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Diversity and functional potential of microbial communities in the hydrocarbon contaminated land-sea continuum

Elise Châtillon

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THESIS

UNIVERSITY OF PAU AND THE PAYS DE L'ADOUR (UPPA)
Doctoral School of Exact Sciences and Their Applications (ED211)

Defended the 30th September 2021

By Élise Châtillon

In order to obtain the Doctor of Philosophy qualification
Of the University of Pau and the Pays de l'Adour
Speciality: Physiology and Biology of Organisms – Populations – Interactions

**Diversity and functional potential of microbial communities
in the hydrocarbon contaminated land-sea continuum**

**Diversité et potentiel fonctionnel des communautés
microbiennes dans le continuum terre-mer contaminé par
les hydrocarbures**

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À Ginette & Gaston

À Juliette & Jean

À Marie-Bernadette & Bernard

Au petit microbe de Camille et Marc ;D

À toute la biodiversité, non détectée, pendant cette thèse !



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Horizontal

- 2 capitale du hygge
- 3 on fait des tisanes parfumées avec ses fleurs et nom de ma résidence
- 8 mon BB à moi
- 9 "cultivons notre ..."
- 11 la Nuit de l'Ours y a lieu pendant le carnaval biarnés
- 12 mon RDV de tous les samedis
- 13 bulles de toutes les occasions
- 14 science des écosystèmes

Vertical

- 1 concept utilisé dans ma thèse (chapitre III)
- 4 bulles de fête !
- 5 des petites choses qui nous rendent bien des services
- 6 cela m'a fait prendre quelques kilos ...
- 7 on les voit bien depuis mon balcon
- 10 belle de Tahiti
- 15 petit félin et homophone de mon surnom





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General introduction

The land-sea continuum is the coastal ecosystem at the interface between terrestrial and marine ecosystems providing high biodiversity and ecological services. However, the escalating human development is a global threat to coasts and affects the structure, the diversity and the functions of biological communities through the habitat conversion and pollution. In coastal ecosystems, in soil as in marine coastal sediment, microbial communities play a pivotal role in the biogeochemical processes and drive ecological services, such as pollutants removal.

The land-sea continuum is particularly subject to chronic and accidental inputs of petroleum hydrocarbons (HC) due to the high concentration of human activities in coastal areas (storage, transport, industries). HC are actually organic pollutants of concern in marine coastal environments because of their toxicity and their persistence. HC undergo different abiotic and biotic processes depending on their chemical properties and the physicochemical conditions in the environment (soil, water, sediment). These hydrophobic compounds aggregate and adsorb on mineral and/or organic particles and thus tend to accumulate in the environment, particularly in marine coastal sediment, which is a sink of organic matter coming from land and sea. Microbial community surveys demonstrated a high catabolic diversity of hydrocarbonoclastic microorganisms in soil and in marine sediment, suggesting that HC are fully biodegraded.

There is growing evidence that human land-based activities significantly disrupt microbial communities and alter microbial processes in marine ecosystems. Surprisingly, very little attention has been paid to the terrigenous inputs derived from soil runoff. Most of studies regarding terrigenous inputs in aquatic environments are limited to the fate of organic matter and have been carried out on rivers, deltas and estuaries. While the riverine network certainly drains a considerable amount of organic matter from the soil to the sea, the terrigenous inputs also reach directly the marine compartment, without dilution in an identifiable streamwater due to soil runoff. In the HC contaminated land-sea continuum, terrigenous inputs that reach coastal marine sediments consist of HC contaminated soil particles hosting soil microbial communities (including possibly hydrocarbonoclastic microorganisms). Thus, microbial communities from soil and marine coastal sediment compartments mix in tandem with their environments, in a process called community coalescence. To our knowledge, this coalescence event and its effect on the resistance/resilience of marine coastal sediment microbial communities facing HC contamination are yet unknown.

There is clearly a need to gain knowledge about the fate of microbial communities from soil runoff and their contribution to the biogeochemical cycles and the HC biodegradation in marine coastal sediment. This information is crucial to assess the impact of HC release from land-based human activities, whether inherent to the activity or accidental, on the downstream marine environments. The knowledge will be useful to develop microbial coalescent-engineered communities to mitigate the effect of HC release into the coastal environment.

The thesis proposes to consider the effect of the terrigenous inputs derived from soil runoff on the structure and the functional potential of the microbial communities in marine coastal sediments contaminated by hydrocarbons (HC). From a fundamental point of view, the expected results will help to characterise the rules regarding which microbial assemblages mix and what they do in marine coastal sediment subject to HC and influenced by terrigenous inputs. From an applied point of view, these results will provide data to link microbial diversity and HC biodegradation potential to apply efficient and suitable bioremediation processes in the land-sea continuum.

This manuscript includes six chapters, three of which (Chapter III, Chapter IV and Chapter V) are presented as publication papers. The research of the thesis was performed within the frame of the project TOTEM (Microbiology of the ecosystems contaminated by hydrocarbons in a land-sea continuum, Microbiologie des écosystèmes contaminés par les hydrocarbures sur un continuum Terre-Mer) funded by LEFE-EC2CO programme of CNRS. The PhD project was also supported by the Community of Communes of Lacq-Orthez (CCLO) and E2S/UPPA.

TOTEM project includes three complementary laboratories:

- IPREM, UPPA
- LIEC, Université de Lorraine
- COBRA, Université de Normandie

Thanks to the international doctoral mobility grant E2S/UPPA, a new collaboration has been initiated with the University of Copenhagen (Denmark) in order to assess plasmid transfer in the land-sea continuum. I was hosted for 6 months by the skilled Molecular Microbial Ecology (MME) group led by Pr. Johannes Søren Sørensen.

French version

Le continuum terre-mer est l'écosystème côtier à l'interface entre les écosystèmes terrestres et marins qui abrite une grande biodiversité et fournit des services écosystémiques. Toutefois, l'intensification du développement humain constitue une menace pour les côtes et affecte la structure, la diversité et les fonctions des communautés biologiques du fait de la conversion et la dégradation des habitats. Dans le sol et les sédiments marins côtiers, les communautés microbiennes jouent un rôle prépondérant dans les processus biogéochimiques et assurent des services écosystémiques comme l'élimination des polluants. Le continuum terre-mer est particulièrement soumis aux apports chroniques et accidentels d'hydrocarbures pétroliers (HC) en raison de la forte concentration des activités humaines sur le littoral (stockage, transport, industries). Les HC sont des polluants organiques largement répandus dans les environnements côtiers et sont préoccupants du fait de leur toxicité et de leur persistance. Après leur émission, les HC subissent différents processus abiotiques et biotiques en fonction de leurs propriétés chimiques et des conditions physico-chimiques du milieu naturel (sol, eau, sédiments). Ces composés hydrophobes s'agrègent et s'adsorbent sur les particules minérales et/ou organiques et ont donc tendance à s'accumuler dans l'environnement, en particulier dans les sédiments marins côtiers qui constituent un puits de matières organiques provenant de la terre et de la mer. L'analyse à large échelle des communautés microbiennes a montré une grande diversité catabolique de microorganismes hydrocarbonoclastes dans le sol et dans les sédiments marins, ce qui suggère que les HC peuvent être entièrement biodégradés.

Il a été montré que les activités humaines terrestres peuvent perturber considérablement les communautés microbiennes et modifier les processus microbiens dans les écosystèmes marins. Il est surprenant de constater que très peu d'attention a été accordée aux apports terrigènes issus du ruissellement des sols. La plupart des études concernant les apports terrigènes dans les milieux aquatiques se limitent au devenir de la matière organique et ont été menées sur les rivières, les deltas et les estuaires. Si le réseau fluvial draine une quantité considérable de matière organique du sol vers la mer, les apports terrigènes générés par le ruissellement du sol atteignent également le compartiment marin, plus directement et sans dilution dans un cours d'eau identifiable.

Dans le continuum terre-mer contaminé par les HC, les apports terrigènes qui atteignent les sédiments marins côtiers sont constitués de particules de sol (contaminées par les HC) et abritant des communautés microbiennes du sol (y compris éventuellement des microorganismes hydrocarbonoclastes). Ainsi, les communautés microbiennes des compartiments du sol et des sédiments marins côtiers se mélangent en tandem avec leur environnement, dans un processus appelé coalescence des communautés. À notre connaissance, cet événement de coalescence et son effet sur la résistance/résilience des communautés microbiennes des sédiments marins

côtiers face à la contamination aux HC restent à étudier. Il apparaît nécessaire d'acquérir des connaissances sur le devenir des communautés microbiennes issues du ruissellement des sols et sur leur contribution aux cycles biogéochimiques et à la biodégradation des HC dans les sédiments marins côtiers. Ces informations sont essentielles pour évaluer l'impact des rejets d'HC provenant d'activités humaines terrestres, qu'ils soient inhérents à l'activité ou accidentels, sur le milieu marin en aval. Ces connaissances seront utiles pour concevoir des communautés microbiennes coalescentes afin d'atténuer l'effet des rejets d'HC dans l'environnement côtier.

La thèse propose de considérer l'effet des apports terrigènes dérivés du ruissellement des sols sur la structure et le potentiel fonctionnel des communautés microbiennes dans les sédiments marins côtiers contaminés par des hydrocarbures (HC). D'un point de vue fondamental, les résultats attendus aideront à caractériser les règles concernant le mélange des communautés microbiennes et leurs fonctions dans les sédiments marins côtiers pollués par les HC et soumis à des apports terrigènes. D'un point de vue appliqué, ces résultats fourniront des données permettant de relier la diversité microbienne et le potentiel de biodégradation des HC afin d'appliquer des processus de bioremédiation efficaces et adaptés au continuum terre-mer.

Ce manuscrit comprend six chapitres, dont trois (Chapter III, Chapter IV and Chapter V) sont présentés sous forme d'articles scientifiques. Le travail de recherche de la thèse a été réalisé dans le cadre du projet de recherche TOTEM (Microbiologie des écosystèmes contaminés par les hydrocarbures sur un cOntinuum TerrE-mer), financé par le programme LEFE-EC2CO du CNRS. Le projet de thèse a également été soutenu par la Communauté de Communes de Lacq-Orthez (CCLO) et E2S/UPPA.

Le projet TOTEM implique trois laboratoires aux expertises complémentaires:

- l'IPREM, UPPA
- le LIEC à l'Université de Lorraine
- le COBRA à l'Université de Normandie

Grâce à la bourse de mobilité doctorale internationale E2S/UPPA, une nouvelle collaboration a été initiée avec l'Université de Copenhague (Danemark) afin d'étudier le transfert de plasmides dans le continuum terre-mer. J'ai été accueillie pendant 6 mois par le groupe Ecologie Microbienne Moléculaire (MME) dirigé par le Pr. Johannes Søren Sørensen.

Structure of the manuscript

This manuscript includes six chapters in which three are written (Chapter III, Chapter IV and Chapter V) as papers. Brief introductions present the background to the studies at the beginning of each chapter.

- ❖ The Chapter I presents the state of the art and the background of the study.
- ❖ The Chapter II describes in particular the specific and original experimental device that has been developed to mimic terrigenous inputs to marine coastal sediment by soil runoff in microcosms.
- ❖ The Chapter III describes and compares microbial communities of soil, runoff and marine sediment in the different microcosms.
- ❖ The Chapter IV describes the functional potential of the microbial communities in the HC contaminated land-sea continuum by using DNA-based microarray
- ❖ The Chapter V explores plasmid-mediated horizontal gene transfer; in particular, the permissiveness of marine sediment toward a known genetically modified plasmid. Experiments have been lead at the University of Copenhagen (Københavns Universitet, KU) thanks to the international doctoral mobility grant E2S/UPPA under the supervision of Pr. Johannes Søren Sørensen (MME laboratory).
- ❖ The Chapter VI concludes and proposes directions for further research.

Chapter I:

Background – State of the art

Chapter I. Background – State of the art

I.1. Soil runoff and the land-sea continuum in coastal marine systems

The land-sea continuum is the coastal ecosystem at the interface between terrestrial and marine ecosystems (Laruelle et al., 2013; Xenopoulos et al., 2017). The land-sea continuum includes diverse forms of linkage between land and sea: estuaries, delta, salt marshes, wetlands etc. Among these diverse ways of connection, the flow resulting from soil runoff is widespread encompassing the entire coastal perimeter. Its contribution to the coastal biodiversity and ecosystem functioning is overlooked (Gounand et al., 2018), appearing then secondary compared to the riverine and fluvial runoffs. However, under the anthropogenic climate change and with the ever-increasing urbanization of coast, runoff is expected to change quantitatively and qualitatively. Scientists and policy makers need robust knowledge about the effect of the human land-based activities via soil runoff to understand the ecology of the coasts and efficiently manage the exploitation of this valuable area.

I.1.1. Soil runoff and terrigenous inputs in marine coastal sediment

I.1.1.1. Definition of soil runoff

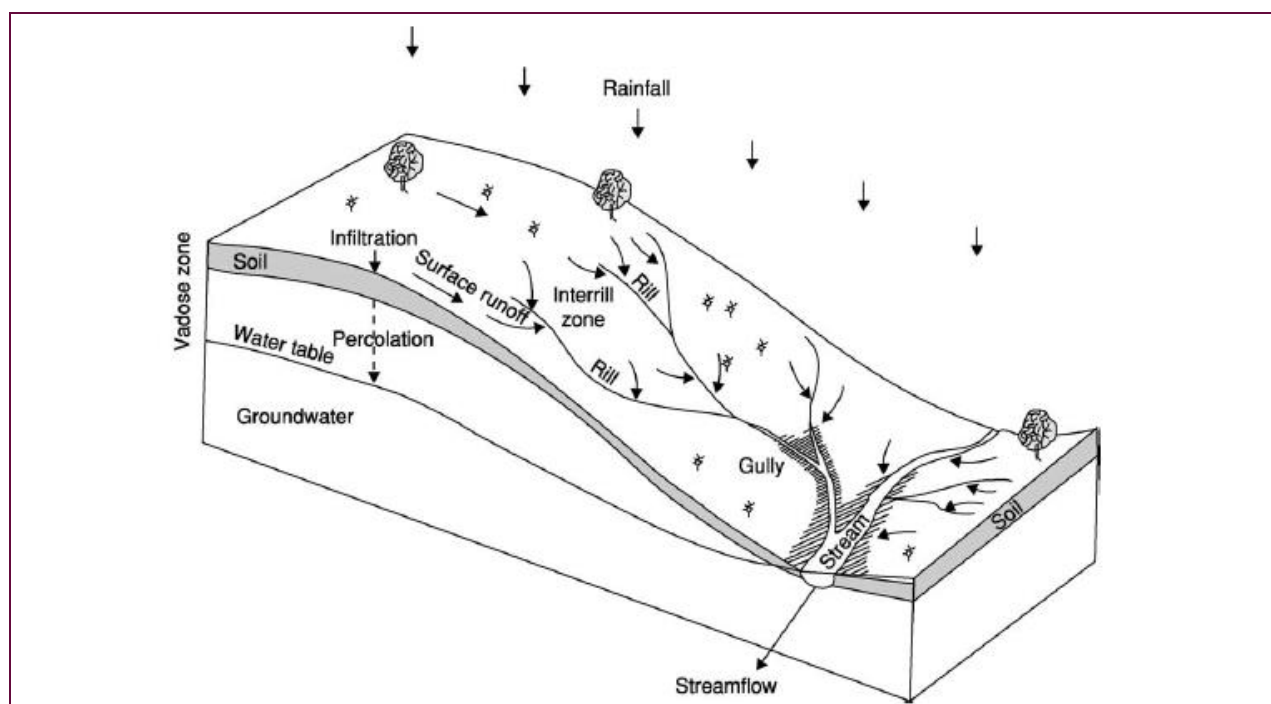


Figure I.1 A sloping area overland flow as well as runoff through rills and gullies (Hillel, 2005).

The term runoff refers to different transport mechanisms and different substances in soil by rainfall. Runoff occurs naturally under different forms: i) “the removal of substances from plants by the action of aqueous solutions, such as rain, dew, mist, and fog” (Tukey, 1970), ii) the solute leaching in soil, iii) during litter decomposition and iv) the non-soluble particles washout. In the present study, runoff is defined as a streamflow that involves surface runoff and groundwater flow that reaches ultimately streams. Runoff typically begins as sheet flow called overland flow and possibly creates channels (rills and then gullies) depending on the topographic relief by acceleration of the flow down the slope and erosion power (Figure 1.1).

Runoff depends on diverse parameters (Montgomery et al., 2000) such as the ground surface (slope and stream channels, type of cover material, presence, and type of vegetation); the soil type, its initial moisture and its permeability; the climatic region with the rain precipitation (intensity, duration and distribution) and the depth of the water table.

The thesis research particularly focuses on the surface runoff that encompass flows of solutes and non-soluble particles from soil due to the rainfall and that directly reach the sea on a stream shape but without dilution in river, directly from the land.

1.1.1.2. Effect of the human activities on runoff

Urbanization implies the removal of trees and vegetation and increases the impervious area by the construction of buildings and road. At the same time, cities require efficient drainage network based on high water flow velocities and reduced detention storage of water.

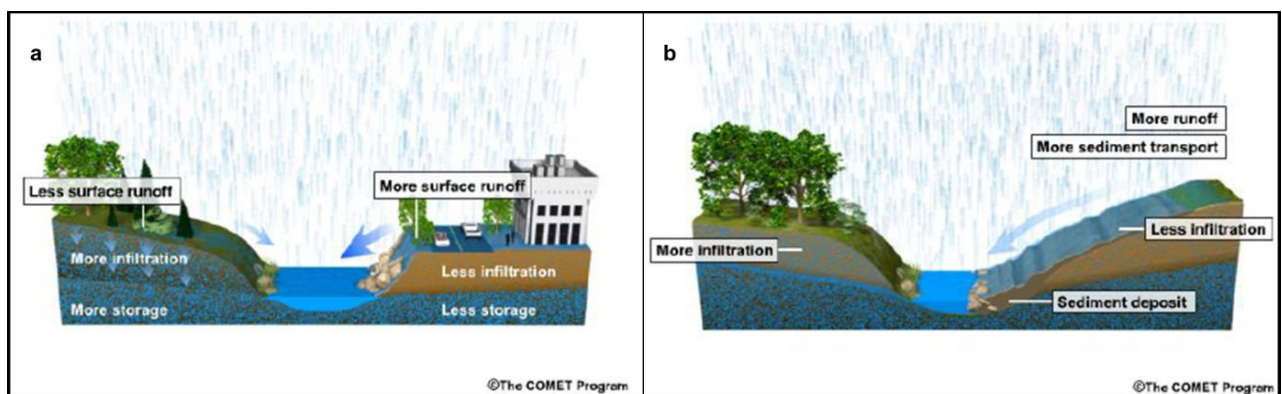


Figure 1.2 Influence of impervious surfaces (a) and deforestation (b) on runoff (COMET Program, 2010). a. Urban infrastructures (roofs, concrete, asphalt etc.) expand surface runoff and reduce water infiltration and water storage in soil. b. Deforestation enhances runoff and is responsible of sediment load in streams nearby.

In addition, many human activities need the importation of water. This way, urbanization changes water flows that results in the increase of the runoff frequency and runoff volume (Trudeau and Richardson, 2015; Walsh et al., 2005; Welsh et al., 2013). The Figure I.2 and the Figure I.3 illustrate these changes: urban runoff is more important and occurs faster in response to rainfall compared to rural runoff, due to the extent of impervious surface (for review see: Ladson, 2019; Leopold, 1968).

It is important to mention that runoff is expected to increase because of climate change in the north hemisphere according to GIEC (Stocker and Intergovernmental Panel on Climate Change, 2013). Lastly, urban heat island effect and urban pollution in the form of fine particles or liquid droplets are associated with the increase of the rainfall intensity (Li et al., 2020; Lin et al., 2011).

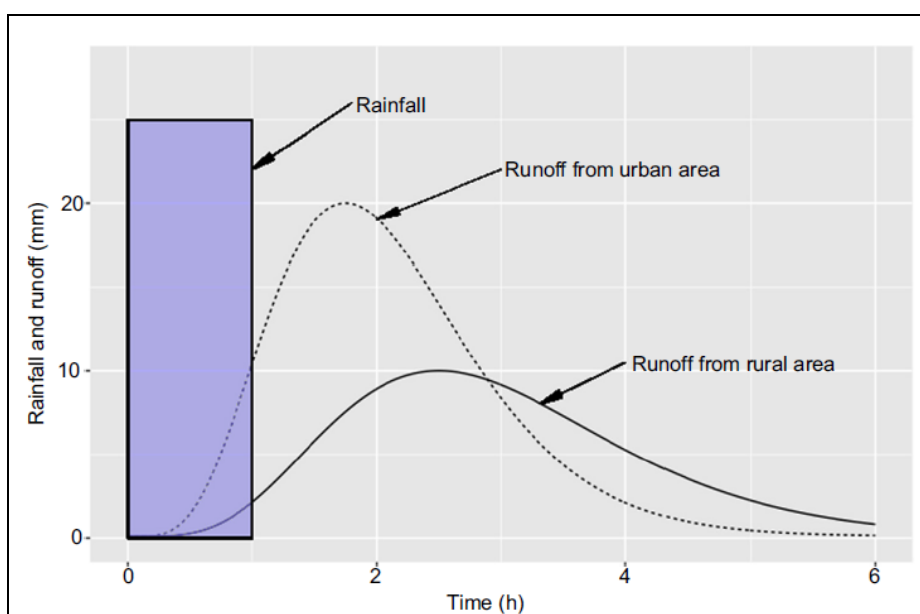


Figure I.3 Comparison of runoff from similar urban and rural catchments (Ladson, 2019).

I.1.2. Composition of the terrigenous inputs

Soil runoff carries what we called in this study terrigenous inputs that include mineral particles material (sand, silt, clay), plant debris, organic matter (Montgomery et al., 2000) that ultimately enter the nearest watercourses and coasts. Numerous studies highlight that human land-based activities affect the associated rivers and more downstream, estuaries and coastal areas. Indeed, clear-cutting of forests, excessive fertilizer application and agricultural practices convey nutrient such as N and P into rivers, seas and oceans and lead to the deterioration of water quality and eutrophication (Xu et al., 2019). In addition, industrial and farm effluents as well as urban and

storm water load runoff with pollutants of concern including heavy metals, pesticides, hydrocarbons (Goonetilleke et al., 2005; Hoffman et al., 1984; Saniewska et al., 2018). Runoff was also evidenced as a source of pathogenic microorganisms (Halliday et al., 2014; Yakirevich et al., 2013).

In land sea continuum, runoff linked thus marine coastal sediment by terrigenous fluxes of resources, pollutants and microorganisms. Globally, the process of community assembly across such habitat boundaries remains to be clarified (Gounand et al., 2018).

The complexity and the dynamic of coastal area are due to the fundamental differences between terrestrial and marine ecosystems. Moreover, the physical-chemical gradients and the flux from land to sea results on spatiotemporal heterogeneity of the abiotic conditions influencing in turn the organization of the inhabiting biota.

I.2. Heterogeneity of habitats within the land-sea continuum

I.2.1. At land side: soil as a succession of microhabitat driven by soil water

I.2.1.1. Soil definition and soil ecosystem services

European Commission distinguishes and defines land and soil as follows: 'Land is the solid surface of the Earth that is not permanently covered by water, while soil is the ecosystem in the uppermost layer of the ground in which plants can grow. Soil is composed of mineral particles, organic matter, water, air and living organisms' (European Commission, 2020). Soil is a non-renewable resource that provide biological services essential to life (FAO, 2020):

- Supporting services: soil formation, nutrient cycling, habitat, carbon sequestration;
- Regulating services: climate regulation, disease and pest regulation, water regulation, remediation, pollination;
- Provisioning services: food, freshwater, fuel, fibre, biochemicals, genetic resources;
- Cultural services: spiritual, recreational, symbolic values of landscapes.

The above essential services that contribute to human well-being are supported by soil functions in which microbial processes account for a lot.

I.2.1.2. Physical-chemical and biological properties of soil microhabitats influenced by soil water

From a mineralogical point of view, soil is characterized by its texture (Figure I.4) i.e. the size and the amount of inorganic particles (sand, silt and clay) and by its structure, i.e. the arrangement of soil particles into aggregate.

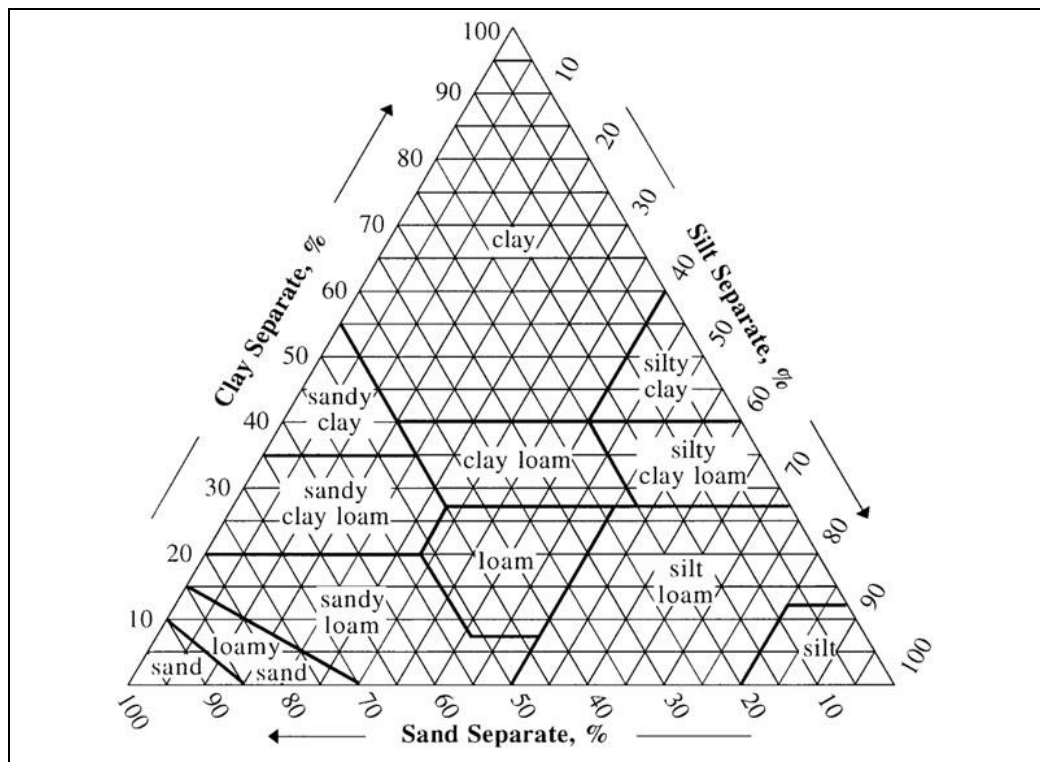


Figure 1.4 Soil texture triangle according to the United State Department of Agriculture nomenclature (U.S. Department of Agriculture, 1993)

A soil aggregate is a unit of soil that consist in solid particles stuck to each other and crossed by pores. The size of the aggregate distinguishes macroaggregates ($> 250 \mu\text{m}$) from microaggregates (large: $20\text{-}250 \mu\text{m}$, small $2\text{-}20 \mu\text{m}$, ultramicroaggregate $<1 \mu\text{m}$; Figure 1.5).

The word aggregatosphere names the total volume of soil aggregates while porosphere names the total volume of soil pores. Three phases coexist in the aggregates: solid, gaseous and liquid. In microaggregate, the solid phase prevails and hosts the majority of the microorganisms (80% to 90%, (Hattori and Küster, 1973), motile or not. The solid phase also adsorbs organic matter, proteins and nucleic acids and protects these macromolecules more or less from abiotic or biotic transformations (Nannipieri et al., 2017). Moreover, the word detritosphere, referring to the total volume of dead plant and dead soil biota, includes these compounds. In the gaseous phase, oxygen decreases from surface downwards to depth and from the outside to the centre of soil aggregates. The proportion of the liquid phase depends on soil moisture that varies according to the climatic events and rainfall. Yet, soil water drives many physical and chemical properties: the diffusion of nutrients, ions and heat; the oxygenation; the pH and redox potential. The water-influenced properties shape soil into a succession of discrete microhabitats (Stotzky, 1997), which provide conditions for the development of microorganisms encompassing various metabolisms (obligate aerobic, facultative aerobic and strictly anaerobic). Nevertheless, less than 5% of the

overall available space in soil (Ingham et al., 1985) is colonized by living microorganisms creating high microbial density areas named hotspots. Similarly, the hot-moment concept was proposed by Blagodatskaya and Kuzyakov (2013) because soil microbiome is dynamic in time. To summarize, soil is the sum of microhabitats of which the chemical and physical properties, and in turn the microbial mobility and activities, are influenced by soil water.

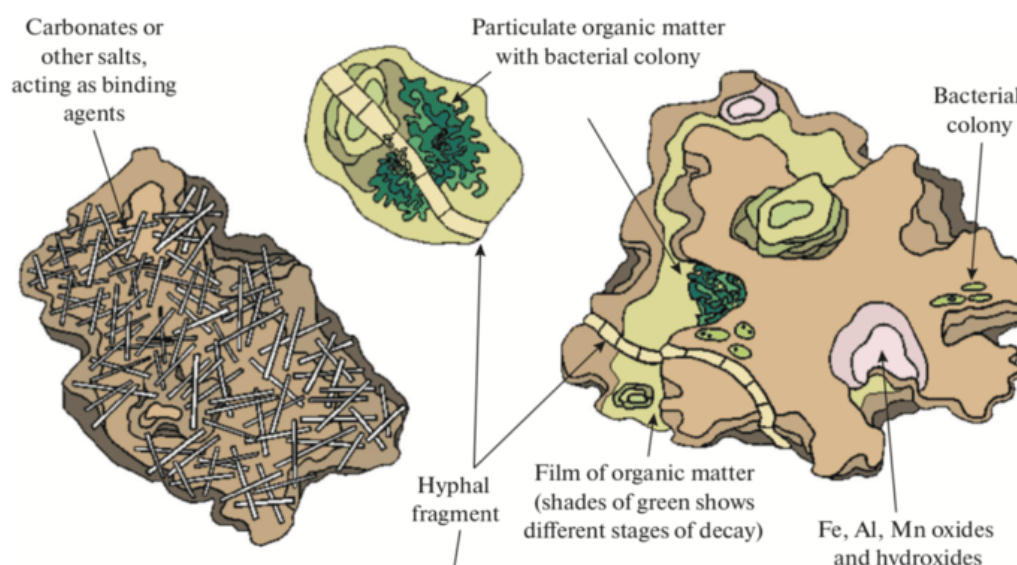


Figure I.5 The structural unit of soil: microaggregate and its inner structure from Yudina et al. (2018).

I.2.1.3. The rhizosphere and the drilosphere: hotspots of activities and biotic interactions

Beside aggregatusphere, the porosphere and the detritusphere, two others soil spheres stem from the activities of living organisms: the drilosphere, namely the soil volume influenced by secretions of earthworms (Brown et al., 2000; Lavelle et al., 1995) and the rhizosphere that is the soil volume influenced by secretions of plant roots (Hartmann et al., 2008; Hiltner, 1904). The soil spheres are dynamic in space and time and contain microbial hotspots of activities and biotic interactions (Figure I.6).

In rhizosphere, roots enhance soil aeration, hence they supply oxygen to heterotroph microorganisms and concurrently redistribute water and substrates in soil (Wenzel, 2009). Roots promote soil microbial growth and activity through exudates and lysates that contain organic compounds such as amino acids, sugars, enzymes, organic acids and carbohydrates (Van Hecke et al., 2005). Root grows preferentially in pre-existing soil pores including earthworm burrows (Passioura, 2002) what illustrates the widespread and close interactions between the soil spheres. Earthworms are considered ecosystem engineers (Jones et al., 1994). Indeed, they create

supportive habitats for other soil organisms through their different activities namely organic matter burial, burrow creation and cast deposition and enhance soil functions (Liu et al., 2019). In the drilosphere, they take part in soil structure stability and nutrient cycling directly and indirectly through their influence on the structure and the activity of soil microbial communities and on the plant growth (Medina-Sauza et al., 2019). It is worth noting that others macro-animals such as enchytraeids and macroarthropods and micro-animals such as microarthropods, mites and collembolan, themselves are part of the drilosphere and contribute to soil particles reworking.

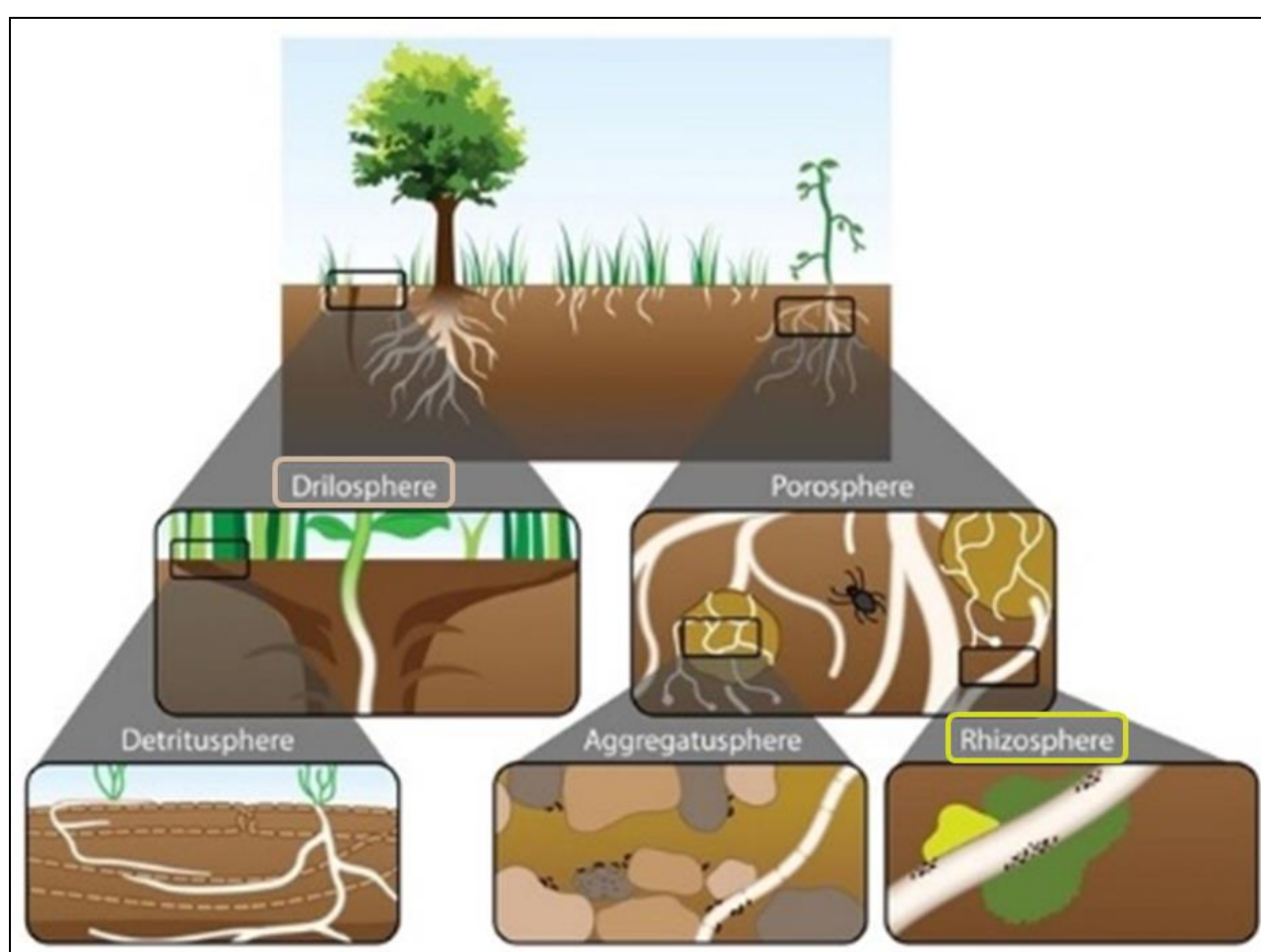


Figure 1.6 Different soil spheres containing hotspots of activities and biological interactions : Drilosphere (light brown surrounded), soil volume influenced by secretions of earthworms; porosphere, total soil pore volume; detritosphere, total volume of dead plant and dead soil biota; agregatusphere, total volume of soil aggregates; rhizosphere (light green surrounded), soil volume influenced by secretions of plant roots (Nature Education, 2012).

I.2.2. At seaside: marine coastal sediment under oxic/anoxic oscillation and constant reworking

I.2.2.1. Marine coastal sediment definition and ecosystem services

Sediment consists of aggregates made up of mineral particles that originate from rock weathering and organic matter that bonds to the mineral surfaces (Fenchel et al., 2012a). The aggregates vary in composition depending on the organic source and the sediment depth. Biogenic particles that derive from calcareous skeletons (foraminifera, coccolithophores) or siliceous tests (radiolarian, diatoms) generally characterize marine sediments. The size of the particles depends on water currents and turbulence. Marine coastal sediment is at the transition of ecosystems where important transformations (carbon, nitrogen, and sulphur cycling) and exchange processes (water column processes, pollutant distribution and fate and transport of matter) take place (Snelgrove, 1997). The active and diverse microbial communities inhabiting marine coastal sediments play a pivotal role in the biogeochemical processes that sustain ecosystem services (Grizzetti et al., 2019), such as:

- Provisioning services: food (fisheries and aquaculture);
- Regulating and maintenance services: water purification;
- Cultural services: recreation.

Defining and quantifying the services provided by ecosystem such as soil and sediment at the land-sea continuum will provide data to justify the cost expended for their protection and restoration. The gained information will also push for more ecological and sustainable management policies (Pouso et al., 2018).

I.2.2.2. Oxic/anoxic oscillation: tidal cycle

Marine coastal sediment from intertidal zone is characterized by environmental conditions that vary constantly due to tidal cycle resulting in oxygen fluctuations. The oxic/anoxic oscillation creates vertical stratification of the redox conditions that influence the repartition of microbial communities depending on their physiological requirements (Fenchel et al., 2012a). As a result of constant variation of the conditions, microbial communities in intertidal sediment host a higher diversity and show a unique pattern compared to freshwater and other marine sediments related to high nutrient concentration (Kim et al., 2008; Wang et al., 2012). In particular, the surface sediment layer has the highest microbial activity (Petro et al., 2019).

I.2.2.3. Bioturbation: reworking of sediments by benthic fauna

The reworking of sediment occurs constantly and alters the vertical stratification pattern of the microbial metabolisms. Waves and flows contribute to disrupt the sedimentary column, though,

bioturbation, the feeding and burrowing activities of benthic fauna (polychaetes, crustaceans, bivalve molluscs) also mix sediment (Cravo-Laureau and Duran, 2014; Duran et al., 2015; Pischedda et al., 2008). The moves of the benthic fauna and the construction through galleries modify the water flux, redistribute the electron acceptors and introduce oxygen and organic matter from the surface at greater depth (Figure 1.7). Like earthworms in soil, benthic fauna in marine sediment is described as ecosystem engineers.

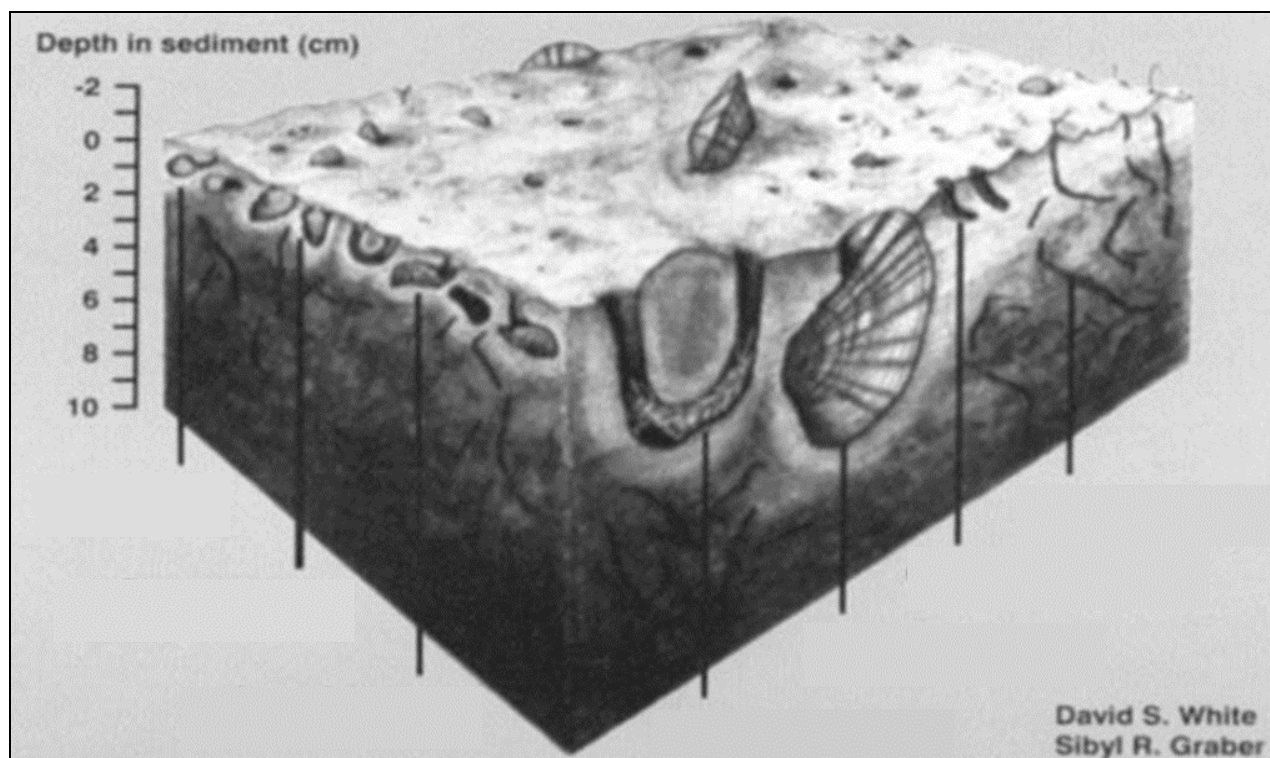


Figure 1.7 Bioturbation of subsurface sediments by benthic organisms (e.g., mollusks, arthropods, and annelids). The structures and activities of those organisms ventilate and extend the depth of oxic conditions in adjacent sediments, illustrated by lighter area. The oxygen gradient is between 1 and 2 cm around the burrow (White and Miller, 2010).

1.2.3. Comparative study of biological and physical-chemical properties between soil and marine coastal sediments

Nealson (1997) noticed that sediment is continuously wet whereas soil undergoes unpredictable drying and rain events. He argued that conditions soil microorganisms have to cope with, such as changes in the level of hydration, temperature and the availability of nutrient (Figure 1.8), cause that soil shows more diversity compared with sediment. Still in connection with water content change, fungal activity is usually considered more important in soil than in sediment (Tate III, 2020) because fungi can grow and be active not only in aqueous medium. Other differences include that the residence time of water is shorter in sediment than in soil and it could affect the biotic and abiotic transformations of compounds (Marín-Spiotta et al., 2014). Salinity is another abiotic

factor that greatly varies between soil and marine coastal sediment and represents one of the main driver of the distribution of microorganisms both in soil and sediment (Dupont et al., 2014; Herlemann et al., 2011; Jeanbille et al., 2016; Thompson et al., 2017). Marine coastal sediment have clearly higher levels of salinity in comparison with most of soils (apart from natural hypersaline soil or overfertilized soil).

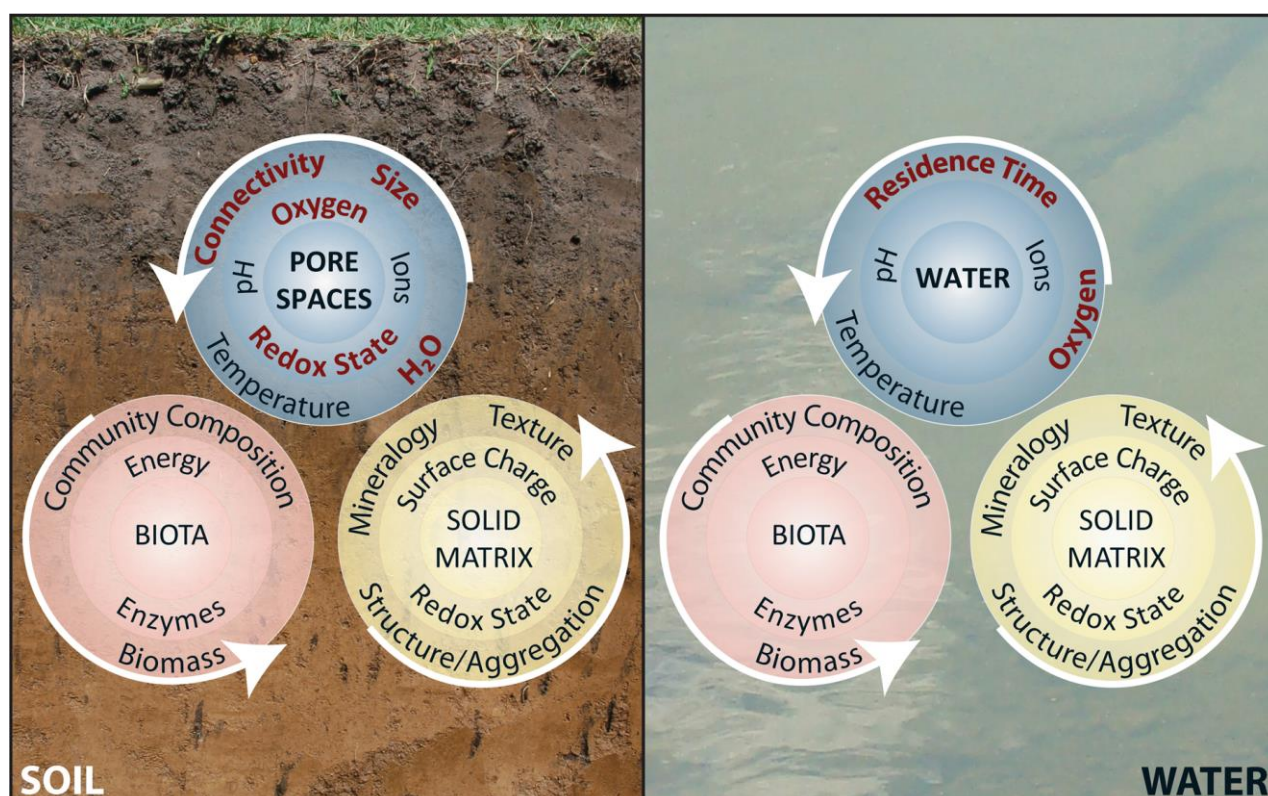


Figure 1.8 Interactions between biota, solid matrix and pore spaces or water (arrows around circles) determining organic matter turnover rates. The main differences between soil and aquatic system are indicated in red (Marín-Spiotta et al., 2014).

Although texture and structure can be determined for soil and sediment, the stability of the structure is not the same. Furthermore, in soil, plant community composition play a major role in the composition and structure of soil microbial communities (Kang and Mills, 2004; Templer et al., 2003) and vice versa (Lau and Lennon, 2011). In both soil and sediment, a stratification of the redox conditions is observed, resulting thus in specific niches for microbial metabolisms, irrespective of the physical disturbance events. The matrices contain also a variety of minerals that could enter in interaction with microorganisms, organic matter and pollutants (Nannipieri et al., 2017).

To summarize, as soil, sediment is characterized by a spatiotemporal heterogeneity of the environmental parameters. This heterogeneity affects the distribution and the function of the microorganisms that, in turn, affect the heterogeneity.

I.3. Carbon cycle and hydrocarbon contamination in the land-sea continuum

I.3.1. Marine coastal sediment: Carbon sink between land and sea

The land-sea continuum is in most cases the focus of an intensive human activity. Approximately one third of the world's population lives in the coasts (Board, 2005) making the area strongly urbanised. Industry, agriculture and fishery are activities of first importance that support the economy of coasts. All those land- or sea-based activities enhance the degradation of the habitat, release pollutants and cause intensive exploitation of natural resources threatening the biodiversity and altering the marine coastal ecosystem services.

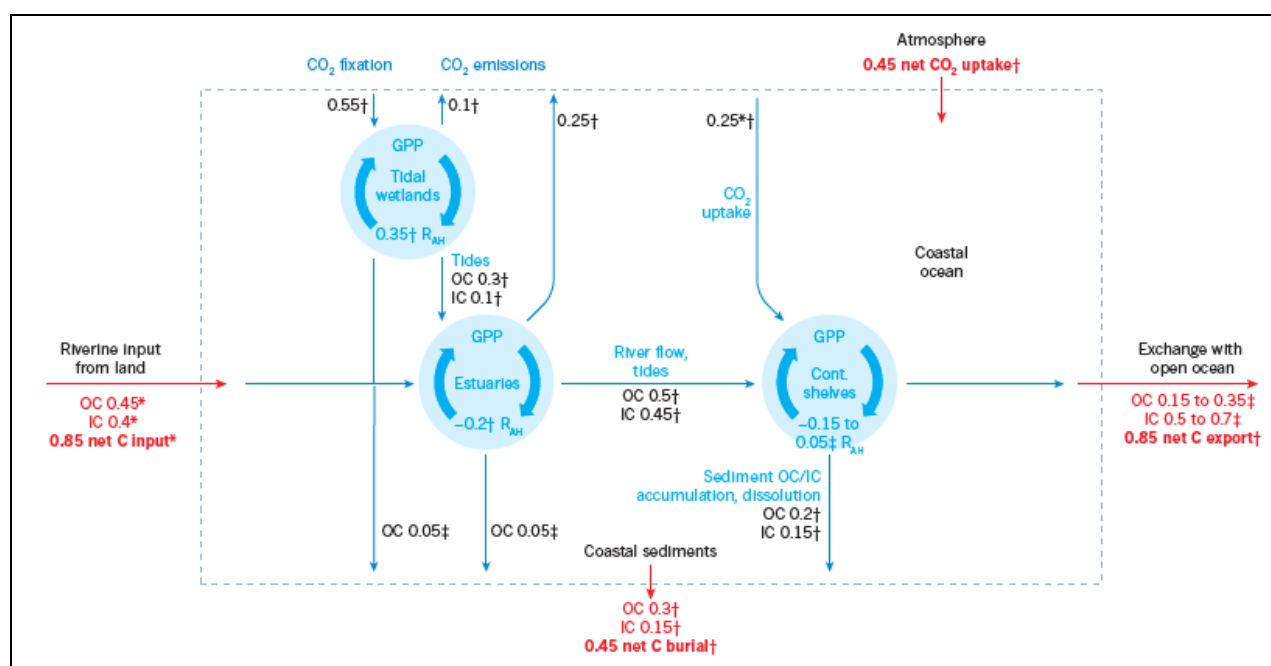


Figure I.9 Organic and inorganic carbon fluxes in the estuarine, tidal wetland and continental shelf subsystems of the coastal ocean (Bauer et al., 2013).

