HAL
open science

Functional redundancy in response to runoff input upholds microbial community in hydrocarbon-contaminated land-sea continuum

Elise Châtillon, Aurélie Cébron, François Rigal, Christine Cagnon, Catherine Lorgeoux, Pierre Faure, Robert Duran, Cristiana Cravo-Laureau

To cite this version:

Elise Châtillon, Aurélie Cébron, François Rigal, Christine Cagnon, Catherine Lorgeoux, et al.. Functional redundancy in response to runoff input upholds microbial community in hydrocarbon-contaminated land-sea continuum. Environmental Pollution, 2023, 335, pp.122330. 10.1016/j.envpol.2023.122330 . hal-04189595

HAL Id: hal-04189595

https://univ-pau.hal.science/hal-04189595

Submitted on 2 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Functional redundancy in response to runoff input upholds microbial community in hydrocarbon-contaminated land-sea continuum

Elise Châtillon[1], Aurélie Cébron[2], François Rigal[1], Christine Cagnon[1], Catherine Lorgeoux[3], Pierre Faure[2], Robert Duran[1], Cristiana Cravo-Laureau[1*]

[1] Universite de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Pau, France

[2] Université de Lorraine, CNRS, LIEC, F-54000 Nancy, France

[3] Université de Lorraine, CNRS, GeoRessources, F-54000 Nancy, France

*corresponding author: cristiana.cravo-laureau@univ-pau.fr; Université de Pau e des Pays de l'Adour, Avenue de l'Université, Bâtiment IBEAS, BP 1155, 64013 Pau Cedex, France; Tel: +33 5 59 40 74 74

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 hypothesized that the coalescent event increases 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 GVs 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-occurence networks, showing a reorganization of the GVs associations with an increase of the network complexity. Different keystone GVs ensuring similar functions were identified in networks with or without terrigenous inputs, further confirming functional redundancy.

We argue that functional redundancy maintains the structure of microbial community in hydrocarbon-contaminated land-sea continuum mixing zone. 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

1. 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 to marine coastal sediments including organic matter, sorbed pollutants and microorganisms (Fagervold et al., 2014; Hoffman et al., 1984; Yakirevich et al., 2013). The composition of terrigenous inputs is influenced by the vegetation cover, which alters water flow (Nagase and Dunnett, 2012; Wenzel, 2009) and regulates the soil microbial communities. The mixing of microbial communities together with the mixing of their environments results in community coalescence (Châtillon et al., 2023; 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 (*e.g.* 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), involving microbial network interactions, which are 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 the best of 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. This microcosm experiment was designed to consider the coalescence events and the effect of plant and HC influencing the runoff. This approach allows the implementation of artificial scenarios by controlling physical-chemical parameters mimicking as close as possible natural environmental conditions (Cravo-Laureau and Duran, 2014; Mazière et al, 2023). 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. DNA-array (Geochip 5S) gene functional analysis was performed to compare microbial communities in sediment receiving or not terrigenous inputs. Different gene families, involved in biogeochemical cycles and organic pollutant remediation, were targeted. Functional genes involved in HC degradation pathways were examined as

candidate indicators of the community coalescence. Their co-occurrence patterns with functional genes involved in the main biogeochemical cycles were established.

2. Material and Methods

2.1. Microcosms

Soil (10 kg) and sediment (2 kg) were collected in the same geographical area, close to petroleum refinery, in southeast of France, in October 2017. Soil, classified as peyrosol (gravelly soil, Baize et al, 2008; Messant et al, 2021), 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). The sampling sites are both near industrial zones, contaminated by HC and enlisted int the BASOL database (Antoni, 2013) for the polluted sites and soils from the French Ministry of the Ecological and Solidary Transition. Soil, rid of vegetation, was collected on surface (maximal depth of 15 cm) and sediment was collected from the surface until 8 to 10 cm depth. 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. Soil was air-dried and stored at room temperature and sediment was stored at 4 °C in a 5 L bottle completely covered with *in situ* seawater, until starting the experiment (one week later). The seawater covering the sediment was submitted to continuous aeration through an aquarium pump. The texture, the pH, the C/N ratio, the cation-exchange capacity, the water holding capacity (WHC), ions and trace metals were quantified on dry soil and sediment (LAS, INRAE Arras, France). Microcosms were set up and incubated for 39 days under controlled conditions, as previously described (Châtillon et al., 2023). The experimental system was designed to highlight community coalescence between soil and coastal marine sediments resulting from terrigenous inputs via soil runoff, considering the effect of plant and HC influencing potentially the runoff. Microcosms were set up in triplicates as follow (Figure 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.

Briefly, the microcosms were composed of glass devices (1 L beaker equipped with a seawater inlet and outlet) containing the equivalent of 90 g of dry sediment and that were connected through Teflon pipes or not to stainless steel devices containing 600 g of moistened soil at 60% WHC. Different conditions were applied in triplicate (Figure 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 and 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. The HC content in soil and sediment before spiking was between 0.8 and 1.8 mg/kg. 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. In both land-sea continuum and control microcosms, peristaltic pumps helped to simulate tidal cycles and renew the seawater (Salt Instant Ocean, Aquarium systems) ensuring conditions close to those prevailing in natural environment. Two tidal cycles were simulated per day: high tide was simulated filling the sediment compartments with 250 mL of synthetic seawater in 6 hours, and low tide was simulated reducing water level in 6h, maintaining sediment covered by 3 cm of seawater.

2.2. Sampling

Samples of the different compartments (soil, runoff, sediment) and of seawater were collected at different times at the beginning (t0) at the end (tf, after 39 days of incubation), and during the incubation (after 23 days of incubation) for subsequent chemical and/or biomolecular analysis. Soil and sediment samples (ca. 10-12 g) were collected in triplicates at the beginning and the 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: 14 mm; 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 soil microcosm specifically set up for this sampling time, one with HC spiking and one without HC spiking, in order to do not alter soil structure or disturb plant germination in the microcosm to be incubated longer. 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). After 23 days of incubation and at tf (39 days), runoff samples were obtained from each land-sea microcosm, by mixing runoff from three consecutive days and filtered on 0.22 µm membrane (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.

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

Runoff water (40 mL) and seawater from the low tides (250 mL) were collected at the beginning, after 23 and 39 (tf) 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 soil and sediment samples (2 g) collected at the beginning (t0) and at the end of the experiment (tf) 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 (Na_2SO_4) to remove remaining water. Samples were extracted at 130 °C, 100 bars and 10 min for each solvent. After adjusting the volume of the DCM extract at 10 mL, an aliquot of 80 µL was spiked with 20 µL 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.

2.4. 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.

2.5. Statistical analysis

All the statistical analyses were performed within the R programming environment (R Core Team, 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 analyses was performed to determine the overall degree of concordance between dissimilarity in all GVs and dissimilarity only remediation category GVs. In the Procruste 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 (i.e. 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 Kruskall-Wallis tests were used to test the effect of the experimental conditions on the removal of the deuterated HC.

2.6. 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).

3. Results and Discussion

3.1. HC removal and impact on the plant biomass and the bacterial abundance

The concentrations of the deuterated HC at the beginning of the experiment (t0) and after 39 days of incubation (tf) were determined in the different microcosms (Table 1). The phenanthrene concentration was reduced significantly 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 (Kruskall-Wallis 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 (Van 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 (for a review, see 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; Jurburg 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 S1) 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 (Wilcox test: $W = 1$, $p = 0.2$) showing that *Catapodium marinum* 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 higher (X 100) than in rainwater controls showing that runoff is enriched with prokaryotic abundance as it passes through the soil. Similarly, runoff was also enriched with DOC irrespective of the condition (Table S2) showing it brings 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 DOC enrichment in the marine compartment, which was even more important in the non-spiked seawater (Sed NC) (ANOVA, interaction runoff: HC; ANOVA, $F=6.534$; $p < 0.05$). 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.

3.2. 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 ± 1751; Table 2) whereas the maximum GVs was found in sediments receiving runoff from non-planted and HC spiked soils (SedR NPC; 45,641 ± 1277; Table 2). The coverage was between 56 % ± 3.0 and 79 % ± 2.2 (Table 2). The GVs richness was significantly higher in sediment than in soil (Exponential Shannon 1D: $p < 0.05$; Table S3) 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 S1) 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 irrespective of the condition indicating that neither plants nor the HC spiking changed the functional diversity. However, the GVs richness was significantly different in planted soil with HC spiking (richness R: $p < 0.05$; Table S3) 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 S3) 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.

To further characterize the effect of runoff on the functional organization, the GVs composition was investigated. The relative abundance of functional gene categories (Figure S1) 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 2 A) showed similar pattern than that obtained by comparing the functional microbial communities based on the GVSs involved in the organic remediation (Figure 2 B), which was further supported by Procrustes ($M^2 = 0.017$, $r = 0.99$, $p < 0.001$). Such observation suggested that the presence of HC, in the soil and sediment contaminated for a long period might explain the functional organization of the microbial communities because they are likely adapted to the presence of HC. Thus, HC probably act as a filter selecting the organisms that are able to survive and establish in this specific condition (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; Hafez et al, 2022). Additionally, NMDS analysis separated clusters according to the compartment, distinguishing the effect of runoff in sediments (Figure 2), 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 2 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).

3.3. Functional gene indicators

3.3.1. 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 3). Soil and sediment 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 S1). 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 3). 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). Noteworthy, in sediment receiving runoff (SedR), any gene family indicator 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.

