, fungi, protists, nematodes that are likely also affected by inundation (Wagner *et al.*, 2015; Abdallah *et al.*, 2019). For instance, it is estimated that one gram of soil can host meters of fungal hyphae and hundreds of thousands of protists, nematodes (10^{1-2}), and microalgae (10^{3-6}) (Dindal, 1990). Since the tolerance and recovery capacity to the flooding conditions depends on species type, there are likely changes within each community. For example, flooding favors colonizers over k-strategist nematodes (Wagner *et al.*, 2015). Thus, it is probable that there is a cascade of effects in other levels of the food web. In the

light of the increase of extreme flooding events projected in the future, it is worth to investigate the interaction of various domains of life with the abiotic soil environment. This approach would take us closer to cracking the unknown functions from the soil “black box.”

- Integration of “omics” with soil enzymatic activities

In **chapter 5**, I based functional inferences upon the genetic potential carried by the communities. This approach does not directly imply that those genes and functions are active in the soil. Further work should include a functional reading of the metagenome, such as transcriptomics, proteomics, and metabolomics. For example, transcriptomics revealed differences in methanogens abundance before and after drainage in paddy soils (Abdallah *et al.*, 2019). Although “omics” technologies are powerful tools to uncover microbial metabolisms and interactions between microbes (Glass *et al.*, 2014; Keegan *et al.*, 2016), the annotation of the genes or proteins relies on the database completeness. A large proportion of novel sequences lack dedicated known functions or insufficient homologs with known taxonomic affiliation (Galperin & Koonin, 2004; Teeling & Glöckner, 2012). Thus, we may overlook rare microbes, transcripts, or proteins crucial for interactions in microbial communities.

Further experiments should also be complemented with metabolic measurements. I suggest including dissolved (in)organic carbon, NO_3^- consumption, NH_4^+ and N_2O productions, and anaerobic ammonium oxidation. I also propose measuring soil enzymes linked to C mineralization such as urease, formate dehydrogenase (NADP⁺-dependent), carbon-monoxide dehydrogenase, laccase, pyruvate synthase, and hexokinase. These data would corroborate or reject the differences found between ecosystem engineering treatments in **chapter 5**.

Concluding remarks

In figure 6.1, I summarize the main findings of my PhD thesis. From my data, I conclude that elevation, as a proxy of inundation frequency, is an important driver of soil properties, affecting both macro- and microbiota community composition. However, the impact of the environmental changes associated with elevation varied depending on the organism’s responses, affecting their microbiome composition. In this thesis, the two focal ecosystem engineers were the litter-feeding burrowing amphipod *O. gammarellus* and the native invasive grass *E. atherica*, both dominant and critical species in the food web of the late-successional stages of European salt marshes. On the one hand, the composition of

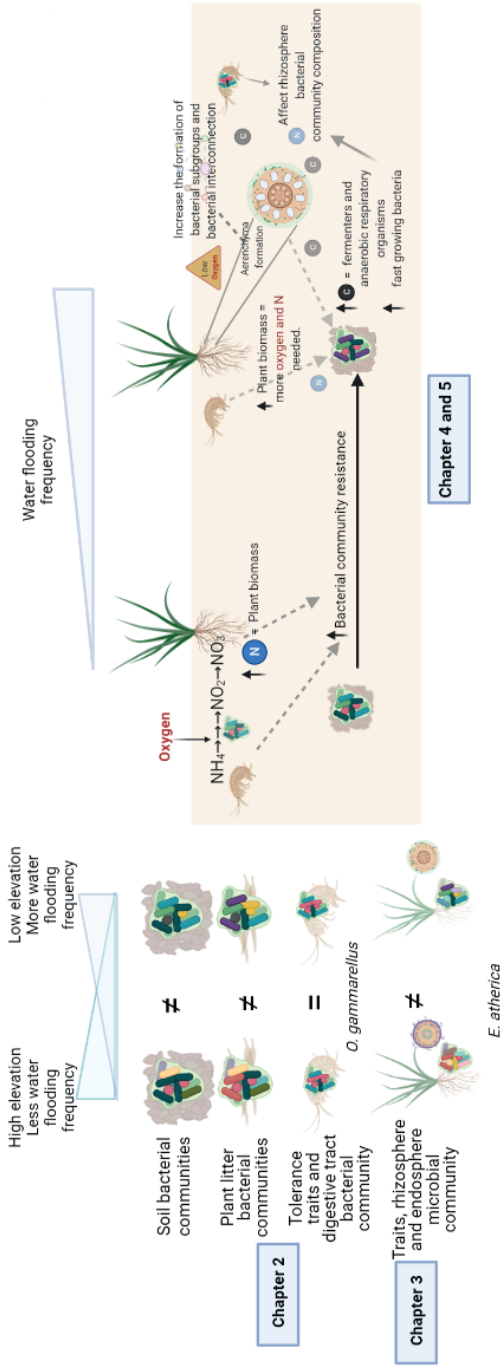


Figure 6.1. Schematic overview of the major findings presented in this thesis. Chapters 2 and 3 showed that salt marsh elevation, as a proxy of different flooding frequencies, affected the physicochemical soil characteristics and soil microbial composition. However, differences between the host-associated bacterial composition from ecosystem engineers, i.e., *O. gammarellus* and *E. atherica*, inhabiting high or low elevation depended on the host type, which was correlated to the impact of the local condition on the host. Further, Chapters 4 and 5 explored the interaction of both ecosystem engineers and their effect on the soil microbial composition in flooded soil. The presence of *O. gammarellus* stimulated the N-metabolism and increased plant biomass. The presence of ecosystem engineers increased the resistance to soil bacterial compositional turnover. Under flooding conditions, plants enhanced the growth of fermenters and some anaerobic respiratory organisms such as methanogens. The presence of *O. gammarellus* stimulated the bacterial assimilation of nitrate and nitrifiers, which may be competing with the plant for N and oxygen. This competence may result in plant stress under anoxic conditions. *O. gammarellus* modulated the rhizosphere composition by an increase in fast-growing bacteria (e.g., *Flavobacteriaceae*) and its associated bacteria, and indirectly by the increase of growth and aerenchyma formation. Image done using biorender.com.

bacterial communities from the digestive tract of *O. gammarellus* was not significantly affected by elevation, which may be related to the low impact of the local environment on host life history and tolerance traits. On the other hand, I observed a strong effect of elevation on *E. atherica* traits, rhizosphere, and root endosphere bacterial community composition. This *E. atherica*'s ability to form facultative associations with local soil communities may promote its invasion across the elevational gradient.

Furthermore, I analyzed the interaction of both ecosystem engineers and their effect on the soil microbial composition in flooded soil. I found that the presence of ecosystem engineers increased the soil bacterial community resistance to composition turnover due to soil anoxia. Moreover, the interaction of *O. gammarellus* with *E. atherica* affected soil bacterial composition differently compared to each ecosystem engineer separately. Specifically, I concluded that such effects were due to the stimulating effect of *O. gammarellus* on the soil bacterial communities associated with the N-cycle or the positive impact of *E. atherica* on the abundance of fermenters and the receptors of those fermentation products, including relevant communities such as methanogens. *O. gammarellus* in the soil affected plant growth and the rhizosphere community composition, which potentially increased the competition for soil N between plants and microbes under flooding conditions. Together, the findings of my thesis make an important step in unveiling several ecological consequences for the interaction between plants, burrowing macro-detrivores, and microbes under different flooding frequencies in salt marsh soils.