In the land-sea continuum, the coupled biogeochemical cycles of nutrient (carbon, nitrogen, phosphorus and sulphur) are affected by human activities: some fluxes of these elements result from perturbation such as fossil fuel combustion, application of fertilizers, use of sewage and wastewater system and other ones already exist and are affected by the human activities and

the land use activities. Mackenzie et DeCarlo (2019) studied these perturbations and modelled these fluxes through their TOTEM II model. Bauer et al. (2013) claimed that coastal ocean is becoming a net sink for atmospheric carbon dioxide during post-industrial times (Figure I.9). Other studies indicate that coastal marine sediment is a burial site for organic and inorganic matter, and contaminants (Dachs et al., 2002; Tolosa et al., 2004; Zhang et al., 2013).

I.3.2. Hydrocarbon properties and flux from land to sea

Hydrocarbons (HC) encompass organic compounds composed of C and H (Moss et al., 1995), functionalized or not by oxygen, nitrogen, sulfur, halogens or phosphorus. A first class includes aliphatic hydrocarbons, such as hexadecane whereas the second one includes aromatic hydrocarbons, with a single benzene ring or several of those, named for that reason, polycyclic aromatic hydrocarbons (PAH). The number of rings distinguishes low molecular weight PAH (three rings or less), such as phenanthrene from high molecular weight PAH (more than 3) such as benzopyrene. HC are both natural and anthropogenic compounds. Natural sources are biogenic (secondary metabolism of plant), petrogenic (diagenesis, natural seepage Figure I.10 d) and pyrogenic (volcanism and forest fires). Anthropogenic sources are pyrogenic (combustion of the fossil fuel Figure I.10 b) and petrogenic (exploitation of the petroleum).

HC are ubiquitous in marine environments, in column water as well as in sediment, especially in coastal ecosystems (Perra et al., 2009). HC inputs in coastal area (Figure I.10 a) are mainly anthropic and pyrogenic and originate from terrestrial runoff, fluvial runoff and atmospheric deposition (Duran and Cravo-Laureau, 2016) and lead to chronic pollution of the ecosystem. Release of petrogenic HC is not occasional due to accidents during petroleum transport or exploitation causing well-mediatized oil spills and subsequent wide acute pollution (non-exhaustive list of such events: Amoco Cadiz in 1978 , Exxon Valdez in 1989 , Erika in 1999, Prestige in 2002, Deepwater in 2010 , Figure I.10Figure I.10 c Wakashio in 2020).

Indeed, HC are hazardous organic compounds due to their hydrophobicity and their relative low solubility, volatility and reactivity. They have non-specific toxic effects on living organisms because they accumulate in and disrupt biological membranes (Heipieper and Martínez, 2010). Other functionalized hydrocarbons, as oxy-PAH show additional specific toxicity and are more mobile and persistent in the environment (Lundstedt et al., 2007). Moreover, the toxicity (mutagenic and/or carcinogenic and/or teratogenic) and the persistence of these molecules pushed the European Chemicals Agency (ECHA) (decision ED/88/2018) and the United States Environmental Protection Agency (USEPA) (Keith, 2015) to qualify 16 PAH as priority pollutants.

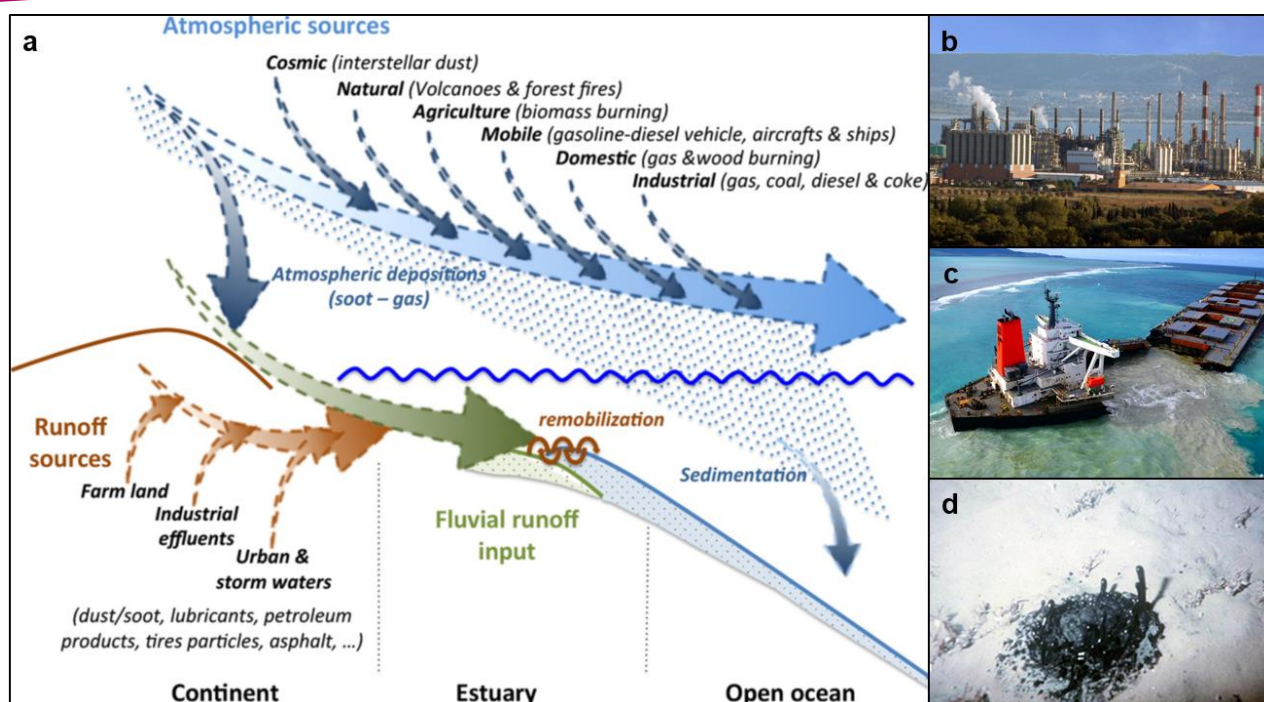


Figure 1.10 a Main sources and fate of HC in the land-sea continuum (Duran and Cravo-Laureau, 2016). b Berre Petrochemical Cluster (Berre l'Etang, France) ; c Wreck of the MV Wakashio pictured on August 17, 2020 (Offshore of Pointe d'Esny, Mauritius); d Seep releasing oil on the seafloor near Coal Oil Point in southern California (Credit: Alan A. Allen).

I.3.3. Fate of hydrocarbons in the coastal environment

Pyrogenic HC emitted as gases or particles pass through the atmosphere and enter the water by deposition (Tsapakis et al., 2006) or direct gas exchange. Then, through the water column, they aggregate and sorb to inorganic or organic materials (including phytoplankton and marine snow) via absorption, adsorption and coagulation and settle at the bottom in sediment (Tolosa et al., 2004). Regarding HC derived from oil seeps and spills, they are partly evaporated in the atmosphere and partly spread in seawater after emulsification or dissolution. Similarly to petrogenic HC, they sink to sediment, sorbed to particles. At the interface between atmosphere and surface water, HC are subjected to abiotic transformation and degradation as photo-oxidation (Lundstedt et al., 2007) whereas in the water column and in sediment they undergo microbial degradation (McGenity, 2014; Timmis, 2010). The biological pump (biological processes of carbon compounds from the surface to the bottom) control HC flux by catching them and transporting them to the sediment (Galbán-Malagón et al., 2012; Jiao et al., 2011; Turner, 2015). HC biodegradation by microbial communities was demonstrated as the main mechanism of HC removal in marine coastal sediment (McGenity et al., 2012).

I.4. Microbial diversity and HC biodegradation in marine coastal sediment

I.4.1. Microbial diversity within the land-sea continuum

In the land-sea continuum (Figure I.11 a), microbial communities in soil and marine coastal sediment show important diversity, which is of high significance at the functional point of view since it underpins the health of this complex ecosystem.

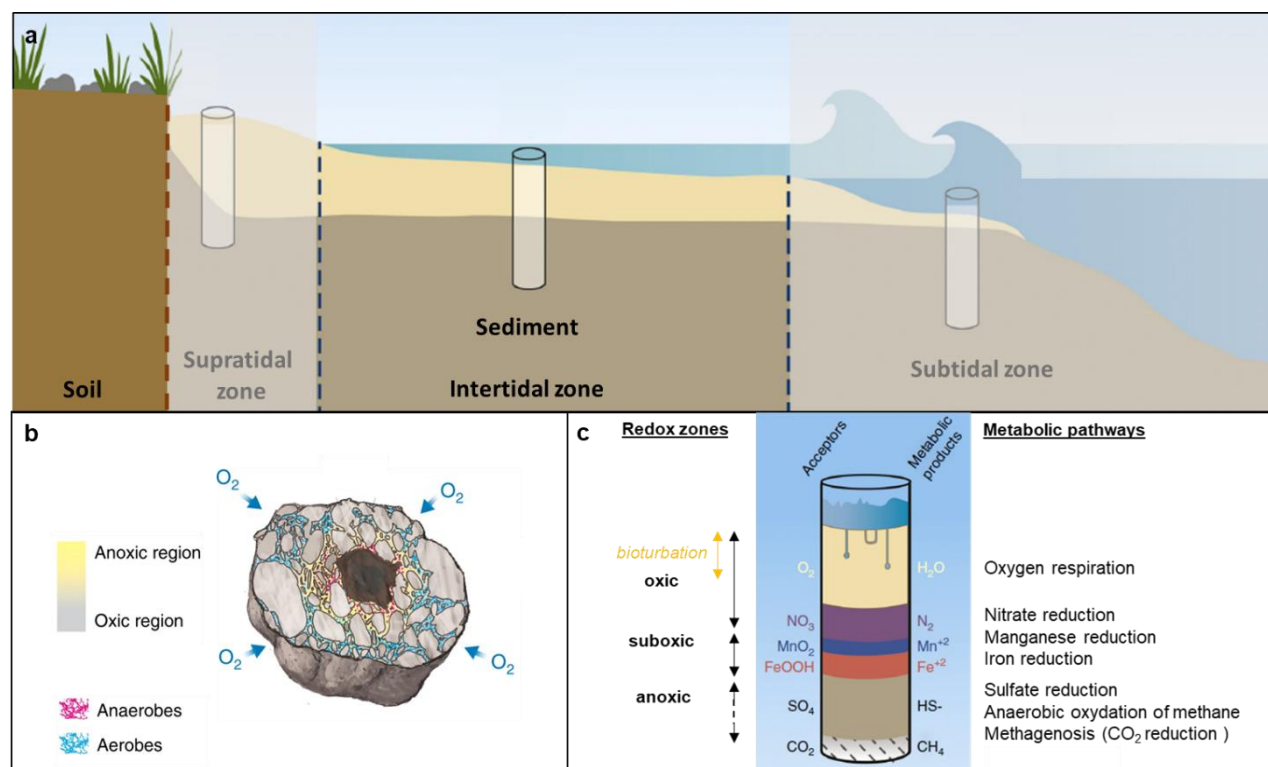


Figure I.11 Redox conditions along the land-sea continuum. a The land-sea continuum and the zonation of the marine coastal sediment produced by tides (Acosta-González and Marqués, 2016); b Spatial segregation of aerobes (blue) and anaerobes (red) in a soil aggregate due to redox conditions. Arrows show the oxygen diffusion from the surface of the aggregate toward the centre containing a carbon source (Borer et al., 2018); c Redox processes present in marine coastal intertidal sediment. The depth profiles show the stratification of different redox zones (oxic zone, suboxic transition zone and anoxic zone) and dominant microbial metabolic pathways depending on the availability of terminal electron acceptors (Acosta-González and Marqués, 2016). The depth scale is arbitrary: oxic zone ranges from few millimetres to several centimetres of sediment, suboxic zone is around 1 decimetre and anoxic zone at depth is meters thick.

In soil as in marine coastal intertidal sediment, microbial functional groups are distributed according to the depth stratification of the electron acceptor gradients (oxygen, nitrate, manganese, iron, sulfate and methane) and the availability of nutrients (Figure I.11 b and c) (Acosta-González and Marqués, 2016; Sørensen et al., 1979; Standing and Killham, 2007). In soil, there is also a spatial organisation of the microorganisms at the scale of the aggregates according to the oxygen gradient (Figure I.11 b) (Borer et al., 2018). Ultimately, the mineralization of the organic matter (complex polymers) releases methane and carbon dioxide with the production of intermediate substrates usable sequentially by microbial functional groups through

soil and sediment trophic networks. The different metabolic groups are the aerobic heterotrophs, the sulfate-reducers, the nitrifiers, the fermenters, the methanogens and the methanotrophs.

1.4.2. HC degrading microorganisms

Although HC undergo adsorption, photolysis and abiotic degradation, biodegradation by microorganisms is the main removal process in the environment. Members of various microbial groups from the different domains of life (archaea, bacteria, fungi and algae) are able to degrade HC (Head et al., 2006; Varjani, 2017; Xu et al., 2018). Some use these compounds as unique carbon source and are called obligate hydrocarbonoclastic microorganisms while HC constitute a facultative carbon source for others. Even high molecular weight PAH generally regarded as recalcitrant can be used by microorganisms for their growth (Álvarez-Barragán et al., 2021; Ghosal et al., 2016). Previous studies identified bacterial genera capable of degrading petroleum hydrocarbons (Prince et al., 2010; Varjani, 2017; Xu et al., 2018).

Proteobacteria, especially Betaproteobacteria and Gammaproteobacteria subdivisions, is usually the dominant phylum in HC contaminated soil, followed by Bacteroidetes and Actinobacteria while Acidobacteria are poorly represented but often detected in PAH contaminated soil (Bourceret et al., 2016; Gołębiewski et al., 2014). Members of the genera *Sphingomonas* (Alphaproteobacteria), *Alcaligenes* (Betaproteobacteria), *Stenotrophomonas* (Gammaproteobacteria), *Pseudomonas* (Gammaproteobacteria), *Flavobacterium* (Bacteroidetes), *Nocardia* (Actinobacteria) and *Rhodococcus* (Actinobacteria) are usually found in HC contaminated soil (Varjani, 2017).

Gammaproteobacteria and Alphaproteobacteria predominate in microbial communities of intertidal sediment exposed to hydrocarbons (Lamendella et al., 2014). Aerobe members from obligate marine hydrocarbonoclastic bacteria (Yakimov et al., 2007) such as *Alcanivorax*, and *Marinobacter* (Duran, 2010) have been shown to dominate the active community of Gammaproteobacteria (Kostka et al., 2011). Among Alphaproteobacteria, *Rhodobacteraceae* has been reported after oil spill (Acosta-González et al., 2015). Archaea (Halophiles) from the genera *Haloferax*, *Halobacterium*, and *Halococcus* (Euryarchaeota) with HC degradation capacities have been described in aerobic and hypersaline habitat (Tapilatu et al., 2010). In anoxic marine sediment, where sulfate is generally not limiting, sulphate reduction is the main anaerobic metabolism (Kleikemper et al., 2002). After HC contamination, enrichment of sulphate-reducing Deltaproteobacteria has been observed with the dominance of *Desulfobacteraceae* in subtidal sediment (Acosta-González et al., 2015; Joye et al., 2014).

In terrestrial as in marine environments, fungi play a pivotal role in the degradation of PAH because they initiate their break down in presence of oxygen, mostly by cometabolism with other carbon sources (Cerniglia et al., 2010). In addition, fungi using PAHs as sole carbon and energy

sources has been described by Rafin et al. (2000). The phyla Ascomycota and Basidiomycota predominate in soil including HC contaminated soil (Ramdass and Rampersad, 2021). Fungi identified from marine environments belong mainly to the phyla Ascomycota and Chytridiomycota and to a lesser extent to the phyla Basidiomycota, Aphelidiomycota, Blastocladiomycota and Mucoromycota (Amend et al., 2019). Among early diverging lineages, such as Chytridiomycota that are dominant in marine sediment, new species are numerous and known only by environmental sequencing survey and could contain HC degraders. Members of the genera *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*, *Mucor* and yeast of genera *Candida*, *Pichia* and *Yarrowia* are commonly reported as HC-utilizing fungi in contaminated environments (Álvarez-Barragán et al., 2021; Daccò et al., 2020).

To sum up, microbial HC degraders are ecologically diverse and phylogenetically widespread. They have generally preference for specific chemical structure (Tapilatu et al., 2010; Whyte et al., 1997) and form a functional network involving cooperative catabolism and cometabolism of HC (Figure I.12 a) (Zhou et al., 2019). After oil spills in marine environments, researchers evidenced a phenomenon of microbial succession (Figure I.12 b) linked to a sequential use of the available substrates (Head et al., 2006; Kimes et al., 2014) (Figure I.12 c). Specialization and succession of bacterial communities were also reported during the bioremediation of HC contaminated soils (Zhen et al., 2021).

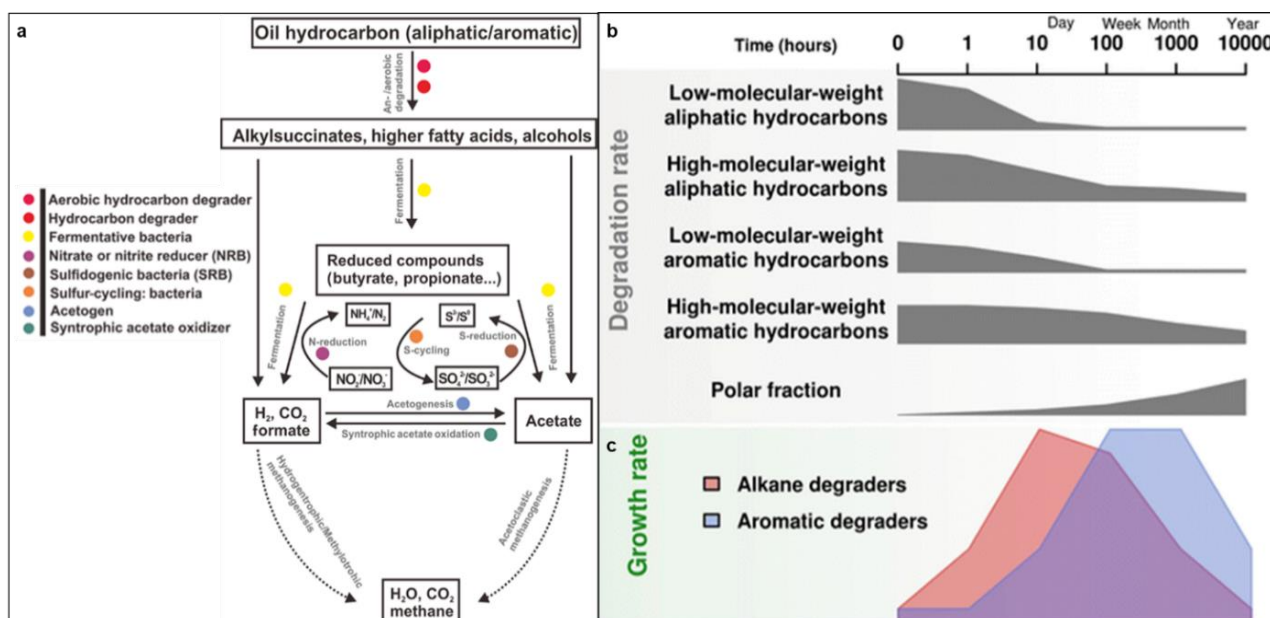


Figure I.12 Dynamic of the hydrocarbon degradation. a HC degradation pathways (Zhou et al., 2019). b The degradation rate of the different type of hydrocarbons shows the aliphatic hydrocarbons as the fastest group being degraded. c The growth rate indicates that the alkane degraders grow up faster than those microorganisms specialized in the degradation of more recalcitrant compounds (Bargiela et al., 2017)

I.4.3. HC degradation pathways and functional genes involved in HC degradation

The processes involved in the HC degradation occur under aerobic and anaerobic conditions (Kimes et al., 2014). Depending on the microbial communities, the type of HC and the presence of dioxygen, various pathways of biodegradation based on selective enzymes can be active (Figure I.13).

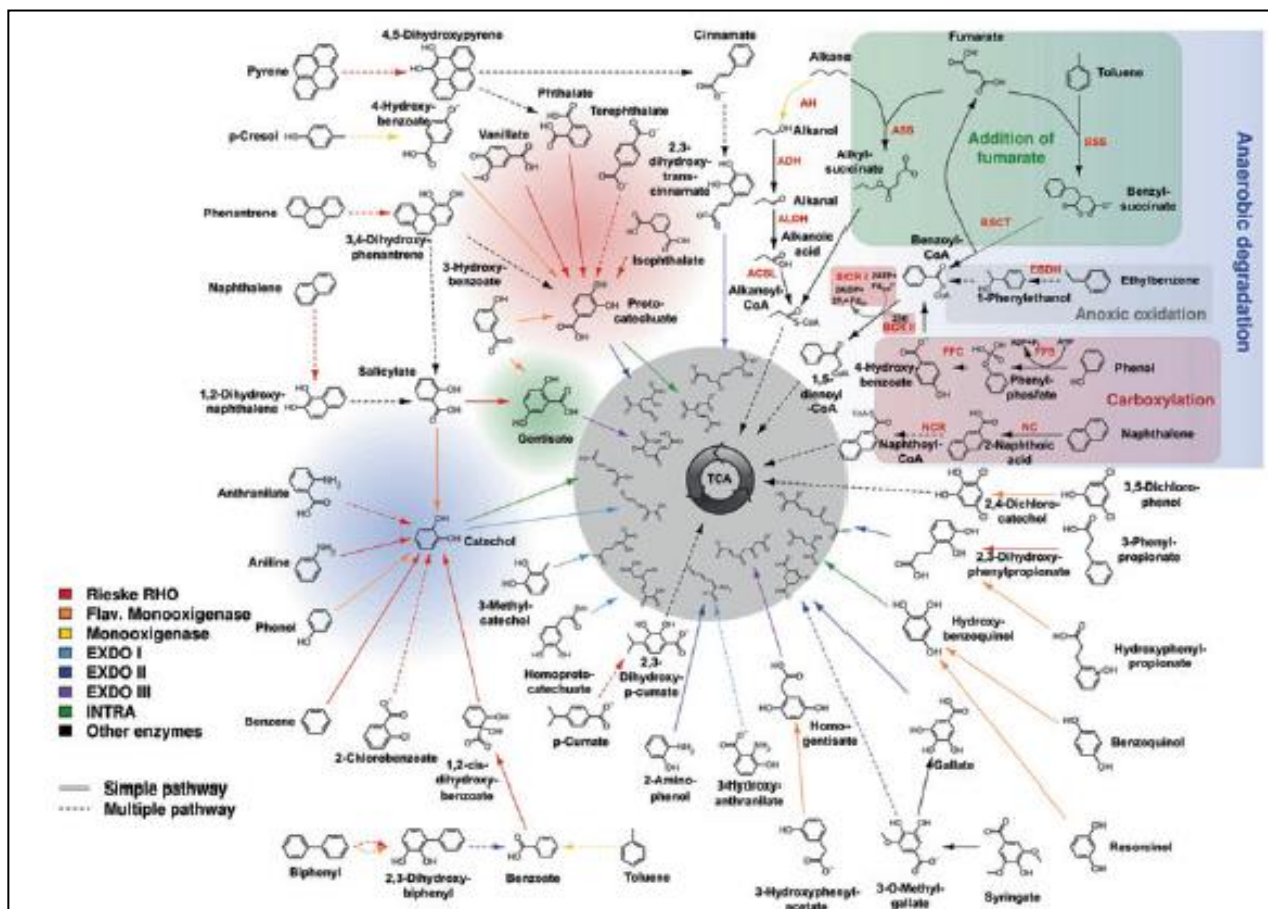


Figure I.13 Representative microbial aerobic and anaerobic degradation pathways (Bargiela et al., 2017). The main anaerobic strategies are summarized on the top right. Reactions ending in the transformation of benzoyl-CoA to 1,5-dienoyl-CoA supported by the benzoyl-CoA reductase (BCR) class I or II with or without ATP consumption respectively, are shown. Specific labels for enzymes involved in anaerobic degradation pathways are indicated as follows: BSS benzylsuccinate synthase, BSCT succinyl-CoA: benzylsuccinate CoA-transferase, BCR I benzoyl-CoA reductase class I, BCR II benzoyl-CoA reductase class II, ASS alkyl-succinate synthase, EBDH 1-phenylethanol ethylbenzene dehydrogenase, NC naphthoate carboxylase, NCR naphthoyl-CoA reductase, FSS phenylphosphate synthase, and FFC phenylphosphate carboxylase. Within aerobic steps, the main reactions feeding three of the main intermediates in aerobic degradation, catechol, gentisate, and protocatechuic acid are highlighted in blue, green, and red color gradients, respectively. The different groups of oxygenases involved in aerobic degradation are shown by different colors in the arrows. Only enzymes for the aerobic alkane degradation pathway are indicated: AH alkane hydroxylase, ADH alcohol dehydrogenase, ALDH aldehyde dehydrogenase, and ACSL acyl-CoA synthase.

I.4.3.1. Aerobic HC degradation

The first step of HC degradation is the activation of the compounds by hydroxylation (alkane as PAH) under aerobic condition before the newly formed compounds embed in central metabolic pathways (Brzeszcz and Kaszycki, 2018; Pérez-Pantoja et al., 2010). In details (Figure I.14), an alcohol is formed from the *n*-alkane by oxydation, which is then oxidized in aldehyde and finally in fatty acid. From fatty acid, acetyl-coA is generated and enters the tricarboxylic acid (TCA) cycle (Figure I.14). Enzymes from different families ensure the oxidation (different monooxygenases, cytochrome P450) and further oxidations require alcohol dehydrogenase (ADH). In the same manner (Figure I.14), benzene ring of PAH is oxidised as activation step by a monooxygenases or a dioxygenases (ring-hydroxylating dioxygenases, intradiol or extradiol dioxygenases, depending on the cleavage position). After some hydroxylation and cleavages process, protocatechuates, gentisate and catechols (central intermediates) are formed and further converted to TCA cycle intermediates.

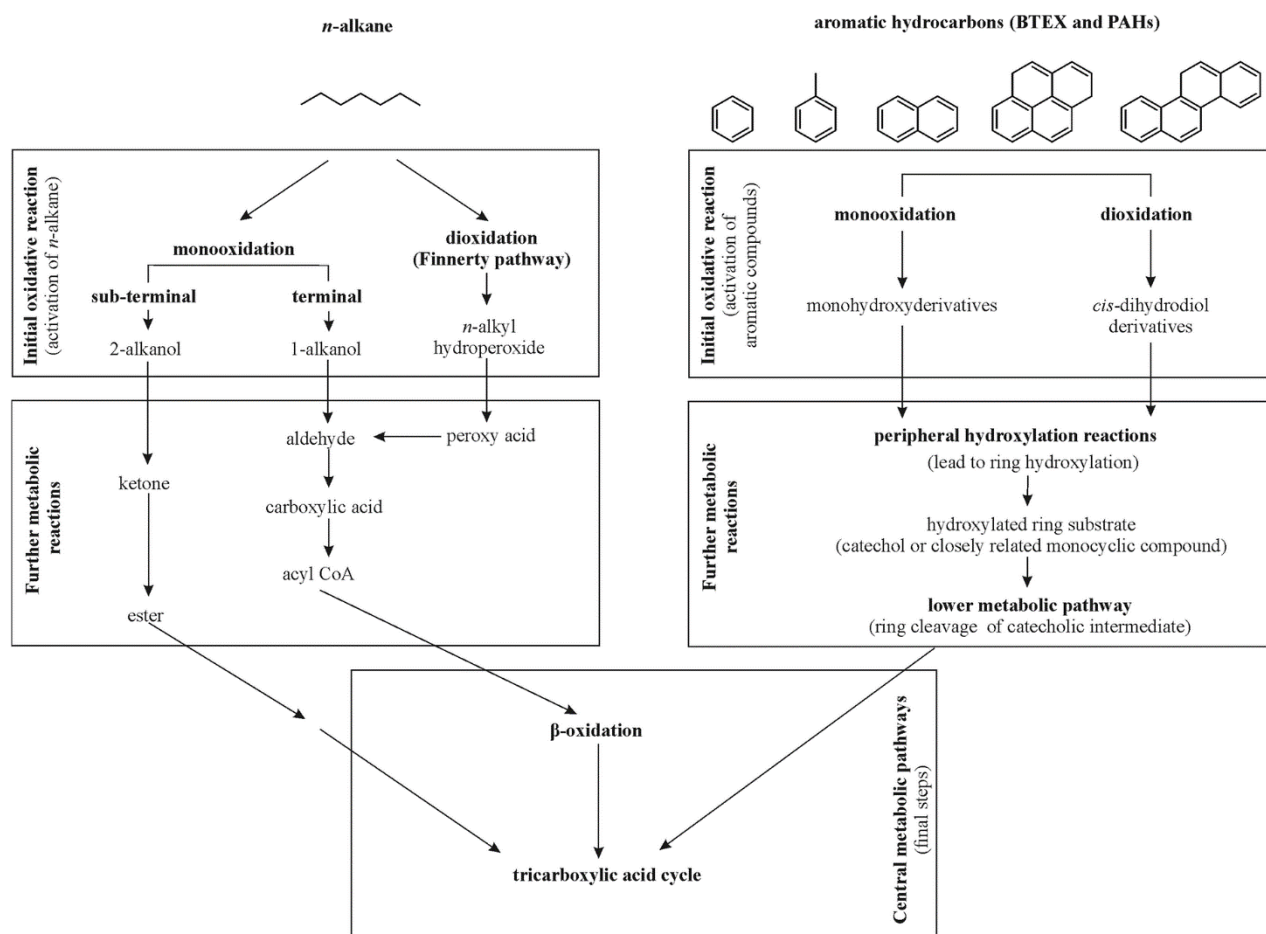


Figure I.14 Aerobic pathways of aliphatic and aromatic hydrocarbons degradation by bacteria (Brzeszcz and Kaszycki, 2018).

The fungi involved in PAH biodegradation act also by oxidation of a benzene ring (Figure I.15) using extracellular peroxidases, lignin peroxidase, manganese peroxidase and laccase (Chen et al., 2001; Scheel et al., 2000), and membrane attached monooxygenases such as cytochrome P450 (Črešnar and Petrič, 2011; Syed et al., 2010).

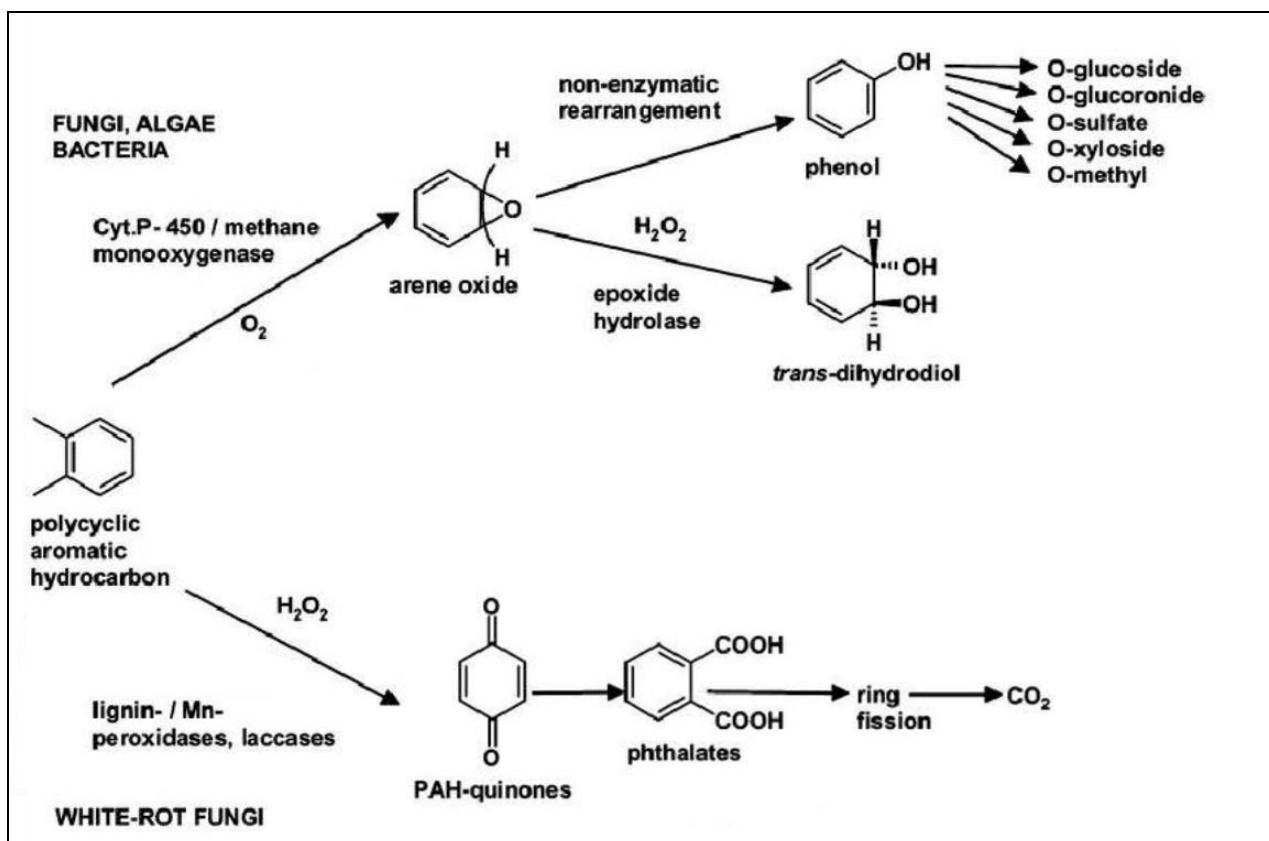


Figure I.15 Initial steps in the degradation pathways of polycyclic aromatic hydrocarbons (PAHs) by fungi (Cerniglia, 1997). Ligninolytic fungi or white-rot fungi produce lignin peroxidase, manganese peroxidase and laccases to degrade the lignin in wood instead non-ligninolytic fungi produce cytochrome P450 monooxygenase like enzymes.

I.4.3.2. Anaerobic HC degradation

In anoxic conditions (Bargiela et al., 2017; Duarte et al., 2014; Rabus et al., 2016), fumarate addition, carboxylation or O_2 -independent hydroxylation (only for PAH) are the main mechanisms to activate HC using respectively succinate synthases (glycyl radical enzymes such as benzylsuccinate synthase or alkyl-succinate synthase), carboxylases and dehydrogenase-like enzymes (Figure I.13 top right). Acyl-CoA derivatives are formed by reductases ATP dependant or not (such as benzoyl-CoA reductase class I and benzoyl-CoA reductase class II) and transformed through beta-oxidation reactions in a TCA cycle intermediates.

Among anaerobic microorganisms able to degrade HC, the sulfate-reducing microorganisms and the denitrifiers play an important role. They can be respectively detected by the amplification of the *dsrA* and *dsrB* genes that code the dissimilatory sulphite reductase (Wagner et al., 2005) and the *nirS* and *nirK* genes which code two different nitrate reductase, key enzymes in the denitrification (Braker et al., 2000).

I.4.4. Determining functional potential

To understand ecosystem functioning, the analysis of the relation structure/function of microbial communities is essential. Different approaches aim to analyse functional potential in environment such as software tools (PiCrust2, Faprotax, Tax4fun and Piphillin) that infer functions from metabarcoding data (Abhauer et al., 2015; Douglas et al., 2020; Louca et al., 2016; Narayan et al., 2020), culture of isolated strain/consortia coupled with measures of enzyme activities, metagenomics (Dombrowski et al., 2016; Segata et al., 2011) and detection of genetic functional markers with DNA array (Shi et al., 2019; Uhlík et al., 2009).

DNA arrays have revealed the functional response of microbial communities in plume during the Deepwater Horizon accident in accordance with metagenomics approach. In particular, the use of the DNA array showed an enrichment in functional genes and a specific functional structure in the plume (Lu et al., 2012; Mason et al., 2012; Rivers et al., 2013). Compared to communities out of the plume, genes involved in HC degradation were numerous and at higher abundance in microbial communities exposed to HC (Lu et al., 2012). Moreover, biogeochemical genes taking part in assimilatory nitrogen reduction, ammonia assimilation, organic phosphorus release, and sulfate reduction were also detected. Thus, DNA array are powerful tool for comparative analysis of functional potential of microbial communities (Shi et al., 2019).

I.4.5. Parameters influencing the HC degradation

The HC degradation depends on inherent properties of the HC compounds, of the matrix (soil and sediment) and of the microbial communities, which influence each other (Figure I.16) (Ghosal et al., 2016). The presence of plants in soil and the oxic/anoxic oscillation in marine coastal intertidal sediment have been also shown to act on the microbial HC degradation (Bourceret et al., 2016; Terrisse et al., 2017).

I.4.5.1. Root exudation as driver of HC remediation

Rhizosphere controls of the bioavailability of organic pollutants (Wenzel, 2009) and contribute to HC contaminated soil remediation (Khan et al., 2013; Rohrbacher and St-Arnaud, 2016). Surfactants produced by plants increase mobility, solubility and availability of organic compounds, including HC (Singer et al., 2003), making them more easily degradable. The

presence of plants increases the abundance and the activity of total PAH degrading microbes. Indeed, exudates production, in addition to release sugars, amino acids and others essential compounds (Bourceret et al., 2016; Cébron et al., 2009; Gerhardt et al., 2009; Newman and Reynolds, 2004), provide co-metabolites required for the biodegradation (Leigh et al., 2002) and secondary metabolites which are analogous to HC (Kamath et al., 2004; Purwanti et al., 2012) that induce biodegradation activity. The Figure I.17 presents certain aspects of the influence of the rhizosphere on the microbial degradation of HC.

I.4.5.2. Oxidic/anoxic oscillations in marine coastal sediment

Studies in microcosms showed that the structure of microbial communities in marine coastal sediment contaminated by HC change in response to oxidic/anoxic oscillations (Duran et al., 2015). Compared to permanent oxidic condition, equivalent or greater degradation capacity of HC was found (Vitte et al., 2013, 2011). The detection of ecotypes in these changing conditions has highlighted the adaptation/tolerance capacities of microbial hydrocarbonoclastic bacteria (namely *Alcanivorax* and *Cycloclasticus* genera) to oscillating oxygen conditions in marine coastal sediments. Furthermore, genes involved in HC degradation were differentially abundant according to oxygen variation showing the influence of anoxic/oxidic conditions in the functional response of microbial communities to HC contamination (Terrisse et al., 2017).

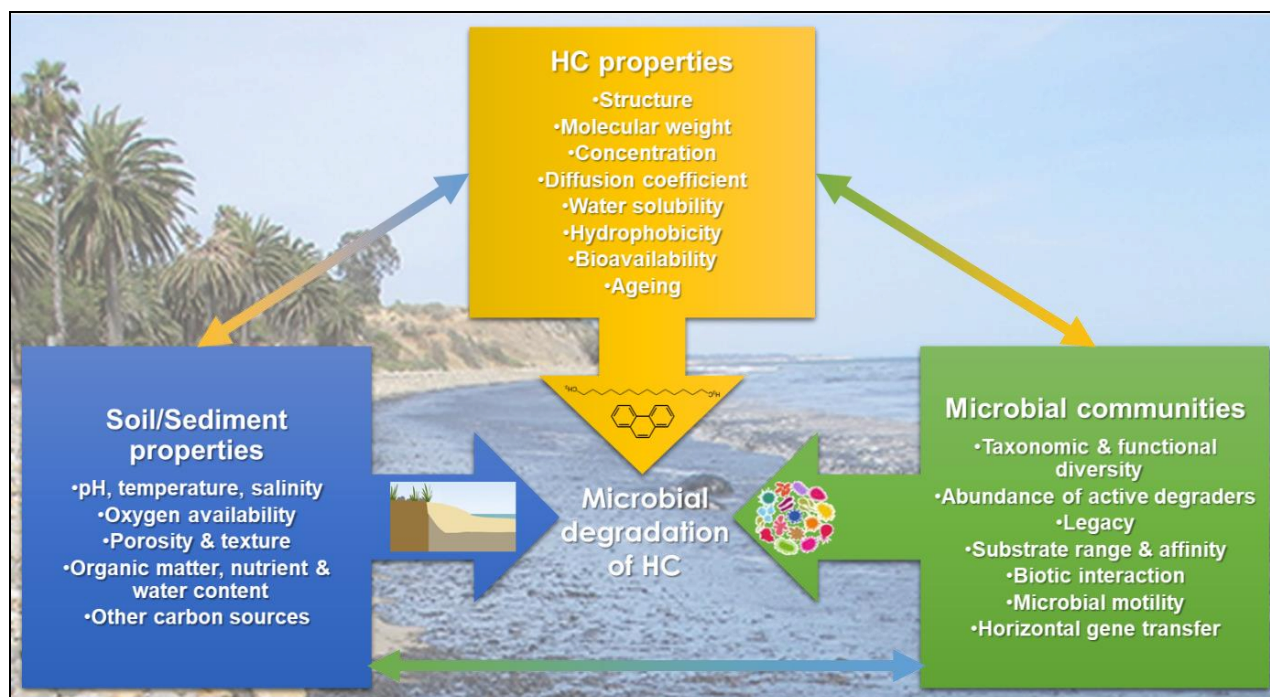


Figure I.16 Abiotic and biotic factors affecting microbial HC biodegradation modified from (Ghosal et al., 2016). Photo (U.S. Coast Guard): Oil on the beach at Refugio State Park in Santa Barbara, California, USA, on May 19, 2015.

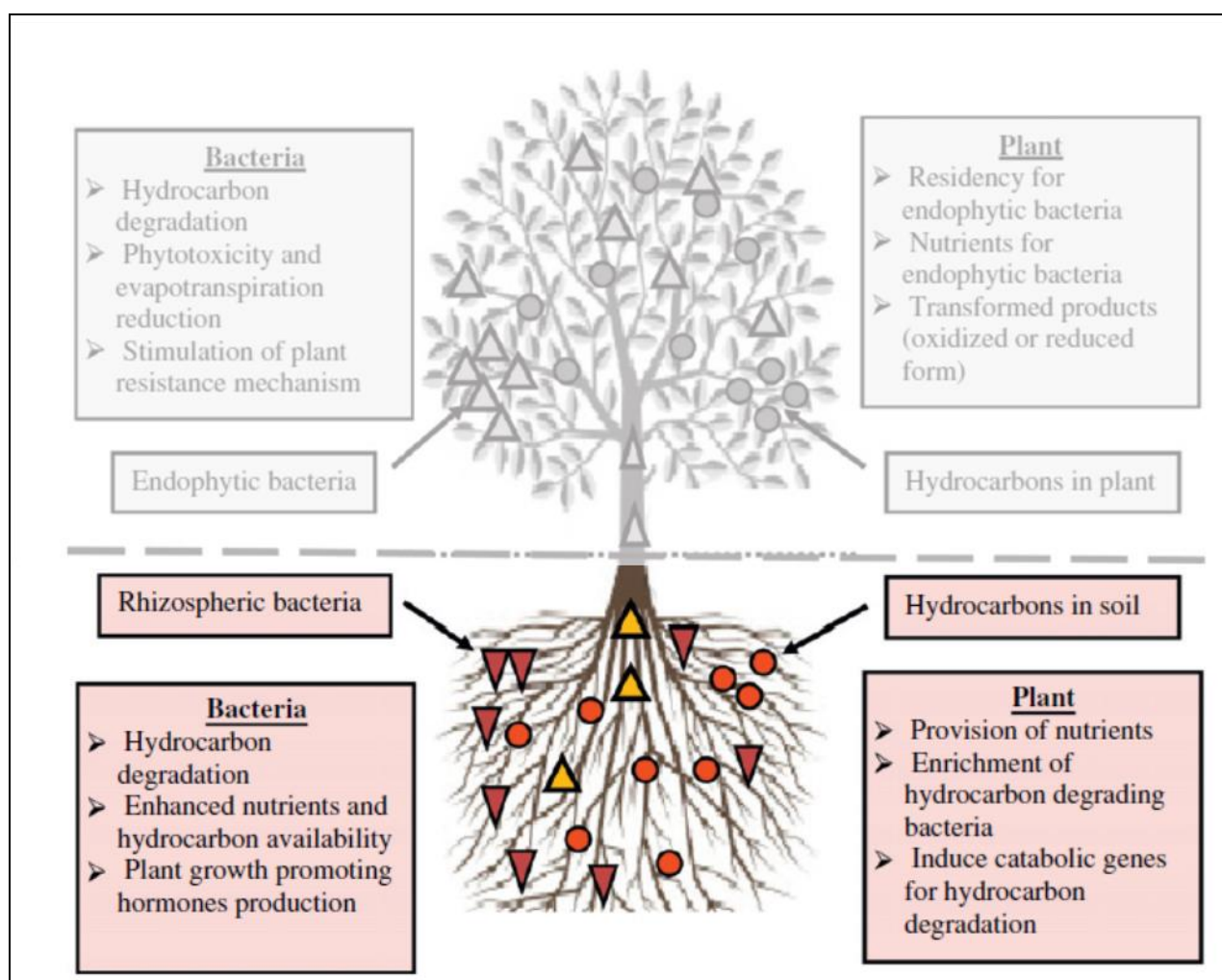


Figure I.17 Plant–bacteria relationships in the rhizosphere and influence on the remediation of hydrocarbon contaminated soil. Orange dots = hydrocarbons, red triangles = rhizobacteria, yellow triangle = endophytes. . The upper part of the schema is shaded because it deals with phyllosphere, a not studied topic in the thesis. Modified from (Khan et al., 2013)

I.5. Microbial ecology in land-sea continuum: Consequence of the terrigenous inputs

I.5.1. Fate of the microbes derived from terrigenous inputs in marine coastal sediment

Considering the biotic connection between soil and marine coastal sediment has the potential to provide novel insights into the dynamic and the functioning of ecosystems from local to global scales, and to predict the consequences of human land-based activities on coastal biodiversity and ecosystem services.

I.5.1.1. Meta-community and terrigenous inputs

Understanding the structure and the function of microbiome in marine coastal sediment requires the incorporation of terrigenous inputs that carry microorganisms. It is typically an objective of the meta-community ecology to consider the exchange of organisms between habitats. A meta-community is a set of local communities connected by the dispersal of multiple species (Leibold et al., 2004; Wilson, 1992). It is the extension of the meta-population concept that takes in account biotic interactions between multiple species (for example predation and competition), in the extinction and colonization processes. Four paradigms are associated to the meta-community theory: patch-dynamic (Townsend, 1989), species sorting (Pimm, 1983), mass-effect (Shmida and Wilson, 1985) and neutral process (Hubbell, 2001). They are presented in the Figure I.18 (Leibold et al., 2004):

- **Patch dynamics perspective** (Figure I.18 a) assumes that patches (habitats) are identical and that each patch is capable of containing populations (same quality) and is thus occupied or not. Spatial dynamics are dominated by local extinction and colonization, whether stochastic or deterministic.
- **Species-sorting perspective** (Figure I.18 b) emphasizes the resource gradients or patch types cause sufficiently strong differences in the local demography of species and the outcomes of local species interactions that patch quality and dispersal jointly affect local community composition. This deterministic perspective is based on the traditional spatial niche segregation theory. Dispersal is important because it allows compositional changes to track changes in local environmental conditions
- **Mass-effect perspective** (Figure I.18 c) focuses on the effect of immigration and emigration on local population dynamics. In such a system species can be rescued from local competitive exclusion in communities where they are bad competitors, by immigrate from communities where they are good competitors. This deterministic perspective emphasizes the role that spatial dynamics affect local population densities.
- In the **neutral perspective** (Figure I.18 d), species have similar competition and dispersal ability. The persistence and the diversity of community result from random species loss (extinction, emigration) and random species gain (immigration, speciation). This stochastic perspective is often used as null hypothesis for microbial assembly.

The theoretical meta-community framework described above, however, overlooks the abiotic component, i.e. the surrounding environment of dispersing microorganisms. From this point of view, the subject matter of our study, the terrigenous inputs, are regarded only as a source of soil communities. However, studies on the assembly and properties of communities have called early for an integration of communities in ecosystems (DeAngelis, 1992; Sterner and Elser, 2002). Another

limitation especially when studying the effect of terrigenous inputs in marine coastal sediment due to soil runoff is that, at first sight, soil and marine coastal sediments do not constitute clearly a meta-community.

