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Summary

This article explores the functional diversity and redundancy in a bacterial metacommunity constituted of three habitats (sediment, water column and fish gut) in a coastal lagoon under anthropogenic pressure. Comprehensive functional gene arrays covering a wide range of ecological processes and stress resistance genes to estimate the functional potential of bacterial communities were used. Then, diversity partitioning was used to characterize functional diversity and redundancy within (α), between (β) and across (γ) habitats. It was showed that all local communities exhibit a highly diversified potential for the realization of key ecological processes and resistance to various environmental conditions, supporting the growing evidence that macro-organisms microbiomes harbour a high functional potential and are integral components of functional gene dynamics in aquatic bacterial metacommunities. Several levels of functional redundancy at different scales of the bacterial metacommunity were observed (within local communities, within habitats and at the metacommunity level). The results suggested a high potential for the realization of spatial ecological insurance within this ecosystem, that is, the functional compensation among microorganisms for the realization and maintenance of key ecological processes, within and across habitats. Finally, the role of macro-organisms as dispersal vectors of microbes and their potential influence on marine metacommunity dynamics were discussed.

Introduction

The ability of natural ecosystems to deliver goods and services requires the realization and maintenance, often combined under the term functioning, of multiple ecological processes across space and time (Mace *et al*., 2012; Harrison *et al*., 2014). Ultimately, ecosystem functioning depends on the presence of species performing key functions, a biodiversity component coined as functional diversity (Tilman *et al*., 1997; Villéger, 2008), while being able to complement each other's across various environmental conditions, a component coined as functional redundancy (Naeem and Li, 1997). According to the ecological insurance theory, species rich communities characterized by high level of functional diversity and functional redundancy should be able to ensure the long-term functioning of ecosystems (Yachi and Loreau, 1999; Oliver *et al*., 2015; Sakschewski *et al*., 2016). However, due to human-induced disturbances and global changes (Mooney *et al*., 2009), sustaining the multiple functions of ecosystems requires higher levels of biodiversity than previously thought (Lefcheck *et al*., 2015; Oliver *et al*., 2015) and the functional vulnerability of macro-organism communities against biodiversity loss has been experimentally demonstrated (Gamfeldt *et al*., 2008; Isbell *et al*., 2011; Cardinale *et al*., 2012; Pendleton *et al*., 2014). Indeed, even in the richest ecosystems, some functions are displayed by a disproportionately high number of species, while others are displayed by few or one species only (Mouillot *et al*., 2014; Dagata *et al*., 2016).

By contrast, the extent to which the long-term functioning of microbial ecosystems is buffered against biodiversity loss is still largely unknown. Some studies suggest that most key bacterial functions benefit from a high functional redundancy among taxa (Nielsen *et al*., 2011) and are thus weakly impacted by disturbances (Girvan *et al*., 2005; Wertz *et al*., 2007; Moya and Ferrer, 2016). However, when considering the wide range of functions performed simultaneously by bacterial communities, and particularly the specialized ones (e.g., decomposition of recalcitrant carbon substrates, heavy metal resistance), the functional redundancy hypothesis can be challenged (Delgado-Baquerizo *et al*., 2016). Indeed, taxa exhibit different trade-offs in their functional abilities and thus tend to show less functional overlap when several functions are considered (Peter *et al*., 2011a, 2011b; Miki *et al*., 2014).

In order to fully appreciate the level of functional diversity and redundancy across the bacterial communities of an aquatic ecosystem we need to consider several habitats. While water and sediment are generally taken into account, macro-organisms and their microbiomes are rarely considered as critical habitats. However, these particular habitats exhibit bacterial communities with different taxonomic composition than the surrounding water (Sunagawa *et al*., 2010; Dupont *et al*., 2013; Li *et al*., 2015) and sediment (Carlos *et al*., 2013; Polónia *et al*., 2014; de Voogd *et al*., 2015). Additionally, there is growing evidence that macro-organism microbiomes harbour a functional potential comparable to those from other habitats

(Dinsdale *et al*., 2008; Lavery *et al*., 2012; Xing *et al*., 2013; Bayer *et al*., 2014). Surprisingly, while these communities are frequently considered from the host point of view (e.g., digestion, transfer of nutrients, immunity), they are still largely ignored in biodiversity assessment at the metacommunity or ecosystem level (Cleary *et al*., 2015; de Voogd *et al*., 2015; Roth-schulze, Zozaya-valdés and Steinberg, 2016) and particularly within a functional context (e.g., nutrients cycling, contaminant degradation, bacterial dispersal). Here, we integrated macro-organisms microbiomes within a metacommunity framework (Mihaljevic, 2012) in order to describe their importance for the functional potential in aquatic ecosystems.

Recent studies in microbial functional ecology proposed to consider individual functional genes instead of species as ecological entities of interest (Boon *et al*., 2014; Krause *et al*., 2014; Miki de Voogd, 2014). This gene-centred approach is appealing in a bacterial context as the unit classically used in functional ecology, that is, the species, is not well defined for bacteria and as lateral gene transfer occurs even between distantly related microorganisms, thus blurring the limits of taxonomic classification (Martiny *et al*., 2013). Furthermore, bacterial communities appear to assemble based on functional gene rather than on the identity of taxa (Burke *et al*., 2011a).

In this study, we explore the levels of functional gene richness and functional redundancy in coastal bacterial metacommunities within and across different habitats, along with the potential contribution of marine macro-organisms microbiomes in this functional biodiversity. To do so, we used a comprehensive functional gene array (FGA; GeoChip 4; Tu *et al*., 2014) to assess the functional potential of bacterial communities from three distinct but interrelated habitats of a coastal aquatic ecosystem: fish gut, sediment and water. Doing so, we benefited from a standardized analysis of the wide spectrum of ecological processes and functions covered by this approach (e.g., C, N, P, S cycles, contaminants degradation, heavy metal resistance; Zhou *et al*., 2015). This gene-centred approach was combined with a conceptual framework in which communities from these three habitats are considered as local communities that constitute a bacterial metacommunity at a larger scale (Leibold *et al*., 2004; Mihaljevic, 2012; Burns *et al*., 2015; Smith *et al*., 2015). Then, we used an original approach of diversity partitioning (Lande, 1996; Belmaker *et al*., 2008; Escalas *et al*., 2013) to characterize functional diversity and redundancy across scales, that is, within (α), between (β) and across (γ) habitats.

Our results show a surprisingly high functional diversity within each community whatever the habitat. We also reveal functional redundancy at several scales within the metacommunity, suggesting the potential functional insurance within this ecosystem, that is, the possibility of functional compensation between microorganisms of different habitats for the realization and maintenance of key ecological processes.

