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Linking initial soil bacterial diversity and polycyclic aromatic hydrocarbons (PAHs) degradation potential

M. Crampon[a, b, *], J. Bodilis[b], F. Portet-Koltalo[a]

[a] COBRA UMR CNRS 6014, Université de Rouen-Normandie, 55 rue saint Germain, 27000 Evreux, France
[b] LMSM, EA 4312, Université de Rouen-Normandie, Place Emile Blondel, 76821 Mont Saint Aignan, France

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ABSTRACT

The aim of this study was to understand the role of indigenous soil microbial communities on the biodegradation of polycyclic aromatic hydrocarbons (PAHs) and to determine whether PAHs degradation potential in soils may be evaluated by analysis of bacterial diversity and potential metabolisms using a metagenomics approach. Five different soils were artificially contaminated with seven selected PAHs and the most abundant bacterial taxa were assessed by sequencing the 16S rRNA gene, and linking them to PAH biodegradation efficiencies. A PICRUSt approach was then led to estimate the degradation potentials by metagenomics inference.

Although the role of bacteria in PAHs degradation is not directly established here, the presence of a large number of bacteria belonging to the *Betaproteobacteria* class correlated to a higher degradation of LMW PAHs. A link with specific bacterial taxa was more difficult to establish concerning HMW PAHs, which seemed to require more complex mechanisms as shown by PICRUSt.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic aromatic compounds containing two or more fused phenyl and/or pentacyclic rings in linear, angular or cluster arrangements [1,2]. They are known to be carcinogenic, mutagenic and genotoxic to both aquatic and terrestrial organisms [3–5]. PAHs are semi-volatile ubiquitous pollutants produced by incomplete combustion of organic material, such as fossil fuel or garbage, and also come partly from petroleum product spillage or from natural sources such as forest fires and volcanic eruptions [6,7]. Among these different sources, the major release of PAHs in the environment is attributable to human activities, and they accumulate in soils after their atmospheric emission and wet or dry deposition [8].

PAHs are known to cause adverse human and ecological health effects, and following their concentration and behavior in soils is essential because soils are considered to be one of the most important natural resources for Human beings [9]. PAH physico-chemical properties (high chemical stability, low vapor pressure, low solubility, high water-organic carbon K_{oc} partition coefficient) make them persistent in soils, this persistence being also dependent on soil physico-chemical properties and soil microbial ecology [10–12]. Among remediation methods, natural bioremediation or enhanced biodegradation are commonly studied approaches for the decontamination of polluted soils [13,14]. Indeed, microorganisms naturally present in soils are able to degrade PAHs, either by metabolism (direct consumption by microorganisms) or by cometabolism (indirect degradation). It is generally admitted that bacteria are able to degrade low molecular weight (LMW) PAHs [15,16], while fungi are more suitable to degrade high molecular weight (HMW) PAHs (>4 aromatic rings) by extracellular enzymatic activities [17–20]. Consequently, the presence of both bacteria and fungi shows better degradation results [21].

Different bacteria have been described as capable of PAH degradation, and several strains that can grow using PAHs as sole carbon source (by the metabolic way) have been isolated from soils. These bacteria belong to different classes such as *α*-, *β*- and *γ*-*Proteobacteria* [14,22,23], *Bacteroidetes* [24], *Actinobacteria* [25], *Nocardioides* [26] or *Firmicutes* [27]. *Betaproteobacteria* have also often been described as capable of PAH degradation [22,28–30]. It has been shown that in an agricultural soil, after 90 days of incubation with phenanthrene (PHE), pyrene (PYR) or a mix of seven PAHs, the proportion of *Betaproteobacteria* increased significantly, showing their degradation capacity [31]. Among *Betaproteobacteria*, the *Burkholderiales* order is particularly known to degrade PAHs [32]. It has also been shown that *Gammaproteobacteria* are capable of PAH degradation [29,31], and particularly the *Xanthomonadales* order [30,33].

Although most of the degrading bacteria already described are culturable, *i.e.* able to grow on culture media, the vast majority of soil micro-organisms are not culturable (*i.e.* from 90% to 99.99% [34]). Several techniques have been described to observe the whole community structure and to quantify the microbial populations (*e.g.* DGGE [35]; ARISA [31]). The recent technological advances in sequencing technol-

* Corresponding author. Present address: Bureau de Recherches Géologiques et Minières (BRGM), 3 avenue Claude Guillemin, 45100 Orléans, France.
Email addresses: m.crampon@brgm.fr (M. Crampon); josselin.bodilis@univ-rouen.fr (J. Bodilis); florence.koltalo@univ-rouen.fr (F. Portet-Koltalo)