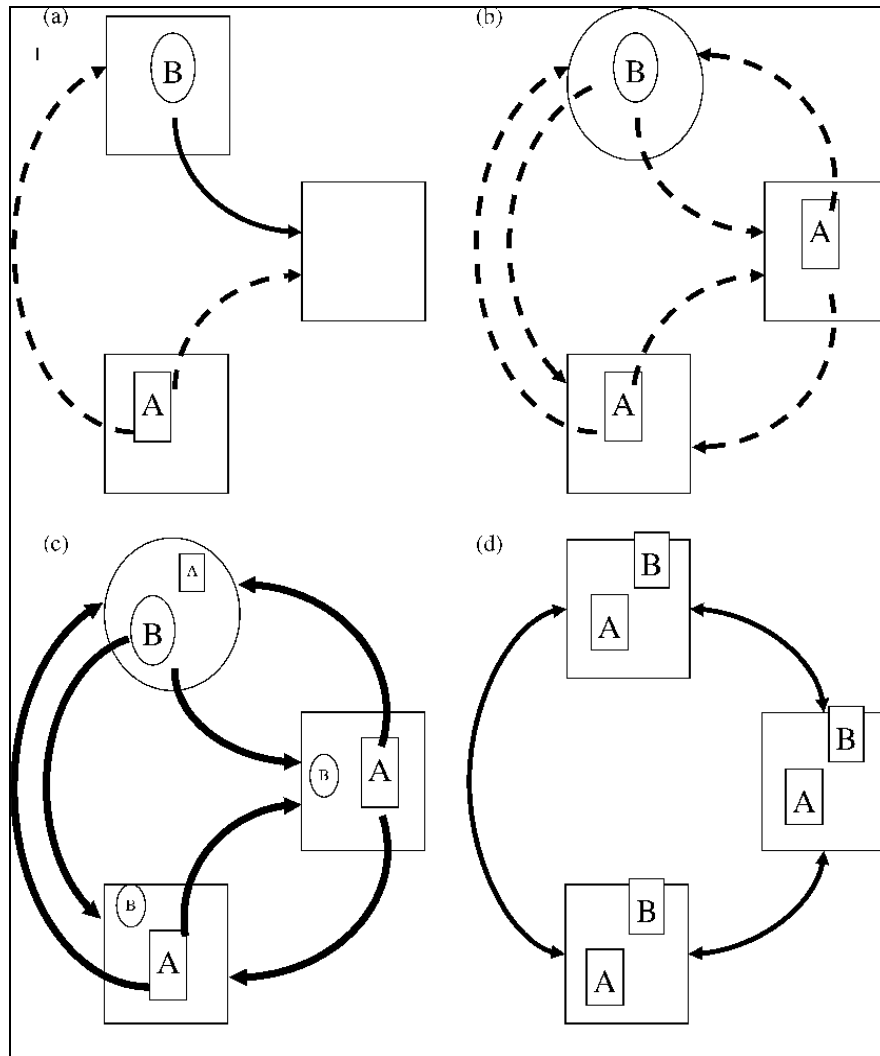


Figure 1.18 Schematic representation of the four paradigms for meta-community theory for two competing species with populations A and B. Arrows connect donor populations with potential colonization sites, shown as large boxes or ovals. Solid arrows indicate higher dispersal than dashed arrows and either unidirectional movement (single-headed arrows) or bidirectional movement (double-headed arrows). The matching of the smaller box or oval (denoting its habitat type niche) with the site symbol shows the degree to which a species is the competitive dominant in a site. The four paradigms illustrated are (a) patch-dynamics, (b) species-sorting, (c) mass-effects and (d) neutral. In (a) the patch-dynamics paradigm is shown with conditions that permit coexistence: a competition-colonization trade-off is illustrated with species A being a superior competitor but species B being a superior colonist; the third patch is vacant and could become occupied by either species. In (b) species are separated into spatial niches and dispersal is not sufficient to alter their distribution. In (c) mass effects cause species to be present in both source and sink habitats; the smaller letters and symbols indicate smaller sized populations. In (d) all species are currently present in all patches; species would gradually be lost from the region and would be replaced by speciation (Leibold et al., 2004).

I.5.2. Community coalescence concept: when communities and their surrounding environments mix

I.5.2.1. Definition of community coalescence

Microbial community interchange between different environments occurs commonly in nature with falling leaves, dust movement, interaction between aquatic and terrestrial ecosystems and even inside organisms as well as in human facilities for example through industrial drainage network (Rillig et al., 2015). This ecological event is termed microbial community coalescence. The macroecologist Wallace (Wallace, 1876) originally used the term community coalescence in 1876 to describe the wholesale exchange of terrestrial fauna between North and South America continents, called "Great America Interchange" during the formation of the Isthmus of Panama. In microbial ecology, community coalescence received only recently a formal definition and essential basis for a systematic study of the phenomenon. Rillig et al. (2015) define microbial community coalescence as the "microbial community interchange event in which a joining of previously separate communities [narrow sense] and even ecosystems [broad sense], forming a new entity that is not easily separable into parts again; this new entity has distinct properties from the parts it unites".

Meta-community theory focuses on the dispersal of individuals into communities while coalescence is interested in the wholesale community interchange that occur when communities encounter. Coalescence concept integrates abiotic factors by considering the concomitant movement and mixing of the environment surrounding the translocated communities. Coalescent events do not necessarily involve communities defined as meta-communities strictly speaking: for example, in case of inefficient dispersal of individuals accompanied by a significant relocation of the environment and in turn a structural change of recipient environment. In this way, community coalescence opens up the understanding of the structure of communities and surrounding environments that collide, without ignoring the meta-community paradigms when conditions are fulfilled.

I.5.2.2. Coalescence outcomes

The coalesced community can be characterized by the extent of convergence toward one of the initial communities and by the extent of new interactions established between members of the initial communities (Castledine et al., 2020) (Figure I.19). The composition of the coalesced community is described as asymmetric in case of the dominance of members of one of the initial communities and, on the contrary, as symmetrical when the members of the inputs coexist in sizeable proportion. Then, the coalesced community is defined as chimeric when new interactions occur between members of the initial communities while the term modular is used when pre-existing interactions between members within the initial communities remain.

It is quite conceivable that communities without a clear organization emerge from a coalescence event due to random mix (stochasticity).

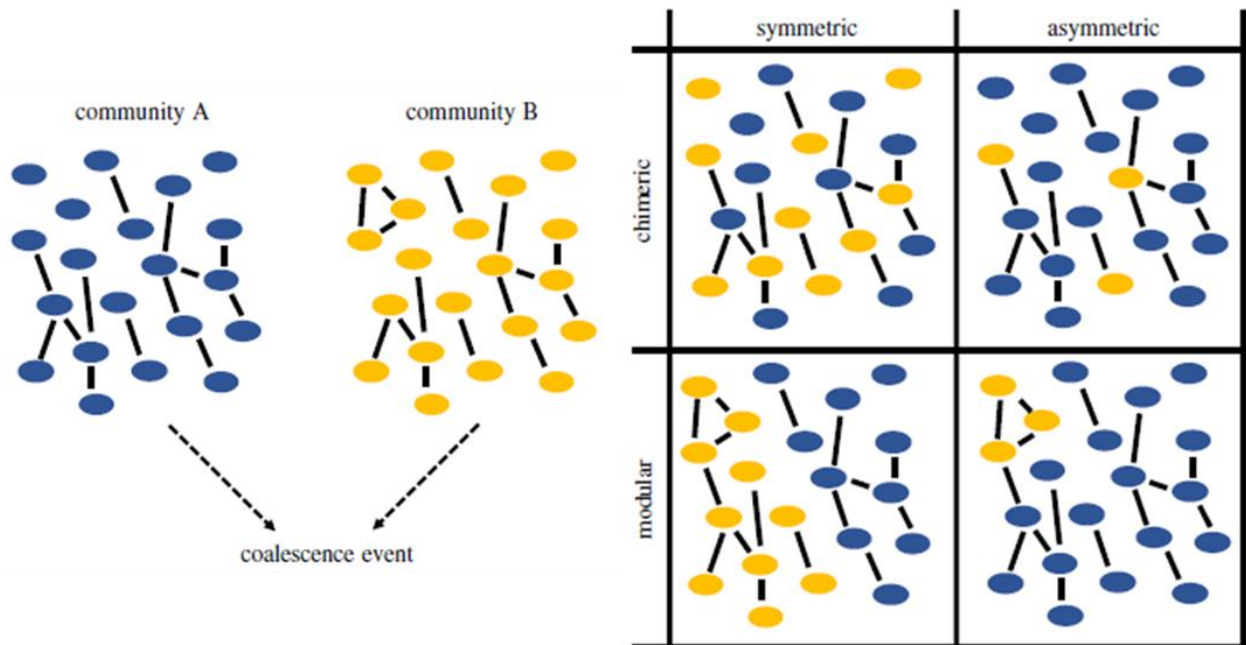


Figure I.19 Different types of communities resulting from coalescence. Each dot represents a species. For simplicity, extinctions post-coalescence and species appearance from rare are not represented. Lines represent interactions within initial and coalesced communities. (Castledine et al., 2020)

I.5.3. Abiotic and biotic perturbations imposed by community coalescence

I.5.3.1. The fate of the microorganisms derived from terrigenous inputs

According to the work of Rocca et al. (2020), the composition of two distinct initial communities that encounter and mix is expected to follow four possible trajectories depending on the coalescence conditions (Figure I.20):

- Symmetric outcomes
 - Species sorting: The mixed community and the new environment are an intermediate between the initial ones conditions because of a symmetric environmental filtering (Figure I.20 a)
 - Community resistance: The two initial conditions remain identical after mixing because of symmetric community resistance (Figure I.20 b).

- Asymmetric outcome
 - Environment or community convergence: The coalesced community converges toward one of the two initial because of strong asymmetric environmental filtering or asymmetric community resistance (Figure I.20 c)
- Stochastic responses: emergence of highly variable communities (Figure I.20 d)

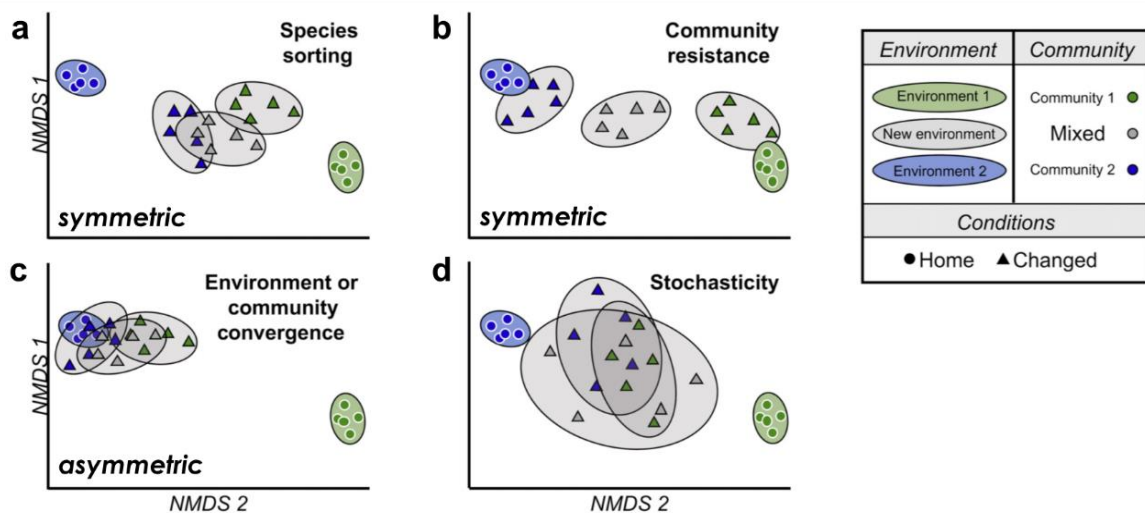


Figure I.20 Potential outcomes of a coalescent event between communities/environments 1 and 2. “Home” conditions correspond to the two distinct initial conditions and “changed” to the final ones. Ellipsoids represent the theoretical variation in community structure or enzyme potential among replicates. Communities may be symmetrically impacted by coalescence (A, B), constrained primarily by environment (A), or resistant to environmental change (B); (C) asymmetrical impact may be driven by either environment or community; or (D) coalescence may result in stochastic shifts in community structure or function (Rocca et al., 2020).

I.5.3.1.1. Species sorting

In the scenario of species sorting, the new abiotic factors may select the most competitive species from the initial communities that form a chimeric coalesced community. It is likely to occur between communities that share traits because of an equal competition for niches and when the newly formed environment is homogenous.

I.5.3.1.2. Community resistance

The resistance of the two initial communities results from their abiotic adaptations for their respective environments and the coexistence of these environments within the new ones. A modular coalescent community may emerge because the new environment is the juxtaposition of the initial ones with physical barriers that limit interactions between members originated from the different communities.

1.5.3.1.3. Community convergence

Community convergence may result from massive post-coalescence extinctions of members from one microbial input due to the new abiotic and/or biotic conditions. It is likely to occur to microorganisms derived from terrigenous inputs since they may experience a harsh transition and be exposed to new physical factors (higher salinity, settling and dilution). When freshwater and marine microbiome collide, strong convergence of the microbial community structure and microbial enzymatic potential was experimentally evidenced toward the marine community (Rocca et al., 2020). In fact, the recipient community may have a resident advantage from priority effect (Meester et al., 2016; Pantel et al., 2015) (monopolization of resources) and abiotic adaptations (already adapted to niches) (Nemergut et al., 2013). Moreover, the input community can suffer from new biotic interactions in which its members are disadvantaged (predation, competition, parasitism, phages) or from pre-existing biotic interactions among residents such mutualism that outcompete them. The corresponding coalesced communities are thus respectively chimeric and modular.

On the contrary, the predominance/ success of the input community may happen if the novel biotic interactions are at the disadvantage of the residents with the introduction of predators and phages (Brown et al., 2006). The concomitant and significant translocation of environment may provide abiotic requirements to dispersing microorganisms (mass effect). In the same way, the multiple microbial inputs can compensate their loss in the recipient community and help in the following establishment. Finally, simultaneous coalescence event and environmental disturbance can affect more the resident (Lear et al., 2020).

1.5.3.1.4. Rare taxa, dormancy and metabolic heterogeneity

Regardless the outcome of the coalescence event, the final community may inherit from persistent taxa and may supply outbreaks of rare taxa, initially at too low abundance to be detected.

Dormancy is the ability to enter a reversible state of low metabolic activity when faced with unfavourable environmental conditions (suboptimal for growth and reproduction) (Lennon and Jones, 2011). Dormancy is common response to environmental stress and is phylogenetically widespread in microorganisms. The physical differentiation into a resting structure (spores, conidia, cysts and akinetes, Madigan et al., 1997) is one of the dormant phenotypes identified among diverse genetic and cellular mechanisms. Dormancy generates a seed bank defined as a reservoir of dormant individuals that can potentially be resuscitated in the future under different environmental conditions (Lennon and Jones, 2011) and replaced taxa that have the potential to fulfil the same metabolic tasks (Kalenitchenko et al., 2018).

Microbial communities are generally characterized by the coexistence of active, slow-growing to dormant microorganisms (metabolic heterogeneity) and are generally dominated by low-abundance taxa (rare taxa) (Galand et al., 2009; Scheckenbach et al., 2010; Sogin et al., 2006).

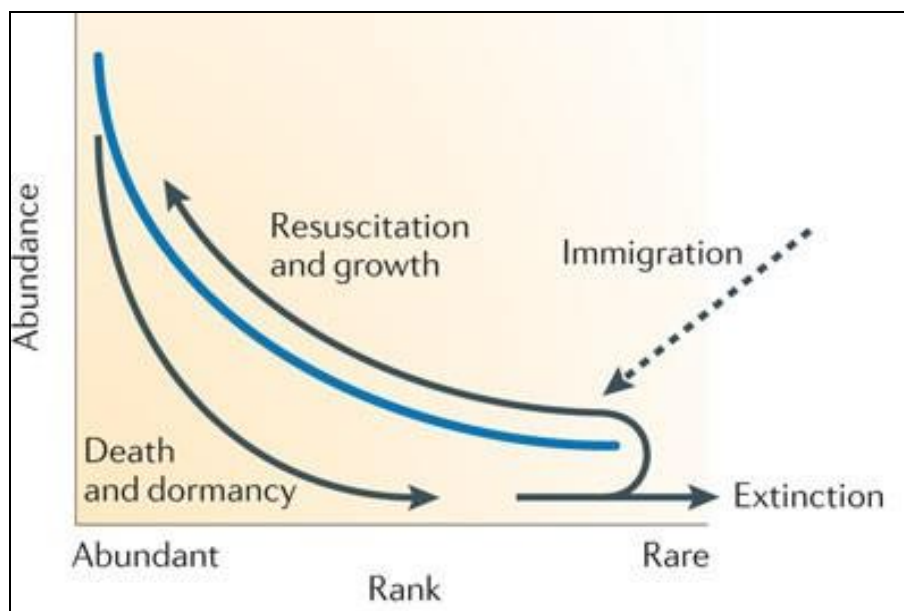


Figure 1.21 A dynamic rank abundance curve for a microbial community that is influenced by dormancy and by immigration (component of dispersal with colonization; dashed line) (Lennon and Jones, 2011) including immigration mediated by community coalescence.

Rare taxa can be regarded as taxa at the edge of the extinction, as taxa maintained by dormancy and/or dispersal (immigration) and as a reservoir of genetic diversity capable of responding rapidly to environmental change. During coalescent event, the mixing of communities and the blending of environment is likely to influence the outbreaks of rare taxa, the transition between the dormant and the active states (and vice versa) and then the relative abundances of taxa (Figure 1.21). Such changes will also change the microbial co-occurrence pattern and all together ultimately affect ecosystem functioning.

1.5.4. Characterization of the community coalescence in the land-sea continuum

The land-sea continuum, at the interface between the terrestrial ecosystem and the marine coastal ecosystem, constitutes a mixing zone between the microbial communities from soil and sediments. The Figure 1.22 describes the community coalescence in this area according to the framework of (Rillig et al., 2015).

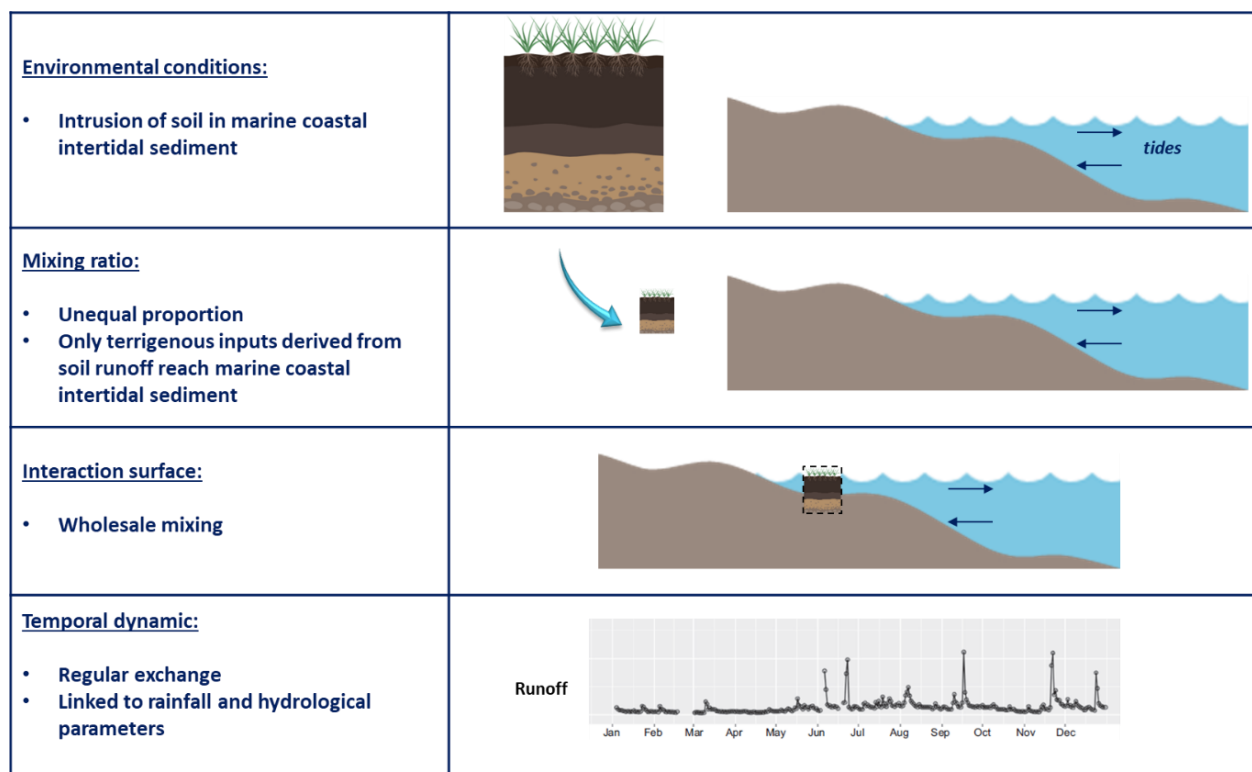


Figure 1.22 Illustration and characterization of the longitudinal community coalescence occurring between soil and sediment under the effect of runoff along land-sea continuum. The graph shown represents flow in Olinda Creek, near Melbourne, Australia, in response to rainfall events over a 12-month period (1988).

1.5.4.1. Lack of data on the community coalescence in land-sea continuum

Coalescence is particularly studied in riverine network, from headwaters to estuaries (Mansour et al., 2018). Transition of communities between freshwater and marine ecosystems is considered infrequent because of the salinity boundary, even if the microbial communities in these environment share similar traits (Logares et al., 2009). However, recent meta-analysis of 16S metabarcoding data from freshwater and seawater revealed the potential for marine and freshwater organisms to transition between habitats with different salinities and adapt to new biotic and abiotic conditions. The analysis also showed that seed bank and rare diversity include communities able to cross the salinity boundary and may contribute to community diversity and functioning (Paver et al., 2018).

The modalities of community assembly in marine coastal sediment in response to terrigenous inputs need to receive interest and could be linked with genotypic traits and activities for a potential use in HC remediation.

I.5.5. Community coalescence and implication on functions

I.5.5.1. Functional redundancy

Functional redundancy (Allison and Martiny, 2008; Lawton and Brown, 1993; Louca et al., 2018; Walker, 1992) occurs when there is an overlap in the metabolic capacities of different species/communities. Functional redundancy stabilizes the function of communities that face environmental perturbations, as long as, redundant species/communities respond differently to the change of the environmental conditions and offset functionally each other (Gonzalez and Loreau, 2008).

Dormancy allows the coexistence of functionally redundant by species/communities and generate seed banks (Lennon and Jones, 2011) thus may contribute to the functional stabilization of microbial communities because a quick recruitment of species/communities involved in the given function may occur in response to environmental perturbation. Following a disturbance, in microbial communities that do not display dormancy (high cost compared to the benefits in a given environment), functional redundancy will draw on the immigration of metabolically similar communities able to cope with new environmental conditions.

Studies confirmed the importance of redundancy, the rare biosphere and the dormancy in response to the HC pollution in marine and soil environment. The biotechnological potential of the rare biosphere to treat oil spill was considered (Pascoal et al., 2020). For example, functional redundancy for anthracene degradation in native community was found in aged PAH-contaminated soil (Dunlevy et al., 2013). High throughput cultivation method lead to the isolation of previously unknown fluoranthene-degrading *Blastococcus* species, members the rare biosphere in HC contaminated soil (Jiang et al., 2016). Smith et al. (2013) characterized the metabolic footprint of hydrocarbon-impacted sediment and observed particularly that genes associated with dormancy and sporulation were overrepresented. This functional shift suggested that microbial communities responded to the HC pollution and allocated energy in survival. After dormancy (in the form of cyst-like cells), a cultivable *Rhodococcus* strain showed an expansion of its ability to degrade HC (Solyanikova et al., 2016).

I.5.5.2. History of contamination and legacy effect

The legacy effect (Cuddington, 2011) is the persistence of the effect/disturbance due to the activity of organisms even after the cessation of such activities. HC released by human activities have been shown to influence community composition, physical-chemical properties of substrates in soil and in marine sediment after oil spill or industries dismantling. In particular, communities keep a long-term resistance to HC link to the long history of contamination (Oil-legacy effect (Thomas et al., 2020) after oil spill). During coalescence, the role of the legacy

effect inherent to the communities encounter in the response to perturbation such as HC pollution is unclear.

1.5.5.3. Horizontal gene transfer in coalescent communities

In the environment, the response of rare taxa to a stress includes also horizontal gene transfer (HGT) of functional traits through different community members (Y. Wang et al., 2017). The terrestrial runoff can influence the microbial community structure in sediment (Fagervold et al., 2014; Le et al., 2016). In addition, the particles subjected to the runoff can bring also microorganisms, bacteriophages, free DNA, and constitute a gene reservoir for horizontal gene transfer (HGT) events that could facilitate HC biodegradation. Indeed, HGT is known as an important force playing a role in the adaptation of microorganisms to their environment (Iwasaki and Takagi, 2009). In particular conjugation seems essential for a relatively rapid response to changing environmental conditions (Thomas and Nielsen, 2005). We need to take into account this process to define the microbial terrestrial component in marine coastal sediment and its effect on the degradation of the hydrocarbons. Recently, Wang et al. (2017) have discovered a *hpah* gene cluster in a halophilic phenanthrene degrading consortium. Results of their study suggest that it has derived from the nah-related gene clusters in the microorganisms via horizontal gene transfer and has adapted to higher salt concentrations. In mangrove contaminated by HC, low abundance plasmids involved in HC degradation became abundant (Gomes et al., 2010).

Further studies are required to know whether the process is common and to clarify the role of the runoff as a pool of functional potential transferable genes.

1.5.5.4. Toward enhancement of beneficial function for human well-being?

Beneficial outcomes from community coalescence in term of functions were reported in medicine and biotechnology. In most described cases, the dominance of the engineered or selected community following the coalescence meant: health recovery in faecal transplant therapy (Wang et al., 2019; Wilson et al., 2019); optimization of an industrial process such as production of methane (Rillig et al., 2016; Sierocinski et al., 2017); or the restoration of altered agricultural soil such as the rescue of N-cycling (Calderón et al., 2017). These studies provided new insights and further studies in natural communities could guide new effective strategy of bioremediation.

In land-sea continuum in which HC release from land-based activities is pervasive and threatens the adjacent coasts, the functional outcome of the multiple coalescence events between HC contaminated terrigenous inputs and marine coalescence sediment must be explored. In fact, a better management of the land-based industrial effluents reaching coast by soil runoff is more realistic than their reduction or stop and they could be designed in order to maintain or even enhance the removal in the coastal recipient.

Thesis objectives

This thesis aimed to consider the impact of the terrigenous inputs on the structure and the functional potential of the microbial communities in marine coastal sediments in the hydrocarbon (HC) contaminated land-sea continuum. A specific and original experimental device mimicking the land-sea continuum in microcosms was developed to test different scenarios: the terrigenous inputs were generated from soil with or without plant and with HC spiking or not in soil and sediment. The specific objectives were:

- To determine whether terrigenous inputs results in microbial community coalescence between soil and sediment in HC contaminated land-sea continuum,
- To define the functional potential of microbial communities in land-sea continuum and the metabolic networks involved in HC degradation in marine sediment under terrigenous inputs and HC contamination,
- To assess the permissiveness of marine sediment microbial communities to plasmids from terrestrial inputs.

Ultimately, the acquired knowledge aim to improve the understanding of microbial processes and actors involved in the restoration of HC contaminated marine coastal ecosystem taking in account terrigenous inputs.

Chapter II:

Experimental ecology: land-sea continuum microcosm experiment

Chapter II. Experimental ecology: land-sea continuum microcosm experiment

This chapter is dedicated to the description of the microcosm system developed for the TOTEM project. It includes the description of sampling sites for soil and sediment and the main results of the preliminary studies lead before the beginning of the thesis. The microcosm set up and the incubation were performed before my recruitment. My contribution to the project started when the incubation stopped (II.3.4).

A detailed material and method is available in each chapter (III.2.3, 0 and V.2) and provides information about the biomolecular, chemical, bioinformatics and biostatistical approaches used to analyse samples.

II.1. Soil and sediment collection

Soil and sediment were collected in the southeast of France (Bouches du Rhône) the 30th and 31st of October 2017 (Figure II.1 a). Soil and sediment came from the same geographical area: soil was collected from the surroundings of the Berre pond (43°29'6.9"N 5°11'18.53"E, Berre-l'Étang, France) and sediment from the Canal Viel cove in the Golf of Fos (43°23'20.3"N 4°59'31.8"E, Lavéra, France) (Figure II.1 b). The sampling sites are both near industrial zones (Figure II.1 c and d), contaminated by HC and enlisted in the database BASOL (Antoni, 2013) for the polluted sites and soils from the French Ministry of the Ecological and Solidary Transition. Samples constituted a model of a realistic HC contaminated land-sea continuum. These sampling sites for soil and sediment were selected following preliminary studies in the area (see II.2).

During the sampling, 10 kg of Berre soil, rid of vegetation were collected with an auger on surface (maximal depth of 15 cm) while 3 kg of Canal Viel marine sediment were collected with a ladle and stored in jars with seawater. Soil was sieved at 4 mm in the field to remove coarse gravels, plant debris and macroorganisms. Then, soil and sediment were transported in cooler (4°C) to the laboratory where sediment was sieved at 1 mm. Subsamples were used to estimate the dry weight (DW) of the two matrices and the water holding capacity (WHC) of the soil. For this purpose, soil and sediment were air-dried at ambient temperature under a fume cupboard. Sediment was previously centrifuged and the supernatant seawater was discarded. In addition, the texture, the pH, the C/N ratio, the cation-exchange capacity, ions and trace metal contents were measured on dried samples for soil and sediment at the Laboratory of Soil Analysis (LAS, INRAE, Arras, France). The rest of soil and sediment was stored at 4°C until the set up of the microcosms. Sediment was covered with *in situ* seawater (~5 cm) in a 5L bottle under oxygenation with an aquarium pump

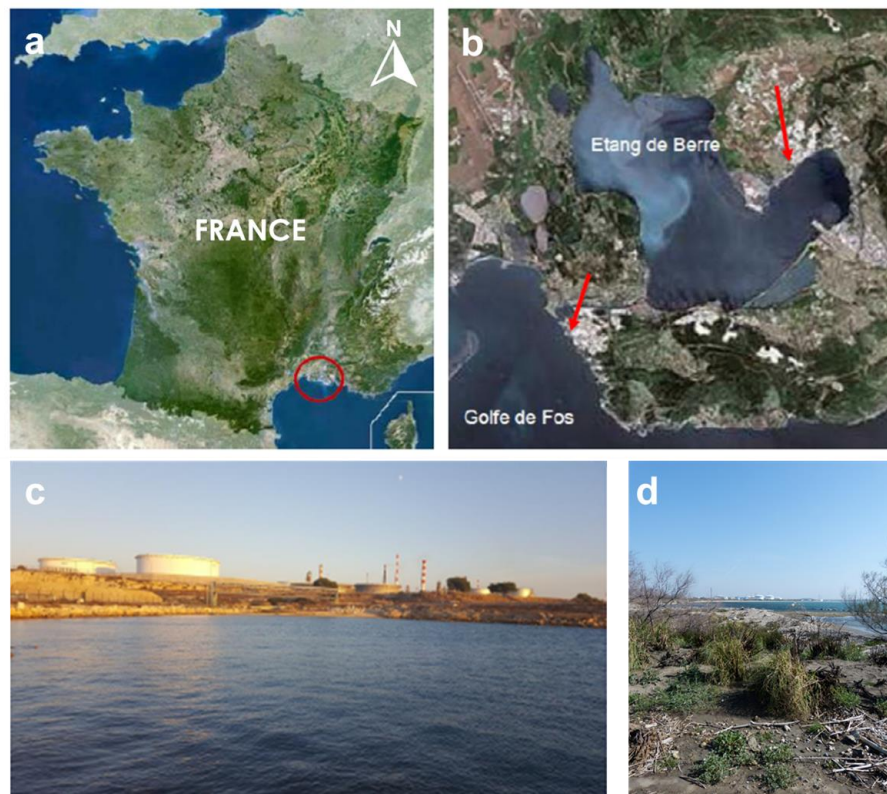


Figure II.1 Geographic localization of the sampling sites. a Samples come from the southeast of France (Provence-Alpes-Côte d'Azur region) b. Arows point toward soil and sediment sampling sites, respectively in Etang de Berre (Berre-L'Étang) and Golf de Fos (Lavéra). c and d Different views on the near petrochemical industrial area.

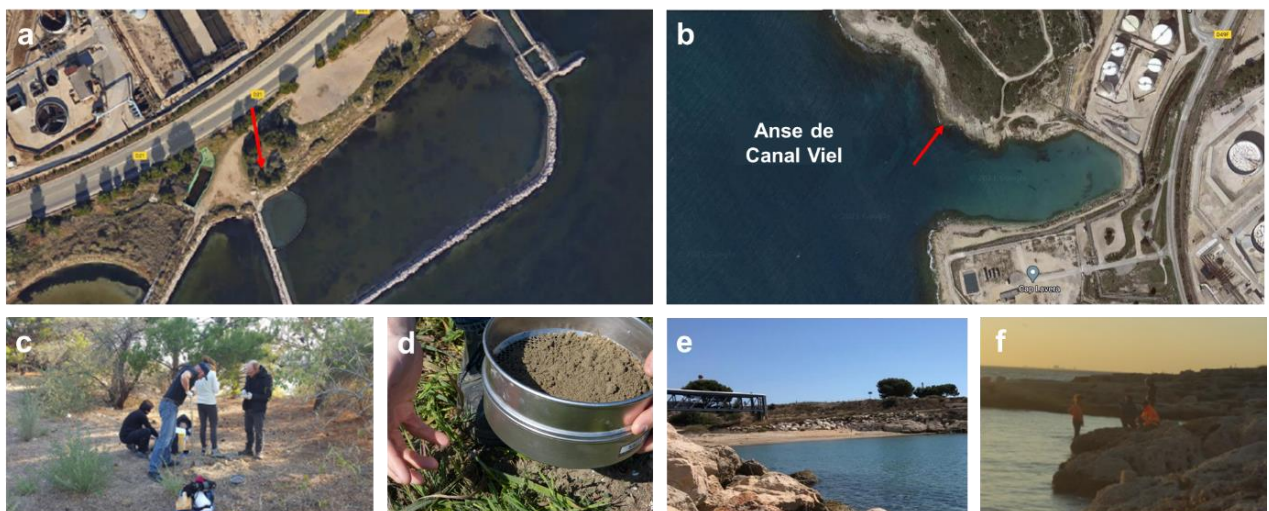


Figure II.2 Soil and sediment sampling. a Localization of soil sampling site. b Localization of sediment sampling site. c Soil collection d Soil sieving. e View on the sediment sampling site. f Sediment collection.

II.2. Preliminary experiments: soil/sediment selection and setting of the experimental device

II.2.1. Sampling and characterization of soil and marine coastal sediment

II.2.1.1. Level of HC contamination

Soils and sediments from the same geographical area were collected in April 2017 to select realistic HC contaminated land-sea continuum samples. The different soils and sediments came from the Gulf of Fos area: from the Canal Vieil cove in Lavéra, the Cavaou peninsula in Fos-sur-Mer, and from the Berre pond in Berre-L'Étang. Sampling sites were close to industrial areas (refineries, coking plants, etc.), contaminated by HC and potentially hosting microbial communities adapted to these organic pollutants.

Briefly, soils and sediments, separated by a distance of ~10 to 50 meters, were sampled in triplicate at the three locations mentioned above. The HC content in soils and sediments was measured after accelerated solvent extractions (ESA) with dichloromethane. The extracts were partly dried to estimate the dry extract rates or directly analysed by gas chromatography and flame ionization detector (GC-FID) to calculate the Hydrocarbon Index (HI).

The different soil and sediment samples were relatively uncontaminated: extractable organic matter contents and HI values ranged between 0.5 and 2 mg/kg of soil or sediment and between 0.8 and 1.8 mg/kg of soil or sediment respectively. Soil and sediment from the Cavaou peninsula showed very low organic contamination close to the detection limit while those from the Etang de Berre were the most contaminated. They were thus excluded for future experiments because it would have been difficult to detect spiked HC in these samples. Finally, sediment from the Canal Vieil cove and soil from Berre pond displayed a background HC contamination compatible with the detection of spiked HC by GC-MS.

II.2.1.2. Potential of HC biodegradation

The potential of HC biodegradation was estimated in the different soils and sediments through a 3 weeks batch experiment. First, soils and sediments were spiked or not with n-hexadecane and phenanthrene to obtain a final concentration of 100 mg/kg. Then, the mineralisation (CO₂ production) were monitored during 3 weeks by infrared analyser. At the end of the incubation, hydrocarbonoclastic bacteria were quantified in microplates using the most probable number (MPN) method within samples of which the mineralisation curves were higher than the basal one (Figure II.3).

Soil from Etang de Berre (Berre_4) and sediment from Canal Viel cove (CV) were selected at the end of experiments because of their higher mineralization rate and the presence of

hydrocarbonoclastic bacteria. In a second step, another batch experiment was carried out with deuterated n-hexadecane and phenanthrene in samples that showed potential of HC biodegradation (each deuterated HC was spiked at 100 mg/kg). Results showed a hexadecane removal of about 10% and a phenanthrene removal of more than 90% in soil Berre_4. In the sediment CV, about 40% of hexadecane and about 65% of phenanthrene were removed.

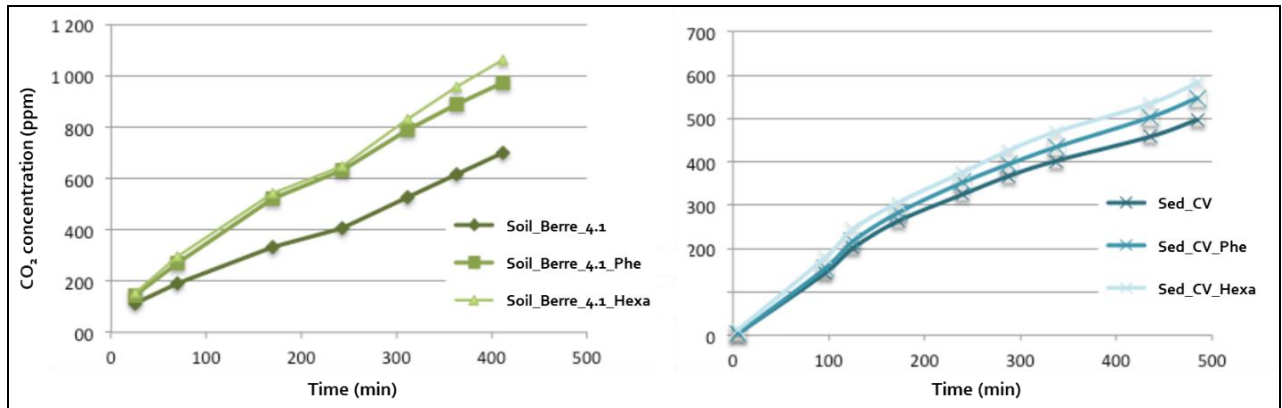


Figure II.3 Batch monitoring of the basal mineralisation and with HC spiking (Phe=phenanthrene and Hexa=hexadecane) for a soil sample (Berre_4) and a sediment (CV). For the other samples the HC mineralisation was negligible, even the presence of HC was toxic and partially inhibited the mineralisation

II.2.2. Experimental device and determination of the incubation conditions

The experimental device was specifically designed and manufactured for the TOTEM project in order to reproduce in a realistic and controlled way the terrigenous inputs to marine coastal sediments by soil runoff in microcosms.

The device consisted of an upper soil compartment (in stainless steel) connected by a Teflon pipe, where soil particles flow, to a lower marine coastal sediment compartment (in glassware). Soil was periodically watered with artificial rainwater (Volvic mineral water), generating a runoff of particles towards the sediment compartment. Marine coastal sediment was subject to tidal cycles by means of valves and pumps that renewed the seawater (sterile synthetic seawater, Sel Instant Ocean, Aquarium systems) and maintained salinity.

In a pilot experiment, 12 land-sea continuum microcosms and six aquatic microcosms without runoff (control) were installed in a phytotronic chamber to verify their conformity. Then, tests were carried out in order to obtain a constant, reproducible, and parallel watering of the 12 soil compartments using syringes.

At the end, the following conditions of incubation were adopted for the thesis experiment:

- The rain regime was adjusted to obtain a significant soil runoff towards the sediment microcosm: 3 hours of watering (73 mL) per day, that represented a runoff volume of about 40 mL and a transfer of between 4 and 15 mg of particles.
- Seeds of *Catapodium marinum*, a grass identified *in situ*, were provided by the seed bank of the French National Museum of Natural History (MNHN, Paris). The presence of HC did not disturb the germination or the growth of the plants, so microcosms with planted soil conditions was possible. Plants were enough developed (foliage growth) after five weeks of culture.
- 24-channel peristaltic pumps were used for the renewal of seawater (simultaneously in the 18 aquatic devices). Two tidal cycles were simulated per 24 hours: sediment compartments were filled with 250 mL of synthetic seawater in 6 hours and then emptied in 6 hours until about 1-2 mm of water is left above the sediment.

Preliminary studies were carried out to select a soil and sediment pair representative to a HC contaminated land-sea continuum and to set the conditions of incubation for experiment in microcosms. The selected soil and sediment were contaminated by HC and hosted microbial hydrocarbonoclastic communities. The level of natural HC contamination was compatible with the detection of HC spiking by GC-MS in microcosms. The rain regime and the settings of the pumps were fixed in order to make the conditions of incubation uniform and repeatable.

The following sections describe the assembly of the land-sea continuum microcosms, the conditions of incubation and the sampling strategy adopted during the thesis experiment.

II.3. Description of the microcosms

Two kinds of microcosms were set up in LIEC lab (Figure II.4 a): the land-sea continuum microcosms (R) that simulated the soil runoff to marine coastal sediment connecting soil and sediment compartments (by a Teflon pipe) and the control microcosms (NR) without runoff of soil consisting of single sediment compartment.

Different conditions were applied in triplicate combining the presence of plant (P) or not (NP) in soil with the spiking of deuterated HC (C) or not (NC) in soil and sediment (Figure II.4 b):

- Soil without plant and without HC spiking in soil and sediment (NPNC)
- Soil without plant and with HC spiking in soil and sediment (NPC)
- Soil with plant (*Catapodium marinum*) and without HC spiking in soil and sediment (PNC)
- Soil with plant (*Catapodium marinum*) and with HC spiking in soil and sediment (PC)

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- Sediment without terrigenous inputs from soil runoff and without spiking of HC (NRNC)
- Sediment without terrigenous inputs from soil runoff and with spiking of HC (NRC)

It is worth noting that glassware, stainless steel or Teflon were used during experiment in order to avoid HC adsorption.

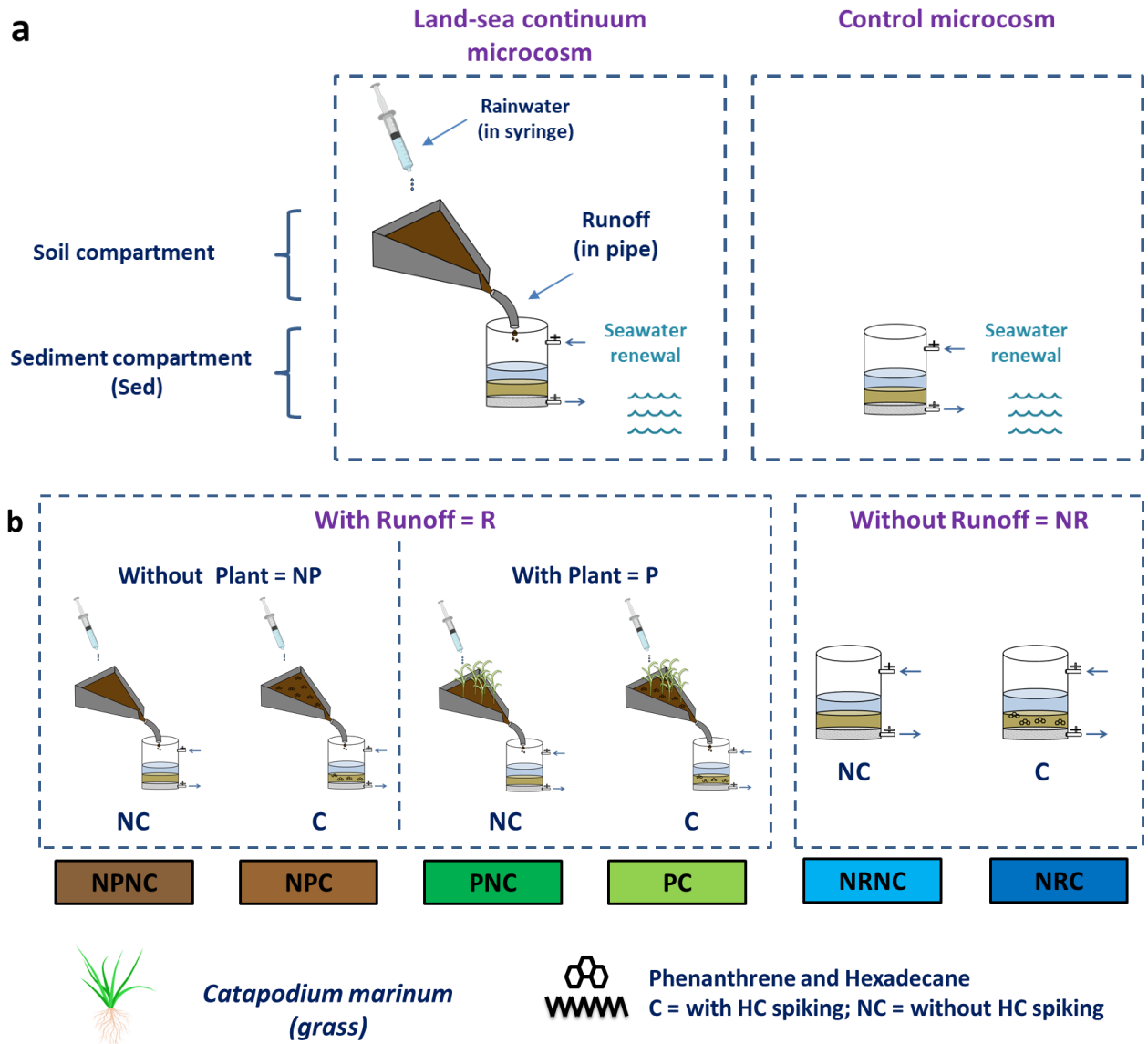


Figure II.4 Experimental device. (a) Schematic representation of the land-sea continuum microcosm and the control microcosm. (b) Schematic representation of the microcosms and the different applied conditions.

II.3.1. Deuterated hydrocarbon spiking solution

The hydrocarbon stock solution was a mix 1:1 of two model compounds of deuterated-hydrocarbons: the alkane, n-hexadecane (D34, 98%, Cambridge Isotope Laboratories, Inc, USA) and the polycyclic aromatic hydrocarbon (PAH), phenanthrene (D10, 98%, euriso-top, France).

Each hydrocarbon was at a concentration of 39,5 mg/mL in hexane. Both compounds were weighted (790 mg) and dissolved in 20 mL hexane. 14 glass bottles for the soil and 18 for the sediment were prepared corresponding to the different conditions. Each bottle contained 60 grams of dry soil or 10 grams of dry sediment (1/10 of the total matrix). They were respectively contaminated by 20 mL or 4 mL of a hexane solution containing 1,519 mL and 253 µL of HC stock solution, respectively. These high volumes of hexane are needed to cover the entire soil or sediment surfaces. In these 1/10 aliquots, the HC concentration is 10 times higher than the final ones. For the unspiked conditions, the hexane was added alone without addition of HC stock solution. After complete evaporation of the hexane solvent under fume hood (during 2,5 days), supplementary soil (540 g dry weight) was added in the bottles containing HC spiked soil to reach a homogenous mixture of 600 g of soil. The 10 g of HC spiked sediment were mixed in aluminium cup with 127 g of humid sediment (corresponding to ca. 90 g dry weight) to reach the equivalent of 100 g of dry sediment. Each HC was thus at a theoretical concentration of 100 mg/kg dry matrix.

II.3.2. Assembly of the devices and ageing

Soil was introduced in stainless steel compartments and humidified with 131 mL mineralised water (Volvic®) corresponding to 60% WHC. Sediment was introduced in homemade geotextile cups deposited on glass beads covering the bottom of glass aquatic devices. 200 mL of artificial seawater (Sel Instant Ocean, Aquarium systems) covered totally the sediment surface in the aquatic compartment (2-3 mm above). 25 seeds of *C. marinum* were sowed in 3-4 rows in the soil with plant (Soil P) conditions. All the devices were stored at 4°C during 4 days for aging period.

II.3.3. Incubation

In the phytotron chamber, the day/night cycle was 16/8 hours and the temperature fixed at 25°C. Individual syringes filled with mineral water (Volvic®) provided daily rain (73mL/day during 5 days and 2 days without rain) to the soil compartment. After germination of *C. marinum*, the number of plants was adjusted to 16 per soil compartment for repeatability. Two peristaltic pumps, one connected to a tank containing artificial seawater (Sel Instant Ocean, Aquarium systems) and the second one connected to a collection tank, created a tidal cycle (6h of rising tide and 6h of down tide) renewing seawater. The experiment took place for 39 days (Figure II.5).

II.3.4. Sampling

Samples of the different compartments (soil, runoff, sediment) and of seawater were collected at different times, at the beginning, at the end, and during the incubation for chemical and/or bimolecular analysis (Figure II.6).



Figure II.5 Photo of the experimental device in the phytotronic chamber during incubation at LIEC lab.