Results

Functional diversity across bacterial communities

We estimated the richness of functional genes detected using the functional gene array in the sediment, water and fish gut communities (see Experimental Procedures). The level of functional richness was not statistically different among habitats (Kruskal–Wallis test; $p = 0.513$, see Table 1) with on average 356 ± 3, 354 ± 12 and 353 ± 3 (mean $\pm$ sd) functional genes detected for fish gut (FGBC), sediment (SBC) and water (WBC) bacterial communities respectively. Additionally, functional richness associated with each of the 12 considered gene categories was not significantly different across habitats ($p > 0.05$; Table 1). At the ecosystem or metacommunity scale ($\gamma_{\text{Ecosystem}}$), that is, when pooling together the 9 bacterial communities from the three habitats, we observed a total functional richness of 399 functional genes, which represents 95% of the microarray potential (i.e., 420 genes; see Supporting Information Appendix S3 for a detailed list of the genes detected in the three habitats, along with their classification into categories, the process, pollutant or stressor they are related with, and the name of the protein). We performed simulations by rarefying the richness of functional genes and gene variants in one habitat and testing the difference with another habitat. Our results suggest that with a sample size comparable to ours, one need a difference of richness equivalent to 25 functional genes (0.06% of the total number) or 4965 gene variants (0.07% of the total number) for the difference to be statistically significant.

Table 1. Richness of functional genes and variants in fish gut, sediment and water habitats.

	Functional genes							Gene variants						
Gene categories	*N*	Fish	Sediment	Water	*df*	Stat	*p* value	*N*	Fish	Sediment	Water	*df*	Stat	*p* value
Antibiotic resistance	12	11.3 ± 0.6	11 ± 0	10.7 ± 0.6	2	0.41	0.816	2728	564 ± 86	581 ± 34	599 ± 24	2	5.54	0.063
Bioleaching	14	10.3 ± 1.5	9.7 ± 2.1	9.3 ± 1.2	2	1.13	0.569	258	35 ± 5	29 ± 10	33 ± 7	2	0.96	0.618
Carbon cycling	55	46.3 ± 0.6	47.3 ± 2.1	45.7 ± 1.2	2	1.71	0.426	9622	1864 ± 317	1868 ± 166	1942 ± 66	2	4.36	0.113
Energy process	15	11.3 ± 0.6	11.3 ± 0.6	11 ± 1	2	0.33	0.846	948	184 ± 32	175 ± 16	189 ± 2	2	5.96	0.051
Metal resistance	49	41.3 ± 0.6	40.7 ± 0.6	40.3 ± 1.5	2	0.30	0.860	7964	1748 ± 267	1798 ± 129	1864 ± 31	2	5.60	0.061
Nitrogen	23	21.3 ± 0.6	21.7 ± 0.6	21.3 ± 0.6	2	2.67	0.264	8070	1551 ± 241	1649 ± 113	1651 ± 37	2	5.60	0.061
Organic remediation	168	141.7 ± 3.8	142.3 ± 3.8	142 ± 2.6	2	2.10	0.350	12 265	3049 ± 518	3350 ± 201	3411 ± 92	2	5.42	0.066
Other	8	5 ± 0	4.3 ± 1.5	5.3 ± 0.6	2	1.32	0.517	2300	315 ± 40	317 ± 24	335 ± 14	2	4.39	0.111
Phosphorus	3	3 ± 0	3 ± 0	3 ± 0	2	NA	NA	1174	234 ± 34	230 ± 25	239 ± 3	2	3.29	0.193
Stress	45	41.7 ± 0.6	42 ± 2	42 ± 0.6	2	0.22	0.896	16 427	2697 ± 481	2802 ± 196	2885 ± 38	2	5.42	0.066
Sulphur	15	9.3 ± 1.5	8.3 ± 0.6	9.3 ± 1.2	2	2.69	0.260	2765	441 ± 80	459 ± 41	490 ± 35	2	4.86	0.088
Virulence	13	13 ± 0	12.7 ± 0.6	13 ± 0	2	2.00	0.368	3107	577 ± 79	569 ± 81	589 ± 35	2	2.49	0.288
Global	420	355.7 ± 2.5	354.3 ± 11.6	353 ± 2.6	2	1.33	0.513	67 628	13 263 ± 2166	13 831 ± 1023	14 227 ± 199	2	5.42	0.066

N is the number of gene or variants from each category. Differences between habitats were tested using Kruskal–Wallis rank sum test.

Using multivariate analysis of variance (PERMANOVA), we showed that the functional gene composition of bacterial communities was not significantly different among habitats ($p > 0.05$; Fig. 1) based either on the whole bacterial community or each gene category separately (Table 2). Similar results were obtained using relative abundance of functional genes (Supporting Information Table S1 in Appendix S2). We also tested for differences in intra-group variance (PERMDISP) and did not observe any significant differences between habitats, whether we used the whole communities or each functional category separately (we observed similar trends using presence–absence or abundance of functional genes, Supporting Information Tables S2 and S3).