3.3.2. 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 4 and Figure 5). GVSs indicators were identified for the 6 conditions (SedR NPNC, SedR NPC, SedR PNC, SedR PC, SedNR NC, SedNR C) (Figure 4). However, sediments receiving runoff (SedR) exhibited more GVs indicators than those without runoff (SedNR), irrespective of the presence of plants and HC spiking (Figure 4 and Figure 5). 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 5). 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 those without (Sed NR) (Figure 5 A). Such observation suggests that runoff enriched sediments with nutrients and microorganisms via coalescence (Châtillon et al., 2023) increasing functional diversity. HC spiked sediments receiving or not runoff exhibited the same categories of GVs indicators (Figure 5 A). Similarly, the pattern of the categories of

GVs indicators was similar in sediments receiving runoff from planted soil or non-planted soils (Figure 5 B). However, the differences between these conditions were observed only at the GVs levels (Figure 4 and Figure 5), supporting the functional redundancy. Such functional redundancy has been previously described in HC polluted marine coastal sediment (Cravo-Laureau and Duran, 2014; Stauffert et al., 2013), which corresponds to known mechanisms involved in adaptive response of microorganisms to environmental perturbation (Allison and Martiny, 2008). 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.

3.4. 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 6). The network without runoff included 173 nodes and 334 significant edges, while the network with runoff included 174 nodes and 445 significant edges (Table 3). The network density, the clustering coefficient, the average number of neighbours and the characteristic path length were higher in the network with runoff (Table 3). 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 S4) 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 6). 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 S4 and Table S5) 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.

4. 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.

Acknowledgments

We thank the TOTEM project team for discussions and technical assistance. The seed bank of the Muséum National d'Histoire Naturelle de Paris - DGD – MJZ is acknowledged for providing seeds of *Catapodium marinum*. The authors are grateful to Manon Runza, Pauline Latour and members of the Experimental phytotronic platform of Lorraine (PEPLor) at the Université de Lorraine (Site FST, Vandoeuvre-les-Nancy) for technical assistance and access to plant growth facilities.

Funding

This work was supported by the French national programme EC2CO-LEFE (TOTEM project). EC was supported by E2S-CCLO grant.

Credit author statement

Elise Châtillon: Methodology, Validation, Formal analysis, Investigation, Writing – original draft. **Aurélie Cébron**: Conceptualization, Methodology, Investigation, Supervision, Writing – review & editing. **François Rigal**: Methodology, Formal analysis, Investigation, Writing – review & editing. **Christine Cagnon**: Methodology, Investigation, Writing – review & editing. **Catherine Lorgeoux**: Methodology, Investigation, Writing – review & editing. **Pierre Faure**: Methodology, Investigation, Writing – review & editing. **Robert Duran**: Conceptualization, Methodology, Investigation, Supervision, Writing – review & editing. **Cristiana Cravo-Laureau**: Conceptualization, Methodology, Investigation, Funding acquisition, Project administration, Supervision, Writing – review & editing.

References

Allison, S.D., Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. Proc. Natl. Acad. Sci. 105, 11512–11519. https://doi.org/10.1073/pnas.0801925105

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Antoni, V., 2013. Basol : un panorama des sites et sols pollués, ou potentiellement pollués, nécessitant une action des pouvoirs publics (CGDD, Études & documents n° 97, novembre 2013).

Avila-Jimenez, M.-L., Burns, G., He, Z., Zhou, J., Hodson, A., Avila-Jimenez, J.-L., Pearce, D., 2020. Functional Associations and Resilience in Microbial Communities. Microorganisms 8, 951. https://doi.org/10.3390/microorganisms8060951

Bader, G.D., Hogue, C.W., 2003. An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinformatics 4, 2. https://doi.org/10.1186/1471-2105-4-2

Baize D., Girard M.C., Jabiol B., Rossignol J.P., Eimberck M., Beaudou A., 2008. Référentiel pédologique. Association française pour l'étude des sols (AFES) - Editions Quae. 435 p

Bauer, J.E., Cai, W.-J., Raymond, P.A., Bianchi, T.S., Hopkinson, C.S., Regnier, P.A.G., 2013. The changing carbon cycle of the coastal ocean. Nature 504, 61–70. https://doi.org/10.1038/nature12857

Bordenave, S., Fourçans, A., Blanchard, S., Goñi, M.S., Caumette, P., Duran, R., 2004. Structure and functional analyses of bacterial communities changes in microbial mats following petroleum exposure. Ophelia 58, 195–203. https://doi.org/10.1080/00785236.2004.10410227

Bourceret, A., Cébron, A., Tisserant, E., Poupin, P., Bauda, P., Beguiristain, T., Leyval, C., 2016. The Bacterial and Fungal Diversity of an Aged PAH- and Heavy Metal-Contaminated Soil is Affected by Plant Cover and Edaphic Parameters. Microb. Ecol. 71, 711–724. https://doi.org/10.1007/s00248-015-0682-8

Bourceret, A., Leyval, C., De Fouquet, C., Cébron, A., 2015. Mapping the centimeter-scale spatial variability of PAHs and microbial populations in the rhizosphere of two plants. PLoS ONE 10, 1–22. https://doi.org/10.1371/journal.pone.0142851

Burdige, D.J., 2011. 5.09 - Estuarine and Coastal Sediments – Coupled Biogeochemical Cycling, in: Wolanski, E., McLusky, D. (Eds.), Treatise on Estuarine and Coastal Science. Academic Press, Waltham, pp. 279–316. https://doi.org/10.1016/B978-0-12-374711-2.00511-8

Caceres, M.D., Legendre, P., 2009. Associations between species and groups of sites: indices and statistical inference, Ecology, 90, 3566-3574. https://doi.org/10.1890/08-1823.1

Calderón, K., Spor, A., Breuil, M.-C., Bru, D., Bizouard, F., Violle, C., Barnard, R.L., Philippot, L., 2017. Effectiveness of ecological rescue for altered soil microbial communities and functions. ISME J. 11, 272–283. https://doi.org/10.1038/ismej.2016.86

Castledine, M., Sierocinski, P., Padfield, D., Buckling, A., 2020. Community coalescence: an eco-evolutionary perspective. Philos. Trans. R. Soc. B Biol. Sci. 375, 20190252. https://doi.org/10.1098/rstb.2019.0252

Cébron, A., Beguiristain, T., Faure, P., Norini, M.P., Masfaraud, J.F., Leyval, C., 2009. Influence of vegetation on the in situ bacterial community and polycyclic aromatic hydrocarbon (PAH) degraders in aged PAH-contaminated or thermal-desorption-treated soil. Appl. Environ. Microbiol. 75, 6322–6330. https://doi.org/10.1128/AEM.02862-08

Cébron, A., Norini, M., Beguiristain, T., Leyval, C., 2008. Real-Time PCR quantification of PAH-ring hydroxylating dioxygenase (PAH-RHD α) genes from Gram positive and Gram negative bacteria in soil and sediment samples 73, 148–159. https://doi.org/10.1016/j.mimet.2008.01.009

Châtillon, E., Duran, R., Rigal, F., Cagnon, C., Cébron, A., Cravo-Laureau, C. 2023. Microbial community diversity in a mixing zone at the interface between land and sea. Microbiological Research 267, 127259. https://doi.org/10.1016/j.micres.2022.127259

Cook, R.L., Hesterberg, D., 2013. Comparison of Trees and Grasses for Rhizoremediation of Petroleum Hydrocarbons. Int. J. Phytoremediation 15, 844–860. https://doi.org/10.1080/15226514.2012.760518

Cravo-Laureau, C., Duran, R., 2014. Marine coastal sediments microbial hydrocarbon degradation processes: contribution of experimental ecology in the omics'era. Front. Microbiol. 5, 39. https://doi.org/10.3389/fmicb.2014.00039

Daryabeigi Zand, A., 2020. Contribution of Dissolved Organic Carbon in Mobilizing and Releasing High Molecular Weight Polycyclic Aromatic Hydrocarbons From Carbonaceous Material-Amended Soil. J. Appl. Biotechnol. Rep. 7, 125–134. https://doi.org/10.30491/jabr.2020.109288

De Cáceres, M., Legendre, P., Moretti, M., 2010. Improving indicator species analysis by combining groups of sites. Oikos 119, 1674–1684. https://doi.org/10.1111/j.1600-0706.2010.18334.x

Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M., Huttenhower, C., Langille, M.G.I., 2020. PICRUSt2 for prediction of metagenome functions. Nat. Biotechnol. 38, 685–688. https://doi.org/10.1038/s41587-020-0548-6

Duarte, M., Jauregui, R., Vilchez-Vargas, R., Junca, H., Pieper, D.H., 2014. AromaDeg, a novel database for phylogenomics of aerobic bacterial degradation of aromatics. Database 2014. https://doi.org/10.1093/database/bau118

Duran, R., Bonin, P., Jezequel, R., Dubosc, K., Gassie, C., Terrisse, F., Abella, J., Cagnon, C., Militon, C., Michotey, V., Gilbert, F., Cuny, P., Cravo-laureau, C., 2015. Effect of physical sediments reworking on hydrocarbon degradation and bacterial community structure in marine coastal sediments. https://doi.org/10.1007/s11356-015-4373-2

Duran, R., Cravo-Laureau, C., 2016. Role of environmental factors and microorganisms in determining the fate of polycyclic aromatic hydrocarbons in the marine environment 1–17. https://doi.org/10.1093/femsre/fuw031

Fagervold, S.K., Bourgeois, S., Pruski, A.M., Charles, F., Kerherve, P., Vetion, G., Galand, P.E., 2014. River organic matter shapes microbial communities in the sediment of the Rhône prodelta. ISME J. 12. https://doi.org/10.1038/ismej.2014.86

Faust, K., Lima-Mendez, G., Lerat, J.-S., Sathirapongsasuti, J.F., Knight, R., Huttenhower, C., Lenaerts, T., Raes, J., 2015. Cross-biome comparison of microbial association networks. Front. Microbiol. 6. https://doi.org/10.3389/fmicb.2015.01200

Faust, K., Raes, J., 2016. CoNet app: inference of biological association networks using Cytoscape. F1000Research 5. https://doi.org/10.12688/f1000research.9050.2