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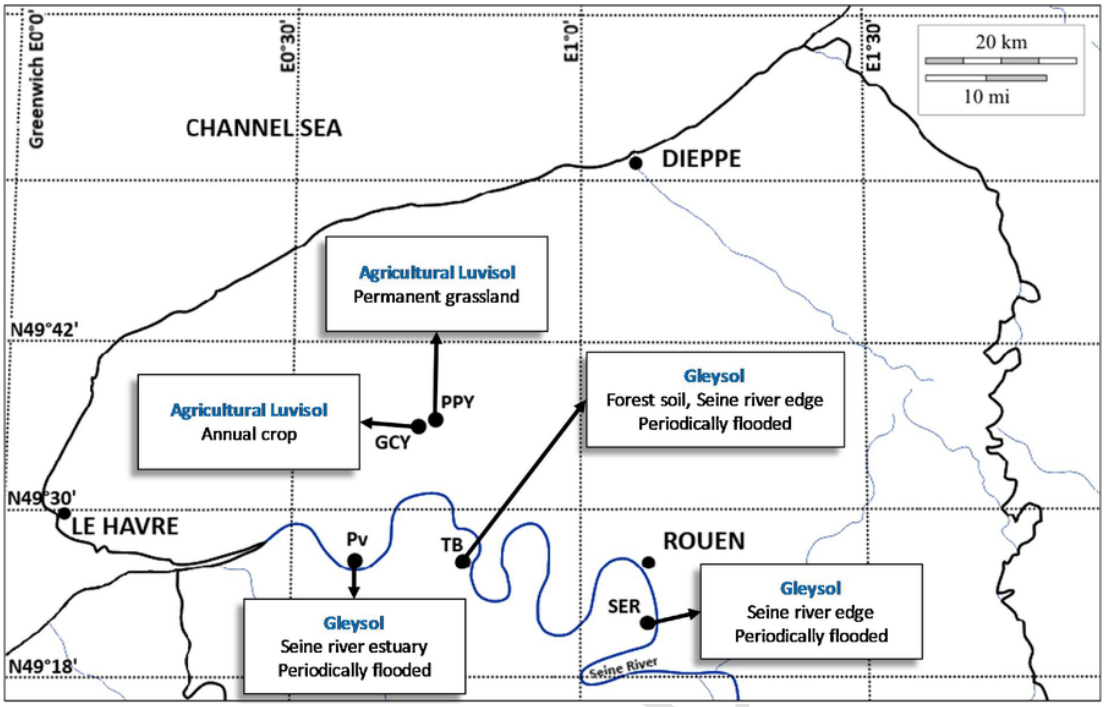

Fig. 1. Location of the five sampling sites in the Seine river watershed, Normandy, France, and type of soil according to the World Reference Base for Soil Resources (WRB).

ogy now allow easier access to metataxogenomics, *i.e.* to read thousands of 16S rRNA sequences per sample. In this context, the analysis of bacterial diversity may help to define the PAH degradation potential in a sample by identifying bacteria known to be able to metabolize PAHs and by determining their abundance [11,12,36,37]. Interestingly, this metataxogenomics approach can be completed by a metagenomics approach [38–42]. Indeed, the genes present (and thus the degradation potential) in the bacterial community could be predicted by inferring the gene content for each OTU (Operating Taxonomic Unit) from the closest sequenced genomes, *via* phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt approach). The aim of our study was to determine if the initial microbiological parameters of natural soils might help to understand the degradation behavior of LMW and HMW PAH in the case of an on-point contamination. In this context, links between soil biological parameters (total abundance, diversity, abundance of some particular bacteria described as capable of PAH degradation in the literature and PICRUSt approach) and PAH biodegradation were compared in five dissimilar freshly contaminated soils.

2. Materials and methods

2.1. Chemicals

All organic solvents were purchased from VWR (Fontenay sous Bois, France) and were of HPLC grade. The seven selected PAH standards were obtained from Sigma-Aldrich-Fluka (St Quentin Fallavier, France): phenanthrene (PHE) and perdeuterated phenanthrene, fluoranthene (FLT), pyrene (PYR), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP) and benzo[*g,h,i*]perylene (BghiP).

2.2. Soils, experiments and PAH analyses

2.2.1. Sampling sites

Five soils were collected from the surface horizon (0–15 cm) in Northern France (Normandy) in spring 2012, and were sieved (2-mm sieve). Sampling site locations are shown in Fig. 1. PPY and GCY soils are agricultural Luvisols, which are located on the Seine river plateau and were under permanent grassland and annual crops, respectively. Pv and SER soils are Gleysols located close to the Seine river (periodically flooded). The soil named TB is a forest soil (Gleysol), also located close to the Seine River, and also periodically flooded [43].

2.2.2. Dissipation time-course studies

Each soil (triplicates for each date) was spiked with a total of 300 mg kg^{-1} of seven selected PAHs. The complete procedure, which gives a good contamination homogeneity in the spiked soils without adverse effects on the organic matter and microorganism communities, is described in Crampon et al. [43]. PAH analyses were carried out just after microcosm preparation and after 8, 30, 60 and 90 days of incubation. Extractions were performed using a microwave-accelerated extraction (MAE) technique on 1 g crushed dried soil [44]. Extracts were analyzed with a 6850 gas chromatographer from Agilent (USA) coupled with a 5975C mass spectrometer. The detection and quantification thresholds, calculated respectively as 3 and 10 times the standard deviation of the blank sample noise, were 1.5/5 µg L^{-1} for PHE, 2.5/8.5 µg L^{-1} for FLT, PYR, BbF and BkF, 3.5/11.5 µg L^{-1} for BaP and 5/16.5 µg L^{-1} for BghiP. Again, the complete procedure can be found in Crampon et al. (2014) [43]. In this study, we decided to compare only the biodegradation of LMW PAH to the biodegradation of HMW PAH in the five soils. Consequently, the LMW PAH degradation corresponds to the mean degradation of PHE, FLT and PYR (≤ 4 aromatic rings) and the HMW PAH degradation corresponds to the mean degradation of BbF, BkF, BaP and BghiP (≥ 5 aromatic rings). Degradation parameters were represented by the percentage dissipated after 90 days (%90), by the initial degradation speed (V_0) and the time necessary for 50% PAH dissipation (DT50) (supplementary material SM1).

2.3. DNA quantification and bacterial identification

Natural soils (in triplicates) were used for soil genomic DNA extractions using PowerSoil DNA isolation kit (Mo-Bio Laboratories) according to manufacturer recommendations. Extracted DNA was quantified

Table 1
Results of bacterial diversity analyses on the five studied soils. The diversity indexes were calculated with data from NGS sequencing (with the total number of observed OTUs, Chao1 being the specific richness index and Simpson the total diversity index) for a similarity threshold of 97%.