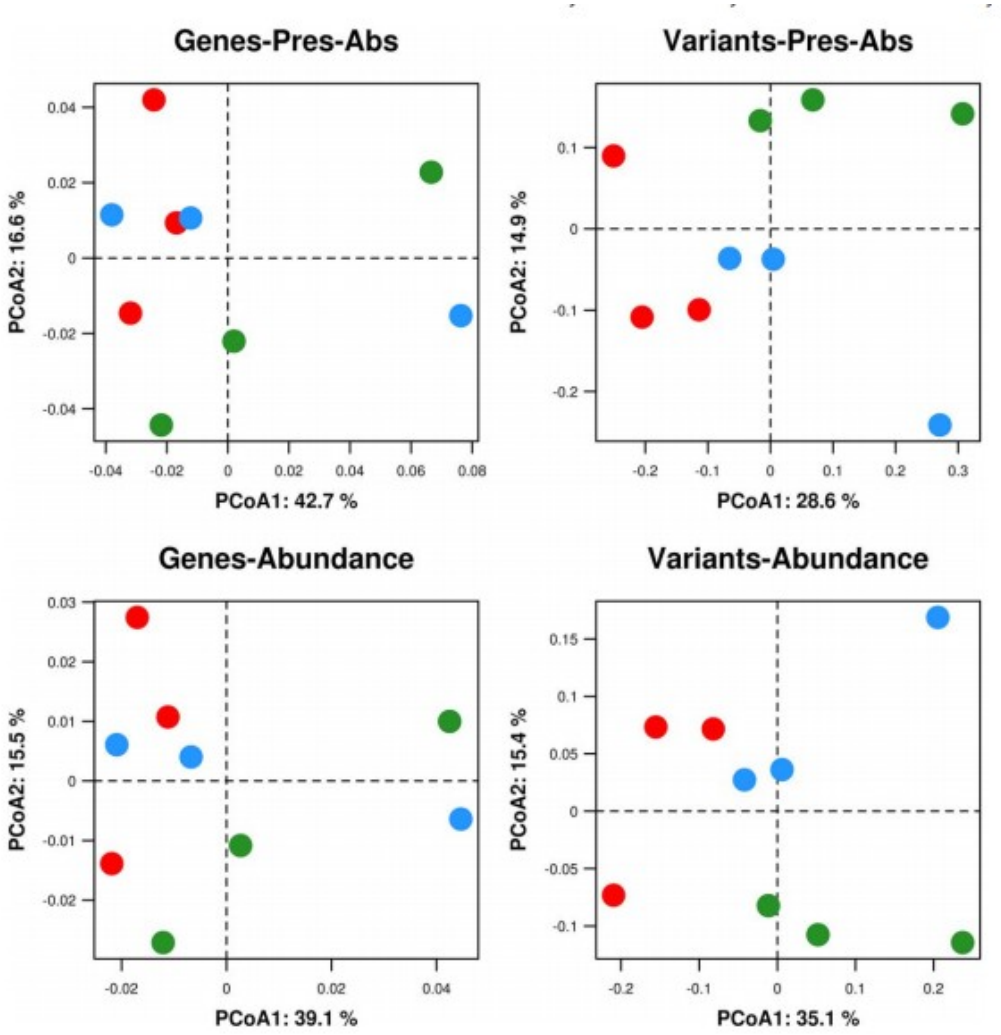

FIGURE 1 Ordination of samples dissimilarity at the functional gene and gene variant levels, using presence–absence (Jaccard) and abundance (Bray–Curtis) data. Fish gut communities are represented by red dots, sediment in green and water in blue. Ordination was realized using the *dudi.pco* function from the *ade4* R package.

Table 2. Analysis of functional composition across habitats for functional genes and gene variants.

		Functional genes			Gene variants		
Gene categories	*df*	*F*	R^2	*p* value	*F*	R^2	*p* value
Antibiotic resistance	2	0.500	0.143	0.770	1.516	0.336	0.015*
Bioleaching	2	0.903	0.231	0.478	1.266	0.297	0.240
Carbon cycling	2	1.439	0.324	0.264	1.470	0.329	0.028*
Energy process	2	1.037	0.257	0.475	1.576	0.344	0.006**
Metal Resistance	2	1.501	0.333	0.304	1.572	0.344	0.024*
Nitrogen	2	0.207	0.065	1.000	1.552	0.341	0.028*
Organic Remediation	2	1.001	0.250	0.470	1.573	0.344	0.023*
Other	2	0.969	0.244	0.572	1.449	0.326	0.028*
Phosphorus	2	NA	NA	NA	1.590	0.346	0.014*
Stress	2	0.488	0.140	0.715	1.455	0.327	0.035*
Sulphur	2	0.135	0.043	0.984	1.556	0.342	0.032*
Virulence	2	1.000	0.250	1.000	1.504	0.334	0.047*
Global	2	0.954	0.241	0.451	1.522	0.337	0.026*

Multivariate analysis of variance (PERMANOVA) was performed for the whole communities and for each gene category separately. Data are presence-absence of functional genes and gene variants. P-values were estimated through random permutation of data (n = 999).

Then, we used additive diversity partitioning of functional diversity across scales (Fig. 2) to determine whether the functional diversity observed at the ecosystem level ($\gamma_{\text{Ecosystem}}$) mainly aroused from a high functional dissimilarity among habitats ($\beta_{\text{InterHabitats}}$), a high functional dissimilarity among communities within each habitat ($\beta_{\text{IntraHabitats}}$) or from a high functional diversity within each local community ($\bar{a}_{\text{LocalCommunities}}$; i.e., each water, sediment or fish gut sample). We observed that $\bar{a}_{\text{LocalCommunities}}$ contribution to $\gamma_{\text{Ecosystem}}$ was 96%, outweighing $\beta_{\text{InterHabitats}}$ (1%) and $\beta_{\text{IntraHabitats}}$ (3%) in its contribution to the bacterial metacommunity functional diversity ($\gamma_{Ecosystem}$). This low contribution of β-diversity to $\gamma_{\text{Ecosystem}}$ was confirmed for each gene category separately (Supporting Information Table S4) and revealed the importance of local communities in generating ecosystem functional diversity, supporting our observation of consistently high functional diversity across local bacterial communities.

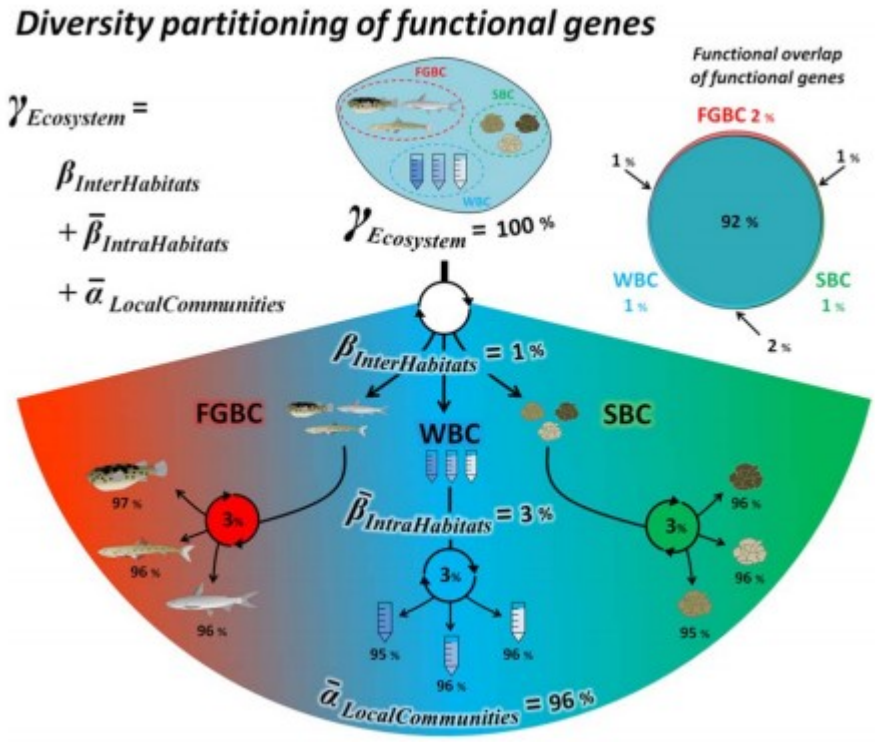

FIGURE 2 Multiscales hierarchical partitioning of functional genes diversity. The total functional diversity of bacterial communities at the ecosystem scale ($\gamma_{Ecosystem}$) was partitioned into the contribution of three habitats, fish gut (FGBC), sediment (SBC) and water (WBC), and three local communities within habitats. Then, we expressed the total functional diversity within the ecosystem as the sum of inter-habitat functional differences ($\beta_{InterHabitats}$), the mean intra-habitat functional difference ($\beta_{IntraHabitats}$) and the mean local functional diversity ($\bar{a}_{LocalCommunities}$) with: $\gamma_{Ecosystem} = \beta_{InterHabitats} + \beta_{IntraHabitats} + \bar{a}_{LocalCommunities}$. The overlap of functional genes among habitats is depicted on the Venn diagram in the top right part of the figure. Percentages are calculated based on the total number of detected functional genes.