Fenchel, T., King, G.M., Blackburn, T.H., 2012a. Chapter 6 - Biogeochemical Cycling in Soils, in: Fenchel, T., King, G.M., Blackburn, T.H. (Eds.), Bacterial Biogeochemistry (Third Edition). Academic Press, Boston, pp. 89–120. https://doi.org/10.1016/B978-0-12-415836-8.00006-2

Fenchel, T., King, G.M., Blackburn, T.H., 2012b. Chapter 7 - Aquatic Sediments, in: Fenchel, T., King, G.M., Blackburn, T.H. (Eds.), Bacterial Biogeochemistry (Third Edition). Academic Press, Boston, pp. 121–142. https://doi.org/10.1016/B978-0-12-415836-8.00007-4

Gerhardt, K.E., Huang, X.D., Glick, B.R., Greenberg, B.M., 2009. Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. Plant Sci. 176, 20–30. https://doi.org/10.1016/j.plantsci.2008.09.014

Ghosal, D., Ghosh, S., Dutta, T.K., Ahn, Y., 2016. Current State of Knowledge in Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.01369

Giorgio, P.A., Davis, J., 2003. Patterns in Dissolved Organic Matter Lability and Consumption across Aquatic Ecosystems. pp. 399–424. https://doi.org/10.1016/B978-012256371-3/50018-4

Groffman, P.M., Bohlen, P.J., 1999. Soil and Sediment Biodiversity: Cross-system comparisons and large-scale effects. BioScience 49, 139–148. https://doi.org/10.2307/1313539

Guo, X., Lu, D., Niu, Z., Feng, J., Chen, Y., Tou, F., Liu, M., Yang, Y., 2018. Bacterial community structure in response to environmental impacts in the intertidal sediments along the Yangtze Estuary, China. Mar. Pollut. Bull. 126, 141–149. https://doi.org/10.1016/j.marpolbul.2017.11.003

Hafez, T., Ortiz-Zarragoitia, M., Cagnon, C., Cravo-Laureau, C., Duran, R. 2022. Legacy and dispersant influence microbial community dynamics in cold seawater contaminated by crude oil water accommodated fractions. Environmental Research, 212. https://doi.org/10.1016/j.envres.2022.113467

Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'Agrosa, C., Bruno, J.F., Casey, K.S., Ebert, C., Fox, H.E., Fujita, R., Heinemann, D., Lenihan, H.S., Madin, E.M.P., Perry, M.T., Selig, E.R., Spalding, M., Steneck, R., Watson, R., 2008. A Global Map of Human Impact on Marine Ecosystems. Science 319, 948–952. https://doi.org/10.1126/science.1149345

Van Hamme, J.D.V., Singh, A., Ward, O.P., 2003. Recent Advances in Petroleum Microbiology. Microbiol. Mol. Biol. Rev. 67, 503–549. https://doi.org/10.1128/MMBR.67.4.503-549.2003

Hervé, M., 2020. RVAideMemoire: Testing and Plotting Procedures for Biostatistics.

Hoffman, E.J., Mills, G.L., Latimer, J.S., Quinn, J.G., 1984. Urban runoff as a source of polycyclic aromatic hydrocarbons to coastal waters. Environ. Sci. Technol. 18, 580–587. https://doi.org/10.1021/es00126a003

Janeau, J.-L., Gillard, L.-C., Grellier, S., Jouquet, P., Le, T.P.Q., Luu, T.N.M., Ngo, Q.A., Orange, D., Pham, D.R., Tran, D.T., Tran, S.H., Trinh, A.D., Valentin, C., Rochelle-Newall, E., 2014. Soil erosion, dissolved organic carbon and nutrient losses under different land use

systems in a small catchment in northern Vietnam. Agric. Water Manag. 146, 314–323. https://doi.org/10.1016/j.agwat.2014.09.006

Jurburg, S.D., Nunes, I., Brejnrod, A., Jacquiod, S., Priemé, A., Sørensen, S.J., Van Elsas, J.D., Salles, J.F., 2017. Legacy Effects on the Recovery of Soil Bacterial Communities from Extreme Temperature Perturbation. Front. Microbiol. 8, 1832. https://doi.org/10.3389/fmicb.2017.01832

Jurburg, S.D., Salles, J.F., 2015. Functional Redundancy and Ecosystem Function — The Soil Microbiota as a Case Study. Biodivers. Ecosyst. - Link. Struct. Funct. https://doi.org/10.5772/58981

Kanehisa, M., Goto, S., 2000. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 28, 27–30. https://doi.org/10.1093/nar/28.1.27

Kappell, A.D., Wei, Y., Newton, R.J., Van Nostrand, J.D., Zhou, J., McLellan, S.L., Hristova, K.R., 2014. The polycyclic aromatic hydrocarbon degradation potential of Gulf of Mexico native coastal microbial communities after the Deepwater Horizon oil spill. Front. Microbiol. 5. https://doi.org/10.3389/fmicb.2014.00205

Khan, S., Afzal, M., Iqbal, S., Khan, Q.M., 2013. Plant-bacteria partnerships for the remediation of hydrocarbon contaminated soils. Chemosphere 90, 1317–1332. https://doi.org/10.1016/j.chemosphere.2012.09.045

Kimes, N.E., Callaghan, A.V., Suflita, J.M., Morris, P.J., 2014. Microbial transformation of the deepwater horizon oil spill-past, present, and future perspectives. Front. Microbiol. 5, 1–11. https://doi.org/10.3389/fmicb.2014.00603

King, G.M., Kostka, J.E., Hazen, T.C., Sobecky, P.A., 2015. Microbial Responses to the \textlessi\textgreaterDeepwater Horizon\textless/i\textgreater Oil Spill: From Coastal Wetlands to the Deep Sea. Annu. Rev. Mar. Sci. 7, 377–401. https://doi.org/10.1146/annurev-marine-010814-015543

Kleindienst, S., Grim, S., Sogin, M., Bracco, A., Crespo-Medina, M., Joye, S.B., 2016. Diverse, rare microbial taxa responded to the Deepwater Horizon deep-sea hydrocarbon plume. ISME J. 10, 400–415. https://doi.org/10.1038/ismej.2015.121

Laruelle, G.G., Dürr, H.H., Lauerwald, R., Hartmann, J., Slomp, C.P., Goossens, N., Regnier, P.A.G., 2013. Global multi-scale segmentation of continental and coastal waters from the watersheds to the continental margins. Hydrol. Earth Syst. Sci. 17, 2029–2051. https://doi.org/10.5194/hess-17-2029-2013

Le, H.T., Ho, C.T., Trinh, Q.H., Trinh, D.A., Luu, M.T.N., Tran, H.S., Orange, D., Janeau, J.L., Merroune, A., Rochelle-Newall, E., Pommier, T., 2016. Responses of Aquatic Bacteria to Terrestrial Runoff: Effects on Community Structure and Key Taxonomic Groups. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.00889

Leahy, J.G., Colwell, R.R., 1990. Microbial degradation of hydrocarbons in the environment. Microbiol. Rev. 54, 305–315. https://doi.org/ 10.1128/mr.54.3.305-315.1990

Levy-Booth, D.J., Prescott, C.E., Grayston, S.J., 2014. Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. Soil Biol. Biochem. 75, 11–25. https://doi.org/10.1016/j.soilbio.2014.03.021

Louca, S., Polz, M.F., Mazel, F., Albright, M.B.N., Huber, J.A., O'Connor, M.I., Ackermann, M., Hahn, A.S., Srivastava, D.S., Crowe, S.A., Doebeli, M., Parfrey, L.W., 2018. Function and functional redundancy in microbial systems. Nat. Ecol. Evol. 2, 936–943. https://doi.org/10.1038/s41559-018-0519-1

Mason, O.U., Hazen, T.C., Borglin, S., Chain, P.S.G., Dubinsky, E.A., Fortney, J.L., Han, J., Holman, H.-Y.N., Hultman, J., Lamendella, R., Mackelprang, R., Malfatti, S., Tom, L.M., Tringe, S.G., Woyke, T., Zhou, J., Rubin, E.M., Jansson, J.K., 2012. Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill. ISME J. 6, 1715–1727. https://doi.org/10.1038/ismej.2012.59

Mazière, C., Duran, R., Dupuy, C., Cravo-Laureau, C. 2023. Microbial mats as model to decipher climate change effect on microbial communities through a mesocosm study. Front. Microbiol. 14:1039658. https://doi: 10.3389/fmicb.2023.1039658

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8, e61217. https://doi.org/10.1371/journal.pone.0061217*

Messant A., Lehmann S., Moulin J., Lagacherie P., Jalabert S., Noraz A., Lemercier B., Chafchafi A., Mure J.-P., Laroche B. et Sauter J., 2021. Diffusion des Référentiels Régionaux Pédologiques sous la forme d'une carte des sols dominants (France métropolitaine – Hors Corse) accessible sur le Géoportail. Etude et Gestion des Sols, Association Française pour l'Etude des Sols, 28, 57-69.

Michas, A., Vestergaard, G., Trautwein, K., Avramidis, P., Hatzinikolaou, D.G., Vorgias, C.E., Wilkes, H., Rabus, R., Schloter, M., Schöler, A., 2017. More than 2500 years of oil exposure shape sediment microbiomes with the potential for syntrophic degradation of hydrocarbons linked to methanogenesis. Microbiome 5, 118. https://doi.org/10.1186/s40168-017-0337-8

Mobilian, C., Wisnoski, N.I., Lennon, J.T., Alber, M., Widney, S., Craft, C.B., 2020. Differential effects of press vs. pulse seawater intrusion on microbial communities of a tidal freshwater marsh. Limnol. Oceanogr. Lett. n/a. https://doi.org/10.1002/lol2.10171

Nagase, A., Dunnett, N., 2012. Amount of water runoff from different vegetation types on extensive green roofs: Effects of plant species, diversity and plant structure. Landsc. Urban Plan. 104, 356–363. https://doi.org/10.1016/j.landurbplan.2011.11.001

Newman, L.A., Reynolds, C.M., 2004. Phytodegradation of organic compounds. Curr. Opin. Biotechnol. 15, 225–230. https://doi.org/10.1016/j.copbio.2004.04.006

Nunes, I., Jurburg, S., Jacquiod, S., Brejnrod, A., Falcão Salles, J., Priemé, A., Sørensen, S.J., 2018. Soil bacteria show different tolerance ranges to an unprecedented disturbance. Biol. Fertil. Soils 54, 189–202. https://doi.org/10.1007/s00374-017-1255-4

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. vegan: Community Ecology Package.