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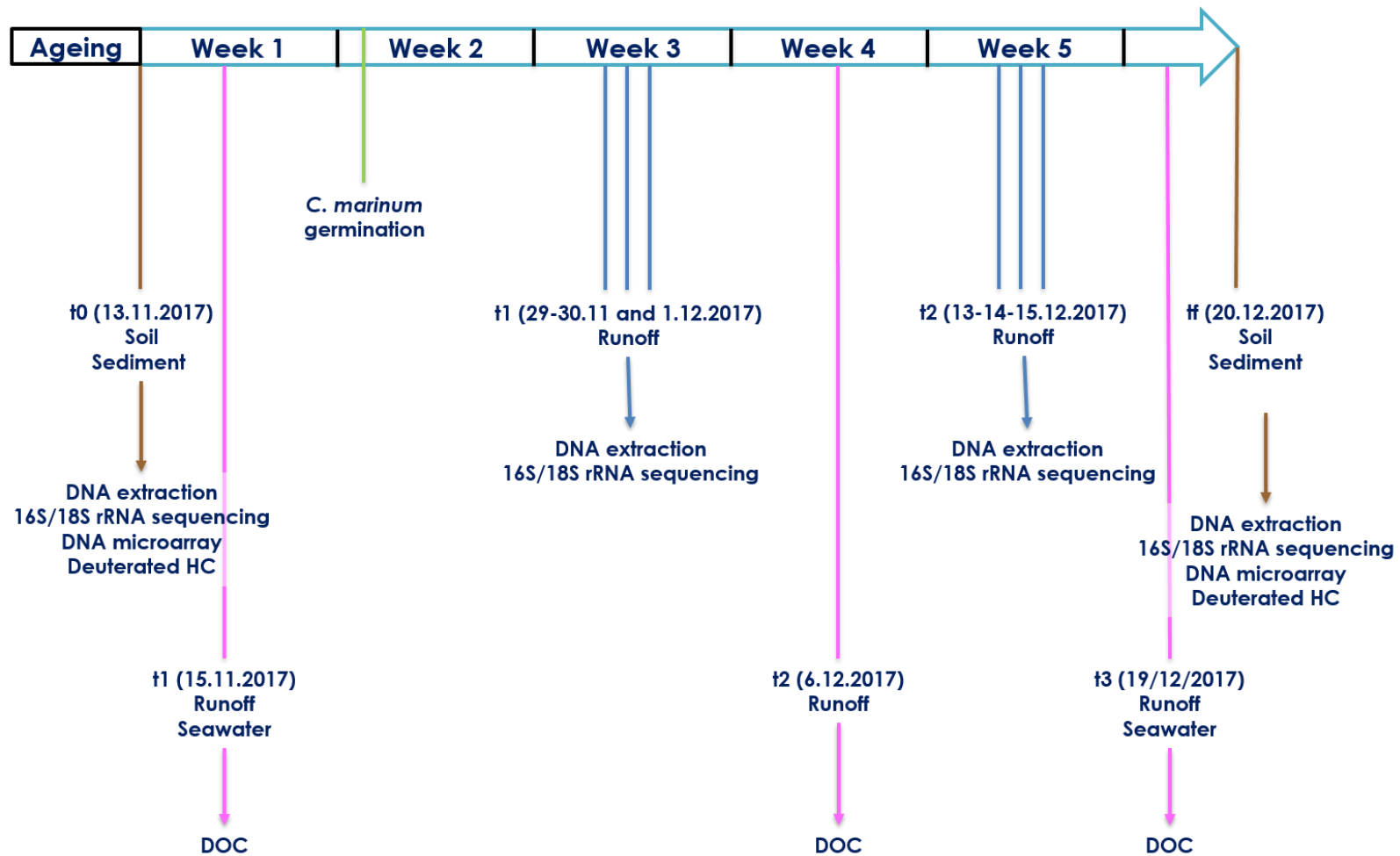


Figure II.6 Sampling schedule for biomolecular and chemical analysis. Samples were collected in triplicates for each condition applied in land-sea continuum and control microcosms (soil with plant or not, HC spiking or not in soil and sediment, with or without soil runoff). Brown arrows represent sampling of soil and sediment dedicated to biomolecular and chemical analysis. Pink arrows represent sampling of soil runoff and seawater dedicated to chemical analysis. Blue arrows represent sampling of soil runoff sampling dedicated to biomolecular analysis. DOC: dissolved organic carbon. Adapted from a schema of Aurélie Cébron (LIEC lab).

Detailed descriptions about the sampling, the biomolecular and chemical approaches and data processing are available in chapters III and IV (III.2.3 and 0). Briefly, soil and sediment were collected in triplicate in the different microcosms at the beginning and at the end of the incubation (respectively after the ageing period at t_0 and at t_f after 39 days of incubation) for further biomolecular and chemical analysis. Soil was sampled using little corer (14 mm diameter and 40 mm high; Bourceret et al., 2015) whereas sediment was collected using a spoon due to its structure (Figure II.7). Runoffs from each land-sea microcosms were collected after rain event 3 consecutive days (15 mL each day) and filtered at $0.22 \mu\text{m}$ (Millex) during the 3rd and the 5th weeks of incubation. Filters were used for further biomolecular analysis (Figure II.6). An aliquot of Volvic water (rainwater) was filtered in the same way, as a control. In addition, runoffs and seawaters (low tides) were collected during the first, the 4th (only runoffs) and the last weeks of incubation for chemical analysis.

Samples dedicated to chemical analysis were stored in glass tubes (Supelco) at -20°C while those dedicated to biomolecular analysis were stored in cryogenic tubes at -80°C .

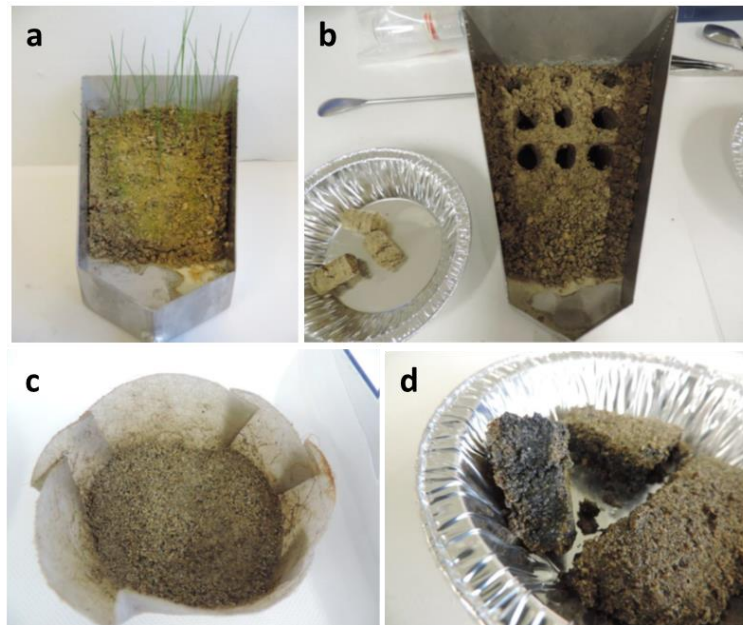


Figure II.7 Disassembly of the microcosms and sampling at the end of the experiment (t_f). (a) Planted soil compartment from a land sea-continuum microcosm at t_f . (b) Soil sampling with a corer. (c) Sediment compartment from a land sea-continuum microcosm at t_f (d) Sediment sampling with a spoon

II.3.5. Synthesis of biomolecular, bioinformatics, statistical and chemical techniques

The project was based on experiment in microcosms simulating the land-sea continuum and the coupling of biomolecular and chemical data using bioinformatics and biostatistics (Table II.1).

In order to describe the microbial communities, 16S and 18S rRNA gene sequencing were performed on samples from the different microcosms, after DNA extraction. Using microarray approach on DNA, the metabolic capabilities of soil and sediment communities were estimated. The taxonomic, phylogenetic and functional diversity profiles were analysed and compared using statistical analyses (Table II.1) implemented within R programming environment (R Core Team, 2020). The analyses described above were done at the IPREM lab.

In order to quantify the dissolved organic carbon (DOC) in runoff and seawater, samples were analysed by TOC-VCSH Analyzer with ASI-V switcher. The removal of the deuterated HC was measured in soil and sediment samples by gas chromatography-mass spectrometry (GC-MS) after dichloromethane (DCM) followed by methanol accelerated solvent extraction, at the LIEC lab. We attempted a detection and quantification of the deuterated HC metabolites by Fourier-transform ion cyclotron resonance-mass spectrometry (FTICR-MS) at the COBRA lab.

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Table II.1 Description of the main biomolecular, bioinformatics and biostatistical methods and techniques used during the thesis.

Description	Details	References/Supplier/External provider
BIOMOLECULAR TECHNIQUES		
DNA extraction	DNeasy PowerSoil Pro Kit	DNeasy PowerSoil Pro Kit Handbook (Qiagen)
Agarose gel electrophoresis	Agarose 1X in TBE with BET	
Qubit DNA quantification	dsDNA High sensitivity Assay Kit	User Guide: Qubit dsDNA HS Assay Kits (Thermo Fisher scientific)
16S/18S rRNA gene PCR	Table II.2 Table II.3 Table II.4	
NGS sequencing	Illumina-MiSeq	Genotoul platform (Toulouse, France)
DNA microarray	GeoChip 5.0S	Glomics Inc., Norman, OK, USA
BIOINFORMATICS TREATMENT		
Equipment	Pyrene: UPPA research computing cluster	UPPA https://git.univ-pau.fr/num-as/pyrene-cluster

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Description	Details	References/Supplier/External provider
Qiime2	The extensible, free, open source, and community developed next-generation microbiome bioinformatics platform	(Bolyen et al., 2018) https://qiime2.org/
Dada2	Package for modeling and correcting Illumina-sequenced amplicon errors	(Callahan et al., 2016) https://github.com/benjjneb/dada2
Silva Database	Ribosomal RNA database (Release 132)	(Pruesse et al., 2007) https://www.arb-silva.de/
STATISTICAL ANALYSIS		
R software		(R Core Team, 2020)
Mean comparisons	wilcox.test and kruskal.test in stats package	-
Correlations	cor.test in stats package	-
Analysis of variance (ANOVA)	anova in stats package	-
Linear Model	Model fitting : gls in nlme package	(Pinheiro et al., 2021)
	Model selection : AICc in AICcmodavg package	(Mazerolle, 2020)
	Least-squares means: lsmeans in lsmeans package	(Lenth, 2016)
Rarefaction	ggrare, rarefy_even_depth in phyloseq package	(McMurdie and Holmes, 2013)

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Description	Details	References/Supplier/External provider
Partitioning of the core and satellite group	Function used at IPREM lab	François Rigal and (Jeanbille et al., 2016)
Phylogenetic tree ultrametrization	chronos in ape package	(Paradis and Schliep, 2019)
Alpha-diversity indices	renyi in vegan package	(Oksanen et al., 2019)
	phyloseq package	(McMurdie and Holmes, 2013)
Distance matrix/dissimilarity	distance in phyloseq package	(McMurdie and Holmes, 2013)
Non-metric multidimensional scaling	metaMDS in vegan package	(Oksanen et al., 2019)
Permutational multivariate analysis of variance	adonis2 in vegan package	(Oksanen et al., 2019)
	RVAideMemoire package	https://CRAN.R-project.org/package=RVAideMemoire
Indicator species analysis	multipatt in Indicspecies package	(De Cáceres et al., 2010)
Graphs	ggplot2 package	(Villanueva and Chen, 2019)

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Description	Details	References/Supplier/External provider
CO-OCCURRENCE NETWORK		
Cytoscape Software		(Shannon et al., 2003)
Construction and analysis	coNet app: network construction analysis	(Faust and Raes, 2016)
	MCODE app : identification of modules	(Bader and Hogue, 2003)
CHEMICAL ANALYSIS		
Dissolved organic carbon (DOC) quantification	TOC-VCSH Analyzer with ASI-V switcher (Shimadzu, Japan)	(Boulangé et al., 2019)
HC extraction	accelerated solvent extraction of the HC in dichloromethane (DCM) and then in methanol	-
Quantification of the deuterated HC in the DCM extract	gas chromatography-mass spectrometry (Agilent GC-MS)	-
Quantification deuterated metabolites	Fourier-transform ion cyclotron resonance-mass spectrometry (FTICR-MS)	COBRA lab (Rouen, France)

Table II.2 Primers used for amplification of the 16S and 18S rRNA genes, targeting Prokaryotes and Fungi respectively.

Domain	Name	Sequence	Reference
Prokaryotes	515F	5'-GTGYCAGCMGCCGCGGTA-3'	(Parada et al., 2016)
	928R	5'-CCCCGYCAATTCMTTTRAGT-3'	(Wang and Qian, 2009)
Fungi	FF390	5'-CGA-TAA-CGA-ACG-AGA-CCT-3'	(Chemidlin Prévost-Bouré et al., 2011)
	FR1	5'-A6C-CAT-TCA-ATC-GGT-A6T-3'	

Table II.3 16S and 18S rRNA gene PCR mix.

Reagents	16S	18S
Normalized DNA (2 ng/μL) μL	1	2
ampliTaq Gold master mix μL	12.5	12.5
Forward primer (20 μM) μL	1	0.5
Reverse primer (20 μM) μL	1	0.5
DEPC water μL	9.5	9.5

Table II.4 16S and 18S rRNA gene conditions.

	Step	Temperature (°C)	Duration
	Initial denaturation	95	10'
35 cycles	Denaturation	95	30''
	Annealing*	60/50	30''/45''
	Elongation	72	40''
	Final elongation	72	10'

* respectively for 16S and 18S rRNA gene primer sets.

Chapter III:

New insights into microbial community coalescence in the land-sea continuum

Chapter III. New insights into microbial community coalescence in the land-sea continuum

III.1. Background of the study

Community coalescence is the wholesale exchange among microbial communities resulting from moving pieces of the environment containing entire assemblages (Rillig et al., 2015). Although this concept has been formalized fairly recently and is receiving increasing interest from microbial ecologists, environmental studies dedicated to community coalescence are still scarce. Most incidental findings from field studies have indicated that the phenomenon occurs frequently and is widespread, notably between terrestrial and freshwater microbial communities (Mansour et al., 2018), benefiting the understanding on the structure of microbial communities in mixing zone. Microbial community coalescence between terrestrial and marine systems has been evidenced in estuaries, well-described mixing zones, where river runoff brings organic and inorganic matter, and transmits impacts of human land-based activities. Noteworthy, these studies focused rarely on microbial benthic communities. In comparison, the coalescence has been neglected in the land-sea continuum, mixing zone where soil runoff (surface flow generated by rainfall) conveys terrigenous inputs (undiluted in river) to marine coastal sediment. In soil, roots limit runoff (Nagase and Dunnett, 2012), influence several parameters, and thus modify the terrigenous inputs. Downstream in the land-sea continuum, marine coastal sediment constitutes a sink for organic and inorganic matter coming from terrestrial and marine ecosystems (Bauer et al., 2013). Microbial communities inhabiting this environment play a key role in the carbon cycle and particularly in the degradation of hydrocarbons (HC), widespread pollutants on coasts. In this context, the objective of this chapter is to collect evidences of community coalescence and find out indicators of the mixing of microorganisms and of the environment. The results are presented as an article in preparation for submission in the ISME Journal.

III.2. New insights into microbial community coalescence in the land-sea continuum

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III.2.1. Abstract

The land-sea continuum constitutes a mixing zone where soil microbial communities encounter, via runoff, those inhabiting marine coastal sediment resulting in community coalescence. Here, we propose an experimental approach, mimicking the land-sea continuum in microcosm, to study the microbial community coalescence event in different situations, by 16S and 18S rRNA genes barcoding. Microbial communities in sediment receiving soil inputs showed different structure from those not receiving soil inputs. For prokaryotes, phylogenetic enrichment and ASVs replacements characterized the community changes in sediment receiving terrigenous inputs. For fungi, despite phylogenetic enrichment was not observed, the fungal ASVs richness was maintained by terrigenous inputs. Comparison of microbial communities revealed ASVs specific to sediments receiving inputs from soil, but also ASVs shared with soil and/or runoff. Among these specific ASVs, four bacterial ASVs and one fungal ASV were identified as indicators of coalescence. Our study provides a set of evidences that coalescence in land-sea continuum involves the mixing of both microorganisms and the environment.

Keywords: microcosm, community coalescence, runoff, marine coastal sediment, indicator analysis

III.2.2. Introduction

The land-sea continuum is a transition zone mixing terrestrial and marine coastal ecosystems, through diverse forms of connections, including direct inputs via rivers and flows resulting from soil runoff. Inputs from the riverine network into marine environment have been shown to significantly change environmental parameters (e.g. nutrients and suspended particulate matter, salinity, pH) (Brannock et al., 2016; Jeffries et al., 2016) and also transport microorganisms (Halliday et al., 2014; Yakirevich et al., 2013), resulting in community coalescence (Rillig et al., 2015). Studies of coalescence in marine environment have only considered the direct inputs via rivers in estuaries (Campbell and Kirchman, 2013; Crump et al., 2004, 1999; Fortunato and Crump, 2015; Mansour et al., 2018; Webster et al., 2015) while the inputs from soil runoff have been neglected within land-sea continuum. However, soil runoff, generated by rainfall, is widespread among coastal areas

and represents an important upload of terrigenous materials (plant debris, organic matter, mineral particles material and pollutants (Duran and Cravo-Laureau, 2016; Hoffman et al., 1984; Montgomery et al., 2000) that reaches directly the sea without dilution in river. The upload of terrigenous materials depend on the vegetation cover that modify water flow (Nagase and Dunnett, 2012; Wenzel, 2009) and control the soil microbial communities. Therefore, it is worth considering the contribution of soil runoff to microbial community structure in marine coastal sediment. Additionally, land-based activities dramatically alter microbial processes (Fredston-Hermann et al., 2016) by disrupting microbial communities influenced by biotic and abiotic factors (Cravo-Laureau and Duran, 2014; Duran et al., 2015; Pischedda et al., 2008), probably affected by terrigenous inputs from soil runoff. Several studies have shown the influence of terrigenous inputs in the organization of microbial communities in water column (Crump et al., 2004, 1999; Logares et al., 2009; Paver et al., 2018; Wisnoski et al., 2020), but information is missing for marine sediment. Microbial communities play a pivotal role in the biogeochemical processes influencing ecological services (Falkowski, Fenchel and Delong 2008; Newton et al.2011; Nelson, Martiny and Martiny 2016). Among ecosystem services, the activity of microbial communities is crucial to mitigate the effect of pollutants (Grizzetti et al., 2019), particularly hydrocarbons (HC) (McGenity et al., 2012) that accumulate in marine coastal sediment (Tolosa et al., 2004).

We hypothesize that soil runoff modifies microbial communities inhabiting HC contaminated marine coastal sediment according to the vegetation cover. In order to test the hypothesis, an experimental ecology approach was set up to mimic soil runoff within land-sea continuum in microcosm. Various situations were considered in soil and sediment compartments (with or without HC addition, soil with or without plant). Microbial communities were described by Illumina sequencing of the 16S and 18S rRNA genes allowing their comparison in the different situations. Specific changes in marine sediment microbial community due to coalescence event were revealed by community statistics.

III.2.3. Material and Methods

III.2.3.1. Soil and sediment collection

Soil (10 kg) and sediment (2 kg) were collected in the same geographical area, in southeast of France, in October 2017. Soil came from the surroundings of the Berre Pond (43°29'6.9"N 5°11'18.53"E, France) and sediment from the Canal Viel Cove (43°23'20.3"N 4°59'31.8"E, France). The sites are closed to petroleum refinery and constitute a model of a realistic hydrocarbons contaminated land-sea continuum. Prior to microcosms setup, soil and sediments were sieved to <4 mm (and <1 mm, respectively to homogenize, remove coarse gravels, plant debris and macroorganisms. The whole soil sample and a part of the sediment sample were air dried under a cupboard. The rest of the sediment was stored at 4 °C in a 5L bottle completely covered with in

situ seawater and with continuous aeration through an aquarium pump. Soil and sediment characteristics were determined on dry samples at the LAS (INRA, Arras, France). The soil had a loamy and sediments a sandy texture (23.4% and 4.7% clay, 38.5% and 4.5% silt, 38.1% and 90.8% sand, for soil and sediment respectively). Both soil and sediment showed basic pH (8.4 and 9.1 for soil and sediments, respectively). The C/N ratio was 30.0 and 46.6 for soil and sediments, respectively. The organic carbon content and the total nitrogen were higher in soil than in sediments (35.4 and 18.6 gC/kg, 1.2 and 0.4 gN/kg). The cation-exchange capacity estimated by cobaltihexamine method is higher in soil than in sediments (15.9 and 1.4 cmol+/kg).

III.2.3.2. Microcosms Setup

An original microcosm system was set up and incubated during 39 days under controlled conditions at the laboratory (Figure III.1). The experimental design was drawn with the aim to test the hypothesis that the soil runoff influences the structure and the composition of microbial communities in downstream sediments depending on the presence of plants and/or on a HC pollution event. For this purpose, two kinds of microcosms were set up (Figure III.1 A): the land-sea continuum microcosms (R) that simulated the soil runoff to marine coastal sediment connecting soil and sediment compartments and the control microcosms (NR) without runoff of soil consisting of single sediment compartment. Briefly, the microcosms were composed of glass devices (1L beaker equipped with inlet and outlet) containing the equivalent of 100 g of dry sediment covered with seawater (Salt Instant Ocean, Aquarium systems). These devices were connected through Teflon pipes or not to stainless steel devices containing 600g of moistened soil at 60% WHC. Different conditions were applied in triplicate combining the presence of plant (P) or not (NP) in soil with the spiking of deuterated HC (C) or not (NC) in soil and sediment (Figure III.1 B and C). The plant used was a grass, *Catapodium marinum* (seed bank of the Muséum National d'Histoire Naturelle de Paris - DGD – MJZ), after seed germination 16 plants were kept per devices. In the HC spiked conditions, soil and sediment (1/10 of the soil and sediment put in the devices) received a mix 1:1 (W:W) of deuterated n-hexadecane (D34, 98%, Cambridge Isotope Laboratories, Inc, USA) and phenanthrene (D10, 98%, euriso-top, France), dissolved in hexane solvent. A stock solution was prepared in order to add each HC at a concentration of 100 mg/kg dry soil or sediment. For the unspiked conditions, the same volume of hexane was added alone without addition of HC stock solution. In land-sea continuum microcosms, soils were watered to reach 60% of their WHC and were daily watered by means of a syringe dispensing 73 ml mineral water (Volvic®) to mimic rainfall and produce soil runoff and in turn terrigenous inputs. Both in land sea continuum and in control microcosms, peristaltic pumps helped to simulate a 12 h tidal cycle. In this way, the seawater was renewed twice a day maintaining sediment covered by 3 cm of seawater during the low tide ensuring conditions close to those prevailing in natural environment.

New insights into microbial community coalescence in the land-sea continuum

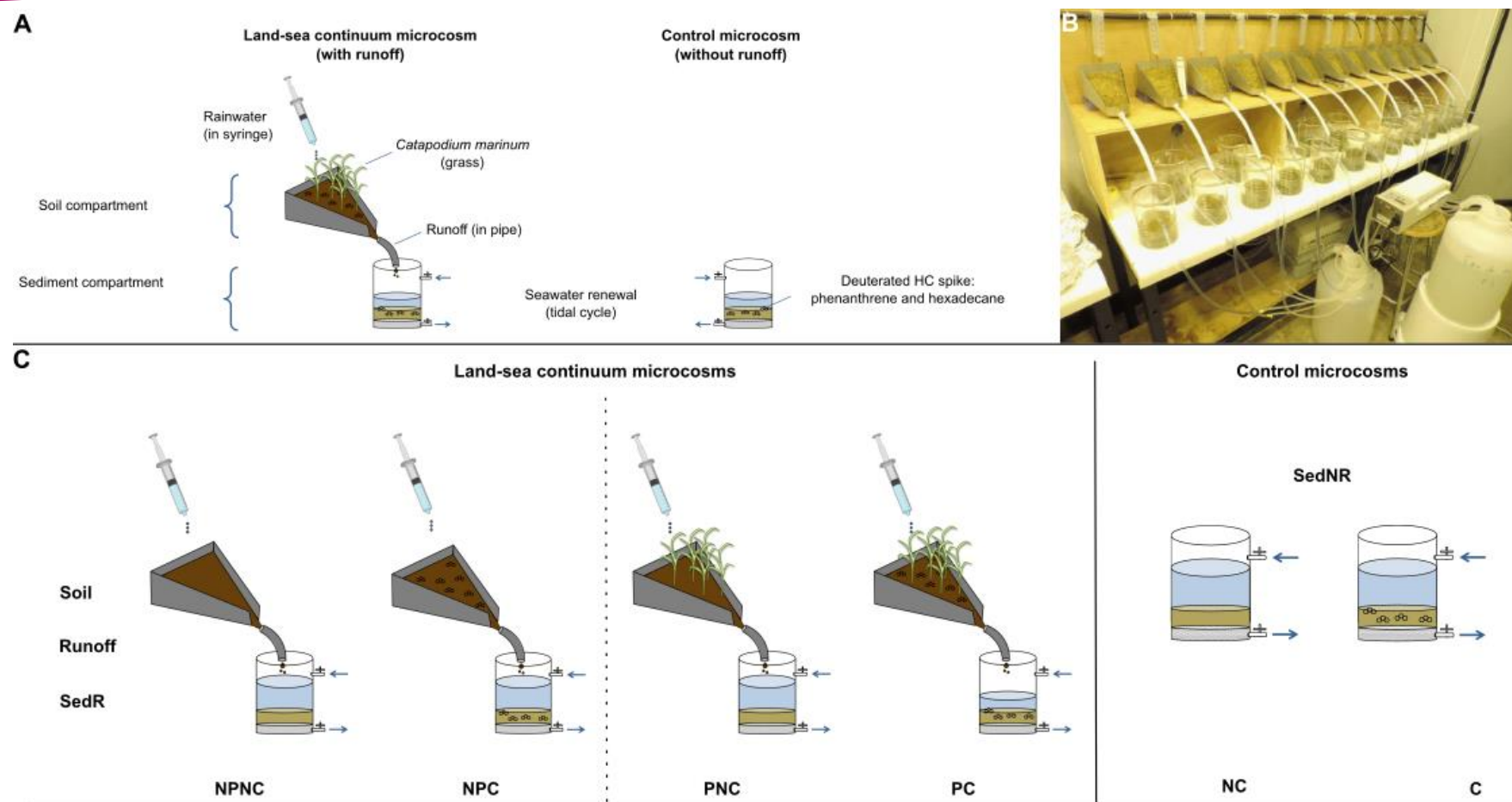


Figure III.1 Experimental design (A) Schema of the land-sea continuum microcosm mimicking the terrigenous inputs to marine coastal sediment by soil runoff (left). Control microcosm (right) corresponds to sediment without soil runoff. (B) Photo of the experiment. (C) Schematic representation of the microcosms with the different conditions applied to the soil and sediment compartments: Soil: soil; Runoff: runoff water (with terrigenous inputs); Sed: sediment; SedR: sediment receiving runoff; SedNR: Sed without runoff; NP: non-planted; P: planted; NC: without HC spiking; C: HC spiking; t0: beginning of the experiment; t1: 16 days of incubation; t2: 30 days of incubation; tf: end of the experiment after 39 days of incubation. Each condition was performed in triplicate.

III.2.3.3. Sampling

Samples of the different compartments (soil, runoff, sediment) and of seawater were collected at different times at the beginning, at the end, and during the incubation for subsequent bimolecular analysis. Soil and sediment samples (ca. 10-12g) were collected in triplicates at the beginning and end of the experiment (t₀ and t_f respectively) for the DNA extraction. Soil samples were collected using a little stainless steel corer (diameter: 14mm; height: 40 mm, Bourceret et al., 2015) and sediment samples using a spoon. At t₀, three soil samples (each being the mixture of 3 cores) were collected from additional soil devices, one with HC spiking and one without HC spiking, so as not to alter soil structure or disturb plant germination in devices that continued the incubation. Three sediment samples (each being the mix of 3 spoons) were collected from the sediment batch prepared for sediment devices with HC spiking (NPC, PC, NRC conditions) and from the one prepared for sediment devices without HC spiking (NPNC, PNC, NRNC conditions). At t₁ and t₂ (after 16 and 31 days of incubation respectively), runoff samples were obtained from each land-sea microcosms, by mixing runoff from three consecutive days and filtered at 0.22 µm (Millex). An aliquot of Volvic water (rainwater) was filtered in the same way as the runoff to serve as a control. At t_f (after 39 days of incubation), *Catapodium marinum* were cut at the collar before soil sampling. Soil samples were collected in triplicate from each land-sea continuum microcosm using a template (three notches per row and three per column) as a guide for the corer. The cores from the same column were mixed and formed one sample. Sediment samples were collected from each microcosm in triplicate after mixing of sediment using a spoon. Thus, the sampling design provided three technical replicates for each of the three biological replicates, for soil and sediment from each microcosm. Soil, runoff (filter) and sediment samples were stored at -80°C.

III.2.3.4. DNA extraction and rRNA gene sequencing

DNA was extracted from 250 mg of soil or sediment sample and from the filter of runoffs using the PowerSoil DNA Isolation Kit (MoBio Laboratories) according to the manufacturer's recommendations. PCR amplification of the V4-V5 hypervariable region of the 16S rRNA genes were performed using the Archaea/Bacteria primers 515F (5'-GTG-YCA-GCM-GCC-GCG-GTA-3') and 928F (5'-CCC-CGY-CAA-TTC-MTT-TRAG-T-3') (Parada et al., 2016; Wang and Qian, 2009). Likewise, 18S rRNA genes were also amplified using the Eukaryotes primers FF390 (5'-CGA-TAA-CGA-ACG-AGA-CCT-3') and FR1 (5'-AIC-CAT-TCA-ATC-GGT-AIT -3') targeting the V7-V8 region (Chemidlin Prévost-Bouré et al., 2011; Vainio and Hantula, 2000). The reverse and forward primers included Illumina adapters. Before amplification, the concentration of DNA was determined with a Quant-It kit in microplate in order to normalize the following amplification. Amplifications were performed in triplicate using AmpliTaq Gold 360 Master Mix (Applied Biosystems) to prepare 25 µL

reactions containing 2 ng (Archaea/Bacteria) or 4 ng (Fungi) of DNA. Cycling conditions were: an initial denaturation step at 95°C for 10 minutes, followed by 35 cycles with denaturation step at 95°C for 30 s, hybridization step at 60°C for 30 s or at 50°C for 45 s (for Archaea/Bacteria primers and Fungi primers, respectively), and elongation step at 72°C for 40 s or 30 s (for Archaea/Bacteria primers and Fungi primers, respectively), after which a final elongation step at 72°C for 10 minutes was performed. Sequencing by synthesis was carried out at the Genotoul platform (Toulouse, France) with Illumina-MiSeq. The 2x 250 paired-end sequencing gave a full length 411 bp fragment with a 87 bp overlap for the V4-V5 region and a 450 bp fragment with a 150 bp overlap for the V7-V8 region.

III.2.3.5. Sequence processing and ASV delimitation

The open source software Qiime2 (Bolyen et al., 2018) was used for 16S and 18S rRNA gene reads analysis. Sequencing resulted in 3,735,622 and 3,716,691 raw reads for prokaryotic and fungal datasets, respectively. The rarefaction curve (Figure S III.1) showed that most of the diversity was captured during the sequencing. The two datasets were first demultiplexed and dada2 (Callahan et al., 2016) was used to control reads quality and infer amplicon sequence variants (ASVs)(Callahan et al., 2017). Default settings were used except that reverse reads were truncated to 230 base pairs before merging. This denoising step resulted in 6,988 and 2,488 ASVs for prokaryotic and fungal datasets, respectively. The taxonomy was assigned to the ASVs with a fixed sequence similarity threshold of 97% using the SILVA database (Quast C et al, 2013) release 132 for 16S and 18S rRNA gene sequences. Then, the datasets were mitochondria, chloroplasts and unassigned (at the domain level) filtered. Bioinformatics treatment resulted in 6,748 prokaryotic ASVs and 705 fungal ASVs. Phylogenetic trees were constructed using FastTree (Price et al., 2010) in the Qiime2 phylogeny plugin. The phylogenetic tree was made ultrametric for the following analyses using the "chronos" function in the R package ape (Paradis, Claude and Strimmer 2004) with branch lengths representing relative time and all tips equidistant from the root. The read abundances for each sample were rarefied to the same number corresponding to the minimum read number per sample (7,578 sequences) for prokaryotic dataset and to the third smallest one for fungal dataset (1,027 sequences) within R software (phyloseq package). The thresholds are trade-offs reflecting correct sampling effort and preserving most of the samples according to the rarefaction curves. The prokaryotic dataset encompassed 66 samples whereas fungal datasets 64 samples. The complete dataset was deposited in the NCBI Sequence Read Archive (SRA) database xxx and is available under the Accession number xxx. The following statistical analyses were implemented within the R programming environment (R Development Core Team, 2019).

III.2.3.6. Alpha and beta diversity of the core microbial communities

First, the correlation between the abundance and the occurrence of each ASV calculated throughout the dataset and their index of dispersion were evaluated in order to partition ASVs into core and satellite group (Hanski, 1982; Magurran and Henderson, 2003; Ulrich and Zalewski, 2006). For that purpose, we followed the method implemented in Jeanbille et al. (2016) where ASVs were considered as core if their index of dispersion deviated from a random Poisson distribution fall outside the 95% confidence interval of the χ^2 distribution. The subsequent analysis were focused on the core group. Microbial alpha diversity and beta diversity were quantified at the taxonomic level with the richness (R) and the Inverse Simpson index (2D). Their phylogenetic equivalents, the Faith's phylogenetic diversity index (Faith's PD) and the Hill2 phylogenetic diversity index (2PD) were used to estimate the phylogenetic alpha diversity. Jaccard and wUNIFRAC distances were calculated taking in account the abundances from the ASV tables square root transformed to measure respectively the taxonomic and the phylogenetic beta diversity. Then, the total β -diversity (β_{tot}) was partitioned into its replacement (β_{repl}) and richness difference (β_{rich}) components according the method developed by Podani & Schmera (2011), Carvalho et al. (2012), and Cardoso et al. (2014) to decipher the different process underlying the change in community assembly. Patterns of community composition were visualized by a non-metric multidimensional scaling (NMDS). PerMANOVA (permutational multivariate analysis of variance) based on 9999 permutations were implemented to test for differences among conditions using the different linear models. Levins' niche breadth index (Feinsinger et al., 1981) was calculated for core and satellite ASVs using levins.Bn function in the R package MicroNiche.

III.2.3.7. Indicator species analysis

An indicator analysis was performed to identify ASVs that were associated with conditions or a combination of conditions using the Dufrene and Legendre indicator value (ie. IndVal) (Dufrene and Legendre, 1997) in the indicpecies package. IndVal index quantifies the strength of the association measuring specificity and fidelity of ASV distribution. Indicator value range from 0 (no association) to 1 (perfect association). Significance of IndVal values were assessed using permutation tests (N=9999) under the null hypothesis of no association.

III.2.4. Results and discussion

III.2.4.1. Partitioning the microbial community into core and satellite groups

The experiment was specifically designed to investigate the microbial coalescence in a soil-sea sediment continuum i.e. the contribution of soil and soil microbial community inputs (via soil runoff) in shaping the marine coastal sediment microbial community. For this purpose, the microbial

community composition of the different compartments (soil, runoff, and sediment), collected at the beginning and at the end of the experimental incubation, was determined by high throughput sequencing. For the prokaryotic and fungal communities, 3 735 622 and 3 716 691 raw reads were respectively obtained (Table S III.1). After trimming and rarefaction, 7578 and 1027 sequences were respectively affiliated to 6064 prokaryotic and 638 fungal ASVs (Table S III.1). The ASVs abundance distribution showed that the microbial communities were distributed into core and satellite ASVs groups, the mean ASVs abundance being positively linked to the occurrence (Figure S III.2A), as already evidenced (Hu et al., 2017; Jeanbille et al., 2016; Lindh et al., 2017). The core and satellite ASVs were identified by examining the relationship between the index of dispersion and occurrence of each ASV (Figure S III.2 B). Most of the microbial ASVs (6064 prokaryotic and 638 fungal ASVs) fitted a random distribution (Poisson model) defining the satellite ASVs. In contrast, 1602 prokaryotic and 173 fungal ASVs formed the non-randomly distributed core ASVs. This core microbial community contained less than 30 % of the total ASVs, accounting for more than 80 % of the total reads. In addition, the core microbial community had a significantly wider (Wilcoxon test, $p < 0.001$) niche breadth (Breadth niche index ~ 0.18) than the satellite community ($0.07 < \text{Breadth niche index} < 0.08$) (Figure S III.3). Wide niche breadth is recognized as a feature of microbial generalists (Brown, 1984, 1984; Jeanbille et al., 2016), thus the microbial core community corresponds to such generalists, able to use widespread resources and cope with environmental disturbances. It has been demonstrated that the core microbial community is not randomly distributed and brings information reporting the effect of the environmental factors (Magurran and Henderson, 2003; Ulrich and Zalewski, 2006). Thus, in order to characterize the microbial communities inhabiting mixing zone influenced by soil runoff, we focused on the core microbial community.

III.2.4.2. Diversity of the core microbial community

Using taxonomic and phylogenetic alpha diversity indices, the effects of plant, HC spiking and soil runoff were investigated in the core microbial communities inhabiting soil and sediment (Figure III.2). Prokaryotic and fungal soil richness (R) and the inverse Simpson (2D) indices remained stable the course of the experiment while the Faith's PD increased (ANOVA 16S: $F = 5.07$, $p < 0.05$; ANOVA 18 S: $F = 12.65$, $p < 0.05$), irrespective of the presence of plants (NP, P), with a significant additional increase (Tukey test: Soil C t0 – Soil C tf, $p < 0.05$) in hydrocarbon spiking samples (Figure III.2A and B). It was surprising to observe that the presence of plants was not affecting the soil microbial diversity although plant roots exudates have been shown to provide nutrients (Van Hecke et al., 2005) and promote the development of microorganisms carrying hydrocarbon degrading genes (Cébron et al., 2011; Toyama et al., 2011). Regarding the sediment microbial diversity, similar trends were observed for the prokaryotic communities with a significant temporal increase of the Hill2 phylogenetic diversity index (2PD) (ANOVA 16S: $F = 6.79$, $p < 0.05$), while the

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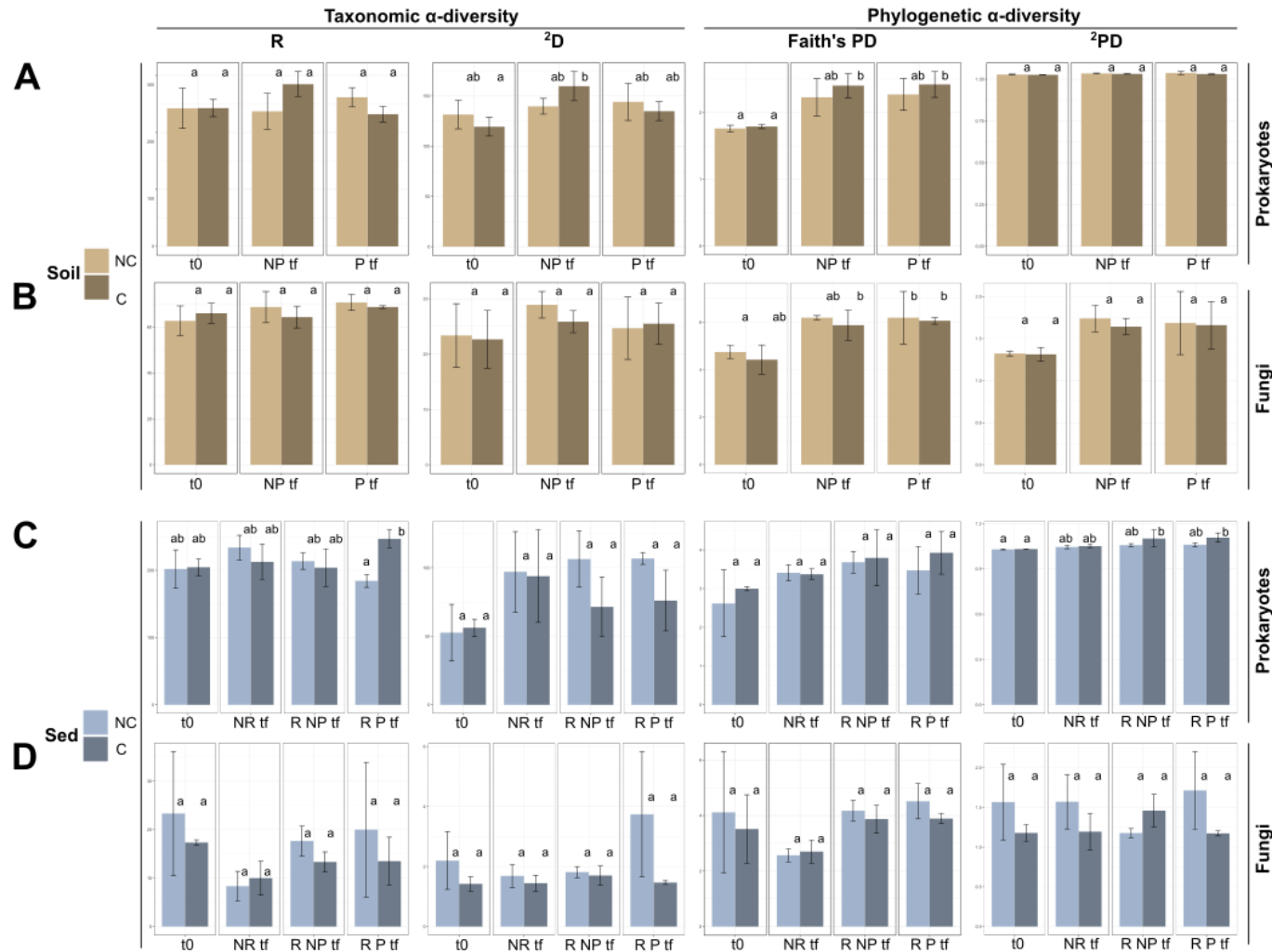


Figure III.2 Microbial α -diversity in soil and in sediment. Taxonomic (left) and phylogenetic (right) α -diversity indices for the prokaryotic (A) and the fungal (B) communities in soil, and the same indices for the prokaryotic (C) and the fungal (D) communities in sediment, depending of the different conditions. Letters represent pairwise contrasts ($p < 0.05$) of sample conditions. R: richness; $2D$: Inverse Simpson index; Faith's PD: Faith's phylogenetic diversity index; Hill 2- PD: phylogenetic diversity index based on Hill 2 number; Soil: soil; Sed: sediment; R: with runoff; NR: sediment without runoff; NC: without HC spiking; C: with HC spiking; P: planted; NP: non-planted; t0: beginning of the experiment; tf: end of the experiment (after 39 days of incubation).

fungal diversity was not affected (Figure III.2 C and D). Sediment receiving terrigenous inputs and with HC spike had the highest prokaryotic 2 PD, showing increased phylogenetic enrichment due to both runoff and HC spiking (Figure III.2 C, Tukey test: Sed t0 - SedR C ff, $p < 0.05$). Such hydrocarbon effect on prokaryotic community was consistent with previous studies showing i/ modification of microbial communities after petroleum addition (Bordenave et al., 2004; Chronopoulou et al., 2013; Stauffert et al., 2014), and ii/ an increase of microbial diversity, especially in historically hydrocarbon contaminated sediments (Duran et al., 2015; Jeanbille et al., 2016; Paissé et al., 2008). The diversity increase was explained by the legacy effect of previously exposed microbial communities to pollutants (Ben Salem et al., 2019) and environmental disturbances (Jurgens et al., 2017; Nunes et al., 2018). In our study, the increased diversity observed in the sediments receiving terrigenous inputs suggested that runoff transferred microorganisms from soil to sediments, which probably resulted in microbial community coalescence as reported for river sediments (Yin et al., 2015; Mansour et al., 2018), relating modifications in the microbial community composition.

III.2.4.3. Microbial composition within the land-sea continuum

In order to have an overview of the composition of the microbial community within the land-sea continuum, the relative abundance of the phyla in the different compartments (soil, runoff, sediment) was examined. Overall, most ASVs were affiliated to nine bacterial and eight fungal phyla (Figure III.3). Proteobacteria dominated the prokaryotic communities (Figure III.3 A) while the Ascomycota dominated the fungal communities, except in runoff in which most fungi were unassigned (Figure III.3 B). As expected, the compartments along the continuum (soil, runoff, and sediment) exhibited different microbial community composition. Regarding the prokaryotic community, soil was characterized by the presence of Acidobacteria, Actinobacteria and Betaproteobacteria (23 %, 21 % and 8 %, respectively). Actinobacteria and Betaproteobacteria are typically found in HC polluted soil (Greer et al., 2010), whereas Acidobacteria is generally more abundant in pristine soil (Xu et al., 2014). Proteobacteria (60 %) and Bacteroidetes (~20%) predominated in runoff and sediment (Figure III.3 A). As in soil, the runoff was dominated by Betaproteobacteria (23 %), whereas in sediment Gamma and Deltaproteobacteria (32 and 19 %, respectively) prevailed. This observation is in accordance with previous studies in HC contaminated coastal sediments (Quero et al., 2015; Sun et al., 2013), where Gammaproteobacteria and Deltaproteobacteria play an important role in HC degradation (Acosta-González and Marqués, 2016; Stauffert et al., 2015). The prevalence of Bacteroidetes in the two aquatic compartments (runoff and sediment) is in line with their life style, characterized by gliding motility and particle attachment (Fernández-Gómez et al., 2013) requiring a solid support, including HC and other organic compounds (Yakimov et al., 2007; Alonso et al., 2007; Musat et al., 2006). Regarding the fungal community (Figure III.3 B), Ascomycota was the dominant phylum in

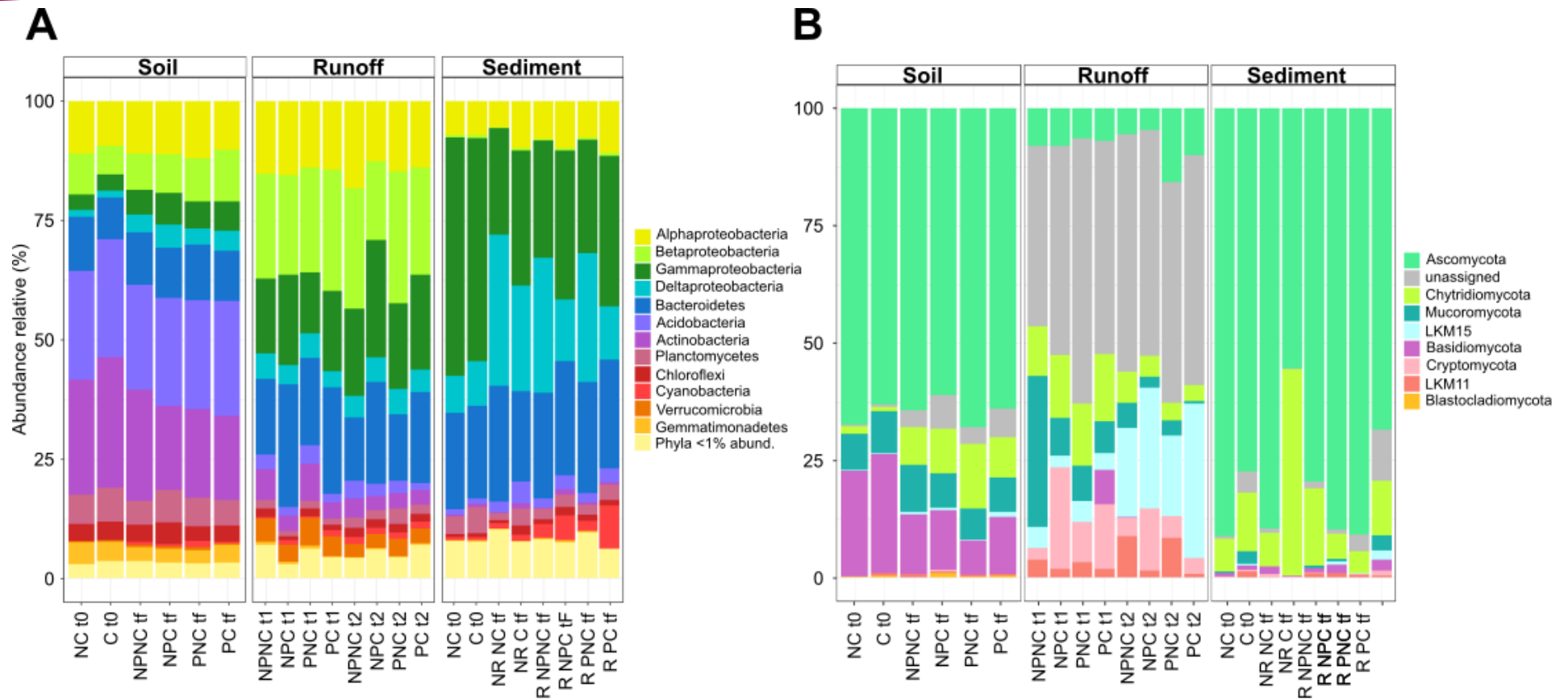


Figure III.3 Composition of the prokaryotic (A) and the fungal (B) communities in soil (Soil), runoff water (Runoff) and sediment (Sed), depending of the different conditions (NC: without HC spiking; C: with HC spiking; NP: non-planted; P: planted; NR: without runoff; R: with runoff; t0: beginning of the experiment; t1: 16 day of incubation; t2: 30; tf: end of the experiment (after 39 days incubation). The analysis was performed at the phylum level with Proteobacteria split into classes. Phyla <1% abund.: phyla with total relative abundance less than 1%, namely, *FBP*, *Cloacimonetes*, *Deinococcus-Thermus*, *WPS-2*, *Epsilonproteobacteria*, *Proteobacteria unassigned*, *Nanoarchaeaeota*, *Zixibacteria*, *Elusimicrobia*, *Dependentiae*, *Dadabacteria*, *Tenericutes*, *Marinimicrobia* (SAR406 clade), *Rokubacteria*, *Calditrichaeota*, *Spirochaetes*, *Patescibacteria*, *Armatimonadetes*, *Latescibacteria*, *Omnitrophicaeota*, *Nitrospirae*, *Fusobacteria*, *Fibrobacteres*, *Firmicutes*, *Thaumarchaeota*, *unassigned* phyla.

soil and sediment (65 and 80 %, respectively), in agreement with previous reports showing that this phylum is dominating a wide range of ecosystems (Amend et al., 2019; Bahram et al., 2018; Kohlmeyer and Kohlmeyer, 1979). The second most abundant fungal phylum was Basidiomycota (15 %) in soil and Chytridiomycota (14 %) in sediment, which is consistent with the fact that this phyla have been found in diverse ecosystems (Amend et al., 2019; Comeau et al., 2016). In runoff, most fungi were unassigned (almost 50 %), showing that a large part of soil fungal diversity transported by soil runoff remains unknown and corresponds to the low abundant soil fungal taxa. Apparently, simulated soil runoff in controlled conditions represents a way to access the undescribed soil fungal diversity. Overall, at the phylum level, distinct microbial communities were identified in each compartment consistent with the α -diversity analysis. Thus, the experimental design allows the study of coalescence by mixing different microbial communities.