Then, we looked at the functional overlap among bacterial communities and found that 92% of the detected functional genes were common to the three habitats (i.e., fish gut, sediment and water) while the proportion of functional genes uniquely found within one habitat was < 5% (see the Venn diagram in the upper right part of Fig. 2).

Diversity of gene variants across bacterial communities

Then, we estimated the richness of functional gene variants detected in each habitat. The richness of gene variants was not statistically different between fish gut, sediment and water communities (Kruskal–Wallis test; $p > 0.05$, Table 1), with on average 13 263 ± 2166, 13 831 ± 1023 and 14 227 ± 199 gene variants for FGBC, SBC and WBC respectively. Additionally, the richness of variants associated with each gene category was not significantly different across habitats ($p > 0.05$; Supporting Information Table S1).

At the ecosystem or metacommunity scale ($\gamma_{Ecosystem}$), the total richness of gene variants represented 43% of the FGA potential (i.e., 29 028 out of 67 628 variants). We also found that several variants were detected for each

functional gene, suggesting intra-gene redundancy. On average, 23% ± 16% of the potential variants were detected, with similar values in the three habitats (Supporting Information Fig. S3).

As opposed to what we observed for functional genes, multivariate analyses of variance revealed significant differences in the functional composition of gene variants among habitats (Table 2 and Fig. 1). This result was confirmed whether we considered the whole bacterial community or each gene category separately (the same trend was observed using abundance data, Supporting Information Table S2). We also tested for differences in intra-group variance (PERMDISP) and did not observed any significant difference between habitats, whether we used the whole communities or each functional category separately but also whether we used presence–absence or abundance of gene variants (Supporting Information Tables S3 and S4).

We then partitioned $\gamma_{\text{Ecosystem}}$ of gene variants across ecosystem scales and we observed, as for functional genes, that local bacterial communities hosted a large proportion of the diversity of variants (Fig. 3; $\bar{a}_{\text{LocalCommunities}} =$ 70%). Nevertheless, the levels of β-diversity among local communities and among habitats were higher than for functional genes ($\beta_{\text{IntraHabitats}} = 18\%$ and $\beta_{\text{InterHabitats}} = 12\%$), suggesting a higher level of dissimilarity in the composition of communities when considering gene variants. This result was consistent for each gene category considered separately (Supporting Information Table S4).

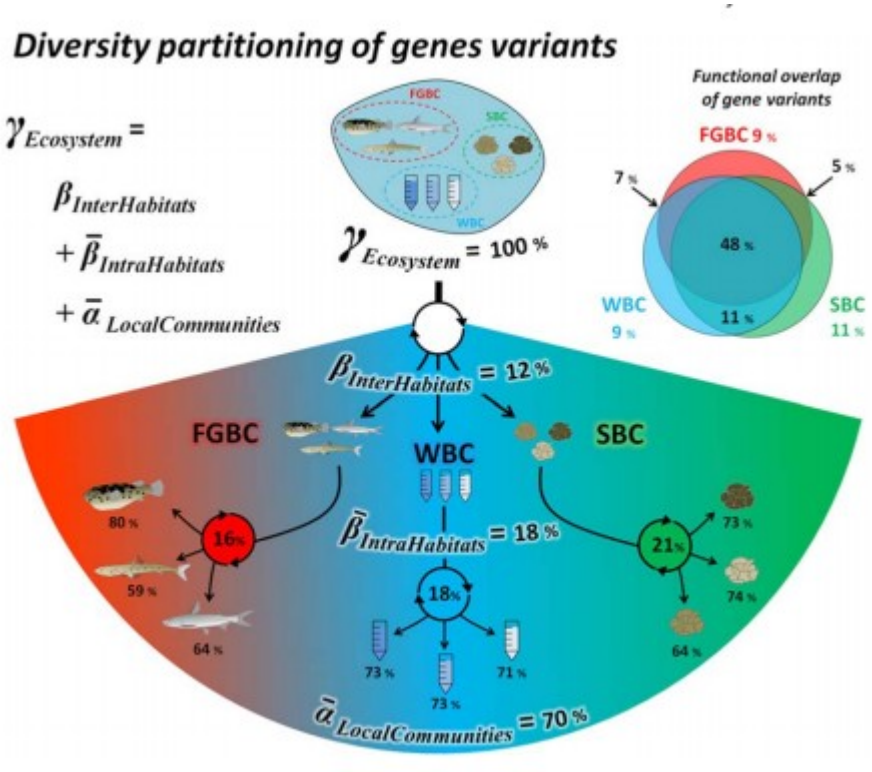

FIGURE 3 Multiscales hierarchical partitioning of genes variants diversity. The total diversity of gene variants in bacterial communities at the ecosystem scale ($\gamma_{\text{Ecosystem}}$) was partitioned into the

contribution of three habitats, fish gut (FGBC), sediment (SBC) and water (WBC), and three local communities within habitats. Then, we expressed this total compositional diversity within the ecosystem as the sum of inter-habitat compositional difference ($\beta_{InterHabitats}$), the mean intra-habitat compositional difference ($\beta_{IntraHabitats}$) and the mean local diversity ($\bar{a}_{LocalCommunities}$) with: $\gamma_{Ecosystem} = \beta_{InterHabitats} + \beta_{IntraHabitats} + \bar{a}_{LocalCommunities}$. The overlap of gene variants among habitats is depicted on the Venn diagram in the top right part of the figure. Percentages are calculated based on the total number of detected gene variants.

The observed compositional differences were reflected in the overlap of variants among habitats as the proportion of habitat-specific variants was higher than for functional genes, from 9% for fish gut and water to 11% for sediment (see the Venn diagram in the upper right part of Fig. 3).