	GCY			PPY			Pv			SER			TB		
Number of observed OTUs	951.79	±	38.75	691.42	±	38.59	779.93	±	189.20	792.92	±	167.58	743.70	±	20.95
Inverted Simpson	238.75	±	17.60	161.08	±	16.89	196.85	±	126.20	169.22	±	118.88	64.78	±	1.86
Chao1	2048.28	±	187.79	1396.29	±	65.94	1630.35	±	380.59	1712.54	±	263.12	1669.40	±	89.32

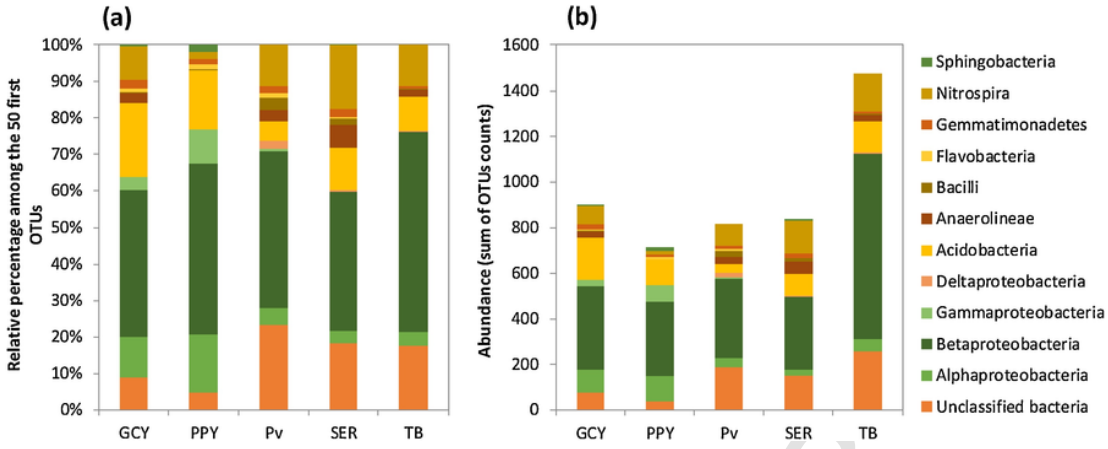

Fig. 2. (a) Proportions of different phyla or classes among the 50 first OTUs in terms of quantity and (b) Quantity of these phyla or classes as a sum of the observed OTUs.

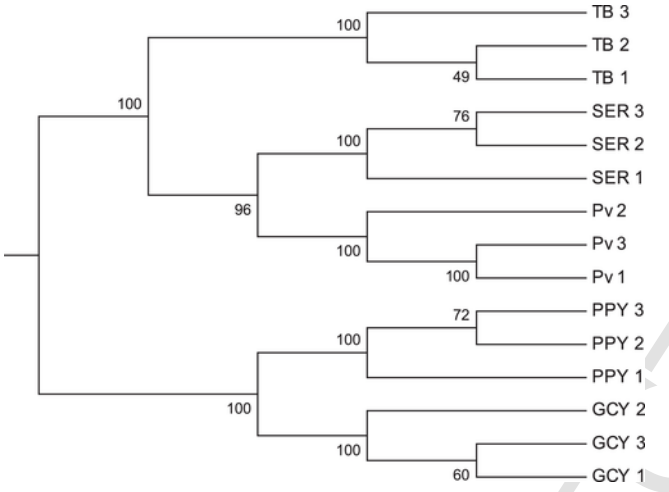

Fig. 3. Dendrogram comparing the structure of the bacterial communities of the five studied soils. Dendrogram was built with UPGMA algorithm and from the Bray-Curtis distances for a similarity threshold of 97%. The values next to the branches correspond to 1000 sub-samplings with normalization at 2463 sequences per sample.

using NanoDrop 2000 (Thermo Scientific) and samples were stored at −20 °C before further analyses.

DNA samples were then prepared for 454 pyrosequencing after amplification using universal primers 27 F (5′ – AGA GTT TGA TCC TGG CTC AG - 3′) and 533R (5′ – TTA CCG CGG CTG CTG GCA C - 3′), extended at the 5′ end by 10 bp multiplex identifiers (MIDs, Roche). Amplifications used GoTaq G2 Green Master Mix (Promega) and were carried out during 30 cycles (30 s at 95 °C, 30 s at 60 °C, 30 s at 72 °C).

After quality checking by agarose gel electrophoresis and quantification using NanoDrop, three independent PCR products per sample were pooled together to minimize PCR bias on sequencing. One equimolar mixture was then made from PCR products from the five soils.

454-pyrosequencing was carried out on a GS FLX 454 (Beckman Coulter Genomics, USA). A sequencing half plate was used, which produced 316.5 megabases corresponding to 716,250 sequences having a median size of 490 bp. Sequence analyses like reducing sequencing error, removing chimeras, grouping into Operating Taxonomic Units (OTUs), aligning to the SILVA database alignment, and classifying using the RDP reference files were performed using MOTHUR software (version 1.36.1), as recommended by its author [44].

As the two primers used for sequencing are tagged by a MID, it was expected that approximately half of the sequences obtained were sequenced from each primer. In addition, because of stringent trimming, we obtained a final sequence size of about 250 bp. Consequently, to facilitate sequence alignment and thus further analyses, it was decided to deal separately with the sequences obtained from the forward primer (16S-0027 F, corresponding to the V1 and V2 16S rDNA regions) to those obtained from the reverse primer (16S-0533R, corresponding to the V3 16S rDNA region). In this paper, only the results from V1 and V2 16S rDNA regions are presented, as no major difference was observed compared to the V3 region. The alpha diversity of the samples was estimated using the Chao1 richness estimators and the inverse Simpson diversity index. To compare the community structures (beta diversity) a dendrogram was constructed using the UPGMA algorithm (Unweighted Pair Group Method with Arithmetic Mean) and the Bray-Curtis index.