III.2.4.4. Comparison of microbial communities

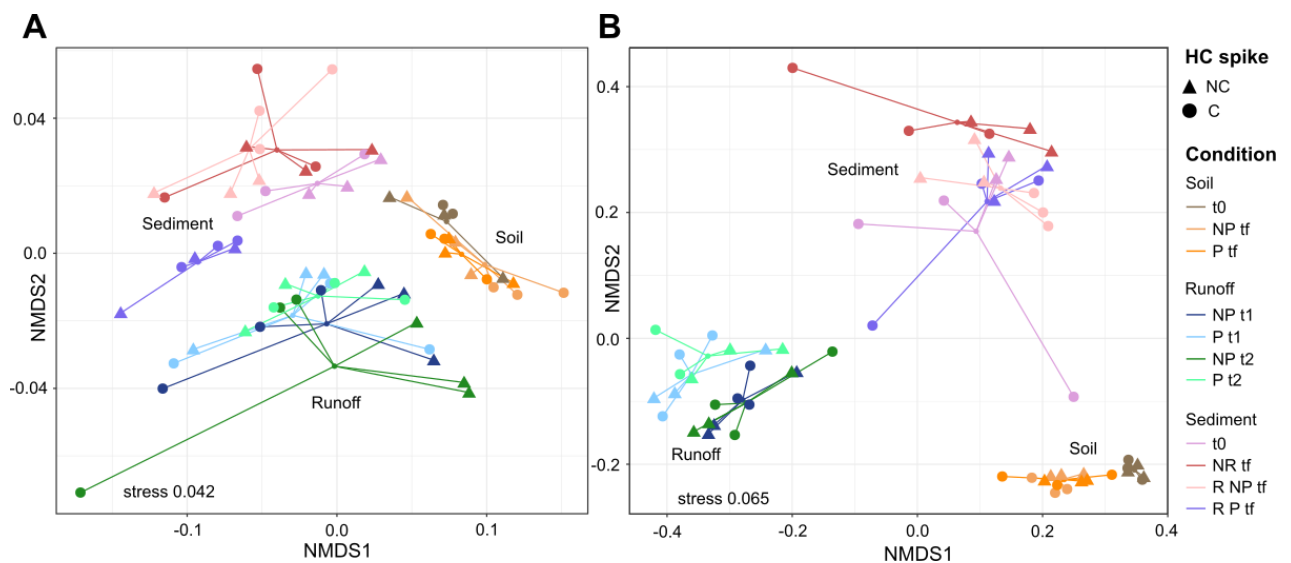


Figure III.4 NMDS analysis based on the weighted UniFrac metrics presenting the differences observed in the prokaryotic (A) and in the fungal (B) community composition of soil, runoff and sediment compartments. Coloured segments connect samples with the same plant condition and sampling date to their average point while shapes indicate if samples were HC spiked (circle) or not (triangle). Soil: soil; Runoff: runoff; Sed: sediment; NR: without runoff (control); R: with runoff; NP: non-planted; P: planted; NC: without HC spiking; C: with HC spiking; t0: beginning of the experiment; t1: 16 days of incubation; t2: 30 days of incubation; tf: end of the experiment.

In order to characterize the coalescence, deeper comparisons between microbial communities were performed, particularly investigating microbial community turnover and/or richness shift due to terrigenous inputs, using β -diversity indices and partitioning. The comparison of the microbial communities (prokaryotic and fungal) showed three clusters corresponding to the three

compartments soil, runoff and sediment (Figure III.4). Significant differences of the taxonomic and phylogenetic microbial composition between the compartments (Figure III.4) were observed. Such observations highlighted that each compartment contained specific ASVs and lineages, in accordance with habitat filtering (Lozupone and Knight, 2007). The fact that runoff microbial communities (prokaryotic and fungal) were distinct from those of soil (Figure III.4) showed that the microbial component of the terrigenous inputs represented only part of the soil diversity. This result suggests that only a subset of soil microorganisms and soil aggregates was able to flow in runoff. In both soil and runoff, the microbial taxonomic and phylogenetic composition changed with time and with the HC spiking, irrespective of the presence of plant (Figure III.4 and Table S III.2). In sediment, the prokaryotic community behaviour was different to that of the fungal community (Figure III.4 and Table S III.2). Two clusters separated the prokaryotic community according to time (Figure III.4 A), while the fungal community was separated into two clusters according to runoff treatment (Figure III.4 B). For prokaryotes, the clusters contained different ASVs corresponding to the replacement of ASVs and lineage changes at the end of the experiment (Table S III.2). The effect of the terrigenous inputs was not observed, despite that α -diversity showed an enrichment, probably hidden by the effect of time. For fungi, the clusters contained different ASVs corresponding to ASV and lineage richness modifications, explained by the loss of richness in sediment without terrigenous inputs (SedNR tf) (Table S III.2). Such observation probably reflects the absence of connection between soil and sediment. In contrast, the fungal ASVs richness was maintained by terrigenous inputs, suggesting that the lack of runoff affect the fungal community. It is likely that the terrigenous inputs simulated in our experiment mimicking in situ coalescent event explained the resilience of the fungal community in sediment observed only with runoff.

The microbial communities from the three compartments were further compared by Venn diagrams in order to identify the shared and specific microbial ASVs (Figure III.5). Any ASVs shared by the compartments was identified for the prokaryotic communities (Figure III.5 A), while five shared ASVs were identified for the fungal communities (Figure III.5 B). This result corroborates the structuration of the microbial communities according to the compartments. The highest number of shared ASVs were observed between soil and runoff for prokaryotic and fungal communities (336 ASVs Figure III.5 A, 27 ASVs Figure III.5 B), as expected since β -diversity analysis showed that runoff is a subset of soil. Interestingly, runoff was mostly constituted of specific prokaryotic ASVs (408 ASVs, Figure III.5 A), supporting the hypothesis that a large part of soil microbial diversity transported by soil runoff corresponded to the low abundant soil taxa. The sediment receiving terrigenous inputs exhibited 48 prokaryotic (Figure III.5 A) and three fungal specific ASVs (Figure III.5 B), which indicates that these ASVs were below the detection threshold in both soil and sediment at the beginning of the experiment (Soil t0 and Sed t0), they emerged in the sediments receiving terrigenous inputs at the end of the experiment (SedR tf).

New insights into microbial community coalescence in the land-sea continuum

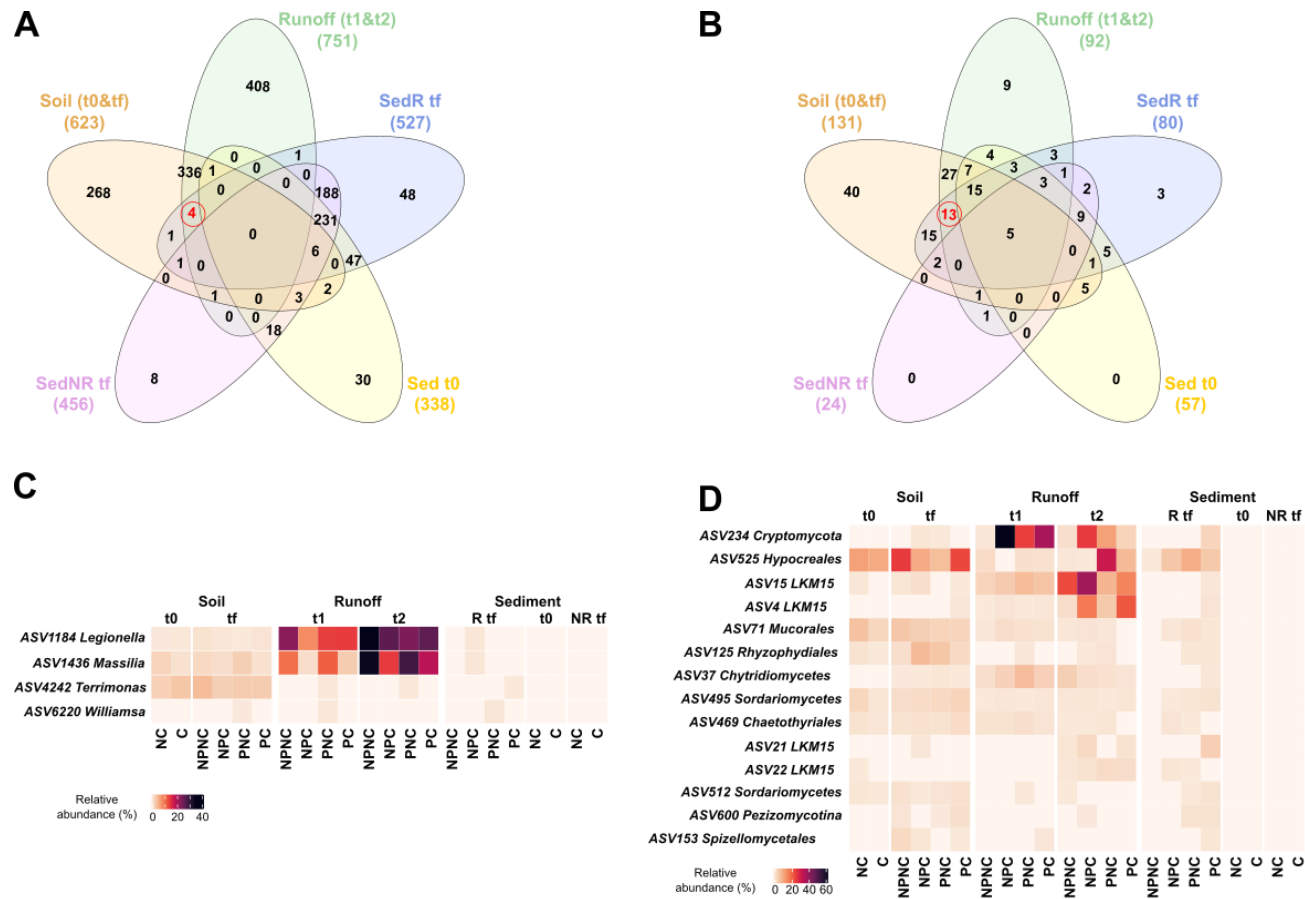


Figure III.5 Venn diagrams of prokaryotic (A) and fungal (B) core communities from the different compartment. Soil (t0&tf): ASVs present in soil at the beginning (t0) and at the end of the experiment (tf); Runoff (t1&t2): ASVs present in runoff after 16 (t1) and 30 (t2) days of incubation; SedR tf: ASVs in sediment with runoff at the end of the experiment; SedNR tf, ASVs present in sediment without runoff at the end of the experiment. The total number of ASVs in each compartment is indicated in parenthesis. The microbial ASVs exclusively shared between soil, runoff and sediment with runoff was coloured in red and were probably transferred from soil to sediment by runoff. Distribution of these transferred prokaryotic (C) and fungal (D) ASVs in soil, runoff and sediment. The data represent the mean of three biological replicates (n=3). Sed: sediment; SedR: sediment with runoff; SedNR: Sed without runoff; NP: non-planted; P: planted; NC: without HC spiking; C: with HC spiking.

We hypothesize that these specific ASVs correspond to i/ sediment taxa benefiting from the terrigenous inputs, and ii/ soil taxa transferred to sediment. It is likely that terrigenous inputs favoured the development of specific ASVs because sediment without terrigenous inputs (Sed NR ff) exhibited only eight specific ASVs. These results are in accordance with previous studies showing the loss of specialists and the emergence of taxa during coalescence event (Logares et al., 2009; Paver et al., 2018; Rocca et al., 2020; Wisnoski et al., 2020). Focusing on the ASVs shared by soil, runoff and sediment receiving terrigenous inputs, 4 prokaryotic (Figure III.5 A) and 14 fungal (Figure III.5 B) ASVs were identified, indicating the potential transfer of ASVs within the soil and marine coastal sediments continuum. This low number of shared ASVs shows that many microbial taxa present in runoff did not persist in the sediment they entered. They probably do not have the capacity to deal with the new conditions (salinity, pH, oxygen) and niche competition, prevalent in sediment. They may have been diluted or washed away during the renewal of seawater.

Among the prokaryotic ASVs, two were affiliated to Gammaproteobacteria (*Legionella* and *Massilia*), one to Bacteroidia (*Terrimonas*) and one to Actinobacteria (*Williamsia*). The fungal ASVs were affiliated to Dikarya (Ascomycota), LKM15 group, Chytridiomycota (Chytridiomycetes), Mucoromycota (Mucoromycotina Mucorales) and Cryptomycota. It is likely that these transferred ASVs characterized the microbial community coalescence event. The distribution of the prokaryotic transferred ASVs in the land-sea continuum showed that *Legionella* (ASV1184) and *Massilia* (ASV1436) were found at low abundance in soils, enriched in runoff, and detected only in HC spiked sediment receiving terrigenous inputs from unplanted soil (SedR NPC ff, Figure III.5 C). Microorganisms affiliated to these taxa are found in various ecosystems (Engleberg, 2009; Ofek et al., 2012), including HC contaminated environments (Gu et al., 2016), and characterized by motile capacity (Engleberg, 2009; Ofek et al., 2012) explaining their enrichment in the runoff. *Terrimonas* (ASV4242) was present in soil compartment in the different microcosms and poorly represented in runoff and sediment receiving terrigenous inputs, which is consistent with the fact that it is a non-motile soil microorganism, sensitive to sodium chloride (Board, 2015). *Williamsia* (ASV6220) was not specific to a compartment (Figure III.5 C). Environmental members from this genus have been isolated from oil-contaminated soil and deep-sea sediments (Pathom-Aree et al., 2006; Stach et al., 2004; Yassin et al., 2007).

Among the 14 fungal transferred ASVs, four ASVs were the most abundant, showing a particular distribution (Figure III.5 D). Noteworthy, Cryptomycota (ASV234) and LKM15 (ASV4 and ASV15) were enriched in runoff. Recent studies revealed the importance of these early diverging lineages in freshwater lakes and marine environments (Rojas-Jimenez et al., 2017). In particular, they produce motile aquatic spores (Money, 2016) and are able to parasitize the phytoplankton, which are advantages for inhabiting aquatic ecosystems (Rojas-Jimenez et al., 2017), supporting their enrichment in runoff. *Hypocreales* (ASV525) was present in soil, runoff and sediment receiving

terrestrial inputs, irrespective of the conditions (Figure III.5 D). *Hypocreales* are common in terrestrial and marine (seawater and sediment) environments (Jones et al., 2015; Torres and White, 2009). Their lifestyle associated to plant, algae, insect or marine animal hosts (as symbiont or pathogen) (Pang et al., 2019; Torres and White, 2009) may explain their persistence along the land-sea continuum. These results, based on the presence/absence of ASVs, indicate that sediment with runoff owned specific microbial population, either coming from or favoured by terrigenous inputs, supporting the microbial community coalescence in the land-sea continuum. In order to deeper characterize the ASVs participating in the coalescence, the identification of indicators based on ASV relative abundances, was performed in sediment receiving terrigenous inputs by runoff (SedR).

III.2.4.5. Identification of community coalescence microbial indicators

Table III.1 ASV indicators of coalescence. (A) ASV indicators, identified among the sediment compartment, specifically associated to sediment with runoff. (B) Distinction of the ASV indicators between taxa benefiting from the terrigenous inputs and soil taxa transferred to sediment.

A

ASV	Taxonomic affiliation			IndVal	p-value
525	Fungi	Pezizomycotina	<i>Hypocreales</i>	0,850	0,015
2716	Bacteria	Alphaproteobacteria	<i>Rhodobacteraceae</i>	0,764	0,045
635	Bacteria	Deltaproteobacteria	NA	0,844	0,025
3543	Bacteria	Bacteroidia	<i>Cyclobacteriaceae</i>	0,857	0,01

B

ASV	Taxonomic affiliation			IndVal	p-value
525	Fungi	Pezizomycotina	<i>Hypocreales</i>	0,850	0,015
2716	Bacteria	Alphaproteobacteria	<i>Rhodobacteraceae</i>	0,764	0,045
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3543	Bacteria	Bacteroidia	<i>Cyclobacteriaceae</i>	0,857	0,01

The ASV indicator value analysis, comparing the distribution of microbial population in the sediment compartment under the different conditions, revealed three bacterial ASVs and one fungal ASV significantly associated (p -value <0.05) to the sediment with runoff (SedR) (We hypothesize that these specific ASVs correspond to i/ sediment taxa benefiting from the terrigenous inputs, and ii/ soil taxa transferred to sediment. It is likely that terrigenous inputs favoured the development of specific ASVs because sediment without terrigenous inputs (Sed NR tf) exhibited only eight specific ASVs. These results are in accordance with previous studies showing the loss of specialists and the emergence of taxa during coalescence event (Logares et al., 2009; Paver et al., 2018; Rocca et al., 2020; Wisnoski et al., 2020). Focusing on the ASVs shared by soil, runoff and sediment receiving terrigenous inputs, 4 prokaryotic (Figure III.5 A) and 14 fungal (Figure III.5 B) ASVs were identified, indicating the potential transfer of ASVs within the soil and marine coastal sediments continuum. This low number of shared ASVs shows that many microbial taxa present in runoff did not persist in the sediment they entered. They probably do not have the capacity to deal with the new conditions (salinity, pH, oxygen) and niche competition, prevalent in sediment. They may have been diluted or washed away during the renewal of seawater.

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III.2.4.6. Identification of community coalescence microbial indicators

Table III.1 A). Interestingly, the distribution of these bacterial and fungal ASV SedR-indicators among all compartments showed that the three bacterial ASVs were associated with sediment, in particular at the end of the experiment (Sed tf) while the fungal ASV was associated with soil (We hypothesize that these specific ASVs correspond to i/ sediment taxa benefiting from the terrigenous inputs, and ii/ soil taxa transferred to sediment. It is likely that terrigenous inputs favoured the development of specific ASVs because sediment without terrigenous inputs (Sed NR tf) exhibited only eight specific ASVs. These results are in accordance with previous studies showing the loss of specialists and the emergence of taxa during coalescence event (Logares et al., 2009; Paver et al., 2018; Rocca et al., 2020; Wisnoski et al., 2020). Focusing on the ASVs shared by soil, runoff and sediment receiving terrigenous inputs, 4 prokaryotic (Figure III.5 A) and 14 fungal (Figure III.5 B) ASVs were identified, indicating the potential transfer of ASVs within the soil and marine coastal sediments continuum. This low number of shared ASVs shows that many microbial taxa present in runoff did not persist in the sediment they entered. They probably do not have the capacity to deal with the new conditions (salinity, pH, oxygen) and niche competition, prevalent in sediment. They may have been diluted or washed away during the renewal of seawater.

Among the prokaryotic ASVs, two were affiliated to Gammaproteobacteria (*Legionella* and *Massilia*), one to Bacteroidia (*Terrimonas*) and one to Actinobacteria (*Williamsia*). The fungal ASVs were affiliated to Dikarya (Ascomycota), LKM15 group, Chytridiomycota (Chytridiomycetes), Mucoromycota (Mucoromycotina Mucorales) and Cryptomycota. It is likely that these transferred

ASVs characterized the microbial community coalescence event. The distribution of the prokaryotic transferred ASVs in the land-sea continuum showed that *Legionella* (ASV1184) and *Massilia* (ASV1436) were found at low abundance in soils, enriched in runoff, and detected only in HC spiked sediment receiving terrigenous inputs from unplanted soil (SedR NPC Tf, Figure III.5 C). Microorganisms affiliated to these taxa are found in various ecosystems (Engleberg, 2009; Ofek et al., 2012), including HC contaminated environments (Gu et al., 2016), and characterized by motile capacity (Engleberg, 2009; Ofek et al., 2012) explaining their enrichment in the runoff. *Terrimonas* (ASV4242) was present in soil compartment in the different microcosms and poorly represented in runoff and sediment receiving terrigenous inputs, which is consistent with the fact that it is a non-motile soil microorganism, sensitive to sodium chloride (Board, 2015). *Williamsia* (ASV6220) was not specific to a compartment (Figure III.5 C). Environmental members from this genus have been isolated from oil-contaminated soil and deep-sea sediments (Pathom-Aree et al., 2006; Stach et al., 2004; Yassin et al., 2007).

Among the 14 fungal transferred ASVs, four ASVs were the most abundant, showing a particular distribution (Figure III.5 D). Noteworthy, Cryptomycota (ASV234) and LKM15 (ASV4 and ASV15) were enriched in runoff. Recent studies revealed the importance of these early diverging lineages in freshwater lakes and marine environments (Rojas-Jimenez et al., 2017). In particular, they produce motile aquatic spores (Money, 2016) and are able to parasitize the phytoplankton, which are advantages for inhabiting aquatic ecosystems (Rojas-Jimenez et al., 2017), supporting their enrichment in runoff. *Hypocreales* (ASV525) was present in soil, runoff and sediment receiving terrigenous inputs, irrespective of the conditions (Figure III.5 D). *Hypocreales* are common in terrestrial and marine (seawater and sediment) environments (Jones et al., 2015; Torres and White, 2009). Their lifestyle associated to plant, algae, insect or marine animal hosts (as symbiont or pathogen) (Pang et al., 2019; Torres and White, 2009) may explain their persistence along the land-sea continuum. These results, based on the presence/absence of ASVs, indicate that sediment with runoff owned specific microbial population, either coming from or favoured by terrigenous inputs, supporting the microbial community coalescence in the land-sea continuum. In order to deeper characterize the ASVs participating in the coalescence, the identification of indicators based on ASV relative abundances, was performed in sediment receiving terrigenous inputs by runoff (SedR).

III.2.4.7. Identification of community coalescence microbial indicators

Table III.1 B), which was in accordance with the Venn analysis (Figure III.5). These results further support that specific bacterial ASVs correspond to sediment taxa benefiting from the terrigenous inputs while the specific fungal ASV corresponds to soil taxa transferred to sediment, showing the

two possible ways resulting in community coalescence. Although the two ways of coalescence, proposed by Rocca et al. (2020) in laboratory experiment, have been suspected in field investigations (Crump et al., 2004, 1999; Logares et al., 2009; Paver et al., 2018; Wisnoski et al., 2020), our experimental ecology approach provide a set of evidences that coalescence in land-sea continuum involves the mixing of both microorganisms and the environment.

III.2.5. Conclusion

Our experimental ecology approach mimicking the land-sea continuum revealed that soil runoff modified the microbial communities inhabiting HC contaminated marine coastal sediment. The comparison of microbial communities, based on high throughput sequencing, identified bacteria and fungi taxa specific to sediment receiving terrigenous inputs via soil runoff. The in-depth analysis of these specific taxa distinguished the two ways of community coalescence: i/ the transfer of soil microorganisms, and ii/ the development of sediment taxa favoured by terrigenous inputs. The community coalescence may result in novel ecological niches constraining the functional organization of microbial communities and their role in biogeochemical cycles. In order to obtain a comprehensive view of microbial communities in coastal marine sediment, we advocate considering coalescence events, particularly for the monitoring and management of HC contaminated areas.

Author Contributions:

CCL, RD, AC designed the study. CCL, RD, AC, FR collected the samples. AC run the microcosm experiment. EC performed all data analyses. EC, CCL, RD wrote the first draft and AC, FR, CC, contributed to the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Supplementary data: Supplementary data to this article can be found online at xxx.

III.2.6. References

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III.2.7. Supplementary data

Table S III.1 Quantitative description of sequencing datasets through bioinformatics processing. The core ASVs group include widely distributed and abundant ASVs opposite to the satellite ASVs. *raw and denoised data include all Eukaryota reads. Fungal sequences were selected from the filtering step.

	Prokaryota		Fungi*
Raw data	Mean reads nb./ sample	51 173	55 473
	Total	3 735 622	3 716 691
Denoised data	Mean reads nb. /sample	14 257	25 683
	Total	1 040 774	1 720 729
	Length (pb)	411	450
Filtering	Total nb of ASVs	6 988	2 488
	Mean reads nb. /sample	13 040	12 977
	Total	951 921	869 479
	Nb ASVs	6 748	705
Rarefaction	Nb reads/sample	7 578	1 027
	Total nb. ASVs	6 064	638
Core ASVs group	Nb. ASVs	1 602	173
Satellite ASVs group	Nb. ASVs	4 462	465

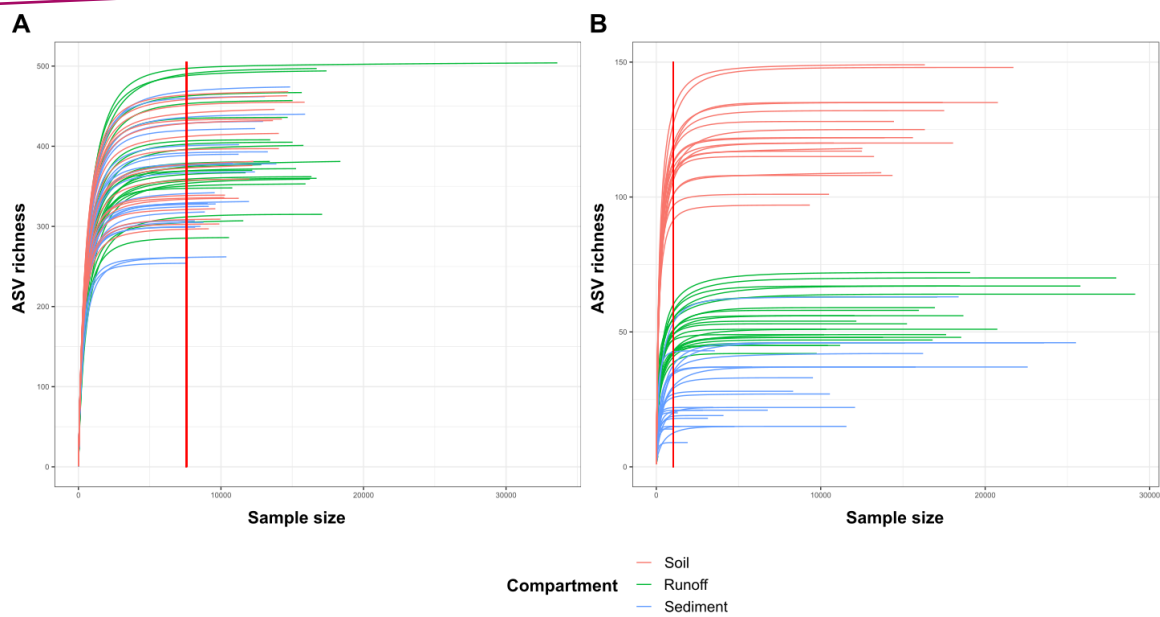


Figure S III.1 Rarefaction curves from 16S (A) and 18S (B) libraries. The vertical red lines represent the chosen subsample sizes for rarefying communities (7578 and 1027 respectively).

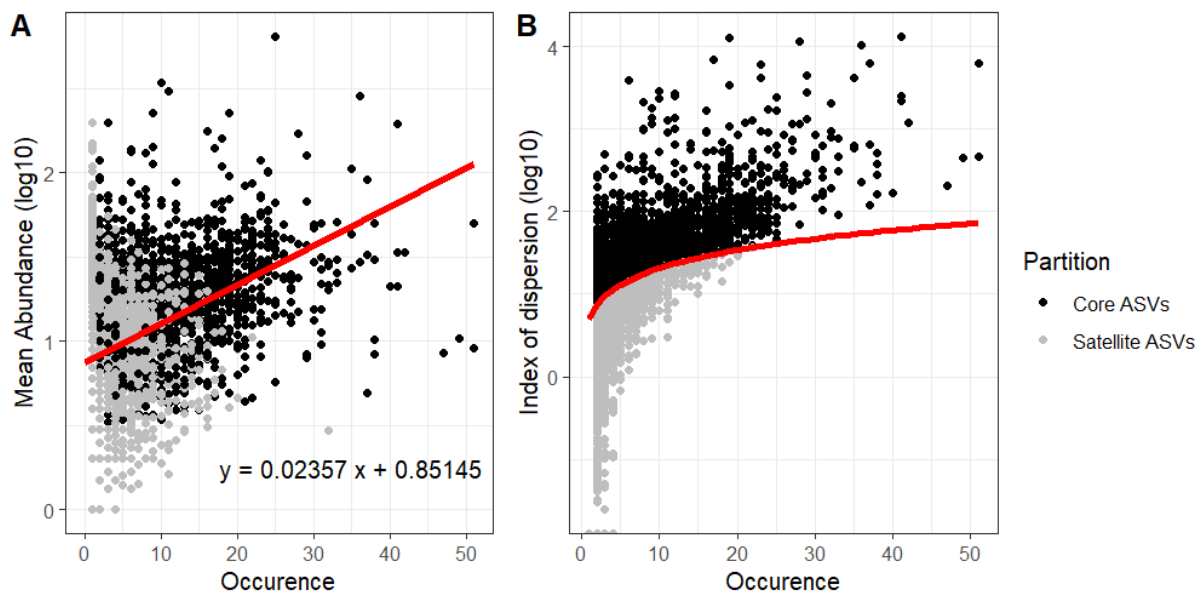


Figure S III.2 Species abundance distribution of microbial ASVs. A Occurrence of ASVs plotted against its mean abundance across samples. The red line shows the correlation between abundance and occurrence (Pearson's correlation coefficient = 0.35, $p < 0.001$). B Occurrence of each microbial ASVs plotted against its dispersion index. The red line represent the 2.5% limit of the χ^2 distribution. Black and grey dots represent core and satellite ASVs, respectively.

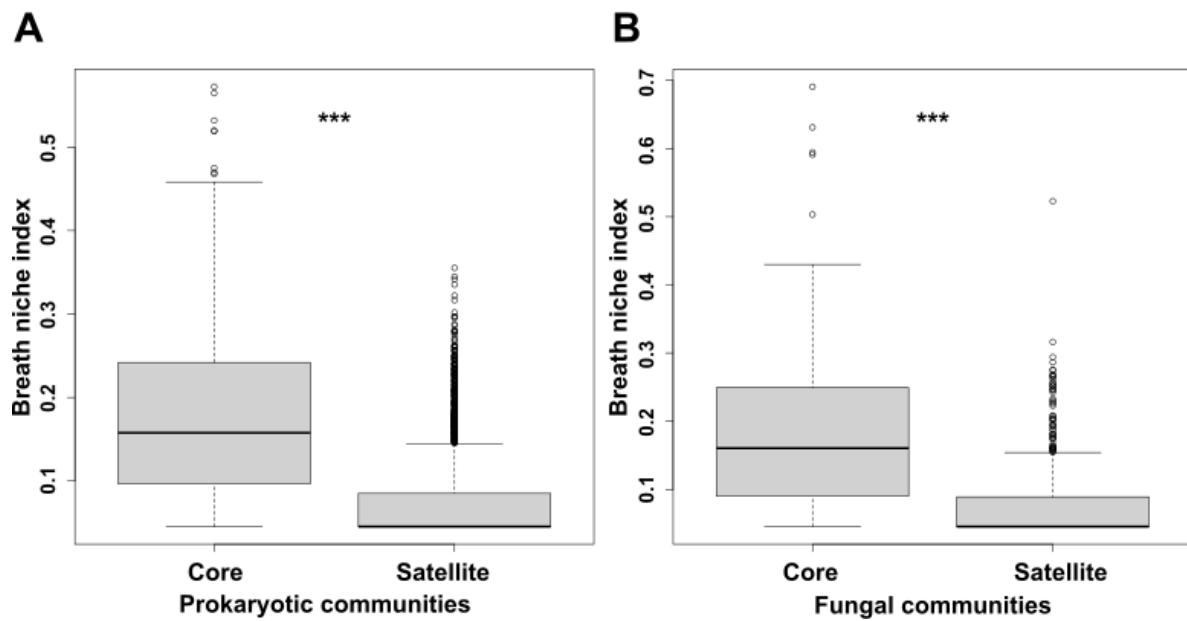


Figure S III.3 Boxplots summarizing mean niche breadth for core and satellite prokaryotic (A) and fungal (B) communities in the land-sea continuum (Wilcoxon sum rank test, $p < 0.001$, ***). The value of niche breadth was calculated using the mean niche breadth of all ASVs in each group.

New insights into microbial community coalescence in the land-sea continuum

Table S III.2 Results of the PerMANOVA testing the effect of the experimental conditions (i.e. compartment, HC spiking, presence of plants, runoff) and their interaction in explaining taxonomic and phylogenetic β -diversity (total, replacement or richness). Degrees of freedom (df), F- statistics (F), R² and associated p-values (P) are shown. Post-hoc test for perMANOVA testing the effect of runoff in sediment compartment via cond2, factor grouping runoff and plant. ns: non significant; tf: at the end of the experiment, day 39; compartment soil or sediment; HC: with spiking of HC (C) or not (NC); plant: with the presence of plant (P) or without (NP).

Compartment		β -diversity	Effect	Df	F	R ²	p-value
SOIL, RUNOFF AND SEDIMENT	16S	Taxonomic total	compartment	2	30.82	0.48	<0.001
			HC spiking	1	2.73	0.02	ns
			interaction	2	2.31	0.04	ns
	18S	Taxonomic total	compartment	2	32.89	0.51	<0.001
			HC spiking	1	1.53	0.01	ns
			interaction	2	1.56	0.02	ns
	Phylogenetic total	compartment	2	84.52	0.73	<0.001	
		HC spiking	1	1.39	0.01	ns	
		interaction	2	1.35	0.01	ns	

New insights into microbial community coalescence in the land-sea continuum

Compartment		β -diversity	Effect	Df	F	R ²	p-value
SOIL	16S	TAXONOMIC TOTAL	HC spiking	1	1.46	0.07	<0.05
			time	1	2.74	0.14	<0.001
			Plant	1	1.09	0.06	ns
			Interaction HC spiking:Plant	2	1.18	0.12	ns
		PHYLOGENETIC TOTAL	HC spiking	1	1.40	0.06	<0.05
			time	1	4.22	0.17	<0.05
			Plant	1	1.08	0.04	ns
			Interaction HC spiking:Plant	2	2.88	0.24	ns
	18S	TAXONOMIC TOTAL	HC spiking	1	1.6	0.07	<0.05
			Time	1	3.8	0.18	<0.001
			Plant	1	1.27	0.06	ns
			Interaction HC spiking:Plant	2	1.41	0.13	<0.05
		PHYLOGENETIC TOTAL	HC spiking	1	0.58	0.02	<0.05
			Time	1	12.4	0.5	<0.001
			Plant	1	0.41	0.02	ns
			Interaction HC spiking:Plant	2	0.70	0.05	ns

New insights into microbial community coalescence in the land-sea continuum

Compartment		β -diversity	Effect	Df	F	R ²	p-value
RUNOFF	16S	TAXONOMIC TOTAL	HC spiking	1	3.99	0.14	<0.001
			Time	1	4.07	0.14	<0.001
			Plant	1	0.99	0.03	ns
			Interaction HC spiking:Plant	1	1.03	0.04	ns
		PHYLOGENETIC TOTAL	HC spiking	1	3.46	0.14	ns
			Time	1	1.04	0.04	ns
			Plant	1	0.24	0.01	ns
			Interaction HC spiking:Plant	1	0.29	0.01	ns
	18S	TAXONOMIC TOTAL	HC spiking	1	2.57	0.09	<0.001
			Time	1	4.53	0.16	<0.001
			Plant	1	1.63	0.06	<0.05
			Interaction HC spiking:Plant	1	1.29	0.05	ns
PHYLOGENETIC TOTAL		HC spiking	1	2.57	0.09	<0.001	
		Time	1	4.53	0.16	<0.001	
		Plant	1	1.63	0.06	<0.05	
		Interaction HC spiking:Plant	1	1.29	0.04	ns	

New insights into microbial community coalescence in the land-sea continuum

Compartment		β -diversity	Effect	Df	F	R ²	p-value		
Sediment	16S	TAXONOMIC REPLACEMENT	cond.sed	3	1.47	0.18	<0.05		
			<i>Post-hoc Sed t0 – SedNR: p-value <0.05; Sed t0 – SedR: p-value<0.05;</i>						
			<i>SedR – SedNR: non significant</i>						
			HC spiking	1	1.53	0.06	<0.05		
			interaction	3	0.74	0.09	ns		
Sediment		PHYLOGENETIC REPLACEMENT	cond.sed	3	7.13	0.48	<0.001		
			<i>Post-hoc Sed t0 – SedNR: p-value <0.05; Sed t0 – SedR: p-value<0.05;</i>						
			<i>SedR – SedNR: non significant</i>						
			HC spiking	1	7.07	0.16	<0.05		
			interaction	3	0.13	0.01	ns		
Sediment	18S	TAXONOMIC RICHNESS	cond.sed	3	4.04	0.34	<0.05		
			<i>Post-hoc Sed t0 – SedNR: p-value <0.05; Sed t0 – SedR: non significant;</i>						
			<i>SedR – SedNR: p-value <0.05</i>						
			HC spiking	1	4.58	0.13	<0.05		
			interaction	3	1.21	0.10	ns		

New insights into microbial community coalescence in the land-sea continuum

Compartment	β -diversity	Effect	Df	F	R ²	p-value
	PHYLOGENETIC RICHNESS	cond.sed	3	4.00		<0.05
		<i>Post-hoc Sed t0 – SedNR: p-value <0.05; Sed t0 – SedR: non significant; SedR – SedNR: p-value <0.05</i>				
		HC spiking	1	4.57		<0.05
		interaction	3	0.92		ns

III.3. Conclusion of the chapter

The community diversity analysis presented in this chapter aimed to determine whether soil runoff was involved in the assembly of microbial communities in marine coastal sediment. This analysis was based on an experimental approach mimicking land-sea continuum in microcosms and 16S and 18S rRNA gene sequencing. Our results provided evidences of community coalescence when marine coastal sediment receives terrigenous inputs via soil runoff.

A large proportion of microbial ASVs was specific to soil runoff, suggesting that these ASVs are probably rare in soil and not surviving in sediment. Nevertheless, significant changes in the microbial structure were observed in sediment induced by soil runoff, demonstrated by the identification of ASV indicators of coalescence in sediment. The results highlighted that soil runoff might have favoured the transfer of microorganisms into sediment as well as the development of autochthonous microorganisms fed with terrigenous inputs.

We expect that such taxonomic changes will modify the functional capabilities of microbial communities. In order to confirm this hypothesis, the functional potential of soil and sediment microbial communities was characterized in the land-sea continuum in the next chapter.

Chapter IV:

Functional redundancy as structural microbial community response in land-sea continuum contaminated by hydrocarbons

Functional redundancy as structural microbial community response in land-sea continuum contaminated by HC

Chapter IV. Functional redundancy as structural microbial community response in land-sea continuum contaminated by hydrocarbons

IV.1. Background of the study

The land-sea continuum, at the interface between terrestrial and marine ecosystems, is increasingly threatened by urbanization, habitat degradation and pollution. In this mixing zone, marine coastal sediment, in particular, receives important quantities of organic matter and pollutants such as hydrocarbons (HC) from human land-based activities by terrestrial runoff (river runoff and direct soil runoff). It has been demonstrated that microbial communities play a major role in the fate of the HC in sediment.

In the previous chapter, community coalescence, mixing of microorganisms and environment, between soil and marine sediment due to soil runoff was highlighted in the HC contaminated land-sea continuum. The evidences of coalescence were collected based on the description of the structure and the composition of the microbial communities. As soil runoff brought into contact dissimilar microbial communities (i.e. terrestrial and marine communities) and moved pieces of environment (nutrients, organic matter, pollutants etc.) in marine coastal sediment, it raises questions about the implications on functions, including HC degradation. We expect that terrigenous inputs modify functionally marine coastal sediment communities either directly by transfer of microorganisms or indirectly by stimulating sediment microorganisms playing a role the biogeochemical cycles and the fate of HC. The present chapter focuses on the functional potential and the functional co-occurrence patterns of sediment microbial communities receiving terrigenous inputs from soil runoff. The GeoChip 5.0 microarray was used for functionally profiling soil and sediment microbial communities in the different microcosms. This high-throughput technology has been recognized as an effective, sensitive, and semi-quantitative tool for examining metabolic capabilities in various ecosystems (Shi et al., 2019). Among other technologies developed to access functional diversity such as NGS sequencing or quantitative PCR (qPCR), this microarray was selected because it is an affordable tool that provides comprehensive datasets, and it is easy and fast to analyse.

IV.2. Functional redundancy as structural microbial community response in land-sea continuum contaminated by hydrocarbons

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IV.2.1. Abstract

An experimental approach mimicking the land-sea continuum in microcosms was developed in order to determine the effect of the terrigenous inputs by soil runoff on the microbial functional potential in hydrocarbon (HC) contaminated marine coastal sediment. We hypothesise that the coalescent event increase the functional potential of microbial communities in marine coastal sediments, influencing the fate of HC in marine coastal ecosystems.

The microbial functional potential including the HC degradation ability was assessed by DNA-array to compare the sediment receiving or not terrigenous inputs. The removal of HC and the functional gene richness in sediment was unchanged with the terrigenous inputs. However, the gene variants (GVs) composition was modified indicating functional redundancy. In addition, functional indicators including GV's related to sulfite reduction, denitrification and polyaromatic degradation were identified in higher proportion in sediment receiving terrigenous inputs. The terrigenous inputs modified the functional co-occurrence networks, showing a reorganization of the GV's associations with an increase of the network complexity. Different keystone GV's ensuring similar functions were identified in networks with or without terrigenous inputs, further confirming functional redundancy.

We advocate that functional redundancy is the main structural microbial community response in land-sea continuum mixing zone contaminated by hydrocarbons. Our results provide helpful functional information for the monitoring and management of coastal environment affected by human land-based activities.

Keywords: functional gene array, bioindicators, runoff, terrigenous inputs, marine sediment, hydrocarbon degradation

IV.2.2. Introduction

Human activities threaten the ecosystem services of coastal areas by introducing contaminants (Halpern et al., 2008). Among these contaminants, petroleum hydrocarbons (HC) are of major concern because of their toxicity. Coastal areas represent a land-sea continuum, mixing terrestrial and marine ecosystems (Laruelle et al., 2013; Xenopoulos et al., 2017). Soil runoff drains terrigenous inputs that convey soil water and aggregates to marine coastal sediments including organic matter, sorbed pollutants and microorganisms (Fagervold et al., 2014; Hoffman et al., 1984; Yakirevich et al., 2013). The mixing of microbial communities together with the blending of their environments results in community coalescence (Châtillon et al., 2021, Chapter I; Rillig et al., 2015). The coalescence events raise questions about the environmental and biotic processes that structure microbial communities, particularly regarding the functional aspect (Rillig et al., 2016; Rocca et al., 2020). Although several studies have investigated functions considering coalescent communities in agriculture, industry and medicine (Calderón et al., 2017; Sierocinski et al., 2017; Wilson et al., 2019), the functional aspect is still unclear in aquatic ecosystems yet limited to some enzymatic activities (Castledine et al., 2020; Mobilian et al., 2020). Regarding HC, the functional potential of degradation has been largely studied, independently in soil (Patel et al., 2016) and in marine coastal environment (King et al., 2015), revealing functional genes as markers of HC degradation (von Netzer et al., 2016), but information in the context of coalescence is lacking. However, the microbial communities inhabiting the land-sea continuum play a pivotal role in determining the fate of pollutants (Bauer et al., 2013; Duran and Cravo-Laureau, 2016), which requires microbial network interactions probably affected by terrigenous inputs (Yan et al., 2021). It has been shown that different metabolic groups, ensuring not only degradation functions but also involved in biogeochemical cycles (Roling and van Bodegom, 2014), compose HC degrading microbial communities. It is thus of paramount importance to investigate the functional component of those communities in order to have a comprehensive view of the functional network in a coalescence context. In order to assess the microbial functionality, different approaches have been developed including measurements of fluxes and activities (Ziervogel et al., 2016), inference from 16S rRNA gene barcoding (Douglas et al., 2020), targeting of functional genes (Duarte et al., 2014), and meta-omics (Mason et al., 2012). Recently, DNA-array approach has been shown to assess the microbial functionality with the advantages to target both structure and functions of microbial communities in various environments (Shi et al., 2019). DNA-array provides the opportunity to follow easily the microbial community functional dynamics that could be useful to monitor the effect of coalescence event. This approach, used in environmental continuum, has provided contrasted results showing either the stability (Avila-Jimenez et al., 2020) or the vulnerability (Yan et al., 2021) of the functional microbial network. To our knowledge, such studies on the functional diversity and the functional networks are still missing within the land-sea

continuum. There is thus need to study the effect of the multiple coalescent events on the microbial functional potential and the functional interactions in marine coastal sediments receiving terrigenous inputs. In order to determine the effect of coalescent event on the functional diversity and the functional interactions in the land-sea continuum, an experimental ecology approach, simulating the continuum, was developed. We hypothesized that terrigenous inputs increase the functional potential of microbial communities in marine coastal sediments and thus influence the fate of HC in coastal ecosystems. Gene functional analysis was performed by comparing microbial communities in sediment receiving or not terrigenous inputs. The DNA-array (Geochip 5S) targeting different gene families, involved for example in biogeochemical cycles and organic pollutant remediation, was used. Functional genes involved in HC degradation pathways were examined as candidate indicators of the community coalescence, as well as their co-occurrence patterns with functional genes involved in the main biogeochemical cycles.



IV.2.3. Material and Methods

IV.2.3.1. Microcosms

Soil (10kg) and sediment (2kg) were collected in the same geographical area, closed to petroleum refinery, in southeast of France, in October 2017. Soil was collected with manual auger from the surroundings of the Berre Pond (43°29'6.9"N 5°11'18.53"E, France) and sediment from the Canal Viel Cove (43°23'20.3"N 4°59'31.8"E, France). Prior to microcosms setup, soil and sediment were sieved to <4 mm and <1 mm respectively, to remove coarse gravels, plant debris and macroorganisms and to homogenise samples. On dry soil and sediment aliquots, the texture, the pH, the C/N ratio, the cation-exchange capacity, the water holding capacity (WHC), ions and trace metals were quantified (LAS, INRAE Arras, France). Soil was air-dried and stored at room temperature and sediment was stored at 4 °C in a 5L bottle completely covered with *in situ* seawater and with continuous aeration through an aquarium pump, until starting the experiment (one week later). An original microcosm system was set up and incubated during 39 days under controlled conditions at the laboratory, as previously described (Châtillon et al., 2021 in submission). The experimental system was designed to highlight community coalescence between soil and coastal marine sediments resulting from terrigenous inputs via soil runoff. We hypothesized that terrigenous inputs generated from soil by runoff select particular microbial communities in downstream sediment, depending on the presence of plants and/or on a HC pollution event and influence the microbial functional potential. For this purpose, microcosms were set up as follow (Table IV.1): land-sea continuum microcosms that simulated the soil runoff to marine coastal sediment connecting soil and sediment compartments, and control microcosms without runoff of soil consisting of sediment compartment.

Functional redundancy as structural microbial community response in land-sea continuum contaminated by HC

Table IV.1 Sample abbreviations. Soil: soil; Sed: sediment; SedR: sediment receiving runoff; SedNR: sediment without runoff (control); NP: without plant; P: with plant; NC: without HC spiking; C: with HC spiking.