Functional overlap among habitat-specific and ubiquitous variants

To determine if habitat-specific variants correspond to functional particularities among habitats, we split these variants into functional categories and compared their distribution to that of ubiquitous variants. We observed that these distributions were not significantly different from each other (Fig. 4A), nor from the null model (χ^2 test, $p > 0.05$, Supporting Information Table S5). Then, we estimated functional overlap across habitats and found that 80% of the 328 functions supported by habitat-specific variants were common to at least two habitats (last row in Fig. 4B). We observed discrepancies across the different gene categories. Indeed, only a third of the bioleaching functions were supported by variants from other habitats. Seven categories (Energy process, Other, Sulphur cycling, Organic remediation, Antibiotic resistance, Metal resistance and Stress) exhibited between 70% and 83% of functions that were supported in more than one habitat. The remaining four categories (C, N and P cycling, Virulence) exhibited high percentage of overlap among habitats (91%–100%). In other words, the functional potential of habitat-specific variants represented a subsample of the functional potential of ubiquitous variants and did not support the functional particularities of each habitat. Thus, habitat-specific variants constituted more a functional seed bank redundant with the pool of ubiquitous variants enhancing the level of functional redundancy across habitats.

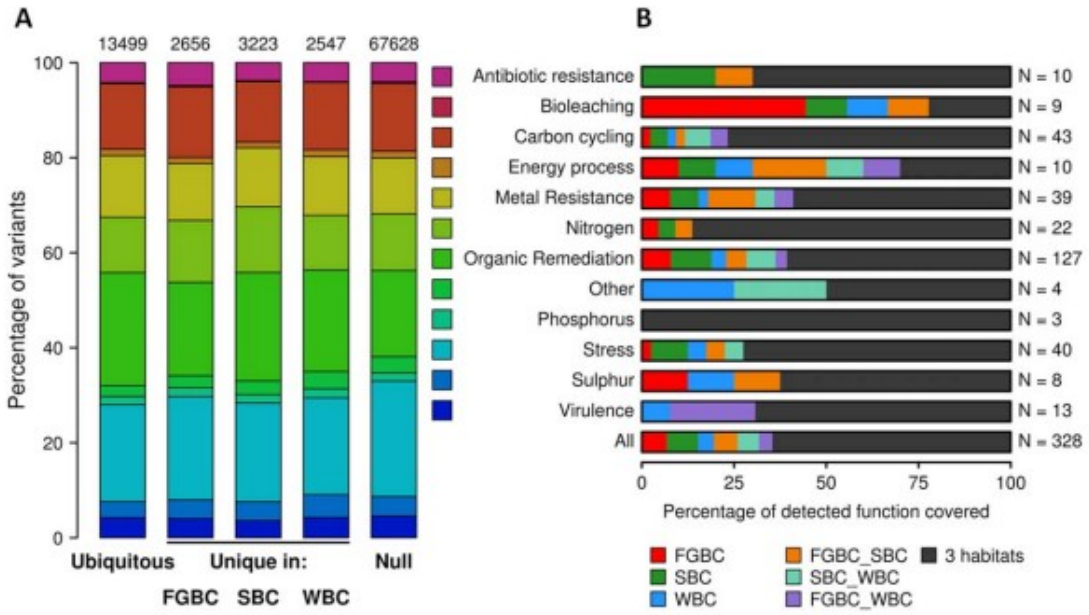

FIGURE 4 Functional overlap among habitat-specific and ubiquitous variants. A. Distribution of ubiquitous and habitat-specific gene variants into functional categories. The null model corresponds to the expected distribution of all potential gene variants on the GeoChip 4. Fish gut: FGBC; sediment: SBC; water: WBC. B. Functional overlap of habitat-specific variants across habitats for each gene categories.

Discussion

High functional diversity and redundancy across bacterial communities

We observed a constantly high level of functional diversity across the metacommunity. The functional potential estimated in individual bacterial communities, within each habitat and ultimately at the metacommunity or ecosystem scale was very close to the potential of the FGA used here (Tu *et al*., 2014). We showed that the functional diversity of the whole metacommunity primarily arises from local communities which have the same functional gene composition, leading to high functional redundancy within and across habitats. These results suggest that bacterial communities from the three sampled habitats in the Terminos lagoon exhibit the potential for the realization of the major metabolic processes essential to ecosystem functioning (i.e., C, N, P, S cycling, energy processes). Besides, these communities display a highly diversified array of functional genes involved in the resistance to environmental stress. We observed genes involved in the resistance to general stressors such as oxygen limitation and antibiotics (Tetracycline, Vancomycin), but more importantly to stressors directly observed in this lagoon, such as osmotic stress (Medina-Gómez *et al*., 2015), heavy metals (Vazquez *et al*., 1993; Benitez *et al*., 2012) and numerous pollutants such as PAH (e.g., Fluorene, Chlorocyclohexane; Norena-Barroso *et al*., 1999) or PCBs (Gold-Bouchot *et al*., 1983; Diaz-Gonzalez *et al*., 2005; Carvalho *et al*., 2009a, 2009b). Altogether, this suggests that the microbial metacommunity has the functional potential to realize important ecological

processes and maintain them within a naturally changing environment (i.e., a lagoon) under strong and multiple anthropogenic pressures. These results are in line with the ones from (Bayer *et al*., 2014) who find that sponge-associated bacterial communities share most of their functional potential with water bacterial communities (88% and 91% of functional genes in common with the Mediterranean and Red sea respectively). These authors detected 627 genes representing 20 273 variants, but using a different version of the FGA (Geochip v4.2). They mentioned that, although there are some methodological limitations such as the applicability of GeoChip outside its original 'soil' context, sponge-associated and water bacterial communities have trustfully most of their functional gene repertoire in common. One of the few other studies reporting the number of detected functional genes is the one from (Paula *et al*., 2014), in which 409 genes were observed in amazonian forest soil communities. These comparisons suggest that our system exhibit a lower level of functional diversity than amazonian forest soil or sponge-associated communities. Direct comparison of our functional gene richness estimates with other Geochip-based studies are difficult because most of these studies only reported the number of gene variants but also as the Geochip design evolves constantly (we used Geochip v4.0 but v4.2, v4.4 and v4.6 also existed). That being said, Bayer *et al*. (2014) reported 392 functional genes associated with sponge and seawater microbiomes, while organic remediation and metal resistance genes have been detected in high proportion in Elbe river sediments (Störmer *et al*., 2013), in aerobic bioreactor (Zhao *et al*., 2014a) and in mangrove sediments (Bai *et al*., 2013). Here, we bring further evidence that bacterial communities associated with macro-organisms exhibit a highly diversified functional potential (Dinsdale *et al*., 2008; Lavery *et al*., 2012; Xing *et al*., 2013; Bayer *et al*., 2014; Polónia *et al*., 2014; Cleary *et al*., 2015; de Voogd *et al*., 2015), which is equivalent to the one observed in water and sediment communities and thus should be accounted for when assessing microbial functional diversity within aquatic ecosystems.