All 16S rRNA gene sequences have been submitted to the NCBI Short Read Archive under the Bioproject accession number PRJNA312913 (native soil).

2.4. PICRUSt approach

Gene prediction with phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) was used here to determine the degradation potential of the bacterial communities of the five soils from 16S rRNA sequences obtained by pyrosequencing. PICRUSt is a computational approach that enables to accurately predict gene family of bacterial communities using 16S rRNA sequences, with the accuracy of 82–95% compared with metagenome. PICRUSt uses evolutionary modeling to predict metagenomes from 16S data compared with a reference genome database.

Thanks to the composition of the bacterial communities in the five studied soils, it was thus possible to predict the mean relative abundances of gene functions, including PAH degradation. In the five studied soils, OTUs (97% identity) deduced from the 16S rRNA sequences were normalized by PICRUSt, *i.e.* each OTU count was divided by the 16S rRNA copy number found in the corresponding ancestral genome inferred. Then, the KEGG profiles (http://www.kegg.jp/) were calculated with the PICRUSt algorithm from the metagenome inferred [38]. KEGG pathways linked to PAH degradation were specifically extracted and compared to soil degradation kinetic parameters for LMW and HMW PAH.

3. Results and discussion

3.1. PAH degradation kinetics

Degradation kinetics of the seven spiked PAHs were monitored during 90 days on the five studied soils and presented in a previous study [43]. The degradation curves for the 7 studied PAHs in the five soils and the summary of degradation results are however provided in the

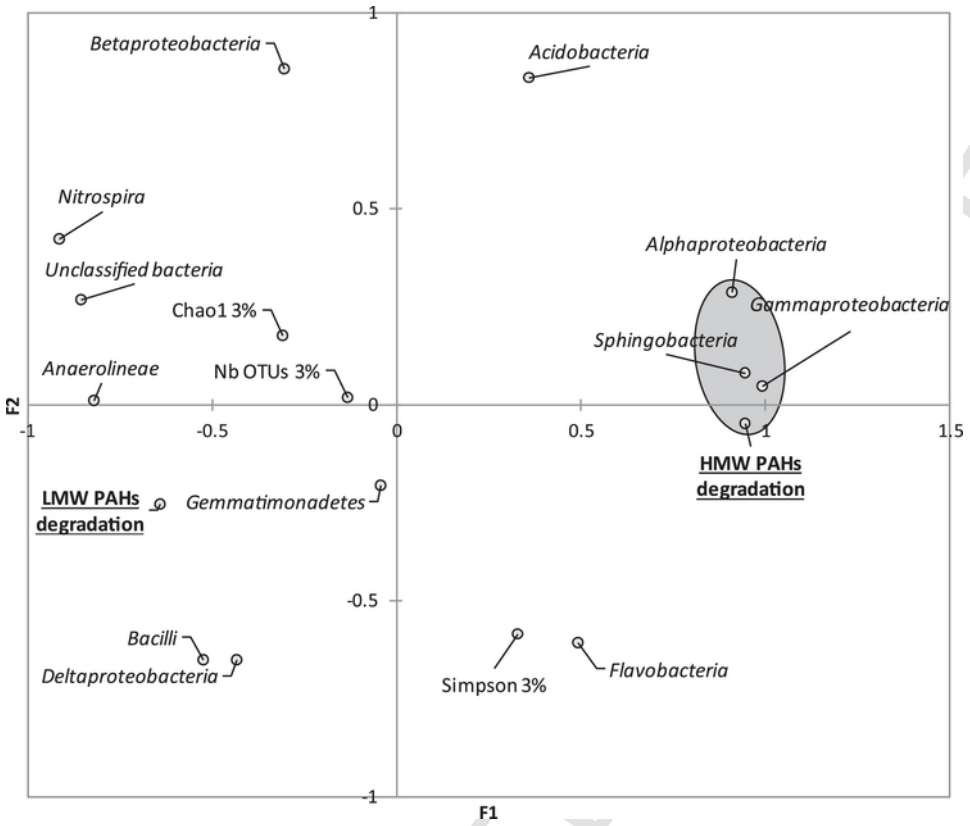

Fig. 4. Principal coordinate analysis (PCoA) showing the correlations between (i) phyla/classes abundance within the 50 first OTUs in terms of abundance in the 5 studied soils, (ii) the diversity indexes calculated with data from NGS sequencing (Nb OTUs is the total number of observed OTUs, Chao1 the specific richness index and Simpson the total diversity index) for a similarity threshold of 97% and (iii) the mean degradation efficiency (%90) of LMW and HMW PAHs in the five studied soils.

supplementary material (SM2). The shapes of these curves seem coherent with the literature. A two-step degradation pattern was observed for LMW PAHs [45], which first degrades rapidly before slowing down after 90 days when all the easily accessible PAHs have been degraded. The remaining PAHs correspond to the low bioaccessible fraction [46].