Microcosm	Compartment	Condition	Description	
Land-sea continuum 	Soil	NPNC	Non-planted soil without deuterated HC spiking	
		PNC	Planted soil without deuterated HC spiking	
		NPC	Non-planted soil with deuterated HC spiking	
		PC	Planted soil with deuterated HC spiking	
	Runoff	NPNC	Runoff water from non-planted soil without deuterated HC spiking	
		PNC	Runoff water from planted soil without deuterated HC spiking	
		NPC	Runoff water from non-planted soil with deuterated HC spiking	
		PC	Runoff water from planted soil with deuterated HC spiking	
	SedR	NPNC	Sediment receiving runoff water from non-planted soil without deuterated HC spiking	
		PNC	Sediment receiving runoff water from planted soil without deuterated HC spiking	
		NPC	Sediment receiving runoff water from non-planted soil with deuterated HC spiking	
		PC	Sediment receiving runoff water from planted soil with deuterated HC spiking	
	Control 	SedNR	NC	Sediment without runoff without deuterated HC spiking
			C	Sediment without runoff with deuterated HC spiking

Briefly, the microcosms were composed of glass devices (1L beaker equipped with a seawater inlet and outlet) containing the equivalent of 90g of dry sediment and that were connected through Teflon pipes or not to stainless steel devices containing 600g of moistened soil at 60% WHC. Different conditions were applied in triplicate (Table IV.1) combining the presence of plant (P) or not (NP) in soil with the spiking of deuterated HC (C) or not (NC) in soil and sediment. The grass *Catapodium marinum* (seed bank of the Muséum National d'Histoire Naturelle de Paris - DGD – MJZ) was used, after seed germination 16 plants were kept per devices. In the HC spiked conditions, soil and sediment (corresponding to 1/10 of the soil and sediment in the devices) were mixed to 1:1 (W:W) of deuterated n-hexadecane (D34, 98%, Cambridge Isotope Laboratories, Inc, USA) and phenanthrene (D10, 98%, euriso-top, France), dissolved in hexane solvent. A stock solution was prepared in order to add each HC at a concentration of 100 mg/kg dry soil or sediment. For the unspiked conditions, the same volume of hexane was mixed to sediment and soil, without addition of HC stock solution. In land-sea continuum microcosms, soils were watered

to reach 60% of their WHC and were daily watered by means of a syringe dispensing 73 ml mineral water (Volvic®) to mimic rainfall and produce runoff waters. Both in land-sea continuum and in control microcosms, peristaltic pumps helped to simulate tidal cycles and renew the seawater (Sel Instant Ocean, Aquarium systems) ensuring conditions close to those prevailing in natural environment. After the 39 days of incubation (tf), *Catapodium marinum* were cut at the collar, put in paper bags, dried and weighted to estimate the aerial dry biomass.

IV.2.3.2. Sampling

Samples of the different compartments (soil, runoff, sediment) and of seawater were collected at different times at the beginning, at the end, and during the incubation for subsequent chemical and/or biomolecular analysis. Soil and sediment samples (ca. 10-12g) were collected in triplicates at the beginning and end of the experiment (t0 and tf respectively) for the DNA extraction and the Geochip analysis. Soil samples were collected using a little stainless steel corer (diameter: 14mm; height: 40 mm Bourceret et al., 2015) and sediment samples using a spoon. At t0, three soil samples (each being the mixture of 3 cores) were collected from additional soil devices, one with HC spiking and one without HC spiking, so as not to alter soil structure or disturb plant germination in devices that continued the incubation. Three sediment samples (each being the mix of 3 spoons) were collected from the sediment batch prepared for sediment devices with HC spiking (NPC, PC, NRC conditions) and from the one prepared for sediment devices without HC spiking (NPNC, PNC, NRNC conditions). At t1 and t2 (after 16 and 31 days of incubation respectively), runoff samples were obtained from each land-sea microcosms, by mixing runoff from three consecutive days and filtered at 0.22 µm (Millex). An aliquot of Volvic water (rainwater) was filtered in the same way as the runoff to serve as a control. At tf (after 39 days of incubation), *Catapodium marinum* were cut at the collar, put in paper bags, dried and weighted to estimate the aerial dry biomass. Soil samples were collected in triplicate from each land-sea continuum microcosm using a template (three notches per row and three per column) as a guide for the corer. The cores from the same column were mixed and formed one sample. Sediment samples were collected from each microcosm in triplicate after mixing of sediment using a spoon. Thus, the sampling design provided three technical replicates for each of the three biological replicates, for soil and sediment from each microcosm. A portion of the soil and sediment samples were dedicated to chemical analyses and were stored in glass tubes (supelco) at -20°C while that dedicated to biomolecular analyses was stored at -80°C.

IV.2.3.3. Quantification of the dissolved organic carbon (DOC) and deuterated HC

Runoff water (40mL) and seawater from the low tides (250 mL) were collected after 16 (t1) and 30 (t3) days of incubation and filtered on GF/F filters. 20 mL were kept at -20°C until DOC analyses. DOC was measured using TOC-VCSH Analyzer (Shimadzu, Japan) with ASI-V switcher. Freeze-

dried spiked soil and sediment samples (2 g) collected at the beginning (t₀) and at the end of the experiment (t_f) were sequentially extracted with dichloromethane (DCM) followed by methanol by accelerated solvent extraction (Dionex, ASE350). ASE cells were filled with activated copper powder (to remove molecular sulfur) and sodium sulfate (Na₂SO₄) to remove remaining water. Samples were extracted at 130 °C, 100 bars and 10 min for each solvents. After adjusting the volume of the DCM extract at 10 mL, an aliquot of 80µL was spiked with 20µL of internal quantifications standards (Fluorene D10 and Fluoranthene D10) to quantify deuterated HC (phenanthrene and n-hexadecane) and injected by gas chromatography–mass spectrometry (GC-MS). The instrument used was an Agilent 7820 gas chromatograph equipped with a DB 5-MS column (60 m × 0.25 mm i.d. × 0.25 µm film thickness) coupled with an Agilent 5975 mass selective detector operating in single ion monitoring mode. The molecules were detected with a quadrupole analyzer following ionization by electronic impact. The temperature program was the following: from 70 to 130 °C at 15 °C min⁻¹, then from 130 to 315 °C at 3 °C min⁻¹ and then a 15 min hold at 315 °C. One microliter of sample was injected in splitless mode at 300 °C. The carrier gas was helium at 1.4 mL min⁻¹ constant flow. The quantitative analyses of PACs were carried out using internal calibration. For each quantified compound, the GC/MS was calibrated between 0.06 and 9.6 µg mL⁻¹ with 10 calibration levels. The calibration curves were drawn and satisfactory determination coefficients were obtained ($r^2 > 0.99$). To verify the quantification, two calibration controls (lower and higher concentrations) were carried out every six samples and only a deviation lower than 20% was accepted.

IV.2.3.4. DNA preparation, functional gene analysis and bacterial abundance

DNA was extracted using the DNA PowerSoil Isolation Kit (MoBio Laboratories) on 0.25 g for soil and sediment samples or on the whole 0.22 µm filter for runoff and rainwater samples. Extractions were performed according to the manufacturer's instructions with a slight modification: samples were ground in liquid nitrogen as prior step. DNA concentration was estimated using Qubit dosage and DNA quality was investigated by spectrophotometry and with agarose gel electrophoresis. DNA extracts were stored at -20°C. GeoChip 5.0S (Glomics Inc., Norman, OK, United States), manufactured by Agilent Technologies (Santa Clara, CA, USA), was used to detect specifically, sensitively and quantitatively key functional genes from soil and sediment microbial communities at the end of the experiment. The DNA-array contains ~57,000 probes covering ~151,000 target genes of 1,517 gene families from functional categories involved in biogeochemical cycling of C, N, S, and P and in other ecological services such as organic remediation (Shi et al., 2019). The probes were designed to detect a single sequence or a similar group of sequences, called a gene variant (GV), corresponding to a target functional gene present in the NCBI databases. Glomics conducted the DNA-array analysis, which consisted of amplification (Wu et al., 2006), labelling hybridization and data pre-processing (normalization and

quality filtering based on the average signal intensity of internal standards and the average background signal) (Van Nostrand et al., 2016) from an aliquot of the DNA extracts. Pre-processed data were loaded under the free software R (R Core Team, 2020) and treated using phyloseq package (McMurdie and Holmes, 2013). Prior to statistical analyses, signals were converted into relative abundances. The bacterial abundance was estimated in runoff and rainwater (at t1 and t2) and in soil and sediment (tf), using quantitative real time PCR to quantify the 16S rRNA gene copy number. The qPCR assay was performed as described in Cébron et al. (2008) by using the primer set 968F/1401R (Felske Andreas et al., 1998) and iQ SYBR green SuperMix (Bio-Rad). Absolute quantification was assessed using a CFX96 Real-Time PCR detection system (Bio-Rad, France) and a 10-fold dilution series from 10^8 to 10^1 copies μl^{-1} of the standard plasmid. Data were then expressed as gene copy numbers per gram of dry weight soil/sediment or per mL of runoff.

IV.2.3.5. Statistical analysis

All the following statistical analyses were implemented within the R programming environment (R Development Core, 2020).

The Hill numbers were calculated at three different orders (q) of diversity with a q value of 0 for species richness with all species having the same weight, $q = 1$ for the exponential of Shannon's index with species being weighted exactly for their abundance in the community and whereas $q = 2$ for the inverse-Simpson's index which favours abundant species. The Simpson's Evenness was calculated as the inverse-Simpson divided by species richness. Analysis of variance (ANOVA) was implemented to determine significant alpha-diversity differences between microbial communities under the different conditions. When the overall ANOVA was statistically significant, the Tukey post hoc test was used to identify statistically significant pairwise differences between communities. Differences in functional gene composition between communities was quantified using Bray-Curtis dissimilarity and further visualized using non-metric multidimensional scaling ordinations (NMDS). To assess the sources of variation (i.e. compartment, HC spiking, presence of plants, runoff) in the functional dissimilarity matrix, a series of permutation multivariate analyses of variance (PERMANOVA; Anderson, (2001)) was carried using permutation tests with 9,999 iterations with the function `adonis` in the R package `vegan` (Oksanen et al., 2019). Post-hoc pairwise comparisons were performed using the package `RVAideMemoire` (Hervé, 2020). A Procrustes analysis was performed to determine the overall degree of concordance between dissimilarity in all GVs and dissimilarity only remediation category GVs. In the Procrustes analysis, a pair of data matrices (i.e. the scores of the communities from two NMDS generated from dissimilarity matrices) is compared by using a rotational-fit algorithm that minimizes the sum of squared residuals between the two matrices. Concordance was then quantified with a correlation index in the symmetric Procrustes rotation. P-values were evaluated with 9999 Monte Carlo permutation tests. In order to identify

gene indicators, a multi-level pattern analysis (MLPA) was conducted using point-biserial correlation coefficient (De Cáceres et al., 2010), suitable to DNA-array abundance data. MLPA calculates the association index of a feature (ie. gene family or GVs) with each particular condition and other combinations of conditions to infer the best association (highest association index value revealing the indicator). The statistical significance of the association index was tested by Monte Carlo method. This correlation index assesses the positive or negative preference of features for the conditions or combinations of conditions compared to the other ones. MLPA was led at the gene family and GVs levels, using the R package *indicspecies* (Caceres and Legendre, 2009). Combinations of conditions were restricted to those with a biological meaning. ANOVAs were also implemented to test the effect of the experimental conditions (i.e. compartment, HC spiking, presence of plants, runoff) on the bacterial abundance, the aerial vegetal dry weight, and the DOC content. Non-parametric Wilcox test and Kruskal-Wallis tests were used to test the effect of the experimental conditions on the removal of the deuterated HC.

IV.2.3.6. Functional co-occurrence network inference

The network analysis was computed with the application CoNet in Cytoscape (Faust et al., 2015; Faust and Raes, 2016). The significant co-presence and mutual exclusion patterns were detected depending on the runoff among the 873 GVs identified as indicators as specified above. Briefly, GVs were filtered with the fewest occurrence (<10) and counts were transformed as relative abundance. Multiple associations were determined by combining correlations (Pearson and Spearman) and dissimilarities (Bray-Curtis and Kullback Leibler) with an initial automatically threshold set, such as the initial network included 1,000 positive and 1,000 negative edges for each coefficient. The significance of edges was assessed by merging permutation and bootstrap distributions (pvalues <0.05, Brown's method) combined with multiple-testing correction (Benjamini and Hochberg's procedure). Modules (highly interconnected nodes) and keynodes in networks were identified using the application MCODE (Molecular Complex Detection) in Cytoscape (Bader and Hogue, 2003).

IV.2.4. Results and Discussion

IV.2.4.1. HC removal and impact on the plant biomass and the bacterial abundance

The concentrations of the deuterated HC at the beginning of the experiment (t_0) and after 39 days of incubation (t_f) were determined in the different microcosms (Table IV.2). The phenanthrene concentration was significantly reduced in soil (up to 95 %) and in sediment (around 68 %) samples whereas hexadecane removal was observed only in soil samples (up to 29 %; Wilcoxon test, $p < 0.05$). The phenanthrene removal was not influenced neither by the presence of plant in soil (Wilcoxon test $W = 4$; $p = 1$) nor by the terrigenous inputs in sediment (Kruskal-Wallis

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test: $H = 1.69$, $p = 0.43$, $df = 2$). The HC removal was higher in soil than in sediment (Wilcoxon tests: $W = 54$; $p < 0.0001$). It was surprising to observe a higher removal of phenanthrene compared to hexadecane because hexadecane is considered as less toxic and more easily used by a wide range of microorganisms (Hamme et al., 2003; Leahy and Colwell, 1990; Yemashova et al., 2007). However, after a contamination, microbial communities able to degrade aromatic compounds could dominate before those able to degrade alkane in coastal sediment, as described during the Deepwater Horizon disaster (Kimes et al., 2014). Such capacity might be explained by the fact that microbial communities were probably adapted to the presence of aromatics compounds (legacy effects; Jurgburg et al., 2017; Nunes et al., 2018; Thomas et al., 2020) and the bioavailability of the compound (Ghosal et al., 2016). In contrast to that previously reported (Bourceret et al., 2016; Khan et al., 2013; Rohrbacher and St-Arnaud, 2016), the presence of plants did not influence the HC removal. Plant biomass and prokaryotic abundance (Table IV.3) were estimated to determine the ecotoxicological effect of the deuterated HC spiking in microcosms. The aerial vegetal dry weight was not significantly different between the non-spiked soil (Soil NC) and the HC spiked soil (Soil C) conditions (Wilcoxon test: $W = 1$, $p = 0.2$) showing that *Catapodium maritimum* was not sensitive to HC contamination. To our knowledge, there is no available information about the effect of the HC on this grass species. However, grasses such as perennial ryegrass or tall fescue are often used in HC phytoremediation (Cook and Hesterberg, 2013) showing enhanced HC removal efficiency (Yavari et al., 2015). The prokaryotic abundance was of the same order of magnitude in the different soil and sediment compartments (around 4.6×10^8 16S rRNA gene copies per g DW), irrespective of the condition (ANOVA within soil samples: $F = 1.05$, $p = 0.42$; ANOVA within sediment samples: $F = 0.4$, $p = 0.84$). Unexpectedly, we did not observe an effect of the plants on the prokaryotic abundance although it has been shown that plants provide substrates and nutrients for microbial growth (Merkl et al. 2004; Inckot et al. 2011; Smith et al. 2006; Shirdam et al. 2008; Hinsinger et al. 2005). Significant lower prokaryotic abundance was found in runoff samples (2.8×10^3 16S rRNA gene copies per mL; ANOVA; $F = 103.63$; $p < 0.001$) than in soils and sediments but richer ($\times 100$) than in rainwater controls showing that runoff is enriched with prokaryotic abundance as it passes through the soil. Similarly, runoff were also enriched with DOC irrespective of the condition (Table S IV.1) showing it bring microorganisms and organic matter to the marine compartment, as it was previously described (Le et al., 2016). Surprisingly, the presence of plant did not change the DOC content contrary to previous report (Janeau et al., 2014). During our experiment, more DOC was found in the seawater receiving runoff (Sed R; ANOVA, $F = 18.150$, $p < 0.001$) than that from control microcosm without runoff (Sed NR). Such observation showed an enrichment with DOC in the marine compartment, which was even more important in the non-spiked seawater (Sed NC) (ANOVA, interaction runoff: HC; ANOVA, $F = 6.534$; $p < 0.05$).

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Table IV.2 Removal percentages of deuterated hydrocarbons spiked in soil and sediment, after 39 days of incubation. The values are presented as mean \pm SD (n=3).

Compartment		Soil				Sed	
		SedR		SedNR			
Condition		NPC	PC	NPC	PC	C	
% removal	Hexadecane	28.8 \pm 10.7	29.7 \pm 1.9	0.0	0.0	0.0	
	Phenanthrene	95.5 \pm 0.6	94.6 \pm 1.6	65.4 \pm 5.9	75 \pm 9.4	64.7 \pm 9	

Soil: soil; Sed: sediment; SedR: sediment receiving runoff; SedNR: sediment without runoff (control); NP: no plant; P: plant; C: HC spiking.

Table IV.3 Plant and prokaryotic abundances at the end of the experiment (tf). The values are presented as mean \pm SD (n=3).

Compartment		Soil				Runoff				Sed					
		SedR		SedNR											
Condition	NPNC	PNC	NPC	PC	rainwater	NPN C	PN C	NP C	PC	NPNC	PNC	NPC	PC	NC	C
Plant biomass¹ (g DW)	-	61.3 \pm 25.3	-	32.9 \pm 12.1	-	-	-	-	-	-	-	-	-	-	-
Prokaryotic abundance² (x10³)	5.97 \pm 1.92x10 ⁵	4.41 \pm 1.54x10 ⁵	5.08 \pm 0.48x10 ⁵	4.22 \pm 0.98x10 ⁵	0.07	1.6 \pm 0.9	2.5 \pm 1	3 \pm 0.8	2.9 \pm 1.1	5.2 \pm 3.39 x10 ⁵	4.03 \pm 1.17 x10 ⁵	3.55 \pm 1.13 x10 ⁵	4.3 \pm 1.19 x10 ⁵	5.14 \pm 1.36 x10 ⁵	4.22 \pm 1.27 x10 ⁵

Soil: soil; Sed: sediment; SedR: sediment receiving runoff; SedNR: sediment without runoff; NP: no plant; P: plant; NC: no deuterated HC spiking; C: deuterated HC spiking; ¹aerial plant biomass; DW: soil or sediment dry weight; ²16S rRNA gene copies per g DW or per mL of runoff.

Such observation was consistent with previous studies indicating that DOC and HC establish complex interactions in soil (Ukalska-Jaruga et al., 2019) influencing the DOC composition in runoff (Daryabeigi Zand, 2020; Janeau et al., 2014), which in turn influence the microbial community structure and activity (Giorgio and Davis, 2003; Le et al., 2016; Pommier et al., 2014; Tian et al., 2015). In order to reveal the effect of the runoff on microbial community in marine compartment, the microbial functional capabilities were assessed by DNA-array analysis.

IV.2.4.2. Diversity, similarity and unique gene sequence variants

The functional gene diversity and composition (non-expressional DNA-array analysis) was explored on soil and sediment from the different microcosms after 39 days. A total of 51 897 gene variants representing 90 % of the 57 498 unique sequences (Shi et al., 2019) were detected on the GeoChip 5.0 S. The minimum GVs was found in sediments receiving runoff (SedR) from planted and HC spiked soils (Sed R PC; $32,274 \pm 1751$; Table IV.4 A) whereas the maximum GVs was found in sediments receiving runoff from non-planted and HC spiked soils (SedR NPC; $45,641 \pm 1277$; Table IV.4 A). The coverage was between $56 \% \pm 3.0$ and $79 \% \pm 2.2$ (Table IV.4 A). The GVs richness was significantly higher in sediment than in soil (Exponential Shannon 1D; $p < 0.05$; Table S IV.2) indicating a prevalence of abundant GVs in sediment, which can be explained by the properties of matrices. However, the relative abundances of main functional gene categories (carbon cycling, metal homeostasis, nitrogen, organic remediation, phosphorus, sulfur, secondary metabolism, virulence, and other) were similar (Figure S IV.1) as previously observed in marine coastal environment (Kappell et al., 2014). Such observation was also consistent with previous studies showing that sediments and soils harbour the same functions but with specific pathways (for review, Fenchel et al., 2012a, 2012b). No significant difference of GV richness was found within soils indicating that neither plants nor the HC spiking changed the functional diversity. However, the GVs richness was significantly different in planted soil with HC spi (richness R: $p < 0.05$; Table S IV.2) indicating that the interaction of the plants and the HC spiking affected the microbial functional diversity in soils. In sediments, the GVs richness was significantly higher in sediments receiving runoff from non-planted soil (SedR NP) (richness R: $p < 0.01$; Table S IV.2) than in sediments receiving runoff from planted soil (SedR P). It is likely that the runoff from planted soils exerted a selection on the functional gene diversity in sediments. The HC spiking had no effect on the GVs richness in sediments as observed in soils confirming that microbial communities were adapted to the presence of HC.

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Table IV.4 Global functional genes diversity: (A) Functional gene variants diversity indices and evenness; (B) Similarity (1-Bray-Curtis dissimilarity*100) and percentage of unique gene variants (**bold and shaded**) among matrices and conditions.

A

Microarray analysis	Soil				Sed					
	NPNC	PNC	NPC	PC	NRNC	NRC	NPNC	PNC	NPC	PC
Coverage (%) ¹	65.1	68.1	65.4	64.6	67.5	68.0	75.3	68.1	79.4	56.1
Richness (R)	37423 ± 695	39173 ± 2103	37616 ± 463	37135 ± 1375	38794 ± 5388	39076 ± 6281	43292 ± 5444	39135 ± 1099	45641 ± 1277	32274 ± 1751
Exponential Shannon (1D)	14491 ± 141	14850 ± 1037	13422 ± 1131	14194 ± 510	14634 ± 1657	15412 ± 2211	16210 ± 1383	15214 ± 694	18252 ± 698	12903 ± 1103
Inverse-Simpson (2D)	8581 ± 148	8791 ± 998	6977 ± 1962	8301 ± 652	7740 ± 875	8717 ± 1459	8483 ± 562	8175 ± 605	10778 ± 366	7020 ± 853
Evenness (E)	0.229 ± 0.002	0.224 ± 0.016	0.185 ± 0.050	0.223 ± 0.010	0.200 ± 0.007	0.224 ± 0.018	0.198 ± 0.025	0.209 ± 0.014	0.236 ± 0.003	0.217 ± 0.015

¹ Coverage = ratio of the detected probes / total number of probes available on GeoChip 5S*100

B		NPNC	PNC	NPC	PC	NRNC	NRC	NPNC	PNC	NPC	PC
Soil	NPNC	0.1									
	PNC	74.1	0.3								
	NPC	73.9	72.5	0.1							
	PC	74.7	73.6	73.2	0.1						
Sed	NRNC	70.4	69.0	69.5	70.9	0.2					
	NRC	72.5	71.6	71.2	71.7	70.4	0.2				
	NPNC	68.2	67.5	66.8	66.8	66.2	69.0	3.0			
	PNC	70.9	70.2	69.5	69.7	68.9	71.5	69.9	0.2		
	NPC	71.2	70.8	69.5	70.3	69.4	71.8	70.2	72.0	0.8	
	PC	70.6	70.5	69.4	69.2	66.5	70.8	67.4	70.5	69.1	0.1

IV.2.4.3. The effect of runoff on the functional structuration in sediment

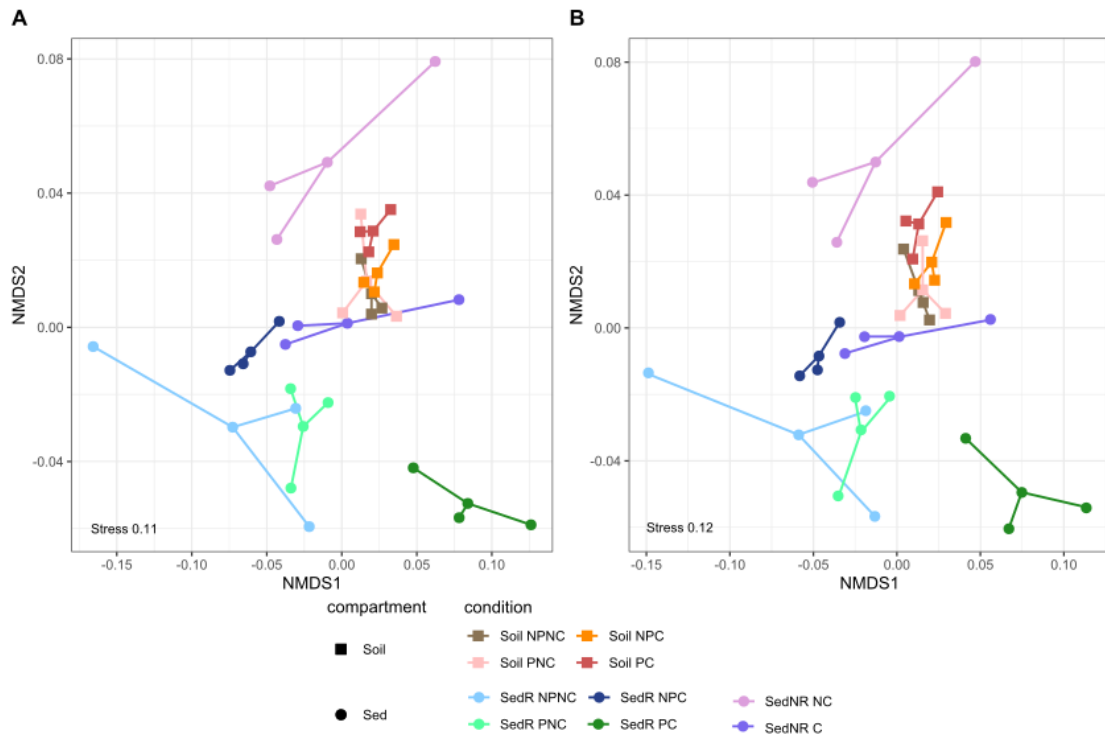


Figure IV.1 NMDS analysis based on the Bray Curtis metrics showing the different conditions of soil and sediment compartments overall the gene sequence variants (A) and for the GVS from the organic remediation category (B). Coloured segments link the three replicates from a condition to their average point. Soil: soil; Sed: sediment; SedR: sediment receiving runoff; SedNR: sediment without runoff (control); NP: no plant; P: plant; NC: no deuterated HC spiking; C: deuterated HC spiking.

To further characterize the effect of runoff on the functional organization, the GVs composition was investigated. The relative abundance of functional gene categories (Figure S IV.1) observed for all conditions revealed the functional stability of microbial communities. However, the comparison of functional microbial communities based on the GVs from all gene categories (Figure IV.1 A) showed similar pattern than that obtained by comparing the functional microbial communities based on the GVSs involved in the organic remediation (Figure IV.1 B), which was further supported by Procrustes ($M^2 = 0.017$, $r = 0.99$, $p\text{-value} < 0.001$). Such observation suggested that the presence of HC, in the historically contaminated soil and sediment, might explain the functional organization of the microbial communities, suggesting that HC act as a filter selecting the organisms that are able to survive and established in this specific conditions (Bordenave et al., 2004; Duran et al., 2015; Stauffert et al., 2013). However, the functional microbial communities structure was not affected by the spiking of HC (PERMANOVA $F(1) = 1.0825$; $p > 0.05$) suggesting a

legacy effect (Jurburg et al., 2017; Nunes et al., 2018). Additionally, NMDS analysis separated clusters according to the compartment, distinguishing the effect of runoff in sediments (Figure IV.1), which indicated functional redundancy, similar function carried by different genes and/or species (Jurburg and Salles, 2015; Louca et al., 2018), as illustrated in Figure IV.1 B for the organic remediation function. PERMANOVA confirmed soil and sediment separate clustering ($F(1) = 3.2066$; $p < 0.05$) unveiling that the ecological niches are the main filter for the organization of the function of the communities as previously proposed (Tringe et al., 2005). Among soil samples, the presence of plant did not affect the functional potential (PERMANOVA $F(1) = 1.1077$; $p > 0.05$). This result was not expected because several studies reported that microbial communities and even specific gene content are affected by the roots (Bourceret et al., 2016; Cébron et al., 2009; Gerhardt et al., 2009; Newman and Reynolds, 2004). Regarding sediments, those receiving runoff (SedR) were significantly different to those without receiving runoff (Sed NR) (PERMANOVA runoff: $F(1) = 1.5180$; $p < 0.05$), showing the influence of the runoff in shaping the functional microbial community structure. In addition, the effect of runoff was different according to presence of plant (PERMANOVA plant: $F(1) = 1.9498$; $p < 0.01$), which was in accordance with previous report showing the influence of plants in the runoff, affecting the amount of water runoff (Nagase and Dunnett, 2012).

IV.2.4.4. Functional gene indicators

IV.2.4.4.1. Gene family indicators

In order to obtain predictive association indices (r.g) correlating the abundance of specific functional gene family to conditions, i.e. gene family indicators, a multi-level pattern analysis (MLPA) was performed. The MLPA revealed that among the 372 genes families present on the DNA-array, 54 gene families (14.5 %) were significantly associated to a particular condition or other combinations of conditions (Figure IV.2). Soils and sediments were characterized by gene family indicators belonging to the following gene categories: carbon cycle, organic remediation, nitrogen cycle and metal homeostasis, the first two being predominantly represented (Figure S IV.1). The carbon and nitrogen cycle categories represent main microbial functions involved in organic matter decomposition and nutrient recycling (Burdige, 2011; Levy-Booth et al., 2014; Trivedi et al., 2016). The two other categories represent functions involved in response to the presence of organic and metallic pollutants (Cébron et al., 2008; Michas et al., 2017) substantiating the legacy effect. Sediments receiving runoff (SedR) had more gene family indicators than soils and sediments without runoff (Figure IV.2). It is likely that sediments exhibit higher diversity of gene family indicators because they are at the mixing land-sea interface area providing a large spectrum of compounds, which require various microbial functions (Groffman and Bohlen, 1999; Guo et al., 2018; Wang et al., 2012).

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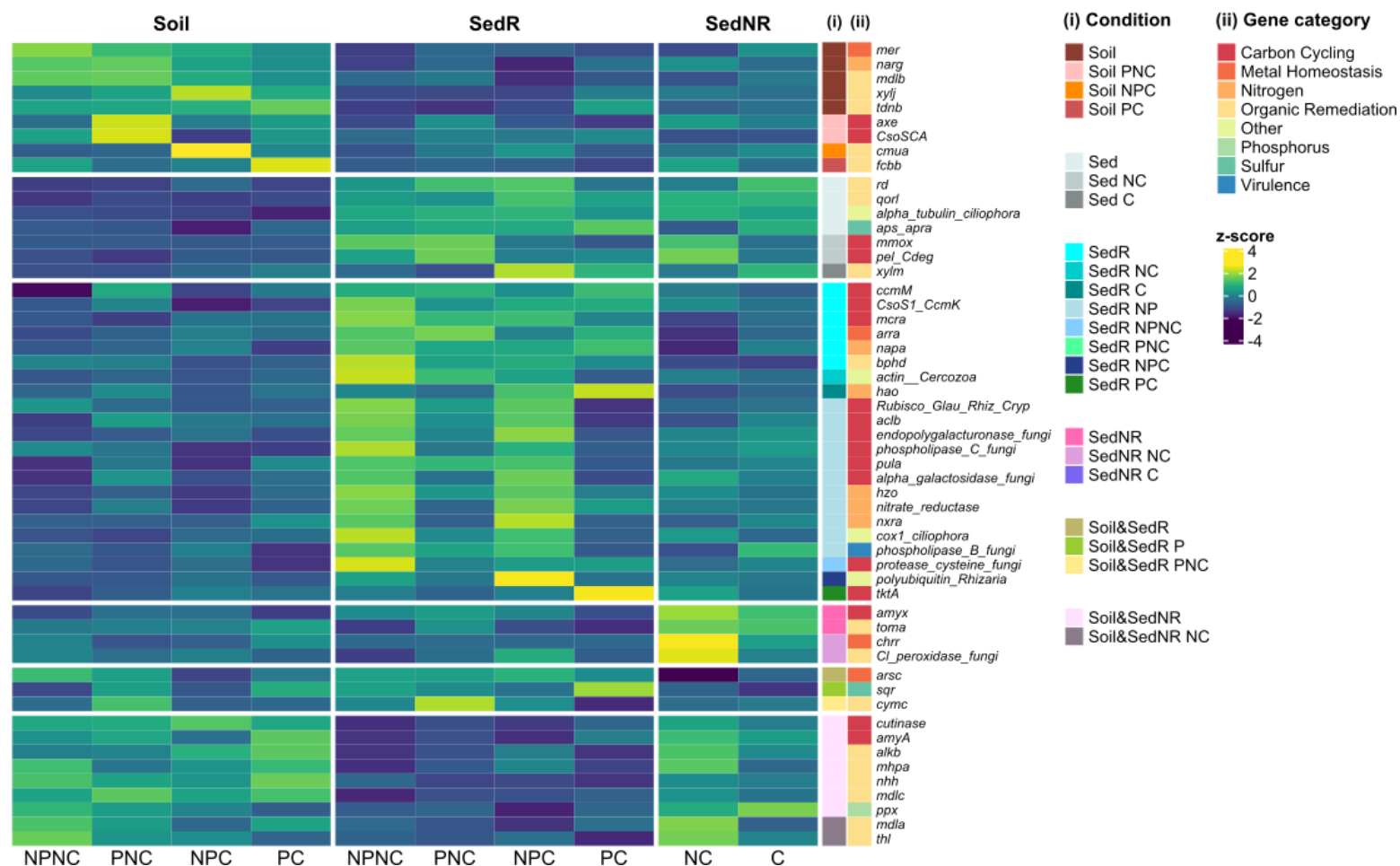


Figure IV.2 Functional gene family indicators for the different conditions of soil and sediment compartment. The heatmap represents the z-scores of indicators at the gene family level. The z-scores were computed on the gene family basis by subtracting the mean and dividing by the standard deviation of the fluorescence intensity (blue for the low fluorescence intensity and orange for the high fluorescence intensity). On the left of the heatmap, the row annotations indicate the condition (i) and the gene family category (ii). The data represent the mean of three biological replicates ($n=3$). Soil: soil; Sed: sediment; SedR: sediment receiving runoff; SedNR: Sed without runoff (control); NP: no plant; P: plant; NC, no deuterated HC spiking; C, deuterated HC spiking.

Noteworthy, in sediment receiving runoff (SedR), any gene family indicators was identified neither for sediment receiving runoff from planted soil SedR P, neither for sediment with runoff from planted and HC spiked soil (SedR PNC) the HC spiking and in presence of plant. Further indicator analyses were performed at the GVs level among the gene families related to the HC remediation, focusing in sediments, which represents the mixing zone in the land-sea continuum.

IV.2.4.4.2. GVs indicators

The identification of community coalescence GVs indicators in sediments was carried out on functional genes involved in the HC remediation including 7374 GVs belonging to 32 gene families which were selected based on DNA-array classification, KEGG pathways (Kanehisa and Goto, 2000) and Aromadeg database (Duarte et al., 2014). Among the 7374 GVs, 873 GVs, distributed in 7 family subcategories, were significantly associated to either a condition or a combination of conditions (Figure IV.3 and Figure IV.4). GVs indicators were identified for the 6 conditions (SedR NPNC, SedR NPC, SedR PNC, SedR PC, SedNR NC, SedNR C) (Figure IV.3). However, sediments receiving runoff (SedR) exhibited more GVs indicators than those without runoff (SedNR), irrespective of the presence of plants and HC spiking (Figure IV.3 and Figure IV.4). The GVs indicators belonged to three main function families involved in sulfite reduction (*dsrA/B*), denitrification (*narG*, *nirK*, *nirS*, and *nosZ*) and polyaromatic remediation (PAH-ring hydroxylating dioxygenases, PAH-rhd) (Figure IV.4). It was not surprising to find GVs indicators related to aerobic and anaerobic conditions because oxic/anoxic oscillations were favored in microcosm experiment through tidal cycles. Such oxic/anoxic oscillations have been described in coastal marine sediments (Cravo-Laureau and Duran, 2014) which influence the organization of sulfate reducers (Stauffert et al., 2015), denitrifiers (Stauffert et al., 2014) and HC degraders (Stauffert et al., 2013).

In addition, higher diversity of GVs indicators was observed in sediments receiving runoff (SedR) than the one without (Sed NR) (Figure IV.4 A). Such observation suggests that runoff enriched sediments with nutrients and microorganisms via coalescence (Châtillon et al., 2021 Chapter III) increasing functional diversity. HC spiked sediments receiving or not runoff exhibited the same categories of GVs indicators (Figure IV.4 A). Similarly, the pattern of the categories of GVs indicators was similar in sediments receiving runoff from planted soil or non-planted soils (Figure IV.4 B). However, the differences between these conditions were observed only at the GVs levels (Figure IV.3 and Figure IV.4), supporting the functional redundancy. Such functional redundancy have been previously describe in HC polluted marine coastal sediment (Cravo-Laureau and Duran, 2014; Stauffert et al., 2013), which correspond to known mechanisms involved in adaptive response of microorganisms to environmental perturbation (Allison and Martiny, 2008).

Functional redundancy as structural microbial community response in land-sea continuum contaminated by HC

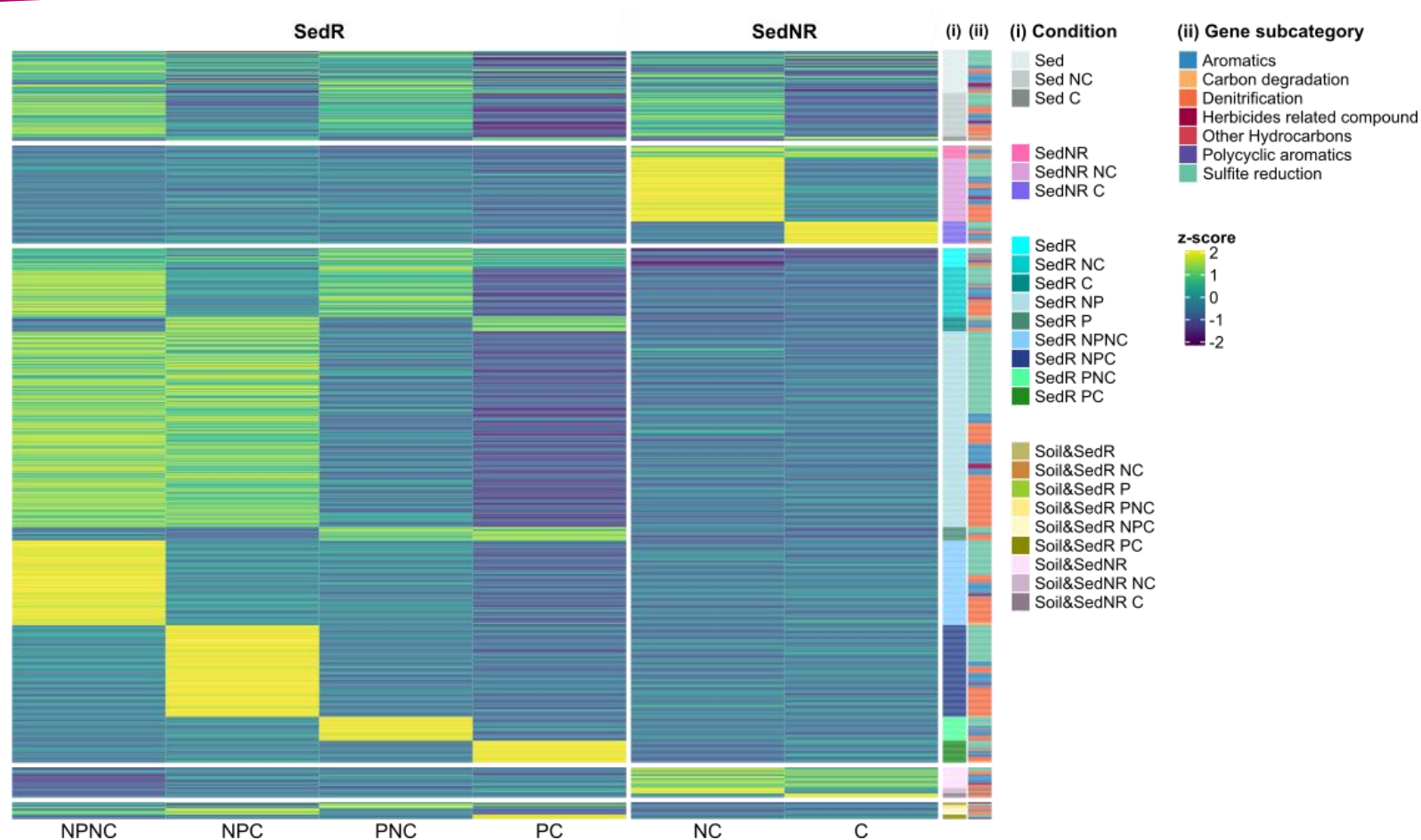


Figure IV.3 Functional HC biodegradation gene indicators for the different sediment conditions. The heatmap represents the z-scores of bioindicators at the GVs level. The z-scores were computed on the GVs basis by subtracting the mean and dividing by the standard deviation of the fluorescence intensity (blue for the low fluorescence intensity and yellow for the high fluorescence intensity). On the left of the heatmap, the row annotations indicate the condition (i) and the gene family subcategory from the organic remediation category (ii). The data represent the mean of three biological replicates (n=3). Sed: sediment; SedR: sediment receiving runoff; SedNR: Sed without runoff (control); NP: no plant; P: plant; NC: no deuterated HC spiking; C: deuterated HC spiking.

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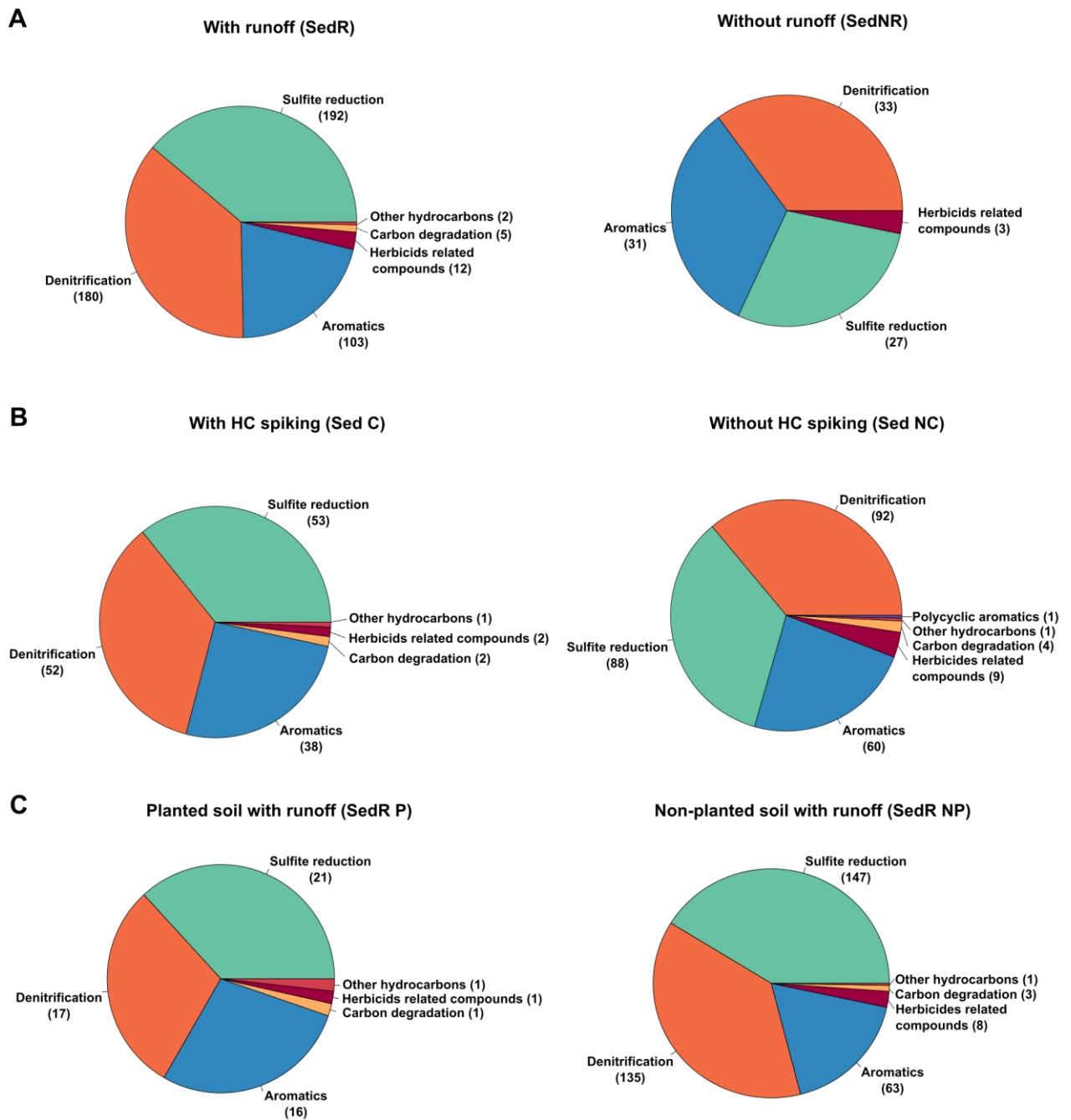


Figure IV.4 Distribution of the different GSV subcategories of organic remediation category in sediment (A) with (SedR) or without (SedNR) runoff, (B) with (Sed C) or without (Sed NC) HC spiking, (C) planted (SedR P) or non-planted (SedR NP) soil with runoff. The number of GVs for each subcategory is indicated in bracket.

In conclusion, our results showed that increasing the resolution of the analysis of indicators at the scale of the GVs was relevant to decipher the influence of the conditions in structuring the microbial functional potential. Consistently, similar observations have been reported for taxonomic analysis showing that environmental perturbations are observed only at lower taxonomic level (Kleindienst et al., 2016; Terrisse et al., 2017). The identified GVs indicators play probably a key role in structuring the microbial functional network.

IV.2.4.5. Functional co-occurrence network

In order to determine the keystones GVs, a co-occurrence network analysis was performed. Based on correlation relationship and dissimilarity indices, a functional co-occurrence network was constructed for the conditions with or without runoff, combining GSV indicators from soil and sediment (Figure IV.5). The network without runoff included 173 nodes and 334 significant edges, while the network with runoff included 174 nodes and 445 significant edges (Table IV.5). The network density, the clustering coefficient, the average number of neighbours and the characteristic path length were higher in the network with runoff (Table IV.5).

Table IV.5 Topological properties of the networks generated for GVs indicators in the conditions without or with runoff.

	Without Runoff	With Runoff
Number of nodes	173	174
Numbers of edges	334	445
Positive edge percentage	93.4 %	95.3 %
Average number of neighbours	3.861	5.145
Characteristic path length	2.163	2.359
Clustering coefficient	0.117	0.135
Network density	0.011	0.015
Connected components	16	9
Number of modules	5	8

These observations suggest that terrigenous inputs resulted in the reorganization of the GSV associations with an increase of the network complexity. The network with runoff showed higher modularity (eight modules representing 25% of the nodes) than the network without runoff (five modules representing 17% of the nodes) further supporting the increase of network complexity due to terrigenous inputs. Such increase in network complexity has been observed in coastal sediment depending on the land-use (Zou et al., 2020) and in PAH polluted sediment (Yan et al., 2021). According to the DNA-array taxonomy, each module in both networks included GVs affiliated to diverse bacterial taxa, suggesting that a module represents communities rather than single populations. The major modules (with highest scores and more than three nodes, Table S IV.3) were characterized by the presence of at least one gene involved in the HC degradation and genes involved in the sulfite reduction and denitrification (Figure IV.5). Although GVs were shared by both networks, they showed different associations, that resulted in different modules, confirming the difference between the networks. Accordingly, the keystone GVs (i.e. highly abundant and connected) identified in the major modules were different in both networks (Table S IV.3) exhibiting similar function. It is likely that the functional redundancy, observed at the GSV level, sustains the main environmental functions despite the reorganization of the networks due to terrigenous inputs.

IV.2.5. Conclusion

This study showed that the removal of HC in sediment was not affected by the soil runoff despite the enrichment in organic matter and microorganisms. However, functional genes were not enriched in sediment receiving runoff but the GV composition was different identifying GV indicators. The terrigenous inputs increased the network complexity and modularity without affecting the overall functions highlighting the functional redundancy. The experimental approach mimicking coalescence zone occurring in the land-sea continuum combined with analyses at the GV level revealed that functional redundancy is the main structural response even in mixing environment. Our results provide helpful functional information for the monitoring and management of coastal environment affected by human land-based activities.