We cannot exclude that at least one part of the observed functional redundancy is due to insufficient sampling effort both in terms of replicates and functional genes on the FGA. We thus certainly underestimated the level of functional β-diversity within and between habitats with this restricted number of replicates. Although our statistical power is low, the results show an impressive lack of variability in functional genes despite the observation of different variants. So we are confident that we sample different microbial communities with a similar functional gene composition. Our results also raise the question of the limitations owing to the microarray approach, notably its inability to discover and characterize novel functional genes since it only offers the possibility to '*find only what we are searching for*' (Zhou *et al*., 2015). The set of functions analyzed here was constrained *a priori* and, consequently, we have certainly missed some uncommon functional genes that could be detected with open-format technologies and which, ultimately,

would have generated more differences in intra- and inter-habitats functional diversity. In other terms, it is likely that our approach overestimates the degree of functional overlap within and between habitats. However, even modern metagenomics approaches may not detect the rarest functional genes and may conclude to a low difference in functional diversity and composition between habitats. Nevertheless, the set of functions assessed with this FGA covers core bacterial metabolic functions (e.g., energy processes), but also functions related to general biogeochemical processes (C, N, P and S cycles) and more specialized non-core functions (e.g., metal resistance, contaminants degradation, stress tolerance; Tu *et al*., 2014). Hence, we are confident that we assessed a sufficiently diversified and ecologically relevant set of functions to tackle questions related to the functional diversity and redundancy within and across bacterial communities. Here we just provide a first step highlighting a common core of functional genes among habitats. The combination of future generation sequencing and high replicated experiments appears necessary to test whether host-associated microbiomes support different taxa and functions from their surrounding environment.

Another limitation of our study is the use of DNA and not RNA. As a consequence, we can only refer to the functional potential of bacterial communities and not to the functions performed by active community members, which could ultimately increase the amount of functional redundancy observed among communities. Unfortunately, we cannot really ruled out whether variants generating intra-gene redundancy were actually present in living cells or were part of environmental ‘dead DNA’. Also, we cannot exclude the possibility that some of the detected genes were in fact ‘fossil genes’, that is, genes that once served in a functional pathway, but have incurred mutations or are part of defunct pathways that led to loss of function (Hittinger *et al*., 2004). Consequently, while the presence of a gene may suggest the potential of organisms to perform a particular function, it might in reality not be the case. However, these limitations are the same whatever the DNA-based approach being used (sequencing or microarray). Additionally, profiling the expression of environmental bacterial communities through hybridization of FGA with cDNA is still challenging and was only recently realized using the same FGA (Xue *et al*., 2016). These authors obtained different and complementary results using RNA- and DNA-based FGA, with the latter being less influenced by short-term expression dynamics resulting from environmental conditions at the time of sampling. Indeed, at a given time only a fraction of the total pool of genes is expressed and the functional diversity estimated using RNA would certainly be lower than the potential functional diversity. Then, at different periods or under alternative environmental conditions, other genes might be expressed, providing a different assessment of bacterial functional diversity and redundancy. This suggests that analyzing the functional potential of bacterial communities appears more relevant in the context of ecological insurance. Ideally, both

potential and expressed bacterial functional diversity should be assessed simultaneously and over time in order to validate the realization of the functional insurance potential highlighted in this study.

That being said, overlapping functional potential across bacterial communities can be explained by different phenomena. First, we made the methodological choice to consider that a given functional gene as present in a community when only one of its variants was detected while previous studies used higher thresholds with the same FGA (Kimes *et al*., 2010; Liang *et al*., 2011; van Nostrand *et al*., 2011; Ding *et al*., 2012). Second, although considered as distinct, the three studied environments are highly connected by physical phenomena such as particles sedimentation from the water column and sediment resuspension. The latter process is expected to be significant in the Terminos lagoon for several reasons: (i) there is a strong current between the two openings which creates a relatively fast water turnover (David and Kjerfve, 1998), (ii) the lagoon has become shallower across the last decades due to sedimentation of land-originating particles and (iii) strong winds blow during the Nortes season and can create surface current resuspending particles (Instituto Nacional de Ecologia, 1997; Parks Watch, 2003). Biological phenomena also participate in habitat connections, for instance saltwater fishes continuously drink the surrounding water and can ingest or resuspend sediment while foraging (Flecker and Taylor, 2004; Brenner and Krumme, 2007). Additionally, it is worth noting that we sampled the transient part of fish gut microbial communities and thus compositional similarities between these communities and those of the surrounding environment can be expected. Third, as suggested by (Bayer *et al*., 2014), one can expect the functional gene repertoire of fish gut, sediment and seawater microbiomes to converge if a common adaptive driving force shape these communities. These three sets of communities are composed of organisms adapted to the aquatic environment. In addition, the presence of numerous organic and chemical pollutants (polynuclear aromatic hydrocarbons: Norena-Barroso *et al*., 1999; pesticides and PCBs: Carvalho *et al*., 2009a, 2009b; heavy metals: Benitez *et al*., 2012), which have accumulated in the Terminos lagoon since the 1970s could have acted as selective forces, leading to the detection in the communities metagenome of genes related to the degradation of these compounds or the resistance to their harmful effects. It was recently reported using a similar tool (i.e., Geochip 5) that functional gene richness increases in heavy metal contaminated sediments (Jie *et al*., 2016), which could be also the case in the Terminos lagoon, leading to the observed functional similarity across habitats.