Patel, V., Sharma, A., Lal, R., Al-Dhabi, N.A., Madamwar, D., 2016. Response and resilience of soil microbial communities inhabiting in edible oil stress/contamination from industrial estates. BMC Microbiol. 16, 1–14. https://doi.org/10.1186/s12866-016-0669-8

Pommier, T., Merroune, A., Bettarel, Y., Got, P., Janeau, J.-L., Jouquet, P., Thu, T.D., Toan, T.D., Rochelle-Newall, E., 2014. Off-site impacts of agricultural composting: role of terrestrially derived organic matter in structuring aquatic microbial communities and their metabolic potential. FEMS Microbiol. Ecol. 90, 622–632. https://doi.org/10.1111/1574-6941.12421

R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rillig, M.C., Antonovics, J., Caruso, T., Lehmann, A., Powell, J.R., Veresoglou, S.D., Verbruggen, E., 2015. Interchange of entire communities: microbial community coalescence. Trends Ecol. Evol. 30, 470–476. https://doi.org/10.1016/j.tree.2015.06.004

Rillig, M.C., Tsang, A., Roy, J., 2016. Microbial Community Coalescence for Microbiome Engineering. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.01967

Rocca, J.D., Simonin, M., Bernhardt, E.S., Washburne, A.D., Wright, J.P., 2020. Rare microbial taxa emerge when communities collide: freshwater and marine microbiome responses to experimental mixing. Ecology 101. https://doi.org/10.1002/ecy.2956

Rohrbacher, F., St-Arnaud, M., 2016. Root Exudation: The Ecological Driver of Hydrocarbon Rhizoremediation. Agronomy 6, 19. https://doi.org/10.3390/agronomy6010019

Roling, W.F.M., van Bodegom, P.M., 2014. Toward quantitative understanding on microbial community structure and functioning: a modeling-centered approach using degradation of

marine oil spills as example. Front. Microbiol. 5, 125. https://doi.org/10.3389/fmicb.2014.00125

Shi, Z., Yin, H., Van Nostrand, J.D., Voordeckers, J.W., Tu, Q., Deng, Y., Yuan, M., Zhou, A., Zhang, P., Xiao, N., Ning, D., He, Z., Wu, L., Zhou, J., 2019. Functional Gene Array-Based Ultrasensitive and Quantitative Detection of Microbial Populations in Complex Communities. mSystems 4, e00296-19. https://doi.org/10.1128/mSystems.00296-19

Sierocinski, P., Milferstedt, K., Bayer, F., Großkopf, T., Alston, M., Bastkowski, S., Swarbreck, D., Hobbs, P.J., Soyer, O.S., Hamelin, J., Buckling, A., 2017. A Single Community Dominates Structure and Function of a Mixture of Multiple Methanogenic Communities. Curr. Biol. CB 27, 3390-3395.e4. https://doi.org/10.1016/j.cub.2017.09.056

Stauffert, M., Cravo-laureau, C., Duran, R., 2015. Dynamic of sulphate-reducing microorganisms in petroleum-contaminated marine sediments inhabited by the polychaete Hediste diversicolor 15273–15284. https://doi.org/10.1007/s11356-014-3624-y

Stauffert, M., Cravo-laureau, C., Je, R., Amouroux, D., Mahdaoui, F., Gilbert, F., Cagnon, C., Duran, R., 2013. Impact of Oil on Bacterial Community Structure in Bioturbated Sediments 8. https://doi.org/10.1371/journal.pone.0065347

Stauffert, M., Duran, R., Gassie, C., 2014. Response of Archaeal Communities to Oil Spill in Bioturbated Mudflat Sediments 108–119. https://doi.org/10.1007/s00248-013-0288-y

Terrisse, F., Cravo-Laureau, C., Noël, C., Cagnon, C., Dumbrell, A.J., McGenity, T.J., Duran, R., 2017. Variation of Oxygenation Conditions on a Hydrocarbonoclastic Microbial Community Reveals Alcanivorax and Cycloclasticus Ecotypes. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.01549

Thomas, G.E., Cameron, T.C., Campo, P., Clark, D.R., Coulon, F., Gregson, B.H., Hepburn, L.J., McGenity, T.J., Miliou, A., Whitby, C., McKew, B.A., 2020. Bacterial Community Legacy Effects Following the Agia Zoni II Oil-Spill, Greece. Front. Microbiol. 11. https://doi.org/10.3389/fmicb.2020.01706

Tian, W., Wang, L., Li, Y., Zhuang, K., Li, G., Zhang, J., Xiao, X., Xi, Y., 2015. Responses of microbial activity, abundance, and community in wheat soil after three years of heavy fertilization with manure-based compost and inorganic nitrogen. Agric. Ecosyst. Environ. 213, 219–227. https://doi.org/10.1016/j.agee.2015.08.009

Tringe, S.G., Mering, C. von, Kobayashi, A., Salamov, A.A., Chen, K., Chang, H.W., Podar, M., Short, J.M., Mathur, E.J., Detter, J.C., Bork, P., Hugenholtz, P., Rubin, E.M., 2005. Comparative Metagenomics of Microbial Communities. Science 308, 554–557. https://doi.org/10.1126/science.1107851

Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I.C., Jeffries, T.C., Zhou, J., Singh, B.K., 2016. Microbial regulation of the soil carbon cycle: evidence from gene–enzyme relationships. ISME J. 10, 2593–2604. https://doi.org/10.1038/ismej.2016.65

Ukalska-Jaruga, A., Smreczak, B., Klimkowicz-Pawlas, A., 2019. Soil organic matter composition as a factor affecting the accumulation of polycyclic aromatic hydrocarbons. J. Soils Sediments 19, 1890–1900. https://doi.org/10.1007/s11368-018-2214-x

Van Nostrand, J.D., Yin, H., Wu, L., Yuan, T., Zhou, J., 2016. Hybridization of Environmental Microbial Community Nucleic Acids by GeoChip, in: Martin, F., Uroz, S. (Eds.), Microbial Environmental Genomics (MEG), Methods in Molecular Biology. Springer, New York, NY, pp. 183–196. https://doi.org/10.1007/978-1-4939-3369-3_11

von Netzer, F., Kuntze, K., Vogt, C., Richnow, H.H., Boll, M., Lueders, T., 2016. Functional Gene Markers for Fumarate-Adding and Dearomatizing Key Enzymes in Anaerobic Aromatic Hydrocarbon Degradation in Terrestrial Environments. J. Mol. Microbiol. Biotechnol. 26, 180–194. https://doi.org/10.1159/000441946

Wang, Y., Sheng, H.-F., He, Y., Wu, J.-Y., Jiang, Y.-X., Tam, N.F.-Y., Zhou, H.-W., 2012. Comparison of the Levels of Bacterial Diversity in Freshwater, Intertidal Wetland, and Marine Sediments by Using Millions of Illumina Tags. Appl. Environ. Microbiol. 78, 8264. https://doi.org/10.1128/AEM.01821-12

Wenzel, W.W., 2009. Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321, 385–408. https://doi.org/10.1007/s11104-008-9686-1

Wilson, B.C., Vatanen, T., Cutfield, W.S., O'Sullivan, J.M., 2019. The Super-Donor Phenomenon in Fecal Microbiota Transplantation. Front. Cell. Infect. Microbiol. 9. https://doi.org/10.3389/fcimb.2019.00002

Wu, L., Liu, X., Schadt, C.W., Zhou, J., 2006. Microarray-Based Analysis of Subnanogram Quantities of Microbial Community DNAs by Using Whole-Community Genome Amplification. Appl. Environ. Microbiol. 72, 4931–4941. https://doi.org/10.1128/AEM.02738-05

Xenopoulos, M.A., Downing, J.A., Kumar, M.D., Menden-Deuer, S., Voss, M., 2017. Headwaters to oceans: Ecological and biogeochemical contrasts across the aquatic continuum. Limnol. Oceanogr. 62, S3–S14. https://doi.org/10.1002/lno.10721

Yakirevich, A., Pachepsky, Y.A., Guber, A.K., Gish, T.J., Shelton, D.R., Cho, K.H., 2013. Modeling transport of Escherichia coli in a creek during and after artificial high-flow events: three-year study and analysis. Water Res. 47, 2676–2688. https://doi.org/10.1016/j.watres.2013.02.011

Yan, Z., Song, N., Wang, C., Jiang, H., 2021. Functional potential and assembly of microbes from sediments in a lake bay and adjoining river ecosystem for polycyclic aromatic hydrocarbon biodegradation. Environ. Microbiol. 23, 628–640. https://doi.org/10.1111/1462-2920.15104

Yavari, S., Malakahmad, A., Sapari, N.B., 2015. A Review on Phytoremediation of Crude Oil Spills. Water. Air. Soil Pollut. 226, 279. https://doi.org/10.1007/s11270-015-2550-z

Yemashova, N., Murygina, V., Zhukov, D., Zakharyantz, A., Gladchenko, M., Appanna, V., Kalyuzhnyi, S., 2007. Biodeterioration of Crude Oil and Oil Derived Products: A Review. Rev. Environ. Sci. Biotechnol. 6, 315–337. https://doi.org/10.1007/s11157-006-9118-8

Ziervogel, K., Joye, S.B., Arnosti, C., 2016. Microbial enzymatic activity and secondary production in sediments affected by the sedimentation pulse following the Deepwater Horizon oil spill. Deep Sea Res. Part II Top. Stud. Oceanogr. 129, 241–248. https://doi.org/10.1016/j.dsr2.2014.04.003

Zou, Y., Yan, J., Hou, S., Yi, Y., Cui, B., 2020. Intensive land uses modify assembly process and potential metabolic function of edaphic bacterial communities in the Yellow River Delta, China. Sci. Total Environ. 720, 137713. https://doi.org/10.1016/j.scitotenv.2020.137713

Table 1. Removal percentages of deuterated hydrocarbons spiked in soil and sediment, after 39 days of incubation.