Some parameters were evaluated thanks to the degradation kinetic curves, especially for the LMW PAHs (PHE, FLT and PYR), for which degradation was faster. DT50, corresponding to the time necessary for 50% dissipation, was measured (for PAHs having reached 50% dissipation only), and the V_0 parameter (corresponding to the degradation rate in the first degradation step) was also measured for the three LMW PAHs on the five soils (SM1). As previously explained, results will be presented here as LMW *vs* HMW PAHs. Overall for the LMW PAHs, the degradation was slow in PPY and GCY soils (low Vo values, high DT50 values), faster in SER and TB soils, and maximum in Pv soil (which presented the highest V_0 value and the lowest value of DT50). The degradation of LMW PAHs was thus slow in Luvisols, and quite fast in the three Gleysols. Trends were very similar between the V_0 DT50 and the dissipated percentage after 90 days (%90) for LMW PAHs ($0.76 > r > 0.98$, Pearson's correlation coefficients). Moreover, V_0 and DT50 values are not relevant for HMW PAHs (because of a too low or no degradation). Consequently, the degradation results are only presented as mean dissipated values after 90 days for LMW *vs* HMW PAHs in the present article. As shown in previous papers, the soil parameters and the bioaccessibility of PAHs could not explain the observed differences in PAHs degradation between the five soils, in the case of a recent PAH contamination [43,47]. The bacterial diversity was hence more thoroughly studied for the five soils, by sequencing the 16S rRNA gene and comparing these results with the degradation efficiency.

3.2. Soil bacteria vs PAHs degradation

3.2.1. Bacterial diversity

To evaluate total bacterial diversity, a metagenomics approach targeting the 16S rRNA gene (*i.e.* meta taxogenomics) was used. Universal primers enable to estimate bacterial diversity through sequencing techniques such as Roche 454 (pyrosequencing or 454 GS FLX, shotgun approach). This approach was applied to the five studied soils (in triplicates for each soil). After demultiplexing and reduction of sequencing errors, we obtained an average of 4000 sequences per replicate of soil, with a median size for the sequences of 231 base pairs, corresponding to the regions V1 and V2 of the ADNr 16S gene. Various analyses were carried out using the Mothur software according the author's recommendations [44]. First, the alpha diversity of each soil was estimated with the inverted Simpson and Chao1 indexes. These indexes estimates the diversity and the species richness of each soil, respectively (Table 1, for 97% similarity corresponding to the species/genus level). The number of sequences per sample (coverage) was sufficient to obtain a good estimation of these diversity indexes. Overall, and whatever the similarity threshold used to define the OTUs, we observed that the GCY soil presented the highest bacterial diversity (inverted Simpson) and the highest species richness (Chao1; Table 1). Consequently, only the results with a similarity threshold of 97%, *i.e.* corresponding to clustering at about the genus level, are presented here.

Pv and SER soils presented an intermediate diversity and species richness compared to the other soils, with however a great variability between the replicates. TB soil presented the smallest diversity but the species richness was comparable to those of Pv and SER soils. Lastly, PPY soil presented a diversity comparable to those of Pv and SER soils, but with the lower species richness.

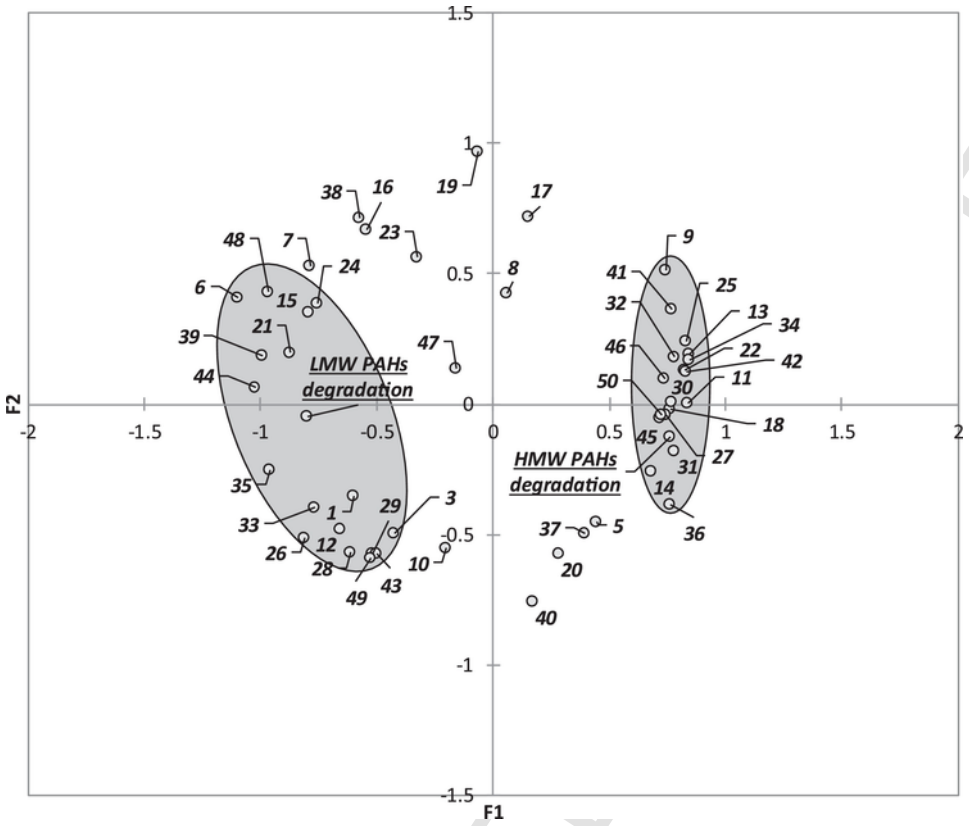

Fig. 5. Principal coordinate analysis (PCoA) showing the correlations between (i) the abundance of the 50 first OTUs in terms of abundance in the 5 studied soils (pls. refer to Table 2 for OTUs correspondence) and (ii) the mean degradation efficiency (%90) of LMW and HMW PAHs.