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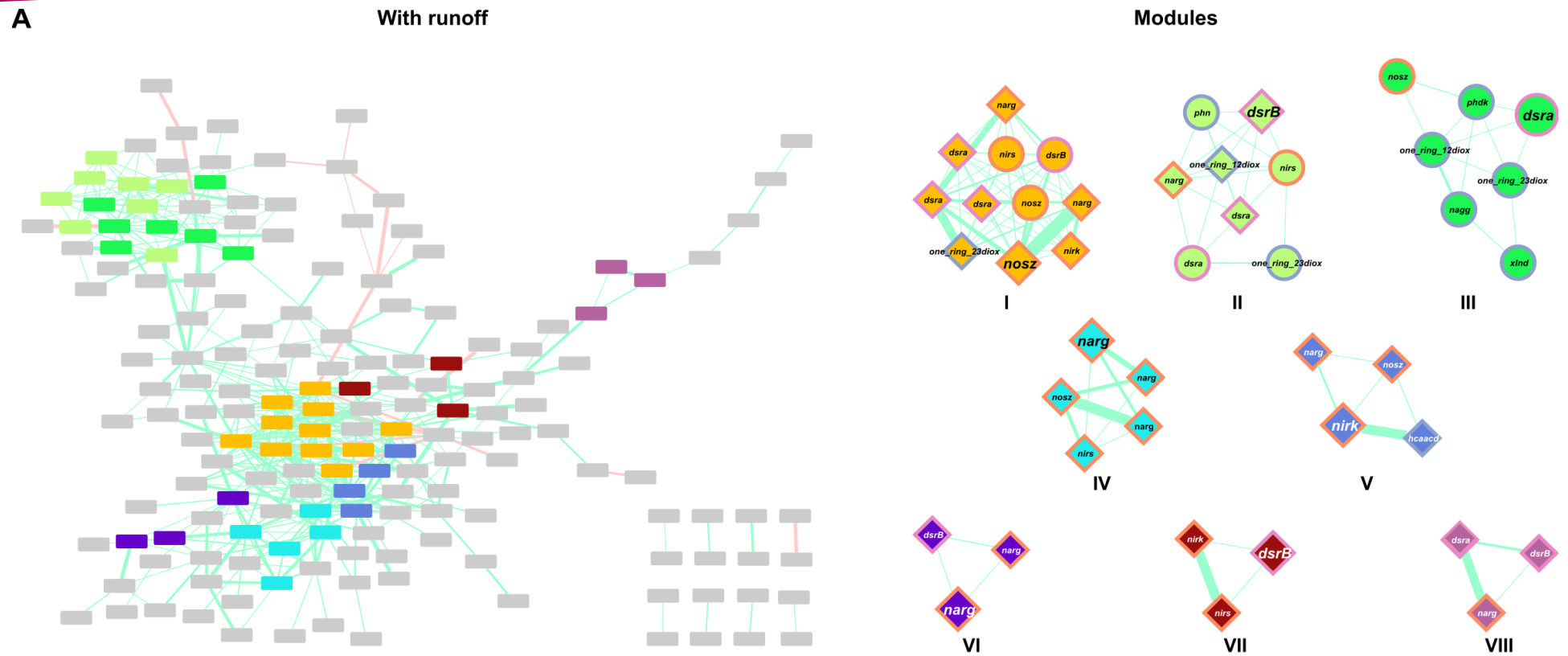
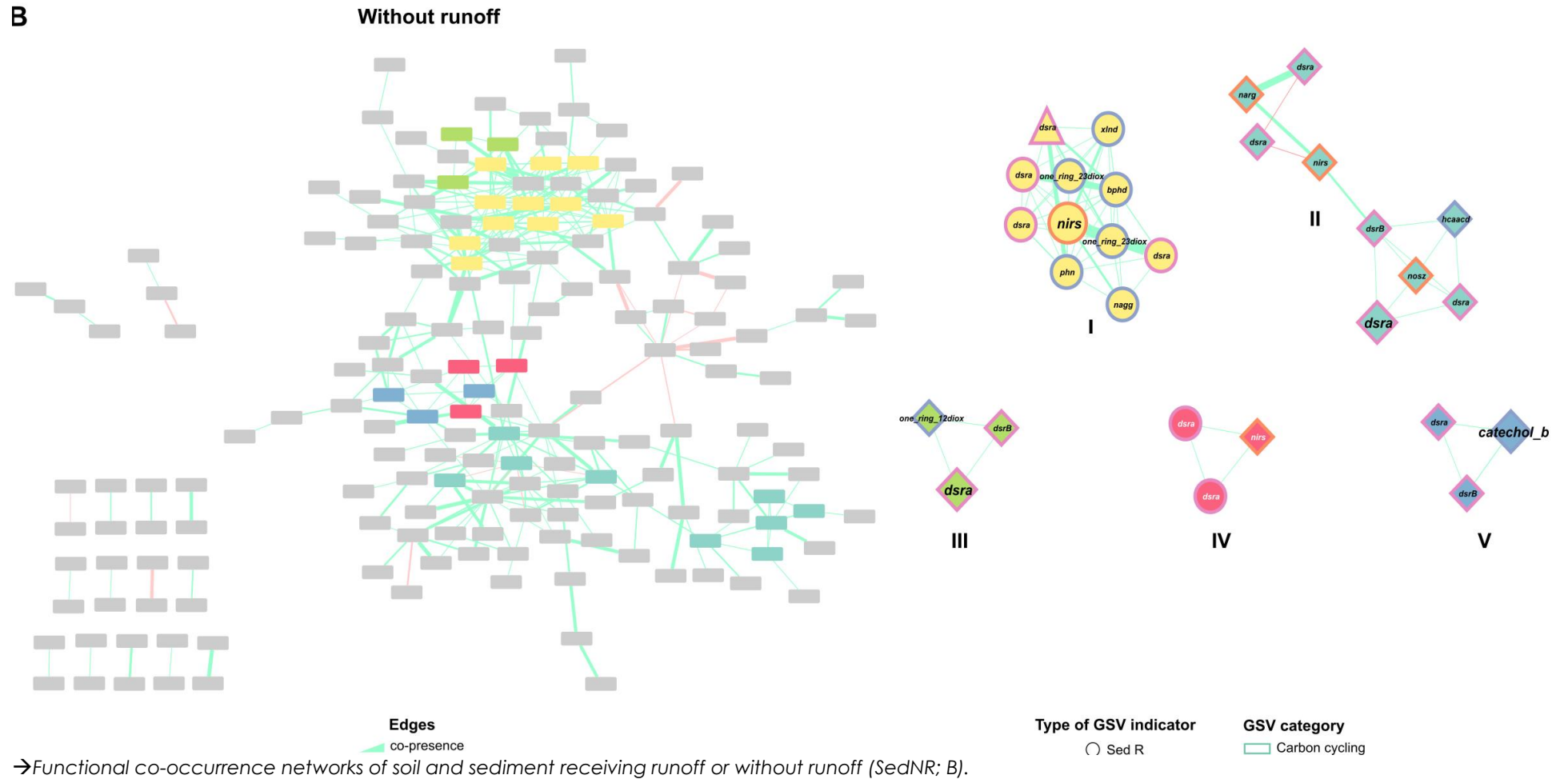


Figure IV.5 Functional co-occurrence networks of soil and sediment receiving runoff (SedR; A) or without runoff (SedNR; B). The nodes were coloured according to the different modules (grey is used for unclustered nodes). The edges represent a robust (convergence of the measures) and significant association (p -value <0.05) between nodes. The size of each nodes is proportional to their weight and the thickness of the edges is proportional to each association coefficient between GVs. On the right, modules of co-occurring microbial GVs of soil and sediment receiving runoff (SedR; A) and in soil and sediment without runoff (SedNR; B) at the end of the experiment based on correlations and dissimilarities analysis. Within modules, the border of the nodes matched the gene categories.

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Author Contributions:

CCL, RD, AC designed the study. CCL, RD, AC, FR collected the samples. AC run the microcosm experiment. EC performed all data analyses. AC, CL and PF performed the chemical analyses. EC, CCL, RD wrote the first draft and AC, FR, CC, contributed to the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Supplementary data: Supplementary data to this article can be found online at xxx.

IV.2.6. References

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IV.2.7. Supplementary data

Table S IV.1 Temporal changes of the dissolved organic carbon (DOC) content in the runoff and in the seawater above sediment.

Runoff													
Time	2				23				36				
Condition	Rainwater		C		NC		C		NC		C		
Cover	NC		C		NPNC	PNC	NPC	PC	NPNC	PNC	NPC	PC	
DOC (mg/L)	80,8 ±				31,8 ±	29,7 ±	36,1 ±	32,4 ±	14,8 ±	21,7 ±			
	2,9	6,8	90,1 ± 16		6,3	4,3	3,2	1,3	1,8	7,7	13,7 ± 2,6		16,4 ± 0,6

Seawater above sediment																
Time (days)	2				23				36							
Condition	Seawater		NR		R		NR		R		NR		R			
HC	N		C		NC		C		NC		C		C			
Cover	NC		C		NP		P		NC		P		NC			
DOC (mg/L)	1,2		1,7	2,9	3 ± 0,3	2,6 ± 0,6	nd	nd	nd	nd	1,2 ± 0,1	1,6 ± 0,5	2,3 ± 0,6	2,1 ± 0,3	2 ± 0,4	2 ± 0,2

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Table S IV.2 Summary of the significance in the ANOVA and post-hoc tests (Tukey tests) based on models with interaction describing the functional gene diversity depending on the condition. Sed: sediment; tf: at the end of the experiment, day 39; matrix: soil or sediment; HC: with spiking of HC (C) or not (NC); cond: factor grouping runoff and cover; cover: with the presence of plant (P) or without (NP).

Samples	Models	Alpha diversity (α) indices					
		Richness (R)		Exponential Shannon (1D)		Inverse-Simpson (2D)	
		Anova	Tuckey	Anova	Tuckey	Anova	Tuckey
All	a~matrix*HC	p>0.05	-	p>0.05	-	p>0.05	-
	a~matrix*cond*HC	cond p<0.01	RunoffxP-RunoffxNP	matrix p<0.05	-	cond p<0.01	RunoffxP-RunoffxNP
				cond p<0.01	RunoffxP-RunoffxNP	HC:cond p<0.05	NC:RunoffxNP-C:RunoffxNP
				HC:cond p<0.05	C:RunoffxP-C:RunoffxNP C:RunoffxP-NC:RunoffxNP	matrix:HC p<0.05	unsolved
Soil tf	aSoil~cover*HC	p>0.05	-	p>0.05	-	p>0.05	-
		cover p<0.01	NP-P	cover p<0.01	NP-P	cover p<0.01	NP-P
Sed tf	aSed~*Runoff*cover*HC			cover:HC p<0.05	P:C-NP:C	cover:HC p<0.01	P:C-NP:C NP:NC-NP:C o:NC-NP:C P:NC-NP:C
Sed tf	aSed~cond*HC	cond p<0.05	RunoffxP-RunoffxNP	cond p<0.01	RunoffxP-RunoffxNP	cond p<0.01	RunoffxP-RunoffxNP
						cond:HC p<0.05	RunoffxP:C-RunoffxNP:C RunoffxP:NC-RunoffxNP:C

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Figure S IV.1 Relative abundance of functional gene categories detected by DNA-array analysis. Each bar corresponds to the average of replicates (n = 3). Soil: soil; Sed: sediment; SedR: sediment receiving runoff; SedNR: Sed without runoff (control); NP: without plant; P: with plant; NC: without spiking of HC; C: with spiking of HC.

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Table S IV.3 Modularity of the network

	Modules	Score*	Nodes	Edges	Node ID
Network with runoff	I	10,2	11	51	gv149360962b, gv32307985, gv94471299, gv109452510a, gv422314413, gv4633574 , gv118424367, gv262089362, gv371486322, gv210153887, gv77820036
	II	5,714	8	20	gv383210500, gv63029731 , gv94471133, gv295413225, gv375302211, gv407788393, gv295412494b, gv146188517
	III	4	7	12	gv63029733, gv2588986, gv206686013b, gv4105709b, gv83723753a, gv430760928, gv302673547
	IV	4	5	8	gv116013294, gv421953160, gv315492406, gv284390194, gv14348604
	V	3,333	4	5	gv199593995, gv334103606b, gv403311954, gv112464033
	VI	3	3	3	gv62512285a, gv190574691 , gv160917801
	VII	3	3	3	gv339787607, gv223585900, gv12667662
	VIII	3	3	3	gv73763010, gv306488215, gv18034308
Network without runoff	Module	Score*	Nodes	Edges	Node ID
	I	8,6	11	43	gv1177721, gv4105709b, gv157679479, gv63029733, gv302673547, gv383210500 , gv375302211, gv295412494b, gv295413225, gv206686013b, gv109452506
	II	3,5	9	14	gv70930934, gv421953160, gv2329993 , gv251848602, gv223585900, gv77820044, gv334103606b, gv116743193, gv18034308
	III	3	3	3	gv229610694, gv87281197, gv187721667
	IV	3	3	3	gv407788393, gv63029731, gv146188517
V	3	3	3	gv13898441, gv7672528 , gv63029767	

*score = density x number of nodes; **identification of the GVs is provided in Table S 4; GVs in bold represented the keystone GVs.

Functional redundancy as structural microbial community response in land-sea continuum contaminated by HC

Table S IV.4 Taxonomic affiliation of the GVs per networks and modules.

Network without runoff											
Modules	I										
GV ID	383210500	295412494b	4105709b	302673547	206686013b	1177721	295413225	109452506	63029733	157679479	375302211
Accession Number	BAM08341.1	ADG06986.1	AAD12763.1	XP_003026460.1	EDZ46495.1	AAB17100.1	ADG07717.1	BAE96491.1	AAY27759.1	BAF80580.1	AFA48345.1
gene	nirs	one_ring_23di ox	one_ring_23di ox	nagg	xlnD	bphd	phn	dsra	dsra	dsra	dsra
Gene category	Nitrogen	Organic Remediation	Organic Remediation	Organic Remediation	Organic Remediation	Organic Remediation	Organic Remediation	Sulfur	Sulfur	Sulfur	Sulfur
Subcategory1	Denitrification	Aromatics	Aromatics	Aromatics	Aromatics	Aromatics	Herbicides related compound	sulfite reduction	sulfite reduction	sulfite reduction	sulfite reduction
Subcategory2		Other aromatics	Other aromatics	Aromatic carboxylic acid	Other aromatics	Polycyclic aromatics					
Species	uncultured bacterium	Kyrpidia tusciae DSM 2912	Pseudomonas fluorescens	Schizophyllum commune H4-8	Rhodobacterale s bacterium Y4I	Rhodococcus sp. M5	Kyrpidia tusciae DSM 2912	uncultured sulfate- reducing bacterium	uncultured sulfate- reducing bacterium	uncultured sulfate- reducing bacterium	Acetobacteriu m woodii DSM 1030
Lineage	Bacteria	Bacteria Firmicutes Bacilli	Bacteria Proteobacteria Gammaproteob	Eukaryota Fungi Basidiomycota	Bacteria Proteobacteria Alphaproteoba	Bacteria Actinobacteria Actinobacteria	Bacteria Firmicutes Bacilli	Bacteria	Bacteria	Bacteria	Bacteria Firmicutes Clostridia

Functional redundancy as structural microbial community response in land-sea continuum contaminated by HC

	Bacillales	acteria	Agaricomycete	acteria	Actinomycetal	Bacillales			Clostridiales
	Alicyclobacilla	Pseudomonada	s Agaricales	Rhodobacterale	es	Alicyclobacilla			Eubacteriaceae
	ceae Kyrpidia	les	Schizophyllace	s	Nocardiaceae	ceae Kyrpidia			Acetobacterium
	Kyrpidia	Pseudomonada	ae		Rhodococcus	Kyrpidia			m
	tusciae	ceae	Schizophyllum			tusciae			Acetobacterium woodii
		Pseudomonas	Schizophyllum	commune					
Modules	II								
GV ID	223585900	77820044	421953160	334103606b	116743193	70930934	251848602	2329993	18034308
accessionNo	ACM91802.1	ABB04158.1	AFX71889.1	AEG51029.1	CAJ84836.1	AAZ15784.1	EES76565.1	AAB66716.1	AAL57452.1
gene	narg	nirs	nosz	hcaacd	dsra	dsra	dsra	dsra	dsrB
geneCategory	Nitrogen	Nitrogen	Nitrogen	Organic Remediation	Sulfur	Sulfur	Sulfur	Sulfur	Sulfur
subcategory1	Denitrification	Denitrification	Denitrification	Aromatics	sulfite reduction	sulfite reduction	sulfite reduction	sulfite reduction	sulfite reduction
subcategory2				Aromatic carboxylic acid					
species	uncultured bacterium	uncultured organism	uncultured bacterium	Sphingobium chlorophenolicum L-1	uncultured sulfate-reducing bacterium	uncultured sulfate-reducing bacterium	Ruminococcus sp. 5_1_39B_FAA	Desulfovibrio simplex	Desulfonatronum lacustre
lineage	Bacteria		Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria

Functional redundancy as structural microbial community response in land-sea continuum contaminated by HC

				Proteobacteria Alphaproteobacteria Sphingomonadales Sphingomonadales Sphingobium Sphingobium chlorophenolicum			Firmicutes Clostridia Clostridiales Ruminococcales Ruminococcus	Proteobacteria Deltaproteobacteria Desulfovibrionales Desulfovibrionales Desulfonatronum	Proteobacteria Deltaproteobacteria Desulfovibrionales Desulfonatronum
Modules	III			IV			V		
GV ID	87281197	229610694	187721667	407788393	146188517	63029731	7672528	13898441	63029767
accessionNo	ABD36703.1	CAQ77272.1	ACD22888.1	ZP_11135524.1	CAL64101.1	AAY27758.1	AAF66550.1	AAK48739.1	AAY27782.1
gene	nirs	dsra	dsra	one_ring_12di ox	dsra	dsrB	catechol_b	dsra	dsrB
geneCategory	Nitrogen	Sulfur	Sulfur	Organic Remediation	Sulfur	Sulfur	Organic Remediation	Sulfur	Sulfur
subcategory1	Denitrification	sulfite reduction	sulfite reduction	Aromatics	sulfite reduction	sulfite reduction	Aromatics	sulfite reduction	sulfite reduction
subcategory2				Other aromatics			Other aromatics		

Functional redundancy as structural microbial community response in land-sea continuum contaminated by HC

species	uncultured bacterium	uncultured sulfate- reducing bacterium	Clostridium botulinum B str. Eklund 17B	Celeribacter baekdonensis B30	uncultured sulfate- reducing bacterium	uncultured sulfate- reducing bacterium	Geobacillus thermogluco- sidius	sulfate- reducing bacterium STC	uncultured sulfate- reducing bacterium
lineage	Bacteria	Bacteria	Bacteria Firmicutes Clostridia Clostridiales Clostridiaceae Clostridium Clostridium botulinum	Bacteria Proteobacteria Alphaproteobacteria Rhodobacterales Rhodobacteraceae Celeribacter Celeribacter baekdonensis	Bacteria	Bacteria	Bacteria Firmicutes Bacilli Bacillales Bacillaceae Geobacillus	Bacteria Proteobacteria Deltaproteobacteria	Bacteria

Monitoring plasmid-mediated horizontal gene transfer in the hydrocarbon contaminated land-sea continuum

Network with runoff											
Modules	I										
GV ID accession No	94471299	32307985	77820036	422314413	4633574	149360962b	262089362	371486322	118424367	210153887	109452510a
	ABF20960.1	AAP79373.1	ABB04154.1	AFX81620.1	AAD26835.1	EDM49413.1	ACY24580.1	AEX31207.1	ABK90703.1	EEA84893.1	BAE96494.1
gene geneCategory1 subcategory1 subcategory2	narg	narg	nirs	nosz	nosz	one_ring_23dio x	nirk	dsra	dsra	dsra	dsrB
	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Organic Remediation	Nitrogen	Sulfur	Sulfur	Sulfur	Sulfur
	Denitrification	Denitrification	Denitrification	Denitrification	Denitrification	Aromatics	Denitrification	sulfite reduction	sulfite reduction	sulfite reduction	sulfite reduction
						Other aromatics					
lineage	uncultured bacterium	uncultured bacterium	uncultured organism	uncultured bacterium	uncultured bacterium 696AB	Marinobacter algicola DG893	uncultured crenarchaeote	uncultured sulfate-reducing bacterium	uncultured sulfate-reducing bacterium	Clostridium hiranonis DSM 13275	uncultured sulfate-reducing bacterium
	Bacteria	Bacteria		Bacteria	Bacteria	Bacteria	Archaea Crenarchaeota	Bacteria	Bacteria	Bacteria	Bacteria
						Proteobacteria Gammaproteobacteria Alteromonadales				Firmicutes	
						Alteromonadaceae				Clostridia	
										Clostridiales	
										Peptostreptococaceae	
										[Clostridium] hiranonis	

II											
Modules	II										
GV ID accession	94471133	383210500	407788393	295412494b	295413225	146188517	375302211	63029731			
	ABF20877.1	BAM08341.1	ZP_11135524.1	ADG06986.1	ADG07717.1	CAL64101.1	AFA48345.1	AAY27758.1			
	narg	nirs	one_ring_12dio	one_ring_23diox	phn	dsra	dsra	dsrB			

Functional redundancy as structural microbial community response in land-sea continuum contaminated by HC

No			x					
gene	Nitrogen	Nitrogen	Organic Remediation	Organic Remediation	Organic Remediation	Sulfur	Sulfur	Sulfur
geneCategory	Denitrification	Denitrification	Aromatics	Aromatics	Herbicides related compound	sulfite reduction	sulfite reduction	sulfite reduction
subcategory1			Other aromatics	Other aromatics				
subcategory2	uncultured bacterium	uncultured bacterium	Celeribacter baekdonensis B30	Kyrpidia tusciae DSM 2912	Kyrpidia tusciae DSM 2912	uncultured sulfate-reducing bacterium	Acetobacterium woodii DSM 1030	uncultured sulfate-reducing bacterium
lineage	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria
			Proteobacteria Alphaproteobacteria	Firmicutes	Firmicutes		Firmicutes	
			Rhodobacterales Rhodobacteraceae	Bacilli	Bacilli		Clostridia Clostridiales	
			Celeribacter	Bacillales	Bacillales Alicyclobacillaceae		Eubacteriaceae	
			Celeribacter baekdonensis	Alicyclobacillaceae	Kyrpidia		Acetobacterium	
				Kyrpidia tusciae	Kyrpidia tusciae		Acetobacterium woodii	

III

Modules	430760928	83723753a	4105709b	2588986	302673547	206686013b	63029733
GV ID	YP_007216785.1	EAP70946.1	AAD12763.1	BAA23265.1	XP_00302646	EDZ46495.1	AAAY27759
accession		one_ring_12dio	one_ring_23dio		0.1		.1
No	nosz	x	x	phdk	nagg	xlnd	dsra
gene	Nitrogen	Organic Remediation	Organic Remediation	Organic Remediation	Organic Remediation	Organic Remediation	Sulfur
geneCategory	Denitrification	Aromatics	Aromatics	Polycyclic aromatics	Aromatics	Aromatics	sulfite reduction
subcategory1		Other aromatics	Other aromatics		Aromatic carboxylic acid	Other aromatics	

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subcategory2	Thioalkalivibrio nitratireducens DSM 14787	Ralstonia solanacearum UW551	Pseudomonas fluorescens	Nocardioides sp.	Schizophyllum commune H4-8	Rhodobacterales bacterium Y4I	uncultured sulfate-reducing bacterium		
lineage	Bacteria	Bacteria	Bacteria	Bacteria	Eukaryota	Bacteria	Bacteria		
	Proteobacteria Gammaproteobacteria	Proteobacteria Betaproteobacteria	Proteobacteria Gammaproteobacteria	Actinobacteria	Fungi	Proteobacteria Alphaproteobacteria			
	Chromatiales Ectothiorhodospiraceae	Burkholderiales Burkholderiaceae	Pseudomonadales Pseudomonadaceae	Actinomycetales	Basidiomycota Agaricomycetes	Rhodobacterales			
	Thioalkalivibrio	Ralstonia	Pseudomonas	Nocardioides	Agaricales Schizophyllum eae				
	Thioalkalivibrio nitratireducens	Ralstonia solanacearum			Schizophyllum commune				

Modules	IV					V				
GV ID accession No	284390194	315492406	14348604	116013294	421953160	199593995	112464033	403311954	334103606b	
gene	narg	narg	narg	nirs	nosz	narg	nirk	nosz	hcaacd	
geneCategory	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Organic Remediation	
subcategory1	Denitrification	Denitrification	Denitrification	Denitrification	Denitrification	Denitrification	Denitrification	Denitrification	Aromatics	
subcategory2									Aromatic carboxylic acid	
lineage	uncultured bacterium	Mobiluncus curtisii subsp. holmesii ATCC 35242	Paracoccus pantotrophus	uncultured bacterium	uncultured bacterium	uncultured bacterium	uncultured bacterium	Riemerella anatipestifer RA-CH-1	Sphingobium chlorophenolicum L-1	
	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	
		Actinobacteria	Proteobacteria					Bacteroidetes	Proteobacteria	
		Actinobacteria	Alphaproteobacteria					Flavobacteriia	Alphaproteobacteria	

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Actinomycetales	Rhodobacterales	Flavobacteria	Sphingomonadales
Actinomycetaceae	Rhodobacteraceae	Flavobacteriaceae	Sphingomonadaceae
Mobiluncus	Paracoccus	Riemerella	Sphingobium
Mobiluncus curtisii		Riemerella anatipestifer	Sphingobium chlorophenolicum

Modules	VI			VII			VIII		
GV ID	160917801	190574691	62512285a	223585900	12667662	339787607	73763010	306488215	18034308
accession No	ABX52696.1	YP_001972536.1	AAX84567.1	ACM91802.1	AAG61235.1	AEK11874.1	AAZ83914.1	ADM93690.1	AAL57452.1
gene	narg	narg	dsrB	narg	dsra	dsrB	nirk	nirs	dsrB
geneCategory1	Nitrogen	Nitrogen	Sulfur	Nitrogen	Sulfur	Sulfur	Nitrogen	Nitrogen	Sulfur
subcategory1	Denitrification	Denitrification	sulfite reduction	Denitrification	sulfite reduction	sulfite reduction	Denitrification	Denitrification	sulfite reduction
subcategory2					uncultured sulfate-reducing bacterium	uncultured sulfate-reducing bacterium			
lineage	uncultured bacterium	Stenotrophomonas maltophilia K279a	uncultured sulfate-reducing bacterium	uncultured bacterium	UMTRAdsr610-13	uncultured sulfate-reducing bacterium	uncultured bacterium	uncultured organism	Desulfonatronum lacustre
	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria
		Proteobacteria			Proteobacteria				Proteobacteria
		Gammaproteobacteria			Deltaproteobacteria				Deltaproteobacteria
		Xanthomonadales							Desulfovibrionales
		Xanthomonadaceae							Desulfonatronum
		Stenotrophomonas							aceae
		as							Desulfonatronum

IV.3. Conclusion of the chapter

In this chapter, the functional profile analysis aimed to determine whether terrigenous inputs from soil runoff increase the functional potential of marine coastal sediment microbial communities and influence the fate of HC. This analysis used the GeoChip5.0 functional array on soil and sediment DNA extracted from the different microcosms. The results show similar HC removal and similar functional richness in sediment, irrespective of the presence of terrigenous inputs. However, sediment receiving terrigenous inputs contained specific GVs resulting in a functional co-occurrence network with different topological characteristics, compared to sediment without terrigenous inputs. Nevertheless, GVs of the network modules were related to the same gene families that support the same functions, irrespective of the presence of terrigenous inputs. Therefore, microbial communities in both sediment with or without terrigenous inputs had the same metabolic capabilities indicating functional redundancy.

Horizontal gene transfer (HGT) allows the transfer of traits including set of metabolic genes between potentially distant bacteria and then contributes to functional redundancy in microbial communities. Thus, the next chapter explores the transfer of plasmids by conjugation in the land-sea continuum. In particular, we are interested in the influence of the terrigenous inputs on the uptake of plasmid by marine bacterial communities.

Chapter V:

Monitoring plasmid-mediated horizontal gene transfer in the hydrocarbon contaminated land- sea continuum

Monitoring plasmid-mediated horizontal gene transfer in the hydrocarbon contaminated
land-sea continuum

Chapter V. Monitoring plasmid-mediated horizontal gene transfer in the hydrocarbon contaminated land-sea continuum

V.1. Background of the study

In this chapter, we were interested in evolutionary implications of coalescence; in particular, we considered plasmid mediated-horizontal gene transfer (HGT). In fact, coalescence event allows new coexistences of microorganisms coming from different habitat and the mixing of environmental conditions (Rillig et al., 2015). In this way, coalescence event may provide both abiotic and biotic environmental factors for conjugation and plasmid dissemination, resulting potentially in modification of the ecosystem functions. In the previous chapters (Chapter I and Chapter IV), we observed that microbial communities in soil were taxonomically, phylogenetically and functionally different from those in marine coastal sediment showing that soil runoff brought into contact very dissimilar microorganisms in the HC contaminated land-sea continuum. In addition, terrigenous inputs modified the functional gene composition and the functional co-occurrence network in marine coastal sediment. In this context, the monitoring of plasmid transfer could bring new insights on the structure and the functional potential in mixing zone.

Compared to other HGT mechanisms (transduction and transformation), the transfer of plasmid by conjugation is recognized as particularly efficient to provide a relatively rapid response to changing environmental conditions (Thomas and Nielsen, 2005). In fact, plasmids carry essential genes undertaking their own maintenance and mobility in bacterial populations (backbone) and accessory genes encoding traits and phenotypes (i.e. resistance to antibiotics and/or heavy metals, virulence factors, degradation of xenobiotics etc.) that potentially give a selective advantage to the host. In particular, plasmids often carry catabolic genes involved in the degradation of organic pollutants (pesticides, HC) or in the antibiotic resistance and thus play an essential role in the adaptation of environmental bacterial communities to new niches (Heuer and Smalla, 2012). Based on their incompatibility (Inc; Thomas, 2021), inability to coexist in the same bacterial recipient (usually model strain such as *Pseudomonas* or *Escherichia coli* strains) over generation, plasmids have been classified into groups. IncP-1 plasmids often contain catabolic genes involved in the degradation of xenobiotics while IncP-7 and IncP-9 plasmids contains genes involved in the degradation of HC (Heuer and Smalla, 2012; Izmalkova et al., 2005; Jutkina et al., 2011).

An additional interesting feature of plasmids is that they transfer between unrelated and ecologically distant microorganisms (Klümper et al., 2015; Musovic et al., 2006). Latha et al. (2001) proved that the transfer and the expression of an HC degrading plasmid from mesophile to halophile bacteria occurs. More recently, Wang et al. (2017) have discovered in a halophilic

phenanthrene degrading consortium a polycyclic aromatic hydrocarbon PAH ring-hydroxylating dioxygenase adapted to higher salinity after its horizontal gene transfer from mesophiles. In the land-sea continuum, the role of the terrigenous inputs from soil runoff as vector of catabolic plasmids from mesophile to halophile bacteria remains to clarify. We hypothesize that terrigenous inputs bring terrestrial plasmids that are transferred to bacteria inhabiting marine coastal sediment.

Several methods have been developed to detect plasmid transfer in various environment. Transfer frequency was primarily measured using culture dependent approaches (Sengeløv and Sørensen, 1998; Shintani et al., 2010; Smalla and Sobczyk, 2002). Typically, mating experiment between pure strains were conducted and involved a donor carrying a plasmid with a known trait (for example resistance to an antibiotic) to identify transconjugants by selective media. However, these approaches are limited on the bacterial cultivable fraction and does not reflect the fact that plasmid transfer occurs in complex communities. The design of fluorescent-based reporter genes to mark plasmids (Dahlberg, 1998) of interest allowed the detection of transconjugants both cultivable and non-cultivable, using epifluorescence microscopy or flow cytometry. Briefly, the expression of the fluorescence is repressed in the modified donor but not in the recipient. From now, the combination of mating experiment using an engineered plasmid donor (fluorescent reporter gene technology) with flow cytometry analysis and with fluorescence activated cell sorting (FACS) represents one of the most powerful technologies to investigate plasmid transfer within indigenous communities (Pinilla-Redondo et al., 2018). Indeed, quantitative and qualitative features acquired simultaneously at single cell discriminate transconjugant populations and offer the possibility to sort them. In addition, after the sorting, transconjugants can be identified by 16S RNA gene sequencing.

Here, the study aimed to determine i/ whether terrigenous inputs from soil runoff are a reservoir of plasmids and ii/ whether they affect the permissiveness of microbial communities in marine coastal sediment.

For the first objectives, the presence of plasmids in the different compartment of the land-sea continuum was investigated by different approaches:

- DNA from soil, runoff and marine sediment samples extracted during the experiment in microcosms (Chapter I), was analysed for the presence of IncP-1, IncP-7 and IncP-9 plasmids using PCR.
- The data from microarray (GeoChip) obtained on the same DNA (Chapter IV) was also examined for the presence of plasmid sequences

- Taking advantages of RefSoil+ (Dunivin et al., 2019), a reference database containing plasmid sequences from soil microorganisms, potential soil donors were searched among the microbial communities of the land-sea continuum described in Chapter I.

For the second objective, mating experiments maximizing cell-to-cell contacts were conducted with a bacterial community extracted from marine sediment and a laboratory donor strain carrying a genetically modified plasmid, in presence or not of terrigenous inputs. The plasmid contains a tagged-gfp gene, expressed from a lac promoter downregulated by a chromosome located repressor. This construction allows the detection and the quantification of the transconjugants by flow cytometry. The experiment were conducted during a doctoral internship in Copenhagen University within the Molecular Microbial Ecology (MME) group lead by Pr. Soren Sorensen.

V.2. Material and method

V.2.1. PCR based detection of IncP-1/7/9 plasmids

In order to detect IncP-1 /7/9 plasmids amplifications of specific regions (Table V.1) were performed from the DNA of all samples of the microcosm experiment (see chapter 0 and Chapter I for sample collection and for the description of the microcosm experiment). PCR were performed in triplicate using AmpliTaq Gold 360 Master Mix (Applied Biosystems) to prepare 25 µL reactions containing 1 µL template DNA (2 ng), 20 µM of each primer. Denaturation was carried out at 95°C for 10 min, followed by 35 cycles of 30 s at 95°C, 20"/30"/20" at 57/47/45°C (primer annealing conditions respectively for IncP-1/7/9) and 35 s at 72°C and a final extension of 10 min at 72°C. Water was used as a negative control. DNA from Port Anglet and of two *Pseudomonas putida* strains, JCM 6156 and JCM 18452, carrying respectively IncP-1, IncP-7 (TOL) and IncP-9 (NAH7) plasmids were used as positive controls for the PCR reactions. Cloning and sequencing of the amplicons obtained with the IncP-1 primer sets were performed in order to verify the amplification and to have a positive control. Prior to cloning, the PCR products obtained for IncP-1 were purified with illustra GFX PCR DNA and Gel Band Purification Kit (Cytiva Life Sciences, USA) and gel band purification kits. Cloning was carried out with TOPO TA Cloning Kit (Invitrogen, CA USA) according to the manufacturer's protocol. Clones were analyzed from each library, amplified with standard M13 forward and reverse primers and sequenced (LightRun) by GATC (Eurofins Genomics).

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Table V.1 Primer sets used for the detection of IncP-1/7/9 plasmids.

Incompatibility groups (Inc)	Subgroup	Targeted regions	Forward primer	Reverse primer	Product size (bp)	Reference
IncP-1	α, β, ϵ	<i>trfA</i>	5'- TTCACSTTCTACGAGMTKGCCAGGAC- 3'	5'- GWCAGCTTGCGGTACTTCTCC CA-3'	281	(Bahl et al., 2009)
	γ		5'-TTCACSTTTTACGAGCTTTCAGCGAC- 3'	5'- GTCAGCTCGCGGTACTTCTCC CA-3'		
IncP-7		<i>rep</i>	5'-CCCTATCTCACGATGCTGTA-3'	5'-GCACAAACGGTCGTCAG-3'	524	Izmalkova et al., 2005
IncP-9		<i>oriV-rep</i>	5'-GAG GGT TTG GAG ATC ATW AGA-3'	5'-GGT CTG TAT CCA GTT RTG CTT-3'	610-637	Dealtry et al., 2014

V.2.2. In silico detection of plasmids using RefSoil+ and Geochip dataset

V.2.2.1. RefSoil+

The RefSoil+, a publicly database for soil plasmid, was downloaded from GitHub (https://github.com/ShadeLab/RefSoil_plasmids). We simply searched matches between species referenced in the database as plasmid host and species in our 16S ASV table obtained from the microcosm experiment. In the Geochip dataset also obtained from the microcosm experiment, the word "plasmid" was searched.

V.2.3. Permissiveness of marine sediment bacterial communities toward Incp-1 plasmid pKJK5 hosted in *Pseudomonas putida*

In Copenhagen University (KU, Denmark), the permissiveness of marine sediment bacterial communities receiving or not terrigenous inputs by soil runoff toward an exogenous plasmid was assessed through solid surface filter matings. The project was based on the implementation of the up-to-date approach coupling fluorescence-activated cell sorting (FACS) and next generation sequencing (NGS) described in Pinilla-Redondo et al. (2018). However, in a first attempt, the workflow (Figure V.1) included a culture dependant step corresponding to the filter matings.

The workflow (Figure V.1) included the following steps: 0/ Soil and sediment samples came from a realistic land-sea continuum contaminated by HC (Berre pond and Canal Viel cove, southeast France see II.1). 1/ A system mimicking rain using a syringe and a 2 mm sieve was used to produce and collect soil runoff. Soil runoff contained a solid and a liquid phases. 2/ For the mating and FACS analysis, communities need to be extracted from the complex matrices (i.e. soil and sediment particles). For this purpose, cell suspensions from the solid phase of runoff and from the sediment were obtained by chemical/mechanical extraction and density-based separation. The liquid phase of runoff was subjected to a mechanical community extraction according to a MME protocol developed for wastewater treatment plant inlet water. 3/ Matings were performed in vitro (filter matings) to maximize cell-to-cell contact, at different donor:recipient ratio and on oligohaline, mesohaline or euhaline media. Recipient, sediment communities with or without runoff communities were challenged the plasmid donor strain (fluorescent red). Recipients might receive the plasmid and display green fluorescence. 4/ Quantification of the donors, the recipients and the transconjugants by flow cytometry. 5/ Transconjugants might be isolated by FACS and their DNA extracted. The diversity and phylogeny of the transconjugants might be resolved by the 16S rRNA gene sequencing. *During my internship, Sorting and NGS sequencing were not performed because any transconjugants were detected.

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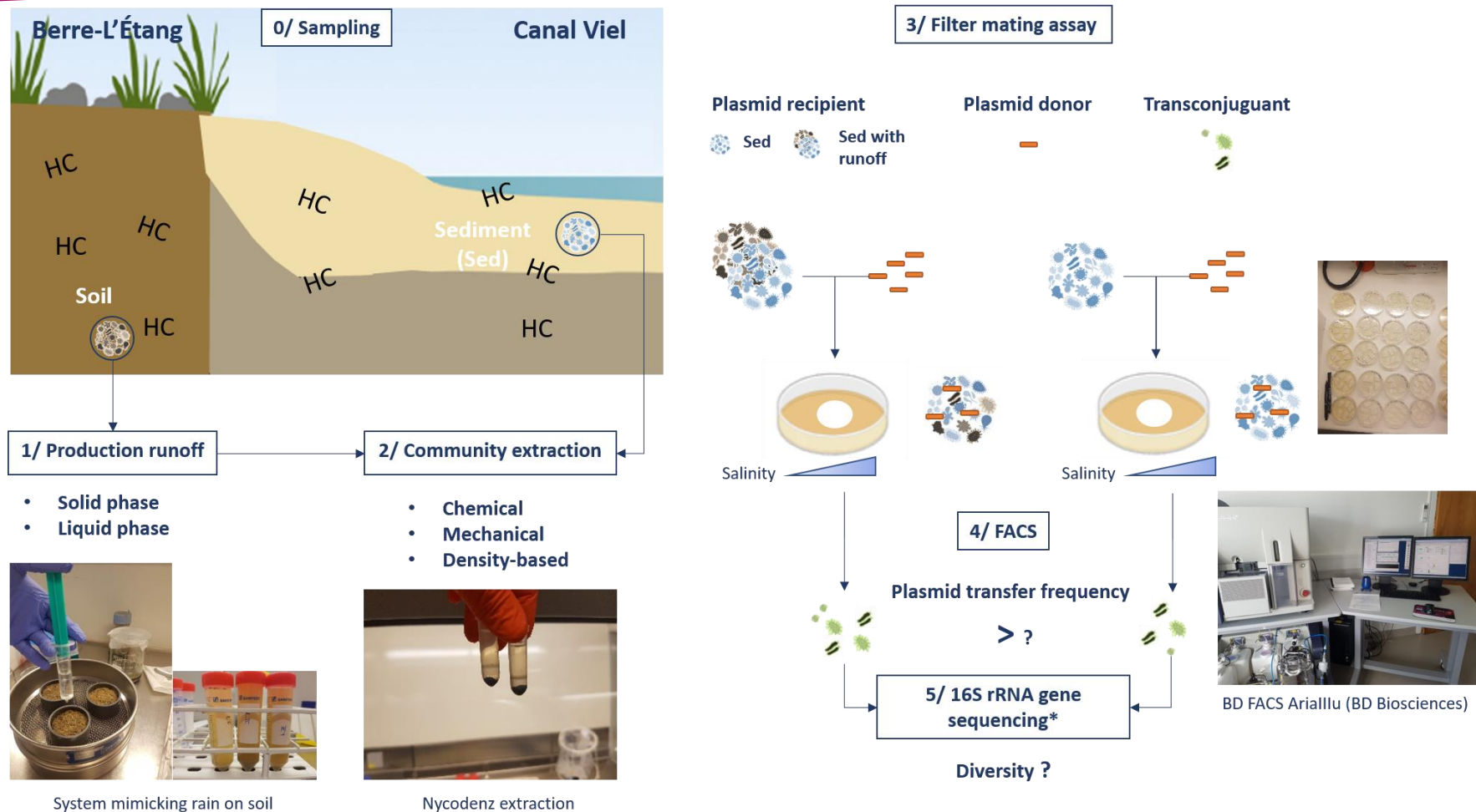


Figure V.1 Workflow for testing the permissiveness of marine coastal sediment to exogenous plasmid depending on the presence or not of soil runoff communities. Plasmid transfer frequency and host range were examined using a fluorescence reporter gene system coupled to FACS and post sorting sequencing.

V.2.3.1. Description of the donor strain

The MME group provided the fluorescent reporter system consisting of a modified Incp-1 plasmid carrying a *gfp* gene of which the expression is conditional and a *Pseudomonas putida* red fluorescent donor strain (sp1089 in MME collection) that constitutively express mCherry gene (Table V.2 Table V.5). In details, the *gfp* gene carried by the plasmid is downstream of *lac* promoter (pA10403), repressible by the *LacIq* protein. In the donor, this *lac* promoter is downregulated by a constitutively expressed *lacIq* repressor gene located on the chromosome. As a result, donors display a red fluorescence while transconjugants, recipients that acquire the plasmid, will develop a green fluorescence, reporting plasmid transfer events. This way, we took advantage of fluorescent marker genes to detect plasmid transfer events in sediments and possibly isolate transconjugants using FACS flow cytometry. The plasmid harbors a trimethoprim resistant gene and thus, it allows selection of the transconjugants by cultural approach (selective plating).

Table V.2 Description of the fluorescent reporter system

Donor	Chromosomal marker		References	
<i>Pseudomonas putida</i> KT2240	lacIq-Plpp-mCherry-KmR		Klümper et al., 2015	
Plasmid	Inc group Insert	Host range	Antibiotic resistance	References
pKJK5-pA10403-gfpmut3-Km-35	IncP-1ε	broad	TMP	Klümper et al., 2015

All overnight cultures of the donor strain were incubated in test tubes with 5 ml of liquid LB broth (VWR International), supplemented with kanamycin (KAN 50 µg/mL) and trimethoprim (TMP 50 µg/mL) at 30 °C and at 250 RPM. LB agar plates were prepared with 1.5% agar (VWR International) and ~ 20 ml per plate. Stock solutions of antibiotics (Sigma-Aldrich) were prepared at the concentration of 10 mg/mL and stored at -20°C until use. Trimethoprim was dissolved in methanol and kanamycin in water. Recipient bacterial community preparation

V.2.3.1.1.1. Soil and marine coastal sediment collection

Soil and sediment were collected in the same geographical area in August 2019. Soil came from the surroundings of the Berre pond (43°29'6.9"N 5°11'18.53"E, France) and sediment from the Canal Viel cove (43°23'20.3"N 4°59'31.8"E, France). Prior to experiment, soil and sediment were both sieved to <4 mm to remove coarse gravels, plant debris and macroorganisms while preserving the structure. Back at the laboratory, soil and sediments were stored at 4 °C in

respectively in a plastic bag and in a 5L becher completely covered with seawater and with continuous aeration through an aquarium pump. The humidity and the water holding capacity (WHC) of soil were evaluated in triplicate on sample of ~12 g.

V.2.3.1.1.2. Preparation of the soil runoff and marine coastal sediment



Figure V.2 Preparation of the soil runoff by mimicking rain. Soil samples subjected to rain laid on a 2 mm sieve in order to collect runoff

Soil runoff were produced in triplicate by mimicking a strong rain (under the climate of the metropolitan France) event on soil. The system consisted of a syringe dispensing water, a cylinder containing soil placed on a 2 mm sieve, under which a cup collected the runoff (Figure V.2). The day before the matings, soil (60 g) was put in each cylinder (height=4 cm, diameter=7 cm) and the WHC was adjusted at 60% with sterile water. Marine coastal sediment (25 g) were collected from the stock and covered with seawater (~2 cm, from the sampling site) overnight at ambient temperature. The day of the matings, soil cylinders were watered with saline solution with a syringe in order to produce an equivalent runoff (1:1 soil:saline solution) mimicking rain event. Runoffs were transferred in 50 mL corner tubes. The volume and the weight of the liquid and the solid phases of runoff were respectively measured.

V.2.3.1.1.3. Runoff and marine sediment bacterial community extraction

The bacterial community from the liquid phase of the runoff was recovered using a MME protocol for wastewater treatment plant inlet water. Briefly, cells were extracted by shaking with metallic beads (80 beads/400 mL samples, 500 rpm, 15') followed by a short sonication (on ice, 1'). After the particles had sunk, the liquid was filtered at 10 μm with a vacuum pump (Figure V.3).

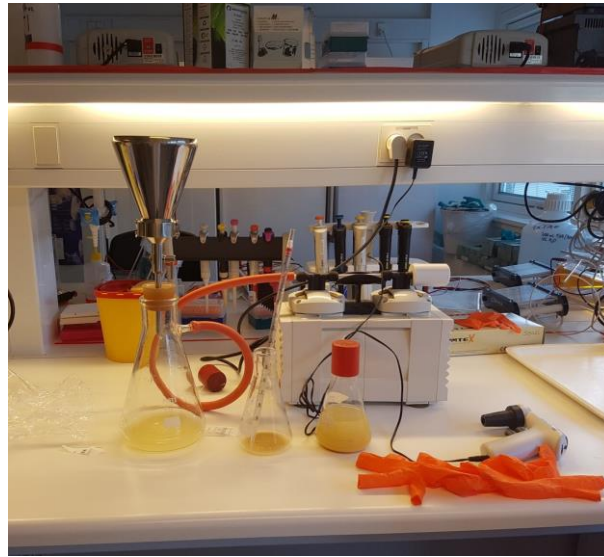


Figure V.3 Filtration of the liquid phase of soil runoff at 10 μm with the help of a vacuum pump after the shaking and the sonication steps.

The bacterial communities of runoff (solid phase) and coastal marine sediments were recovered by combining a chemical and mechanical cell detachment with a Density based Nycodenz extraction. c) Pyrophosphate buffer (0.05 M $\text{Na}_4\text{O}_7\text{P}_2$ with 0.05% Tween 80) and shaking at 250 rpm (15 minutes, 4°C) detached the microorganisms from the particles and the Nycodenz solution separated, based on density differences, a top cell layer from residual solid particle layer (Musovic et al., 2010). The density gradient extraction was performed according to the protocol of Klümper et al. (2017). Briefly, runoff and marine sediment suspensions obtained after the chemical/mechanical extraction were carefully transferred on top of the Nycodenz solution. After centrifugation, the dense particles were on the bottom of the tube whereas bacteria, denser than water were at the interface of the between water and Nycodenz solution (Figure V.4). The cell layers were carefully collected by pipetting and transferred in 50 mL tubes.

The filtrate derived from the liquid phase of runoff and the cell layers from the solid phase of runoff and sediment were centrifuged at 10000 g for 10' at 4°C. The supernatant was discarded and the

cells were resuspended in 0.9% solution for the runoffs and in artificial seawater for sediment. An aliquot of each suspension was filtered at 10 μm before quantification of the cell by cytometry.

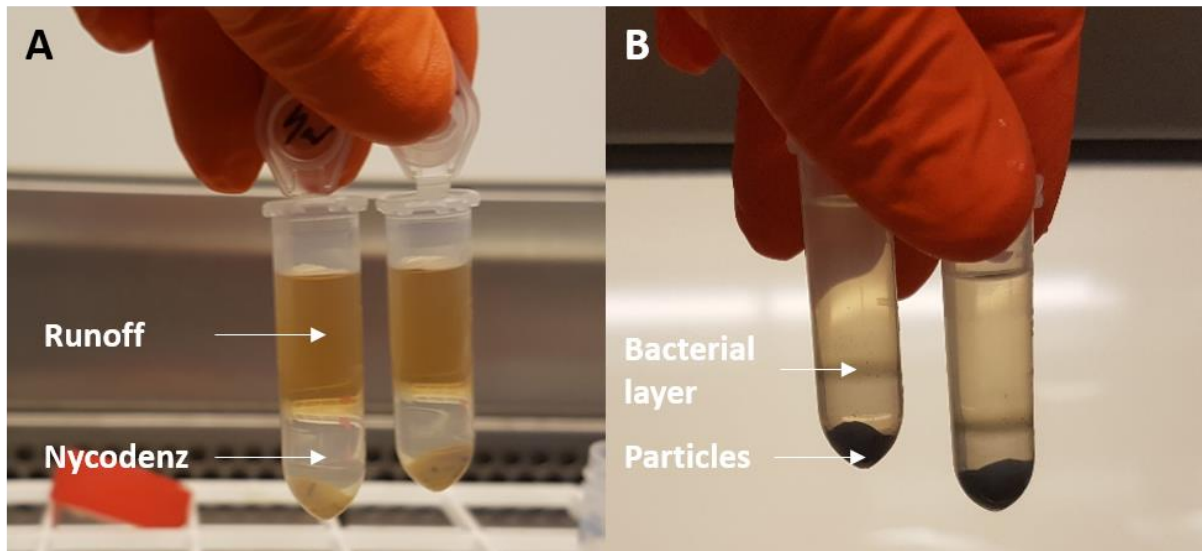


Figure V.4 Density based Nycodenz extraction of bacterial community. A Before centrifugation: runoff or sediment were placed on the top of a Nycodenz solution. (here runoff). B After centrifugation, particles sunk into the bottom. Bacteria cells were at the interface between water and Nycodenz (here marine coastal sediment).

V.2.3.1.2. Solid surface mating assay

The extracted sediment microbial communities were challenged with the exogenous plasmid donor in presence or not of extracted soil runoff communities via solid surface matings.