The values are presented as mean ± SD (n=3).

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

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

Table 2. Functional gene variants diversity indices and evenness

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 (^{1}D)	14491 ± 141	14850 ± 1037	13422 ± 1131	14194 ± 510	14634 ± 1657	15412 ± 2211	16210 ± 1383	15214 ± 694	18252 ± 698	12903 ± 1103
Inverse-Simpson (^{2}D)	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

Table 3. 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

Figure caption

Figure 1. Schematic representation of the microcosms and the different applied conditions. Soil: soil; Sed: sediment; SedR: sediment receiving runoff; NR: sediment without runoff (control); NP: without plant; P: with plant; NC: without HC spiking; C: withHC spiking.

Figure 2. NMDS 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.

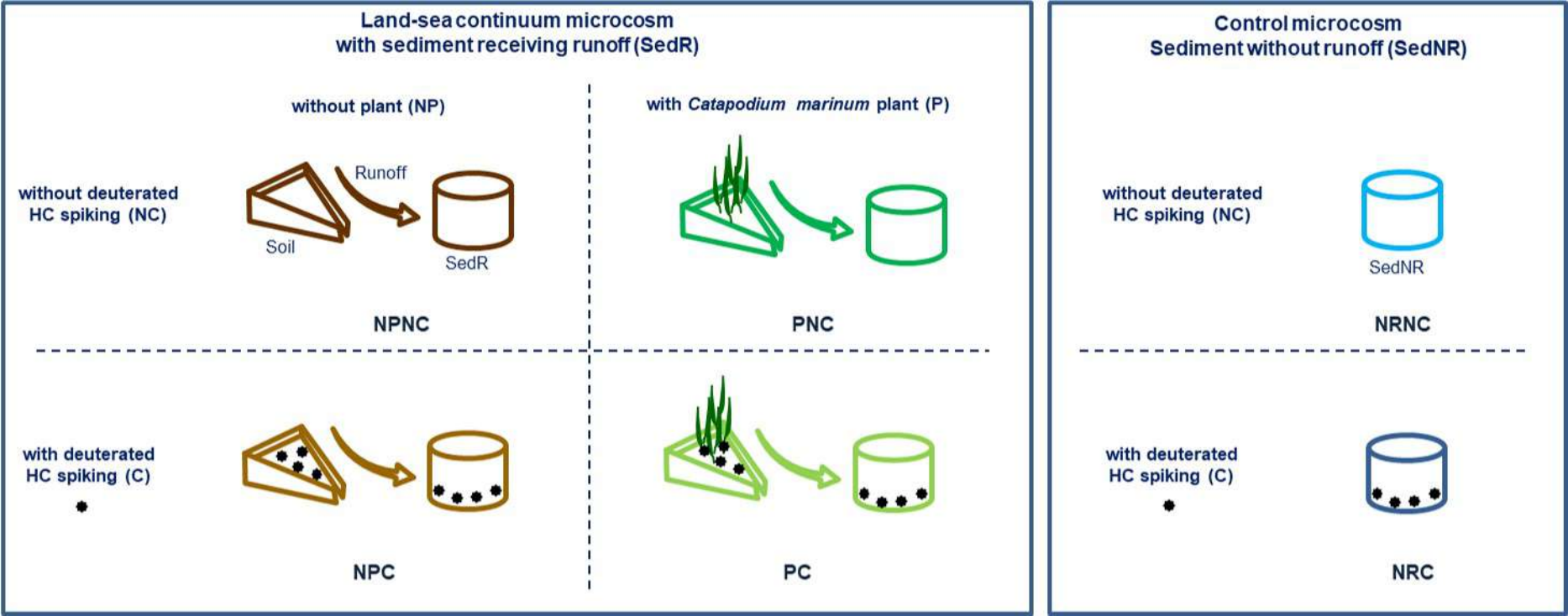
Figure 3. Functional gene family indicators for the different conditions of soil and sediment compartments. The heatmap represents the z- scores of indicators at the gene family level. The z-scores were computed on the gene family basis by subtracting the mean and dividing by the standard deviation of the florescence 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.

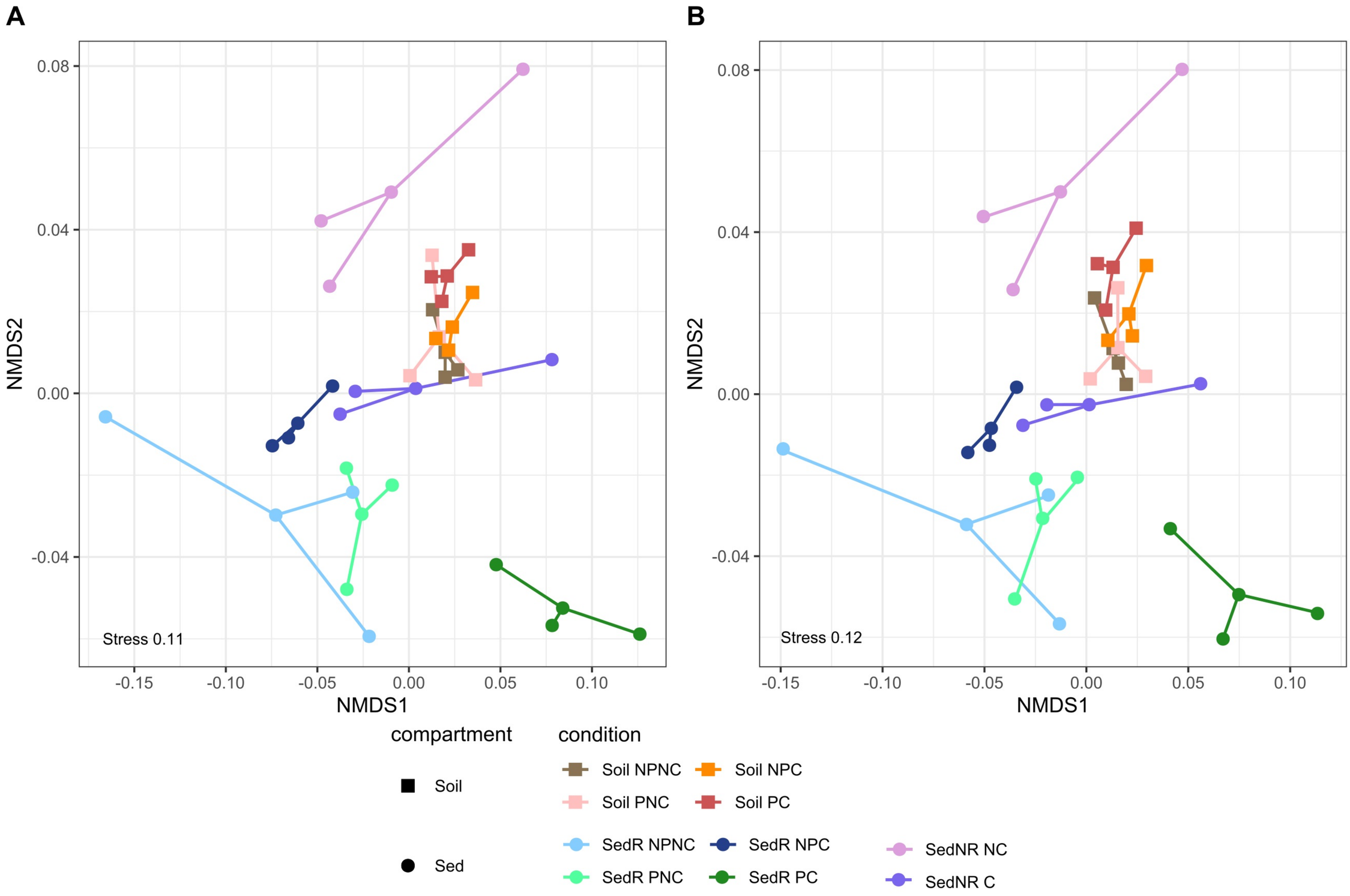
Figure 4. Functional HC biodegradation gene indicators for the different sediment conditions. The heatmap represents the z- scores of bioindicators at the GVs level. The z-scores were computed on the GVs basis by subtracting the mean and dividing by the standard deviation of the florescence intensity (blue for the low fluorescence intensity and yellow for the high