A comparison of soil diversity at a high taxonomic rank (phylum or class) among the 50 more abundant OTUs showed only few differences between the five soils (Fig. 2). It can be noted that the proportions of *Alpha-*, *Beta-* and *Gammaproteobacteria* were quite similar for all the studied soils (Fig. 2a). However, in terms of quantity, the TB soil has a lower diversity, more *Proteobacteria* represented in the 50 first OTUs (Fig. 2b), and also more *Betaproteobacteria*. Finally, the structures of the bacterial communities were compared by building a dendrogram from Bray-Curtis distances at the threshold of 97% similarity (Fig. 3). This showed good similarities between soils. Moreover, as expected, PPY and GCY soils, which are physico-chemically and geographically close, showed similar bacterial community structures. The three Gleysols were more similar to each other and formed a group of three soils differing from the Luvisols. The similarities of the bacterial communities between SER and Pv soils were greater than with the TB soil.

The diversity results were compared to PAH degradation parameters using principal coordinate analysis (PCoA) (Fig. 4). On one hand, quite a good correlation ($r = 0.619$, Pearson's correlation coefficient) was observed between the Chao1 index and the degradation (%90) of LMW PAHs. This correlation with LMW PAH degradation percentage after 90 days of soil ageing confirms that the abundance of some bacteria, those capable of metabolizing these molecules, was an important factor controlling their degradation. On the other hand, total diversity, represented by the Simpson index, as well as the total number of OTUs, had little importance on PAH degradation, as observed in Fig. 4.

A higher degradation of HMW PAHs seemed to correlate here with a higher abundance of three taxa: *alpha-* and *gamma-proteobacteria* classes, and *sphingobacteria* phylum. The degradation of HMW PAHs was indeed higher in Luvisols, which are those containing the higher numbers of these taxa. The abundance of the other phyla/classes was hard to correlate here with PAH degradation after 90 days (Fig. 4). The capacity to metabolize PAHs is often strain dependent, thus the links between degradation and soil bacteria were then analyzed for a finer phylogenetic classification using another PCoA analysis (Fig. 5). Each OTU number is represented in the PCoA (50 first OTUs in terms of abundance), and the correspondence of these OTUs with their taxonomic classification (obtained by comparison with the Genbank database, for a 97% similarity threshold) is presented in Table 2. We could first observe from Fig. 5 that the abundance of more OTUs correlated to a higher degradation of LMW PAHs compared to the phylum/class phylogenetic range presented in Fig. 4.

Indeed, concerning the degradation of LMW PAHs, the abundance of 17 OTUs out of 50 correlated well together (OTUs n ° 1, 3, 6, 12, 15, 21, 24, 26, 28, 29, 33, 35, 39, 43, 44, 48 and 49; Fig. 5). Concerning HMW PAH degradation, the percentage degraded after 90 days correlated well to the abundance of 18 other OTUs (9, 11, 13, 14, 18, 22, 25, 27, 30, 31, 32, 34, 36, 41, 42, 45, 46 and 50). Of note, the number of OTUs that correlated to degradation, whether LMW or HMW PAHs, was almost identical whatever the similarity threshold 90, 95 or 97% (data not shown). Consequently, there were only a few species/genus per family that correlated to PAH degradation. The adaptation to the degradation of the PAHs seems therefore both a strain / species specific property but is also distributed widely throughout the Bacteria domain.

It is interesting to note that for LMW PAHs, the best correlations were observed for OTUs belonging to the *Betaproteobacteria* class, of which members have been described many times as capable of degrading PAHs [22,28–31], even if correlation was not evident at the phylum/class rank (Fig. 4). This observation shows that PAH degradation capacity is probably not common to the whole *Betaproteobacteria* class. The *Burkholderiales* order (and in particular the genus *Polaromonas*) has been described as capable of degrading LMW PAHs [32], and is commonly found in the literature. The abundance of *Polaromonas* genus (OTU 26) actually showed a good correlation with LMW PAHs degradation. In addition, the abundance of OTUs 43 and 44 (from the *Rhodocyclaceae* family, in the *Betaproteobacteria* class) correlated to a

Table 2
Taxonomic correspondences of the 50 first OTUs in terms of abundance in the five studied soils at a similarity threshold of 97% with percentages of similarity by comparison with GenBank database.