Mating experiments were conducted with extracted microbiomes from sediments, soil runoff or sediments with soil runoff and the donor lab (Table V.3). Different salinity conditions (oligohaline, mesohaline and euhaline media) were used for the filter matings to evaluate the influence of the salinity on the gene transfer. In fact, in the land-sea continuum, soil runoff mix with marine sediment in an unequal ratio, so the environment of the plasmid transfer is expected brackish or euhaline. Oligohaline, mesohaline and euhaline solid media were based on the recipe of the solid LB 10%. Oligohaline medium corresponds to a solid LB (10%). Mesohaline and euhaline solid media were prepared combining the classical ingredients of solid LB 10% (tryptone and yeast extract) apart from NaCl that was replaced sea salts (Sigma-Aldrich), respectively at 18 and 40 g/L. LB was diluted to limit the overgrowth of the donor and the fast growing microorganisms. In addition, the media were supplemented with cycloheximide (50g/mL) to inhibit the development of fungi. The matings were performed in four replicates on cellulose ester filter (Advantec) placed on oligohaline, mesohaline or euhaline solid LB (10%) medium. Filters were loaded with 40 μL a cell suspension mixing the lab donor and the sediment recipient (sediment or sediment with runoff

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communities) in a ratio of 1:100 (~ 10⁷ cells/mm²). The donor was washed free of antibiotics and resuspended in 0.9 % NaCl solution. Controls were set up with either recipient or donor cells alone. Following incubation (25°C, 18 h), cells were harvested by vortexing filters (3 min, 2 mL, 0.9% NaCl). The cell suspension was stored in a box with ice at 4°C.

Table V.3 Description of the matings. *solid and liquid phase of runoff in a ratio 1:1.

Donor	Recipient communities (extracted)	Abbreviation
<i>P. putida</i> pKJK5	Runoff (solid and liquid phases in a ratio 1:1)	DxR
<i>P. putida</i> pKJK5	Sediment	DxS
<i>P. putida</i> pKJK5	Sediment with runoff* (in a ratio 1:1)	DxS+R
<i>P. putida</i> pKJK5	-	CTL D
	Runoff (solid and liquid phases in a ratio 1:1)	CTL R
	Sediment	CTL S
	Sediment with runoff* (in a ratio 1:1)	CTL S+R

V.2.3.1.3. Enumeration of the donor, the recipient and the transconjugants by selective plating and fluorescent activated cell sorting (FACS)

The drop plate method and selective media were used to quantify donor and recipient as first step for the matings and to quantify the potential transconjugants after the mating. Dilution series were made in Deepwell Plates (Eppendorf) by transferring 100 µl samples (donor, recipient or cell suspension from matings) into 900 µl 0.9% NaCl solution or seawater for euhaline condition, until the sample were diluted by a factor of 10⁷. For each sample dilution (10⁰-10⁷), three drops of 10 µl were spotted on selective LB agar plates (oligohaline, mesohaline or euhaline) supplemented cycloheximide. Spots were distributed upon two plates. Trimethoprim (TMP 50 µg/ml) was used for quantification of donor plus transconjugants under UV lamp.

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Cell detection and fluorescence counting were done using a FACS Aria IIIu (BD Biosciences, San Jose, CA, USA) as previously described (Jacquiod et al., 2018; Kalckar Olesen, 2018; Klümper et al., 2017). The following thresholds and voltages were used during the analysis: forward scatter (FSC) = 1200, side scatter (SSC) = 200, with the photomultiplier tubes (PMT) values FSC = 505 V, SSC = 308 V. green fluorescent (gfp) cells were excited by a 488 nm laser (20 mW) and detected on the FITC channel with 530/30 nm bandpass filter. Red fluorescent cells (mCherry) were excited by a 561 nm laser (50 mW) and detected on the PE-Texas Red channel with a 610/20 nm bandpass filter. The enumeration of the donor and the recipient prior to the matings was done on aliquot (10 μ m filtered) after a 200X dilution and the events were recorded for 60 sec at flowrate 1. For transconjugants detection, samples were diluted until reaching \sim 3000 evt/s. The different cell populations (donor, recipient transconjugants) were visualized on dot plots: i/ a first plot (exponential axis) with forward scatter area (FSC-A) at the x-axis, side scatter area (SSC-A) on the y-axis was used for size discrimination, with the parent gate called "Bacteria" (all bacteria event); ii/ a second plot (exponential axis) with PE-Texas Red-Area at the x-axis and FITC-Area on the y-axis, was used for color segregation of the forwarded events from the "bacteria" gate, with a quadrant gate, dividing events into "Green", "Red+Green", "No Color" and "Red".

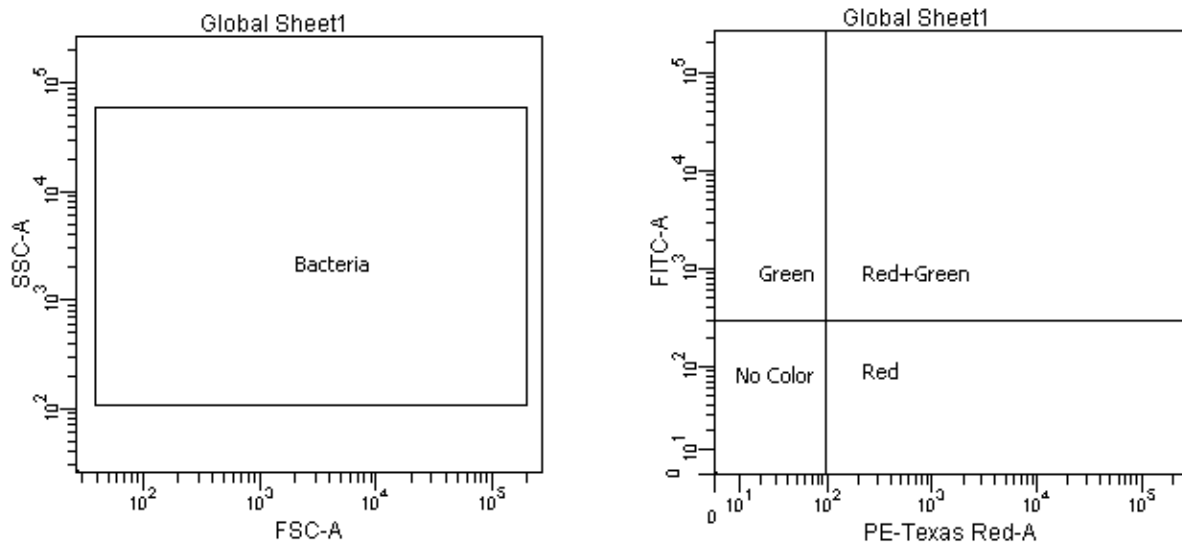


Figure V. Dot plots and the applied gating to visualize the different cell populations during the data acquisition. From the master thesis of Kalckar Olesen (2018).

V.2.4. Results and discussion

V.2.4.1. Soil runoff plasmid reservoir

The presence of plasmids was investigated in the different compartments of the land-sea continuum microcosms, including all conditions (soil with plant or not, HC spiking or not in soil and sediment, with or without runoff). For this purpose, three approaches were followed: i/ PCR based detection of IncP-1/7/9 plasmids, ii/ *in silico* detection of plasmids and bacteria hosting plasmids and iii/ detection of plasmids using microarray (GeoChip).

V.2.4.1.1. PCR based detection of IncP-1/7/9 plasmids

In order to have an overview of the plasmid content in the land-sea continuum, samples of the microcosms were analysed for the presence of IncP-1/7/9 plasmids by PCR.

Table V.4 PCR based detection of IncP-1/7/9 plasmids. Numbers indicate the number of replicates (3 per condition) in which plasmid groups were detected. Blank indicates no detection.

Samples			IncP-1		IncP-7	IncP-9
			α, β and ε subgroup	γ subgroup		
SOIL	t0	NC			1	
		C	1		2	
	tf	NPNC	1	2		2
		PNC	2			
		NPC	3			
PC	3		2	3		
RUNOFF	t1	NPNC	1			3
		PNC	3			3
		NPC	3			3
		PC	2			2
	t2	NPNC	3			
		PNC	3			3
		NPC	3			
		PC	3			
SEDIMENT	t0	NC	3		1	3
		C	3			
	tf	NPNC	3			
		PNC	3		2	
		NPC	3		1	
		PC	2		1	1
		NRNC	3			
		NRC	3		1	1

Among IncP-1 plasmid group, α , β and ϵ subgroups were detected in soil, runoff and coastal sediment compartments (in 54 of 66 samples) while γ subgroup was only detected in two samples (Table V.4). This is in accordance with previous studies that reported its detection in various environment including soil, salt marshes, water, and sediment (Jechalke et al., 2013). In soils, IncP-1 α , β and ϵ subgroups were more detected at the end of the experiment, especially in samples with HC spiking. In our runoff samples, they were found in almost all samples (21 of 24 runoff samples). In sediments, they were detected at the beginning of the experiment as well as at the end of the experiment irrespective of the runoff treatment. Incp-7 plasmid group was detected in 11 of 66 samples in soil and sediment compartments but not in runoff compartment (Table V.4). Incp-7 plasmid group is often reported in environment contaminated by oil (Dealtry et al., 2014). IncP-9 plasmid group was detected in 24 of 66 samples, in soil, runoff and sediment compartments (Table V.4). In soil compartment, IncP-9 plasmids were detected only in samples at the end of the experiment. In runoff, the IncP-9 group was detected in almost all samples (11 of 12) at day 16 of incubation and in 3 of 12 of the samples at day 30 of incubation. In sediments, this group was detected in samples at the beginning of the experiment, all without HC spiking. At the end of the experiment, it was found only in two samples, one receiving terrigenous inputs by soil runoff and one without such inputs. IncP-9 group is often described in soil contaminated by oil (Dealtry et al., 2014; Izmalkova et al., 2006). Our results show that soil, runoff and sediment compartments contained plasmids of the different incompatibility groups. IncP-1 (subgroups α , β and ϵ) and IncP-9 plasmids prevailed in samples, including runoff samples. Thus, runoffs were a source of plasmids for sediment receiving terrigenous inputs by soil runoff. These inputs may enrich sediment with these plasmids known to carry genes encoding enzymes involved on the degradation of xenobiotics. However, further analysis providing quantitative information such as qPCR are required to validate this hypothesis.

V.2.4.1.2. *in silico* detection of plasmids and bacteria hosting plasmids

As exploratory studies, we exploited 16S rRNA gene sequencing data acquired during the experiment in microcosms mimicking the land-sea continuum in order to detect plasmids and bacteria hosting plasmids. In particular, we looked for bacteria known to carry plasmids among the amplicon sequence variants (ASVs) found in the microcosms using RefSoil+ plasmid and host database. Among the 6669 prokaryotic ASVs detected in the microcosm, 18 were affiliated to species level. However, the RefSoil+ database provides the taxonomy of the plasmid hosts until the strain level. Therefore, we inferred the presence of plasmids in the microcosms based on the species level. Among the 18 species found in microcosms, four matched species from the RefSoil+ database making them candidates for hosting plasmids (Table V.5): *Rhodococcus erythropolis* (Actinobacteria), *Paenibacillus polymyxa* (Fimicutes), *Ralstonia pickettii* and *Methylobacterium radiotolerans* (both Proteobacteria).

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Table V.5 Description of the strains and plasmids belonging to species found as candidates for hosting plasmids in microcosms.

RefSoil+ ID	Species	Plasmid.ID	Plasmid Name	Features	References
RefSoil_236	<i>Methylobacterium radiotolerans</i> JCM 2831	NC_010510.1	pMRAD01	NA	
		NC_010509.1	pMRAD02	NA	
		NC_010514.1	pMRAD03	NA	
		NC_010517.1	pMRAD04	NA	
		NC_010518.1	pMRAD05	NA	
		NC_010502.1	pMRAD06	NA	
		NC_010504.1	pMRAD07	NA	
		NC_010507.1	pMRAD08	NA	
RefSoil_427	<i>Paenibacillus polymyxa</i> SC2	NC_014628.2	pSC2	Chloramphenicol resistance	(Schwarz et al., 1989)
RefSoil_616	<i>Paenibacillus polymyxa</i> M1	NC_017543.1	pPPM1a	DNA topoisomerase VIII	(Gadelle et al., 2014)
RefSoil_257	<i>Ralstonia pickettii</i> 12J	NC_010683.1	pRPIC01	ATPases involved in chromosome partitioning	Bacmap genome atlas

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RefSoil+ ID	Species	Plasmid.ID	Plasmid Name	Features	References
RefSoil_335	<i>Ralstonia pickettii</i> 12D	NC_012855.1	pRp12D01	Orphan protein	P2CS genoBrowser
		NC_012849.1	pRp12D02	in environment with heavy metals	(Nojiri et al., 2013)
		NC_012851.1	pRp12D03	NA	
RefSoil_800	<i>Rhodococcus erythropolis</i> CCM2595	NC_022125.1	pRECF1	NA	

NA, not available.

In RefSoil+, the four species of interest encompass 12 strains carrying plasmids of which the features (host range, incompatibility group, and encoded traits) are unknown. The four species were detected at low abundance mostly in runoff compartment. This result shows that runoff displayed species that were rare in soil and then undetected there. As indicated by its name the RefSoil+ database lists soil plasmids and their hosts what can explain the lack of correspondence with species found in sediment. Admittedly, the research of bacteria carrying plasmids in microcosms was limited by the lack of taxonomic resolution at the species and strain levels inherent to sequencing approach, but the results suggest that bacterial communities of the land-sea continuum hosted a diversity of plasmids.

V.2.4.1.3. Detection of plasmids using microarray (GeoChip)



Figure V.5 Relative abundance of the plasmid detected by microarray (GeoChip5S) on the DNA extracted from soil and sediment compartments at the end of the microcosm experiment. Soil: soil; Sed: sediment; SedR: sediment receiving runoff; SedNR: sediment without runoff (control); NP: without plant; P: with plant; NC: without HC spiking; C: with HC spiking.

The dataset obtained from the microarray (GeoChip) applied to the DNA extracted from microcosms was analysed for the direct detection of plasmids. Five different plasmids were detected by the microarray. They were found at low abundance (relative abundance <0.02 %) in soil and sediment compartments (

Figure V.5). They carry genes involved in organic remediation, metal resistance and antibiotic resistance (Table V.6). These plasmids have been described in terrestrial and freshwater environments: rhizosphere for pSB102 (Schneiker et al., 2001), river epithelion for QKH54 (Haines et al., n.d.) and wastewater treatment plant (WWTP) for pB3 (Heuer et al., 2004). The plasmids pWW0 and pEST1226 have been detected in *Pseudomonas putida* bacteria found in soil, water and also marine habitat (Greated et al., 2002; Kivisaar et al., 1991; Marinho et al., 2009). The plasmids pB3 and QKH54 belong to IncP-1 group (beta and gamma respectively) while pWW0 is an Incp-9. The plasmid pSB102 is broad host range plasmid unrelated to the known incompatibility groups of bhr plasmids IncP-1, IncW, IncN and IncA/C (Schneiker et al., 2001).

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Table V.6 Information available for the detected plasmid based on the Geochip classification.

GeochipID	accessionNo	gene	species	annotation	geneCategory	subcategory1	subcategory2
15722312	CAC79204.1	<i>mer</i>	Plasmid pSB102	MerA protein	Metal Homeostasis	Mercury	Detoxification
108524633	YP_619855.1	<i>mer</i>	Plasmid QKH54	MerA mercuric ion reductase	Metal Homeostasis	Mercury	Detoxification
15722310	CAC79202.1	<i>merg</i>	Plasmid pSB102	MerG protein	Metal Homeostasis	Mercury	Transport
151725	AAA26053.1	<i>xylg</i>	Plasmid pWW0	2-hydroxymuconic semialdehyde dehydrogenase	Organic Remediation	Aromatics	Aromatic carboxylic acid
145129	AAC64900.1	<i>catechol</i>	Plasmid pEST1226	catechol 1,2-dioxygenase	Organic Remediation	Aromatics	Other aromatics
55418027	YP_133927.1	<i>b_lactamase</i>	Plasmid pB3	beta-lactamase LCR-1 precursor	Virulence	Antibiotic resistance	Degradation

V.2.4.2. Permissiveness of microbial communities from marine coastal sediment

In order to investigate the permissiveness of microbial communities from sediment, mating experiments maximizing cell-to-cell contacts were conducted. Bacterial communities extracted from marine sediment were challenged by a laboratory donor strain carrying a genetically modified plasmid, in presence or not of terrigenous inputs.

V.2.4.2.1. Suitability of *Pseudomonas putida* KT2240 carrying pJK5 as donor in marine conditions

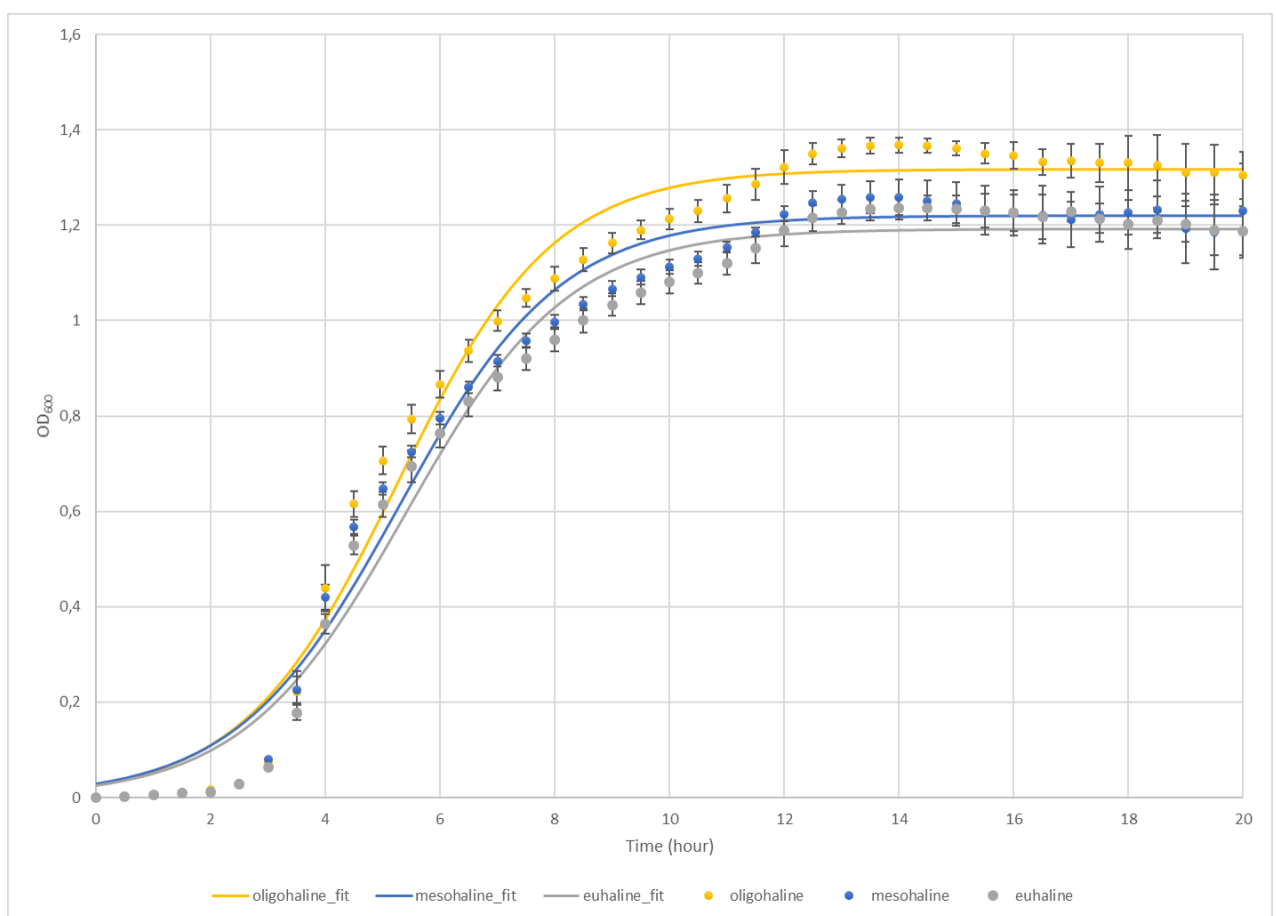


Figure V.6 Growth curves of *Pseudomonas putida* on liquid LB 10% supplemented with seasalt or not to mimic different salinity conditions A/ oligohaline (10 g/L NaCl according to the classic LB recipe) B/ mesohaline (20 g/L sea salt) C/ euhaline (40 g/L sea salt). The growth curves (mean of 6 replicates) are drawn with dot and their corresponding fit logistic curves are drawn with solid lines.

To assess the suitability of the lab donor for the filter matings at salinity closed to marine environment, growth was followed in microplate with oligohaline, mesohaline and euhaline LB

10% media. In fact, we wanted to make sure that *Pseudomonas putida* survive during mating at higher salinity and eventually transfer the pKJK5 plasmid to marine sediment recipient.

The growth curves in mesohaline and euhaline conditions were similar to the growth curve in classic LB medium 10% (Figure V.6), showing that the donor is able to grow in presence of sea salt. During the exponential growth, the three curves almost overlapped. There was a light delay to reach stationary phase in salty conditions. The parameters of the growth curves (Table V.7) indicated that the donor is ready to use after ~5h of incubation (30°C) corresponding to OD600=0.8.

Table V.7 Parameters describing the growth of *P. putida* KT2240 carrying pKJK5 by fitting logistic model to the experimental data. *k*: carrying capacity; *r*: growth rate; *n0*: initial absorbance reading; *t_mid*: time at the inflection point of the logistic curve (occurs at half of the carrying capacity); *t_gen*: generation time ; *sigma*: residual standard error from non-linear least squares fit of the model to the data .

Media	k	n0	r	t_mid	t_gen	sigma
oligohaline	1.318	0.027	0.736	5h15'	56'	0.058
mesohaline	1.220	0.028	0.708	5h17'	59'	0.056
euhaline	1.192	0.026	0.705	5h24'	59'	0.054

V.2.4.2.2. Development of the mating conditions

In order to fix the ratio between the donor and the recipient for the filter matings, cell suspensions of donor and bacterial communities from extracted runoff and sediment communities were quantified by FACS. In addition, this step allows to i/ validate the gating and ii/ verify that the extracted communities do not display an autofluorescence inferring with the red and green fluorescence reporter system.

The Figure V.7 presents typical cytometry profiles obtained for the donor and the recipients before the matings. The donor presented a cell heterogeneity (Figure V.7 A) indicated by a large oval group of dot. As expected, the donor displayed red fluorescence as seen in Figure V.7 B. Donor cells uncoloured were also detected. These cells probably correspond to dead or inactive cells. Recipients, from runoff as sediment, showed an expected heterogeneity based on the scatter parameters (Figure V.7 B), inherent to coexistence of multiple populations (i.e. communities). Still in the profiles based on scatter, recipients showed a top right plume of dots, typical of particles. The plume was pronounced for the liquid phase of runoff, calling a better removal of the soil particles during the community extraction. The recipients did not display neither red nor green autofluorescence (Figure V.7 B), making possible the detection of any transconjugants after the filter matings.

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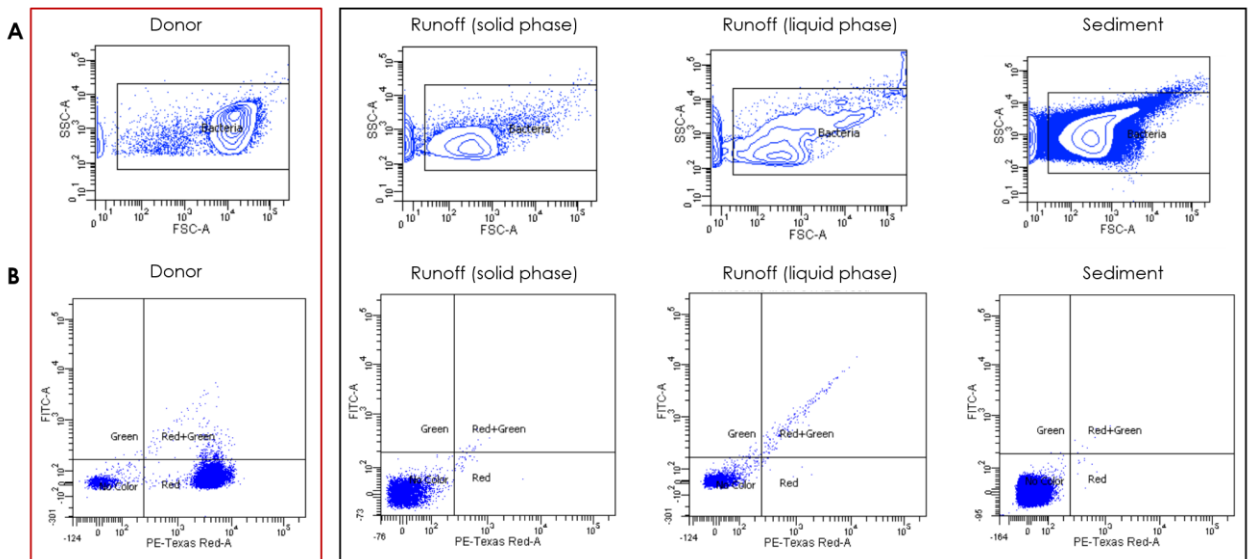


Figure V.7 FACS profile of the donor and the recipient during the enumeration before the filter matings. A Discrimination of the bacterial cells based on scatter parameters (A) and on fluorescence (B). Red fluorescence was measured by PE-Texas Red-A and green fluorescence was measured on the FITC-A.

The Figure V.8 (A and B) presents representative cytometry profiles obtained for cell solutions prepared from the filter matings. We distinguished the recipient communities from the donor but no transconjugants were detected. The Figure V.8 C gives an example of matings that generated transconjugants (top left group of green dots).

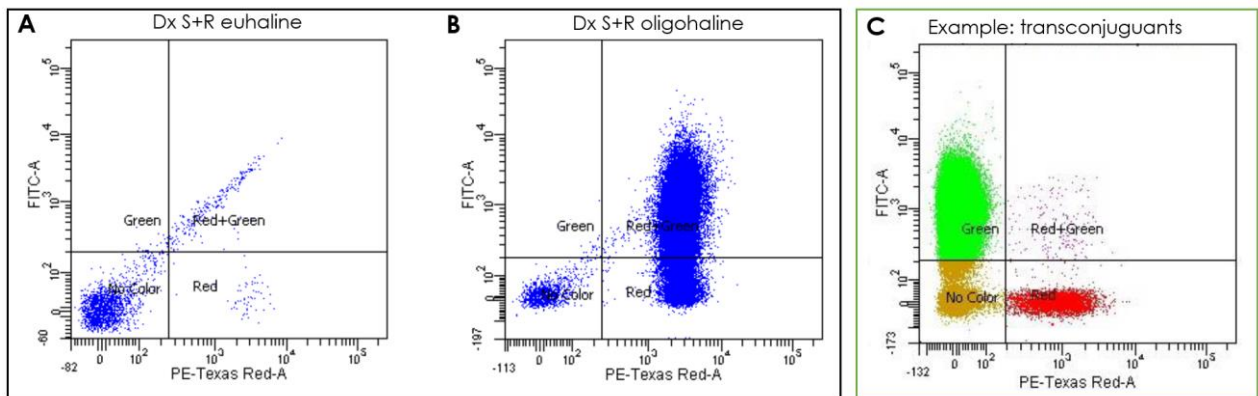


Figure V.8 FACS profile after incubation of the filter matings. Cell suspensions obtained from filter mating between the lab donor and the recipient sediment with runoff communities in LB 10% oligohaline medium (A) and in LB 10% euhaline (B). In C, example of successful matings revealing donor, recipient and transconjugant populations (Kalckar Olesen, 2018). Red fluorescence was measured by PE-Texas Red-A and green fluorescence was measured on the FITC-A.

V.2.5. Adjustments for the protocol to assess permissiveness of sediment communities

The study aimed to determine whether soil runoff affect the permissiveness of microbial communities in marine coastal sediment. The protocol included community extraction, filter mating and a fluorescence reporter-gene approach coupled to FACS. In view of results, adjustments of the protocol are required. In particular, the community extraction and the choice of the ratio are critical steps, as they will condition the fraction of the bacteria that will be in contact.

We propose to assess the efficiency of the community extraction from the liquid phase of soil runoff and from marine sediments by Nycodenz density gradient as well as the mechanical extraction used for liquid phase of soil runoff. Indeed, community extractions present some disadvantages. For example, Nycodenz extraction is high effective to separate cells from particles, making cytometry profiles clearer of non-cell event, and preventing of clog the FACS. However, it is biased toward certain bacterial phyla. Other available methods have been demonstrated suitable for extracting cells from wastewater, manure and coastal sediment (Frossard et al., 2016; Lavergne et al., 2014). The comparison of the efficiency of the different methods by quantifying the cultivable bacteria will allow the select the best method to recover microbial communities from sediments. We also recommend sampling aliquots before and after community extraction. In case of the detection of transconjugants, NGS sequencing of community before and after extraction and of the transconjugants will provide interesting information about the abiotic conditions of mating *in vivo* compared to *in vitro*. Following the community extraction, the viability of the bacteria should be assessed by DNA staining and quantification through the cytometer. In parallel, the number of the cultivable bacteria should be estimated by plating on the different media (oligo-, meso- and eu-haline media). The results will be essential to fix and adjust the donor:recipient ratio, considering the viable and the cultivable fraction of the communities. In a second step, we propose to the test different donor :recipient ratio for each media.

The Danish team (MME lab) suggested also to regrow overnight i/ the extracted communities before mating or ii/ the suspension derived from mating in order to i/ have active extracted bacteria during mating and ii/ optimize the detection of transconjugants. The use of a selective medium will help to select most probable recipients on the extracted communities (*Pseudomonas*).

V.2.6. Conclusion

Soil runoff has been shown to be a reservoir of IncP-1 (α , β , and ϵ subgroups) and IncP-9 plasmids, known to carry genes involved in xenobiotic degradation. Catabolic gene transfer in sediments between mesophilic and halophilic bacteria via soil runoff is therefore conceivable, which probably affect the microbial communities at the functional level. The permissiveness of marine coastal sediment to plasmid transported via soil runoff and the diversity of the recipients constitute key parameters to understand and predict the fate of catabolic genes.

In order to gain first knowledge and experience on plasmid transfer in the land-sea continuum, fluorescence reporter-gene approach coupled to FACS was used. To the best of our knowledge, it is the first implementation of the method with complex communities from soil runoff and marine coastal sediment collected in HC contaminated areas. A protocol was developed to produce soil runoff. To maximize cell-to-cell contact and avoid interference from abiotic environments, bacterial communities were extracted from soil runoff and marine coastal sediment. For the same reasons and also because plasmid transfer more likely occur when bacteria needs are fulfilled, the encounter of the donor and the extracted communities cells was done on mating filters that laid on media (oligo-, meso- or eu-haline). The growth curve analysis of the donor showed that the donor strain is able to grow under salty conditions. It makes it a suitable tool for study the plasmid transfer in the land-sea continuum. Transconjugants were not detect from the matings between the extracted marine sediment microbiomes and the modified lab strain, irrespective the salinity of media and with a 1:100 donor: recipient ratio. It is likely that under oligohaline condition, the donor outcompeted the environmental recipient whereas in salty conditions, donor is less fit and experience colonization resistance of the marine sediment communities (van der Waaij et al., 1971). The event of transfer that we wanted to highlight is probably rare, even below the detection threshold but we assumed that adjustments of the protocols are required to validate this hypothesis. In fact, such events had been reported between the donor and natural complex communities from river sediments (Jacquiod et al., 2018). The strategy for further experiments should be to oversimplified the matings until detection of transconjugants and only then shift to more and more complex experimental designs mimicking as closer as possible the environmental conditions.

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Chapter VI:

Conclusion – Discussion - Perspectives

Chapter VI. Conclusion – Discussions – Perspectives

Coastal areas are typically mixing zone between terrestrial and marine ecosystems forming a land-sea continuum. These areas, that provide valuable ecosystem services, concentrate populations and human activities and are therefore particularly vulnerable to habitat degradation (urbanization) and pollution (gasoline/diesel combustion, oil spill). By their location in the land-sea continuum, marine coastal sediments constitute an important sink for organic matter and pollutants (including hydrocarbons HC) coming from atmospheric deposition, farm and industrial effluents and fluvial runoff. Marine coastal sediment are continuously subjected to fluctuating environmental conditions and own high biodiversity in which microbial communities play a pivotal role in the carbon cycle and the degradation of pollutants such as HC. In situ studies provided key information for understanding the fate of HC in such environments, but all parameters cannot be integrated because of the environmental complexity. To our knowledge, soil runoff, which conveys terrigenous inputs to sediment without dilution in river, has not been yet considered as parameter bringing heterogeneity in marine coastal sediment. Therefore, we proposed to dissect the effect of soil runoff on coastal marine sediment microbial communities through microcosm experiments, mimicking as close as possible the natural environment under controlled conditions.

This thesis aimed to consider the impact of the terrigenous inputs on the structure and the functional potential of the microbial communities in marine coastal sediments within a HC contaminated land-sea continuum. In this context, the specific objectives of the thesis were:

- To determine whether terrigenous inputs result in microbial community coalescence in HC contaminated land-sea continuum,
- To define the functional potential of microbial communities in land-sea continuum and the metabolic networks involved in HC degradation in marine sediment under terrigenous inputs and HC contamination,
- To assess the permissiveness of marine sediment microbial communities to plasmids from terrestrial inputs.

The innovative character of the project is represented by i/ the type of ecosystem addressed focusing on marine coastal sediment receiving runoff, ii/ the consideration of the mixing of both microbial communities and their surrounding environments, iii/ the context of HC contamination. In addition, the project was based on the implementation of a new and original experimental device mimicking the land-sea continuum in microcosms applying different scenarios under controlled conditions. The terrigenous inputs were generated from soil with or without plant and with the addition of HC or not in soil and sediment.

Microbial diversity analysis revealed that soil runoff was involved in the assembly of microbial communities in marine coastal sediment. Our results provided evidences of community coalescence when marine coastal sediments receive terrigenous inputs via soil runoff.

Interestingly, the two ways of community coalescence were evidenced in the microcosms: new biotic cohabitation due to transfer of microorganisms and the creation of novel niches due to the mixing of the environmental conditions. Indeed, the mixing condition likely promoted the exchange and renewal of organic matter, nutrients, freshly spiked HC, leading to an increase of resources in quantity and quality for microbial communities in sediment. We argue that coalescence event due to soil runoff cannot be ignored in future HC remediation studies in land-sea continuum. The knowledge of rules that govern coalescence event could even help to develop monitoring tools and steer HC natural attenuation.

Studies on microbial coalescence in marine environment mainly focused on prokaryotic communities and, to a limited extent, on fungal communities, encouraging us to acquire data on eukaryotic microbial communities. Therefore, **we contributed to the acquisition of environmental sequences from fungal group, historically neglected in marine sediment environment.** Our data showed the importance of the early lineages of fungi in the composition of sediment communities, in accordance with recent environmental survey studies (Amend et al., 2019; Rojas-Jimenez et al., 2017). In the other hand, runoff was composed in majority of unassigned fungi showing that a part of rare soil microorganisms remains unknown. It also highlight limitations of molecular approaches to describe communities (detection threshold and non-exhaustive databases).

The beta diversity partitioning analysis showed that **the processes underlying prokaryotic and fungal community organization differed in coastal marine sediment subjected to soil runoff.** In prokaryotic communities, turnover predominated, whereas in fungal communities, changes of richness predominated. These results are probably linked to different traits of life between the two domains of life (Chen et al., 2020). Because fungi play also a crucial role in the biogeochemical processes and the degradation of the organic matter (Amend et al., 2019; Gladfelter et al., 2019), it is a requirement to include them systematically in environmental studies. In addition, specific biotic interactions were described between fungi and prokaryotes in marine sediment (Booth et al., 2019), and the loss, the gain or the replacement of species may affect microbial processes. In disturbed environment, in particular during coalescence event, the organization of microbial communities depends on the multiple factors including the adaptations and changes in biotic interactions such as competition, trophic interactions and mutualisms. Finally, overall, this study highlights that efforts are still required to conduct microbial ecology studies considering different microbial groups such as prokaryotes, fungi, viruses, diatoms, algae and protists, and their interactions (McGenity et al., 2012) .

Microbial ecology aims also to develop bioindicators for monitoring ecosystems that are threatened and impacted by humans (Aylagas et al., 2017). Metabarcoding provide information for the development of biomonitoring tools, which may have the advantages to be cost effective, automatized and standardised. During our study, **amplicon sequence variants (ASVs) were identified as indicators of sediment receiving terrigenous inputs by soil runoff in a context of HC contamination.** Their robustness remains to be demonstrated, by *in silico* meta-analysis, *in situ* detection in different land-sea continuums (targeted PCR) or using our microcosm system with soils and marine sediments from different continuums. Information about the metabolic activity of bioindicators is also required, especially their functional role in the degradation of organic matter and HC. In previous laboratory work, CARD FISH (catalysed-reported deposition fluorescence *in situ* hybridization; (Pernthaler et al., 2002)) and microautoradiography (Cottrell and Kirchman, 2000) techniques have shown promise in confirming the link between the bioindicator diversity and the ecological services they provide in HC contaminated marine coastal sediments (Jeanbille, 2015). This technique may be used to confirm the specificity of our indicators through the development of specific probes.)

The unit used for the diversity analysis is another point worth discussing. Indeed, the latest denoising algorithms are powerful to discriminate two sequences based on the assumed biological change of one nucleotide in the sequence and then cluster sequences into unique amplicon sequence variants (ASVs; Callahan et al., 2017). We chose to work with ASVs rather than OTUs because they are a taxonomic unit reusable across studies, not limited by database and efficient to discriminate ecological patterns. That way, merging the ASV tables with those from future experiments reusing of our microcosm system will thus extend and validate the list of coalescence bioindicators. In the same way, **merging the ASV tables with those from previous and future surveys of coastal ecosystem will allow to study community coalescence at geographical scale.** On such large datasets, the study of β -diversity and the use of null models are promising to determine mechanisms driving outcomes of coalescence.

During the thesis, we determined the closest taxonomic affiliation by comparing our ASVs to the sequences in the Silva database (Pruesse et al., 2007) in order to benefit from the available bibliography on the lifestyle and functions of the detected microorganisms. **By side of diversity analysis based on ASVs (taxonomic level), phylogenetic indices were also used in order to decipher changes in the relatedness within and between communities, regardless of the level of the classification.** Therefore, we considered both ecotype-based (ASV) and higher-level community changes.

In our microcosm system, the small amount of soil runoff compared to the marine sediment compartment, combined with the short residence time of the soil runoff (tidal cycle), gave

probably limited time for establishment of terrestrial microorganisms, but enough to functional adaptation of the autochthonous. This is in accordance with the fact that we found more taxa benefiting from the coalescent environment than taxa transferred by soil runoff in sediment receiving terrigenous inputs. **The functional profile analysis and the quantification of deuterated HC provided evidences of functional redundancy in marine coastal sediment.** Indeed, the results showed similar metabolic capabilities and similar HC removal in sediment, irrespective of the presence of terrigenous inputs. Gene variants involved in carbon cycling and organic remediation were specifically associated to sediment receiving terrigenous inputs, and thus identified as indicators of community coalescence. It raises questions about their roles in the adaptation of sediment microorganisms to terrigenous inputs and in biogeochemical processes and HC degradation. **Studies at the transcriptome level and on their metabolic activities are required to clarify link between functional diversity and HC removal.** In our study, we deduced the effect of terrigenous inputs in microbial assemblages of coastal marine sediments from taxonomic/phylogenetic and functional diversity patterns, respectively by 16S/18S metabarcoding and microarray (GeoChip), both based on DNA. We are aware that these methods, used on DNA, cannot distinguish active, slow-growing, dormant and dead microorganisms. For example, this may explain why we found high compositional similarity (taxonomic, phylogenetic and functional) between microbial communities from bare soil and planted soil. **The same approaches mentioned above (metabarcoding and microarray) used on RNA or complementary DNA (cDNA after reverse transcription) will refine our description of the different communities of the land-sea continuum, as these sequences are commonly associated with the active fraction of microorganisms.** Furthermore, Wisnoski et al (2020) showed the importance of introducing data on metabolic heterogeneity to describe structure of microbial communities in the mixing zone by sequencing 16S rRNA genes (DNA) and transcripts (RNA). Among the RNA-based techniques, metatranscriptomics simultaneously provides information on taxonomy and function by detecting transcripts of genes not detected by metagenomics due to their low abundance in the ecosystem.

Other tools are available for distinguishing living from dead microorganisms (Emerson et al., 2017) such as techniques based on cellular metabolism (stable isotope probing, SIP), on proteins (proteomics) or on metabolites (metabolomics). These techniques are helpful to study the implications of coalescence on microbial processes in land-sea continuum. In the context of HC contamination, the investigation of biodegradation of these pollutants, using the SIP (Lemmel et al., 2019; Neufeld et al., 2007) with ¹³C-labelled HC, will help to identify the active microorganisms (DNA containing ¹³C). In parallel, attempt to isolate HC degraders among the active fraction are possible in order to study their metabolisms and identify specific functional traits. Regarding metabolomics, during the thesis, the removal of deuterated HC and the production of

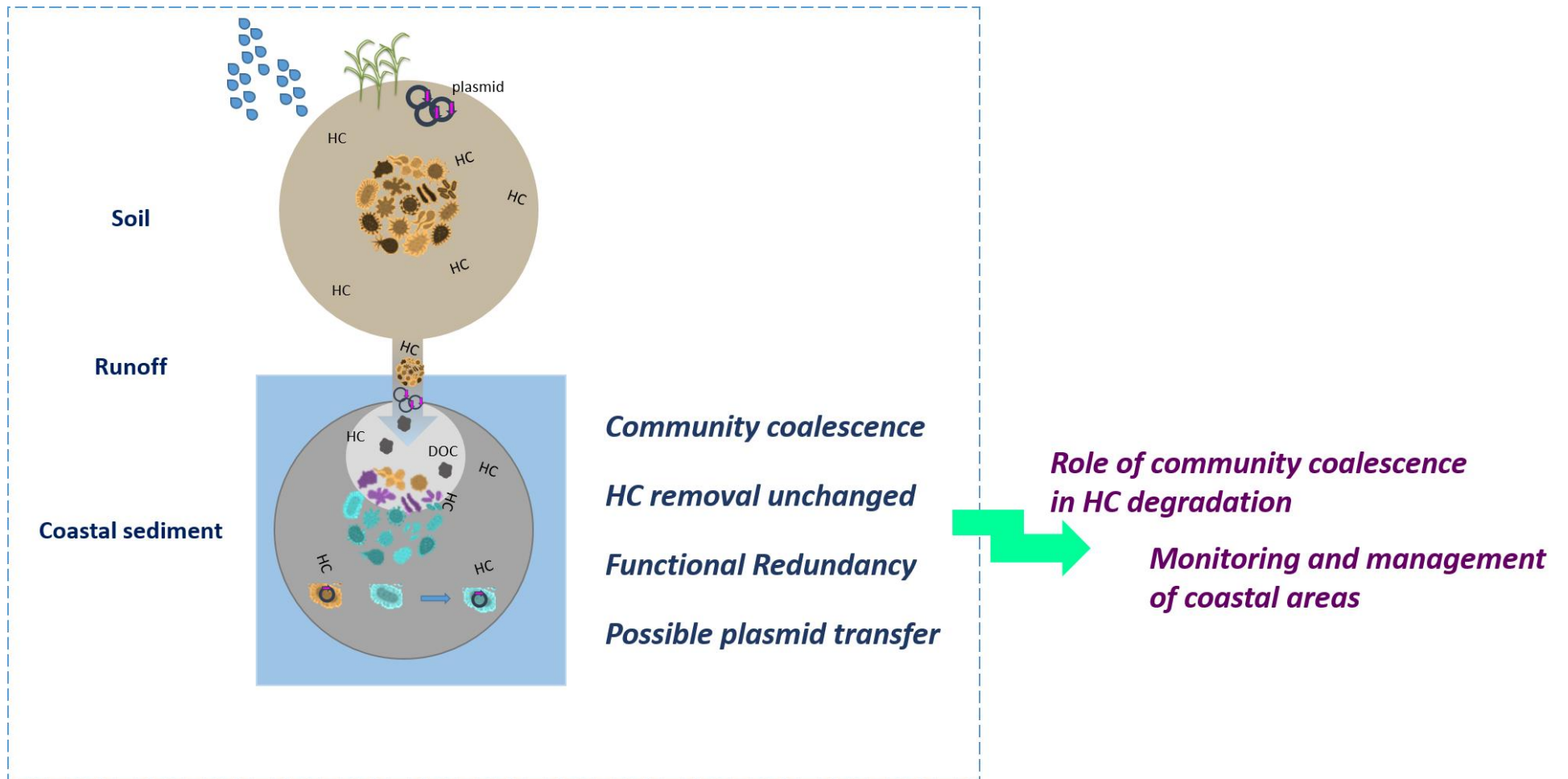


Figure VI.1 Graphical conclusion and perspectives

deuterated metabolites were characterized by gas chromatography-mass spectrometry (GC-MS) and Fourier-transform ion cyclotron resonance-mass spectrometry (FTICR-MS). **As result, the removal of HC occurred in soil and sediment under all scenarios. However, any metabolites of HC degradation was detected making impossible the connection between HC removal, specific microbial pathways and specific microbial communities.** The development of extraction and chemical analysis methods (including detector calibration) is still needed for the detection of deuterated FTICR, particularly in complex matrices such as marine sediment.

Soil runoff was found to be a source of plasmids (α , β , and ϵ subgroups of IncP-1 and IncP-9) for sediments receiving terrigenous inputs. Horizontal gene transfer mediated by plasmid are possible during coalescence event. We attempt to investigate the influence of the terrigenous inputs on the uptake of an exogenous plasmid by marine bacterial communities using fluorescence reporter-gene approach coupled to FACS. Preliminary studies have allowed us to identify required adjustments to optimize the use of this approach for plasmid transfer detection. The plasmid transfer frequency, the host range and the recipient diversity will provide information on the role of coalescence in the adaptation of the sediment bacterial communities.

The results of the thesis show that community coalescence play a crucial role in the organization of microbial communities in marine coastal sediment (Figure VI.1). In coastal environment, consideration of coalescence events should provide a better understanding of microbial community structure and functioning in response to disturbances such as oil pollution. A thorough understanding of coalescence mechanisms will provide useful information for the development of coastal-specific monitoring and management tools.

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Abstract

Microbial communities in marine coastal sediment provide ecosystem services by driving essential processes including biogeochemical cycles, organic matter and pollutant degradation. In the land-sea continuum, we hypothesize that the microbial community structure and function inhabiting marine coastal ecosystems are influenced by soil runoff they receive. In order to verify the hypothesis, an experimental ecology approach was set up mimicking soil runoff within hydrocarbon (HC) contaminated land-sea continuum in microcosms. The community diversity analysis provided evidences of community coalescence (i.e. mixing of entire communities and their surrounding environments) when marine coastal sediments receive terrigenous inputs via soil runoff. The functional potential of sediment microbial communities and the removal of HC were unchanged by the terrigenous inputs. The gene variants (GVs) composition and the co-occurrence network of sediment microbial communities receiving soil runoff were modified. However, GV encode the same function found in sediment microbial communities not influenced by soil runoff, revealing functional redundancy. Amplicon sequence variants (ASVs) and GV were specifically associated to sediment receiving terrigenous inputs, and thus identified as indicators of community coalescence. Soil runoff was demonstrated as a plasmid reservoir, suggesting possible horizontal gene transfer mediated by plasmid during coalescence event. Our results show that community coalescence play crucial role in the organization of microbial communities in marine coastal sediment. Better understanding the coalescence mechanisms will provide useful information for the monitoring and management of coastal environment affected by human land-based activities.

Résumé

Les communautés microbiennes des sédiments marins côtiers fournissent des services écosystémiques en régissant des processus essentiels comme les cycles biogéochimiques, la dégradation de la matière organique et des polluants. Dans le continuum terre-mer, nous émettons l'hypothèse que dans les écosystèmes marins côtiers, la structure et la fonction des communautés microbiennes sont influencées par les apports terrigènes provenant du lessivage des sols. Afin de vérifier cette hypothèse, une approche d'écologie expérimentale a été mise en place pour simuler en microcosmes le ruissellement des sols dans le continuum terre-mer contaminé par les hydrocarbures (HC). L'analyse de la diversité des communautés a mis en évidence la coalescence des communautés (c'est-à-dire le mélange de communautés entières, en tandem avec leurs environnements) lorsque les sédiments marins côtiers reçoivent des apports terrigènes via le ruissellement des sols. Le potentiel fonctionnel des communautés microbiennes des sédiments et l'élimination des HC n'ont pas été modifiés par les apports terrigènes. En revanche, la composition en variants de gène (VG) et le réseau de co-occurrence des communautés microbiennes sédimentaires recevant le ruissellement du sol ont été remaniés. Cependant, les VG codent les mêmes fonctions retrouvées dans les communautés microbiennes sédimentaires non influencées par le ruissellement du sol ce qui révèle la redondance fonctionnelle. Des variants de séquence d'amplicon (VSA) et des VG ont pu être spécifiquement associés aux sédiments recevant des apports terrigènes, et ont donc été identifiés comme des indicateurs de coalescence des communautés. Il a été démontré que le ruissellement du sol est un réservoir de plasmides ce qui suggère que le transfert horizontal de gènes médié par les plasmides est possible pendant l'événement de coalescence. Nos résultats montrent que la coalescence des communautés joue un rôle crucial dans l'organisation des communautés microbiennes dans les sédiments marins côtiers. Une meilleure compréhension des mécanismes de coalescence fournira des informations utiles pour la surveillance et la gestion de l'environnement côtier affecté par les activités humaines terrestres.