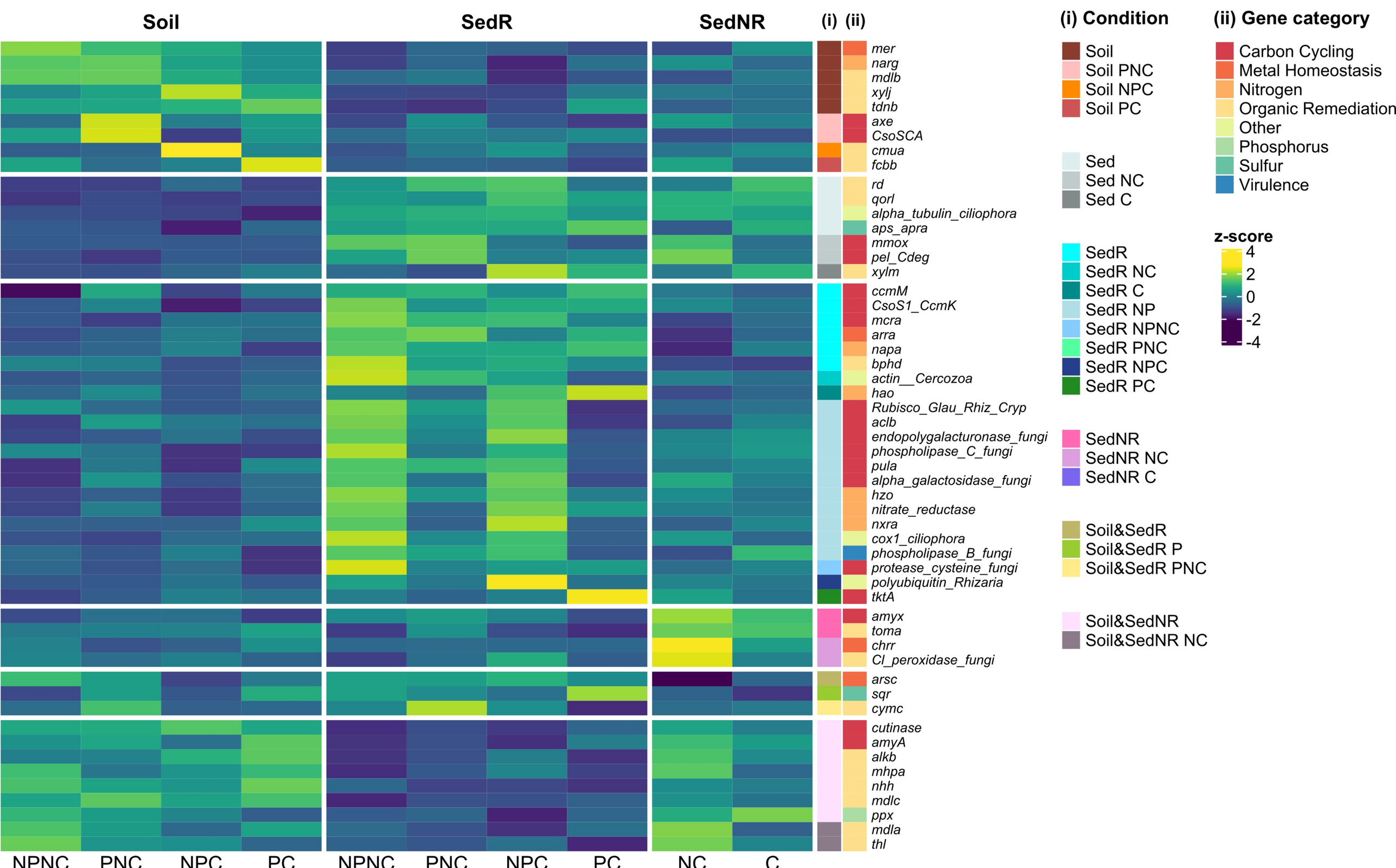
fluorescence intensity). On the left of the heatmap, the row annotations indicate the condition (i) and the gene family subcategory from the organic remediation category (ii). The data represent the mean of three biological replicates (n=3). Sed: sediment; SedR: sediment receiving runoff; SedNR: Sed without runoff (control); NP: no plant; P: plant; NC: no deuterated HC spiking; C: deuterated HC spiking.

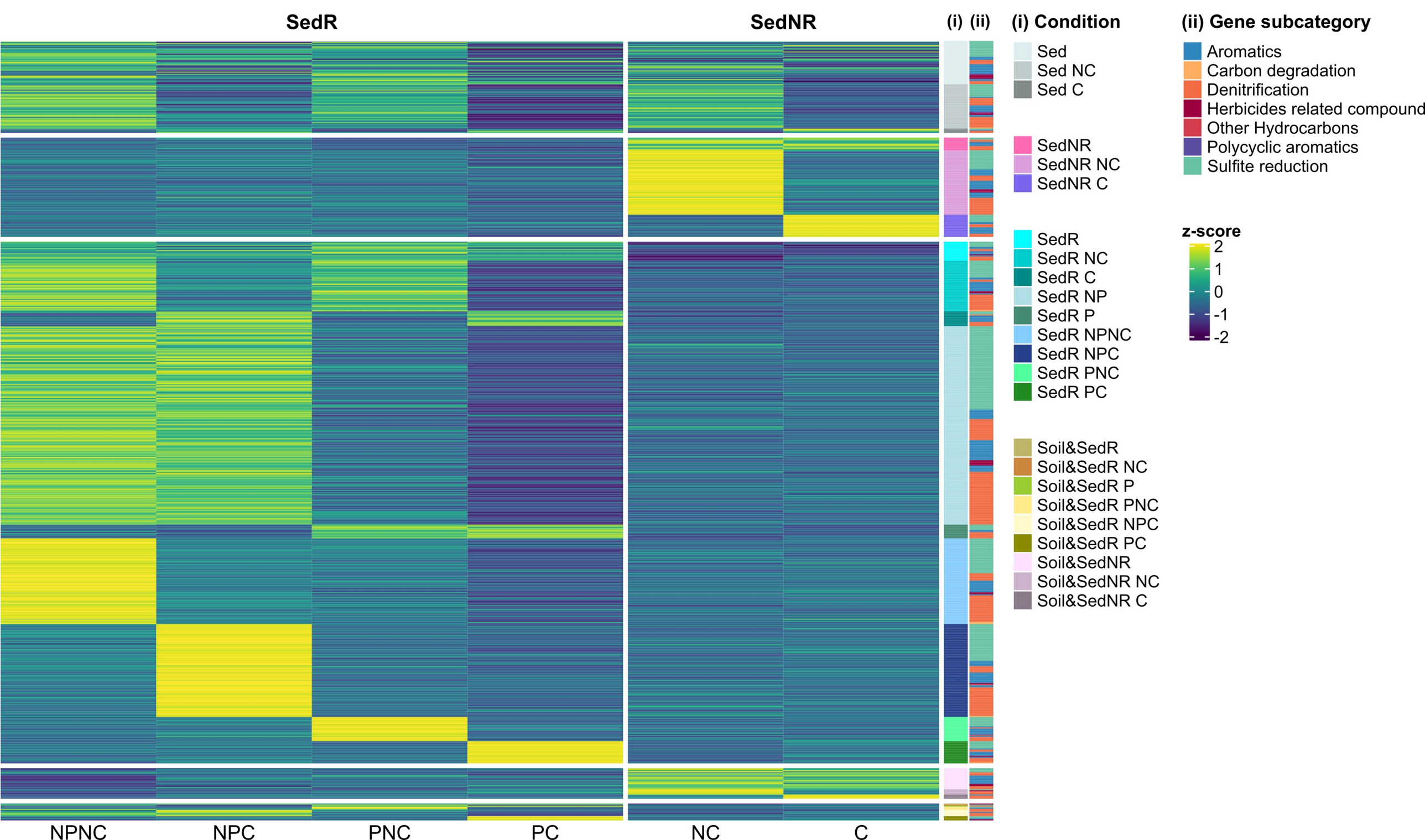
Figure 5. Distribution of the different GSV subcategories of organic remediation cateory in sediment (A) with (SedR) or without (SedNR) runoff, (B) with (Sed C) or without (Sed NC) HC spiking, (C) planted (SedR P) or non-planted (SedR NP) soil with runoff. The number of GVs for each subcategory is indicated in bracket.

Figure 6. 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 ($p < 0.05$) between nodes. The size of each node 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.


Land-sea continuum microcosm
with sediment receiving runoff (SedR)
without plant (NP)
with *Catapodium marinum* plant (P)
without deuterated HC spiking (NC)
Runoff
Soil
SedR
NPNC
PNC
with deuterated HC spiking (C)
NPC
PC
Control microcosm
Sediment without runoff (SedNR)
without deuterated HC spiking (NC)
SedNR
NRNC
with deuterated HC spiking (C)
NRC

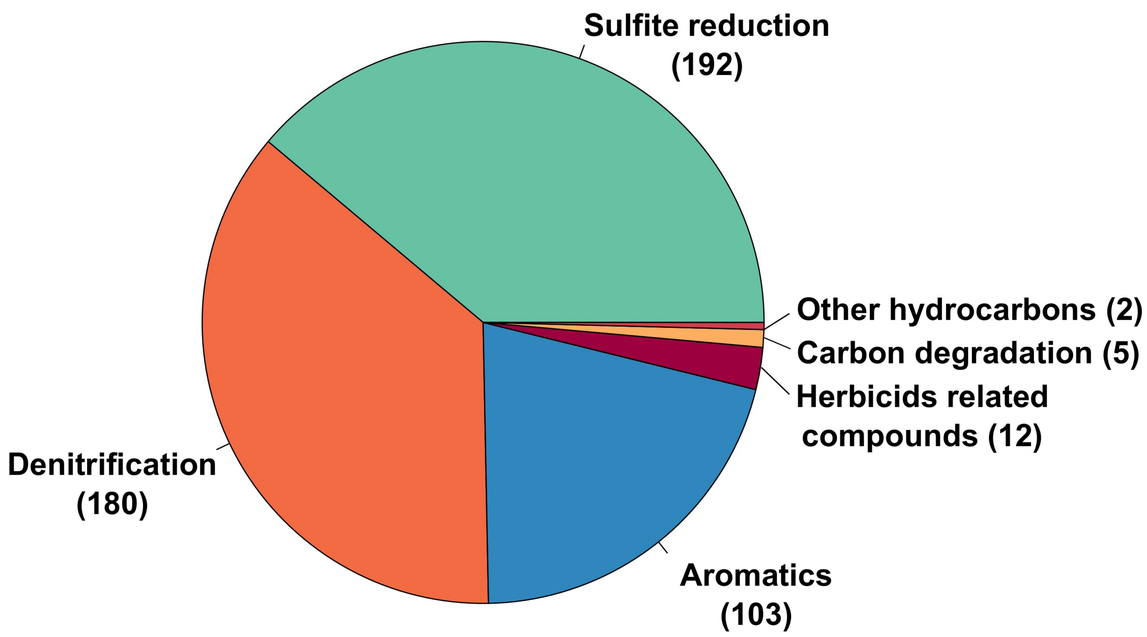

A
B
0.08
0.04
0.00
-0.04
NMDS2
Stress 0.11
Stress 0.12
-0.15
-0.10
-0.05
0.00
0.05
0.10
NMDS1
compartment
Soil
Sed
condition
Soil NPNC
Soil NPC
Soil PNC
Soil PC
SedR NPNC
SedR NPC
SedR PNC
SedR PC
SedNR NC
SedNR C