	Embranchment	Phylum	Class	Order	Family	Genus
Otu00001	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	–	–	–
Otu00002	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	–	–	–
Otu00003	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Xanthomonadales(100)	Sinobacteraceae(100)	Nevskia(100)
Otu00004	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	unclassified(100)	–	–
Otu00005	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Comamonadaceae(100)	Albidiferax(100)
Otu00006	Bacteria(100)	Nitrospira(100)	Nitrospira(100)	Nitrospirales(100)	Nitrospiraceae(100)	Nitrospira(100)
Otu00007	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	–	–	–
Otu00008	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Oxalobacteraceae(100)	Undibacterium(100)
Otu00009	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Rhizobiales(100)	Bradyrhizobiaceae(100)	Bradyrhizobium(89)
Otu00010	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	–	–
Otu00011	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Rhizobiales(100)	–	–
Otu00012	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Burkholderiales_incertae_sedis(100)	–
Otu00013	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(99)	–	–	–
Otu00014	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Oxalobacteraceae(100)	Collimonas(100)
Otu00015	Bacteria(100)	Proteobacteria(100)	–	–	–	–
Otu00016	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(95)	–	–	–
Otu00017	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	–	–	–
Otu00018	Bacteria(100)	Acidobacteria(100)	Acidobacteria_Gp1(100)	Acidobacteria_Gp1_order_incertae_sedis(100)	Acidobacteria_Gp1_family_incertae_sedis(100)	Gp1(100)
Otu00019	Bacteria(100)	Acidobacteria(100)	Acidobacteria_Gp4(100)	Acidobacteria_Gp4_order_incertae_sedis(100)	Acidobacteria_Gp4_family_incertae_sedis(100)	Gp4(100)
Otu00020	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Xanthomonadales(100)	Xanthomonadaceae(100)	Dyella(100)
Otu00021	Bacteria(100)	Proteobacteria(64)	–	–	–	–
Otu00022	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Xanthomonadales(100)	Xanthomonadaceae(100)	–
Otu00023	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	–	–	–
Otu00024	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	–	–	–
Otu00025	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	–	–	–
Otu00026	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Comamonadaceae(100)	Polaromonas(100)
Otu00027	Bacteria(100)	Acidobacteria(100)	Acidobacteria_Gp1(100)	Acidobacteria_Gp1_order_incertae_sedis(100)	Acidobacteria_Gp1_family_incertae_sedis(100)	Gp1(100)
Otu00028	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Burkholderiales_incertae_sedis(98)	–
Otu00029	Bacteria(100)	Bacteroidetes(100)	Sphingobacteria(100)	Sphingobacteriales(100)	Chitinophagaceae(100)	–
Otu00030	Bacteria(100)	Firmicutes(100)	Bacilli(100)	Bacillales(100)	Bacillaceae_1(93)	Bacillus(87)
Otu00031	Bacteria(100)	Proteobacteria(99)	–	–	–	–
Otu00032	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Alphaproteobacteria_order_incertae_sedis(75)	Alphaproteobacteria_family_incertae_sedis(75)	Rhizomicrobium(75)
Otu00033	Bacteria(100)	–	–	–	–	–
Otu00034	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Xanthomonadales(100)	Xanthomonadaceae(100)	–
Otu00035	Bacteria(100)	–	–	–	–	–
Otu00036	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(95)	–	–	–
Otu00037	Bacteria(100)	Bacteroidetes(100)	Flavobacteria(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	Flavobacterium(100)
Otu00038	Bacteria(100)	Acidobacteria(100)	Acidobacteria_Gp6(100)	Acidobacteria_Gp6_order_incertae_sedis(100)	Acidobacteria_Gp6_family_incertae_sedis(100)	Gp6(100)
Otu00039	Bacteria(100)	Chloroflexi(99)	Anaerolineae(99)	Anaerolineales(99)	Anaerolineaceae(99)	–
Otu00040	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	–	–	–
Otu00041	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Caulobacterales(100)	Caulobacteraceae(100)	Phenylobacterium(100)
Otu00042	Bacteria(100)	Acidobacteria(100)	Acidobacteria_Gp3(100)	Acidobacteria_Gp3_order_incertae_sedis(99)	Acidobacteria_Gp3_family_incertae_sedis(99)	Gp3(99)
Otu00043	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Rhodocyclales(98)	Rhodocyclaceae(98)	–
Otu00044	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Rhodocyclales(100)	Rhodocyclaceae(100)	Dechloromonas(98)
Otu00045	Bacteria(100)	Acidobacteria(100)	Acidobacteria_Gp1(100)	Acidobacteria_Gp1_order_incertae_sedis(100)	Acidobacteria_Gp1_family_incertae_sedis(100)	Gp1(100)
Otu00046	Bacteria(100)	Proteobacteria(100)	Deltaproteobacteria(100)	Desulfuromonadales(100)	Geobacteraceae(93)	Geobacter(93)
Otu00047	Bacteria(100)	Acidobacteria(100)	Acidobacteria_Gp22(100)	Acidobacteria_Gp22_order_incertae_sedis(100)	Acidobacteria_Gp22_family_incertae_sedis(100)	Gp22(100)
Otu00048	Bacteria(100)	Acidobacteria(100)	Acidobacteria_Gp6(100)	Acidobacteria_Gp6_order_incertae_sedis(100)	Acidobacteria_Gp6_family_incertae_sedis(100)	Gp6(100)
Otu00049	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	–	–	–
Otu00050	Bacteria(100)	Acidobacteria(100)	Acidobacteria_Gp1(100)	Acidobacteria_Gp1_order_incertae_sedis(100)	Acidobacteria_Gp1_family_incertae_sedis(100)	Gp1(100)
–	Unclassified					

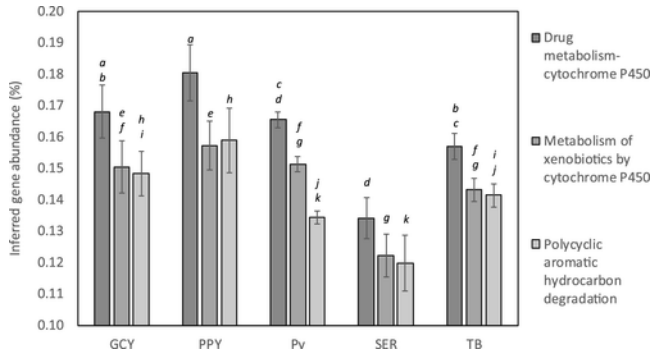

Fig. 6. Results of PICRUSt analyses on the sequences of the five soils presented as PICRUSt inferred abundances (%) of genes controlling drug and xenobiotics metabolism *via* cytochrome P450 and PAHs degradation. By PICRUSt test, histograms with different letters are significantly different from each other ($P < 0.05$, KW test with Conover-Iman comparison).