α-Diversity of gene variants supports high insurance potential

The richness of gene variants estimated at the ecosystem scale (43% of the potential, i.e., 29 028 out of 67 628 variants) was comparable to those estimated with the same tool in bacterial communities from marine (18 7987; Störmer *et al*., 2013) and mangrove (35 000; Bai *et al*., 2013)

sediments, anaerobic reactors (28 575; Zhao *et al*., 2014b), sponge-associated communities (20 273; Bayer *et al*., 2014) and in grassland soils (49 520; Yang *et al*., 2014). The variants richness was around two orders of magnitude higher than the richness of functional genes and many variants were detected for each gene. This corresponds to intra-gene redundancy and suggests that each function can be realized by several taxa. According to the ecological insurance theory, taxa redundant for a given function but with asynchronous responses to environmental fluctuations are likely to show complementarity across several spatio-temporal contexts (Yachi and Loreau, 1999; Isbell *et al*., 2015). Hence, the decline or extinction of one taxa can be compensated by others, thus enhancing ecosystem resistance and resilience to disturbances and should ultimately result in a limited impact of environmental variability and perturbations on ecosystem functioning (Yachi and Loreau, 1999; Shade *et al*., 2012; Mori *et al*., 2013; Oliver *et al*., 2015). Our result might be considered as conservative regarding the level of intra-gene redundancy since, with a closed device like a FGA where the set of probes is define *a priori*, we probably missed some gene variants that would increase even more intra-gene redundancy. However, one pitfall of this approach is that the taxonomic resolution is not the same among gene variants and, consequently, that we do not have a precise information about the identity of taxa harbouring the genes. This should constitute a way of improvement for next generations of FGAs. In order to further explore the insurance potential provided by functionally similar but taxonomically different taxa, both FGA and 16S sequencing should be applied to the same samples; an approach that could unfortunately not be achieved in this study due to limited collection of DNA.

β-Diversity of gene variants supports even higher insurance potential

The functional compensation discussed above has been defined for an isolated system (i.e., a community or an ecosystem) over time. However, in nature, local communities are connected by dispersal and, together, constitute a metacommunity (Leibold *et al*., 2004). In such a spatially defined system, local communities experience asynchronous fluctuations in response to heterogeneous environmental conditions and are expected to host different species (Loreau *et al*., 2003). Then, when environment change in one community, dispersal between communities ensures that species adapted to the new environmental conditions can thrive and replace less adapted but functionally redundant ones. This concept has been coined as the spatial component of the ecological insurance hypothesis (Loreau *et al*., 2003) and is expected to allow the maintenance of ecosystem processes over large spatiotemporal scales and to reduce their variation in changing environmental conditions (Hector *et al*., 2010; Shanafelt *et al*., 2015). It has been experimentally tested and validated in aquatic bacterial communities (Baho *et al*., 2012).

We observed that contribution of β-diversity intra- and inter-habitats constitutes a higher proportion of the gene variants diversity in the

metacommunity ($\gamma_{Ecosystem}$) compared with functional genes. We found significant differences in variants composition across habitats and showed that a third of detected variants were habitat-specific (~10% from each habitat) which may reflect differences in the taxonomic composition of communities. These habitat-specific variants were functionally redundant across habitats (although we found some discrepancies across functional categories) and represented the same functional potential as variants shared across habitats. This another level of redundancy as the potential differences in taxa composition across habitat are not translated in differences in their functional potential. Patterns of higher taxonomic than functional dissimilarity among bacterial communities are due to functional redundancy between organisms and have already been observed in epibiotic communities of macroalga (Burke *et al*., 2011b), microbiome of mammals (Muegge *et al*., 2011), fish (Mouchet *et al*., 2012) or sponge (Fan *et al*., 2012) and soil communities (Wertz *et al*., 2006).

However, and as previously mentioned for the α-diversity, the potential of the metacommunity for the realization of ecological insurance could have been further validated by showing the presence of different taxa harbouring the same gene pool. Recently, Roth-schulze *et al*. (2016) applied a similar across-scales biodiversity partitioning approach on both taxonomic and functional diversity of seawater and epibiotic bacterial communities living on algae and inanimate substrates. Using a sequencing approach, they show that the majority of taxonomic diversity ($\gamma_{Ecosystem}$) corresponds to inter-habitat differences ($\beta_{InterHabitats}$) while, as seen in our study, most of the metacommunity functional diversity ($\gamma_{Ecosystem}$) was attributed to local communities ($\bar{a}_{LocalCommunities}$). To conclude, the distribution of the functional potential across habitats provides several levels of functional redundancy intra- and inter-habitats. In such a metacommunity, the spatial component of the ecological insurance is expected to promote the resistance and resilience of bacterial processes at large scale in a context of environmental variability (Hector *et al*., 2010; Pasari *et al*., 2013; Wang and Loreau, 2014).

The potential role of macro-organisms on the spatial dynamics in bacterial metacommunities

It was recently showed that dispersal from temporal and spatial refuges enhance both the resistance and resilience of bacterial metacommunities (Baho *et al*., 2012). Such results support directly the spatial insurance hypothesis, highlighting the importance of spatial dynamics and dispersal between patches for the maintenance of metacommunity scale processes. As mentioned earlier, the mixing and dispersal of microbial communities in the Terminos lagoon is under influence of currents and climatic factors. A recent review of the literature suggests that, beside abiotic factors such as currents, motile macro-organisms play an important role in dispersal and spatial dynamics of marine microbes (Troussellier *et al*., 2017) notably at local (e.g., between coral reefs or habitats within a lagoon) or mesoscale (e.g., between coastal lagoons or islands). These authors estimated the

dispersal potential of gut microbes in 16 different fish species and reported values ranging from 2 to 190 km. Additionally, the recent literature describes marine animals as bioreactors favouring the growth of marine aquatic microbes (Hentschel *et al*., 2006; Smriga *et al*., 2010; Beardsley *et al*., 2011; McFall-Ngai *et al*., 2013). There is, thus, increasing evidences that macro-organisms microbiomes participate in source-sink population dynamics and dispersal across microbial communities (Mihaljevic, 2012; Troussellier *et al*., 2017). Such dynamics have been shown to allow communities to recover after disturbance and perform ecological processes on a long-term basis (Shade *et al*., 2014; Aanderud *et al*., 2015; Shade and Gilbert, 2015). In this perspective, it appears important to determine whether the erosion of fish biodiversity observed in the Terminos lagoon (Villéger *et al*., 2010), or in other marine coastal ecosystems (Lotze *et al*., 2006; D'agata *et al*., 2014), might affect the metacommunity dynamics across bacterial communities. The answer of such question is undoubtedly complex as it depends at the same time on the spatial scale considered but also on the level of functional redundancy in fish communities themselves. In the light of our results, it appears necessary to explore further the recently suggested role played by marine macro-organisms as dispersal vectors of microbes (Troussellier *et al*., 2017) and the potential consequences of marine systems defaunation on microbial metacommunities functioning.

Conclusions

In this study, we were interested in exploring the levels of functional diversity and redundancy in coastal bacterial metacommunities. We integrated macro-organisms, water column and sediment microbial communities in a metacommunity framework and used a diversity partitioning approach in order to estimate the level of functional diversity and redundancy across scales (within communities, across communities within a habitat and across habitats within the ecosystem). We showed that all local communities exhibit a highly diversified potential for the realization of key ecological processes and resistance to various environmental conditions, supporting the growing evidence that macro-organisms microbiomes harbour a high functional potential and thus should be considered as integral components of functional gene dynamics in aquatic bacterial metacommunities. We observed several levels of functional redundancy within and across habitats, and discussed the implications in the light of the spatial ecological insurance hypothesis along with the potential role of fish in microbial metacommunities dynamics. The next challenge is to determine whether this ecological insurance potential will result in actual benefits and allows the maintenance of ecosystem functioning and services in a changing world experiencing global biodiversity erosion.