Soil
SedR
SedNR
(i)
(ii)
mer
narg
mdlb
xylj
tdnb
axe
CsoSCA
cmua
fcbb
rd
qorl
alpha_tubulin_ciliophora
aps_apra
mmox
pel_Cdeg
xylm
ccmM
CsoS1_CcmK
mcra
arra
napa
bphd
actin__Cercozoa
hao
Rubisco_Glau_Rhiz_Cryp
aclb
endopolygalacturonase_fungi
phospholipase_C_fungi
pula
alpha_galactosidase_fungi
hzo
nitrate_reductase
nxra
cox1_ciliophora
phospholipase_B_fungi
protease_cysteine_fungi
polyubiquitin_Rhizaria
tktA
amyx
toma
chrr
Cl_peroxidase_fungi
arsc
sqr
cymc
cutinase
amyA
alkb
mhpa
nhh
mdlc
ppx
mdla
thl
NPNC
PNC
NPC
PC
NPNC
PNC
NPC
PC
NC
C
(i) Condition
Soil
Soil PNC
Soil NPC
Soil PC
Sed
Sed NC
Sed C
SedR
SedR NC
SedR C
SedR NP
SedR NPNC
SedR PNC
SedR NPC
SedR PC
SedNR
SedNR NC
SedNR C
Soil&SedR
Soil&SedR P
Soil&SedR PNC
Soil&SedNR
Soil&SedNR NC
(ii) Gene category
Carbon Cycling
Metal Homeostasis
Nitrogen
Organic Remediation
Other
Phosphorus
Sulfur
Virulence
z-score
4
2
0
-2
-4

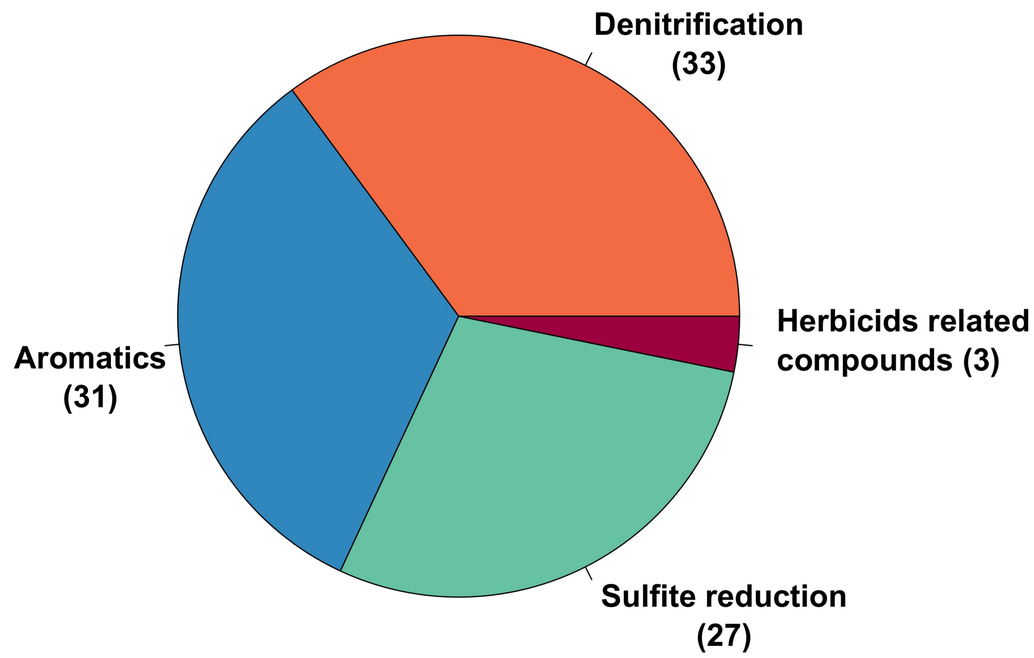

SedR
SedNR
(i) (ii)
(i) Condition
Sed
Sed NC
Sed C
SedNR
SedNR NC
SedNR C
SedR
SedR NC
SedR C
SedR NP
SedR P
SedR NPNC
SedR NPC
SedR PNC
SedR PC
Soil&SedR
Soil&SedR NC
Soil&SedR P
Soil&SedR PNC
Soil&SedR NPC
Soil&SedR PC
Soil&SedNR
Soil&SedNR NC
Soil&SedNR C
(ii) Gene subcategory
Aromatics
Carbon degradation
Denitrification
Herbicides related compound
Other Hydrocarbons
Polycyclic aromatics
Sulfite reduction
z-score
2
1
0
-1
-2
NPNC
NPC
PNC
PC
NC
C

A

With runoff (SedR)

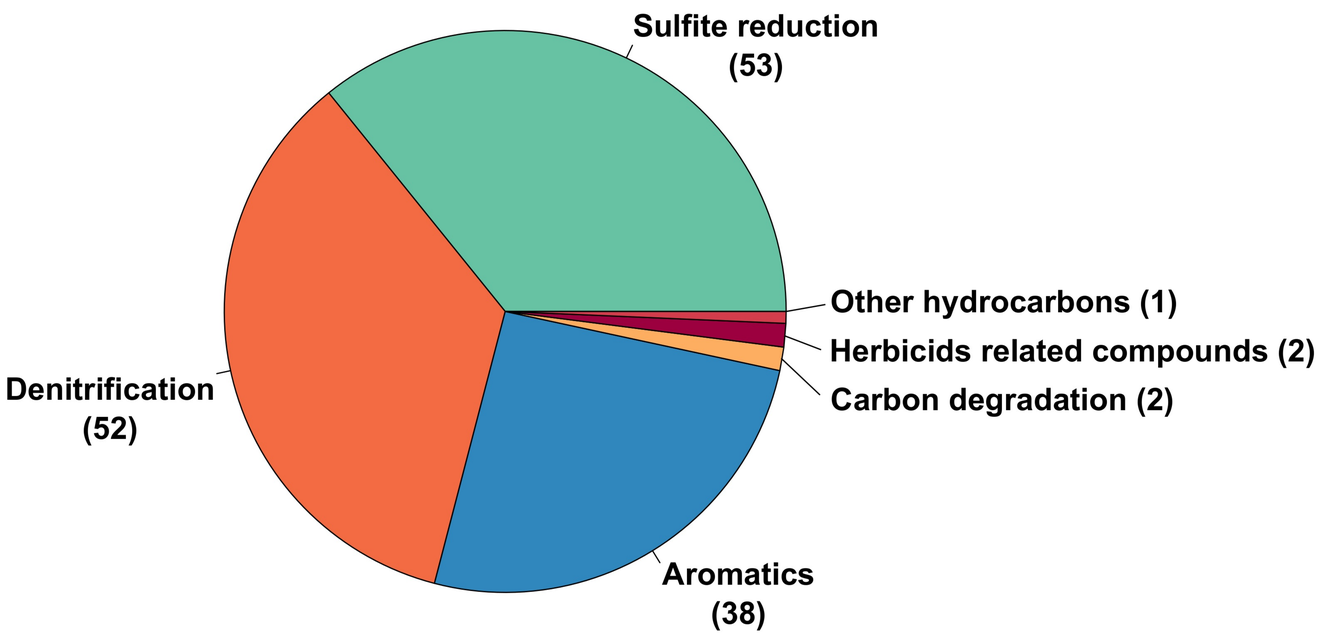


Without runoff (SedNR)

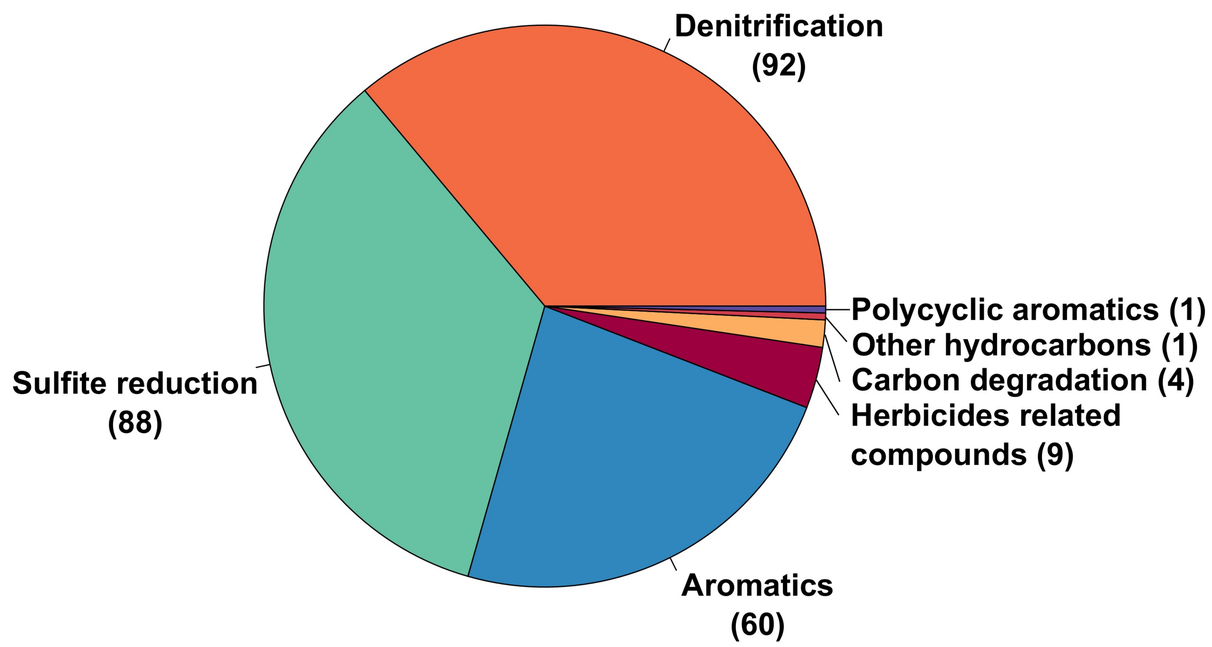


B

With HC spiking (Sed C)

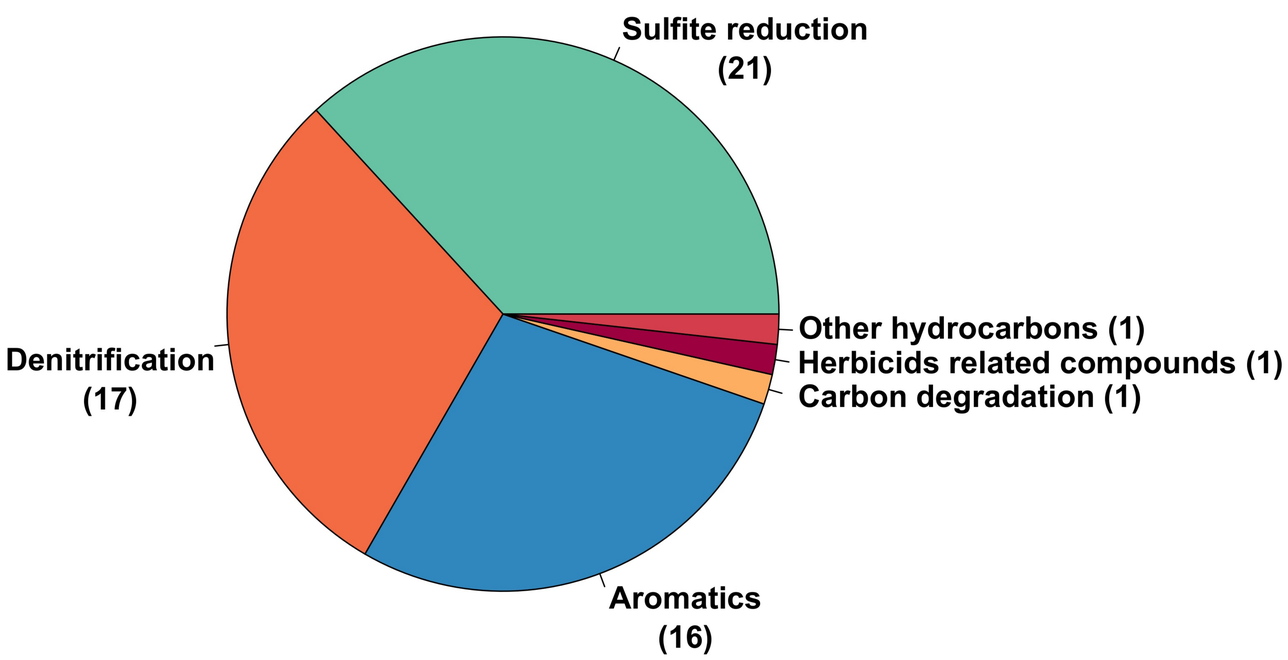


Without HC spiking (Sed NC)

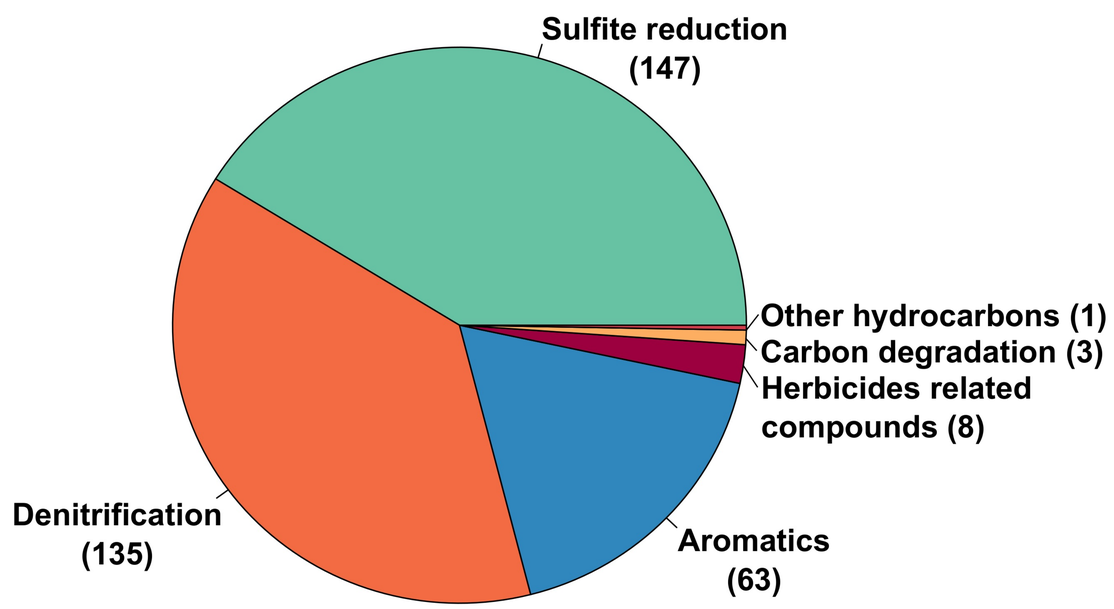


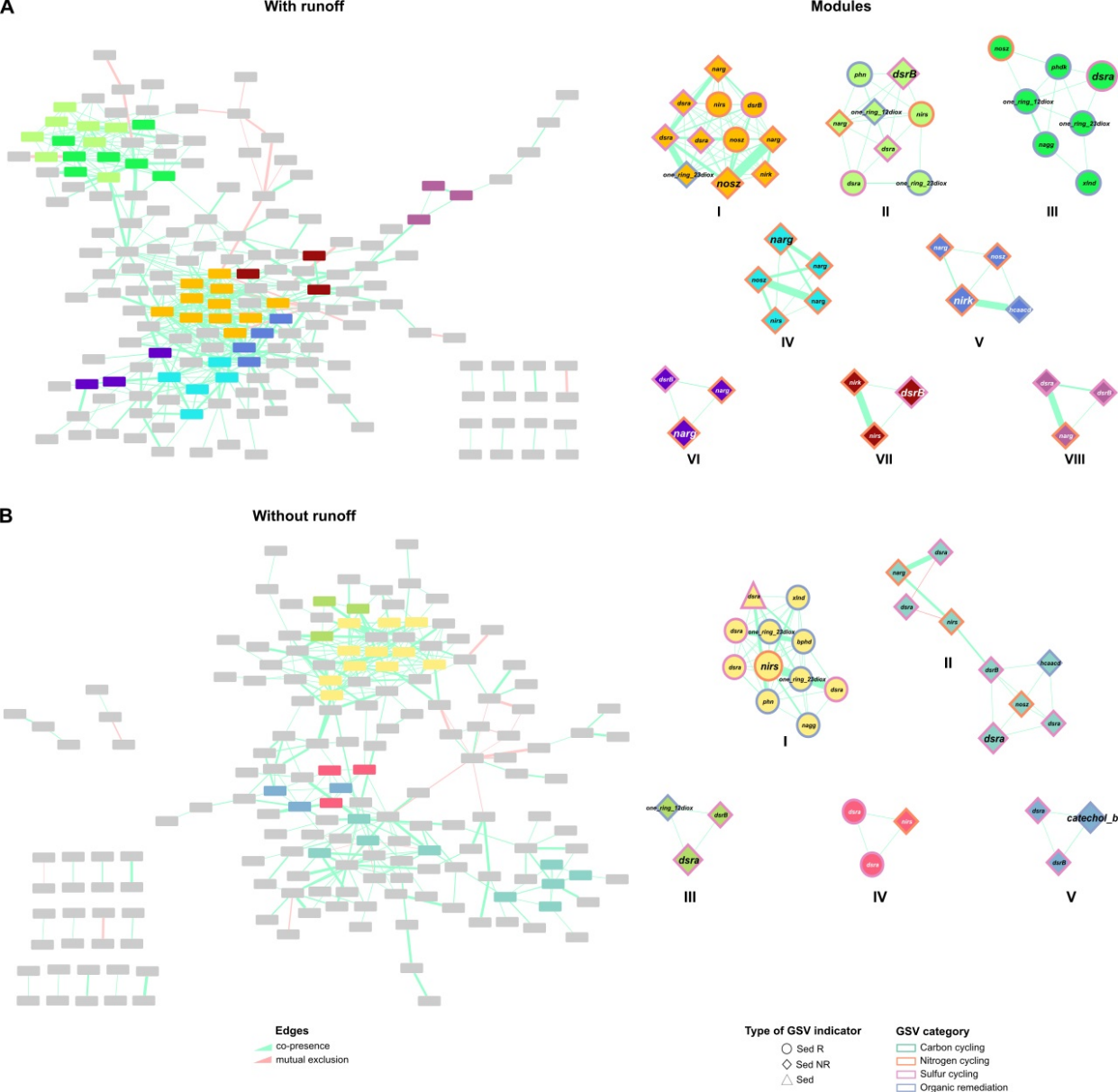
C

Planted soil with runoff (SedR P)



Non-planted soil with runoff (SedR NP)




A
With runoff
Modules
I
II
III
IV
V
VI
VII
VIII
B
Without runoff
Edges
co-presence
mutual exclusion
Type of GSV indicator
Sed R
Sed NR
Sed
GSV category
Carbon cycling
Nitrogen cycling
Sulfur cycling
Organic remediation