Experimental procedures

Study area and sampling

The study area was located in the southern part of the Gulf of Mexico in the Terminos lagoon, which has experienced high anthropogenic pressures since the last three decades (see Supporting Information Appendix S1 for further details). This includes domestic and industrial pollution by compounds such as polynuclear aromatic hydrocarbons (Norena-Barroso *et al*., 1999), pesticides, chlorinated compounds (Carvalho *et al*., 2009a, 2009b) and heavy metals (Benitez *et al*., 2012). In addition, recent studies showed that anthropogenic pressure and environmental change have impacted the biomass of fish communities along with their taxonomic and functional diversity (Ramos Miranda *et al*., 2005; Villéger *et al*., 2010). This was particularly the case in one part of the lagoon, which was selected for our study (see Supporting Information Appendix S1 for further details).

Bacterial communities from the three habitats (fish guts, sediment and water) were sampled in October 2011. Water (100 ml) and sediment (first 10 cm) were sampled in three stations and immediately stored in ice. We selected three fish individuals representing three different species, orders and trophic guilds: *Synodus foetens* (Siluriform, piscivorous), *Ariopsis felis* (Aulopiform, detritivorous) and *Sphoeroides testudineus* (Tetraodontiform, zoobenthivorous). Fish were instantly killed, individually placed in plastic bags and immediately stored in ice before dissection (within 5 h after sampling). During dissection, prey items were removed and gut mucus was processed as done in Mouchet *et al*., (2012) to assess sample bacterial communities as they are expected to be more involved in metacommunity dynamics than gut wall associated bacterial communities. All samples were frozen and stored at −80°C until DNA extraction was performed according to Mouchet *et al*. (2012). Briefly, total DNA was extracted using lysozyme-based lysis buffer, followed by incubation with proteinase K and SDS before precipitation using isopropanol (further details in Supporting Information Appendix S1).

Functional diversity of bacterial communities

The functional potential of bacterial communities was determined using GeoChip 4 (Tu *et al*., 2014), which is composed of 67 268 probes (i.e., protein-coding genes here called ‘gene variants’), corresponding to 420 functional genes distributed into 12 functional categories. This approach was chosen in the present study as it is: (i) standardized and reproducible (Tu *et al*., 2014), which are key features in narrowly defined, hypothesis-driven, quantitative and comparative studies (Zhou *et al*., 2015); (ii) less subject to random sampling artefact, that can lead to *β*-diversity overestimation, compared with sequencing-based approaches (Zhou *et al*., 2013, 2015); (iii) designed with several functional genes corresponding to heavy metal resistance, contaminant degradation and ecosystem functioning (C, N, P, S cycles) which is in line with the questions addressed here; (v) less subject to

host-DNA contamination compared with sequencing-based approaches (Gevers *et al*., 2012; Kuczynski *et al*., 2012).

To determine the levels of functional diversity and functional redundancy we applied a gene-centred approach using two different resolution levels: functional genes and gene variants. A functional gene is defined as a gene coding for a given function or chemical reaction and their richness is considered as a measure of functional diversity. A gene variant corresponds to a particular form of a functional gene found in different microorganisms and detected by its unique nucleotide sequence (also called probe; Tu *et al*., 2013). The presence of several variants for a given gene indicates that several bacterial taxa harbour this gene in the community (Bai *et al*., 2013; Tu *et al*., 2014) and consequently gene variants can be used to estimate functional redundancy (Miki *et al*., 2014).

To estimate the abundance of gene variants, noise data were removed using a hybridization signal cutoff of 1500 intensity unit and a signal to noise ratio > 2 (Wu *et al*., 2006). Data were normalized using the mean-ratio approach as described elsewhere (He *et al*., 2007). All the genes on the array are not represented by the same number of probes and thus summing the abundance of all the probes from a given gene will give higher importance to 'probe-rich' genes in abundance-based analyses. Hence, we used the average intensity of probes from a given gene to give an equivalent weight to all functional genes. All experimental procedures related to the GeoChip 4 approach are described in Supporting Information Appendix S1. Differences in functional gene and gene variant richness across ecosystem habitats (fish gut, sediment and water) were tested using the Kruskal–Wallis rank sum test while differences in composition and structure were tested by non-parametric permutation-based multivariate analysis of variance (PERMANOVA, Anderson, 2001), using presence-absence (Jaccard) and abundance-based (Bray–Curtis) dissimilarity metrics respectively. We also tested whether observed differences were not due to difference in intra-groups variances using multivariate homogeneity of variance test (PERMDISP).

Multi scales hierarchical diversity partitioning

We applied an additive partitioning framework (Belmaker *et al*., 2008; Escalas *et al*., 2013) to separate the total diversity of functional genes and of gene variants at the ecosystem level ($\gamma_{\text{Ecosystem}}$) into contributions at smaller scales from habitats to local communities. More precisely, total ecosystem functional diversity was expressed as the sum of inter-habitat difference, the mean intra-habitat difference and mean local community diversity with: $\gamma_{\text{Ecosystem}} = \beta_{\text{InterHabitats}} + \beta_{\text{IntraHabitats}} + \bar{a}_{\text{LocalCommunities}}$. The approach is presented in greater detail in Supporting Information Appendix S1. β-diversity estimates allow us to determine the functional dissimilarity among habitats and among bacterial communities within them, providing information on the amount of functional redundancy that exists at different levels of spatial organization in

the ecosystem. This partitioning was performed for both functional genes and gene variants, for the whole community, and separately for each gene category.

Functional overlap of habitat-specific and ubiquitous gene variants

To determine if the differences in variants composition across habitats have a functional significance, we replaced the habitat-specific and ubiquitous (i.e., present in all habitats) gene variants into their corresponding functional categories and functional genes. We then compared their distributions to a null expectation which corresponds to the distribution of all the possible variants present on the GeoChip 4, using the Chi-squared test for given probabilities. Then, we estimated whether the functional potential of habitat-specific variants overlapped. This was performed for the whole set of habitat-specific variants and for each gene categories separately using a Venn diagram approach.

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Data accessibility

The data set supporting the results of this article is available in the IEG microarray data repository, unique persistent identifier and hyperlink to dataset(s) at http://ieg2.ou.edu/NimbleGen/analysis.cgi

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