higher LMW PAHs degradation. Representatives of these taxa have also been described as able to degrade PAHs and to possess specific PAH RHD enzymes [28,29,48–50], and a cultivable member of this *Rhodocyclaceae* family, that degrades PAHs, has been recently sequenced [51]. Interestingly, in a previous study using DNA Stable Isotope Probing, we showed that all the bacteria implied in PHE degradation in Pv soil belonged to the *Betaproteobacteria* class, especially in the *Rhodocyclaceae* family [52]. *Gammaproteobacteria* have also been described as capable of degrading PAHs [29], in particular the *Xanthomonadales* order [30,33]. The abundance of the OTU 3 (*Gammaproteobacteria*, order *Xanthomonadales* of the genus *Nevskia*) also showed a good correlation with the degradation of LMW PAHs. As previously shown [52], *Nevskia* was found as the main PHE degrader in PPY soil, with about 24% relative abundance among bacteria labelled with ^{13}C PHE (DNA-SIP). Among these different OTUs, 6 were dominantly found in the PHE degraders of Pv and PPY soils (from the *Rhodocyclaceae* family (OTUs 43 and 44), *Polaromonas* (OTU 26), *Nevskia* (OTU 3), *Burkholderiales* order (OTUs 5, 8, 10, 12, 14, 26, 28), *Collimonas* (OTU 14) and *Dyella* (OTU 20)). Their abundance should then logically lead to higher PHE (and LMW PAH in extent) degradation. Finally, for LMW PAH degradation, good correlations were also observed with abundance of *Nitrospira* (genus *Nitrospira* OTU 6), *Acidobacteria* (genus *Gp6,* OTU 48) and unclassified bacteria or *Proteobacteria*. Overall, a large proportion of OTUs whose abundance presented a good correlation with LMW PAHs degradation belonged to the *Betaproteobacteria* class (7 over 17).

Concerning HMW PAH degradation, OTUs correlating with a higher degradation belonged to a larger number of phyla and classes (out of 18 OTUs: 4 *Alpha-*, 4 *Beta-*, 2 *Gamma-* and 1 *Delta-Proteobacteria*, 5 *Acidobacteria* (genus *Gp1* and *Gp3*), 1 *Firmicutes* (*Bacilli Bacillus* OTU 30), and unclassified *Proteobacteria*). For *Alphaproteobacteria*, correlations were observed with the abundance of genus *Bradyrhizobium,* (OTU 9) and *Rhizobiales* order (OTU 11). In particular, a correlation was observed for *Firmicutes* (genus *Bacillus,* OTU 30) and *Deltaproteobacteria* (genus *Geobacter* (OTU 46). Some *Deltaproteobacteria* are known to degrade PAHs [53], but it is globally difficult to find literature about bacteria which effectively degraded HMW PAHs (5 or more aromatic rings) [54], especially because of the low biodegradability of these molecules. Comparison of our results with known strains able to degrade HMW PAHs was therefore more difficult than for LMW PAHs, and the correlation with the abundance of bacteria belonging to *Betaproteobacteria* was finally much less apparent than for LMW PAHs. The few strains described in the literature as capable of degrading HMW PAHs were not found in the 50 first OTUs of our soils (for example *Mycobacterium sp* [55–57], *Sphingomonas paucimobilis* [58], or *Stenotrophomonas maltophilia* [59,60]).

3.2.2. Metagenomics inference

For the five soils, the functional profiles of bacterial communities associated to PAH degradation was assessed by inferring the gene content for each OTU from the closest sequenced genomes (*i.e.* the PICRUSt approach). The presented results concern the three mean KEGG pathways linked to PAH degradation: "drug metabolism *via* cy-

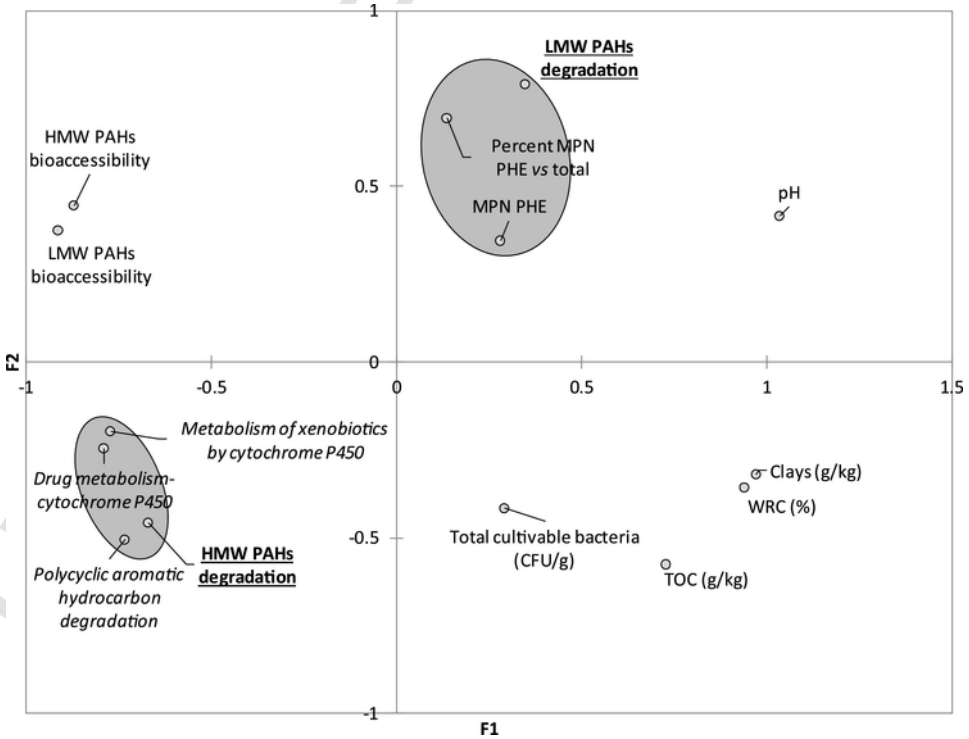

Fig. 7. Principal coordinate analysis (PCoA) of the different physico-chemical/biological parameters of the 5 studied soils. WRC = water retention capacity, TOC = Total Organic Carbon, MPN PHE = most probable number of bacteria degrading PHE, Percent MPN PHE *vs* total = percentage of PHE degrading bacteria compared to total bacteria, PICRUSt inferences are represented in italic, PAHs degradation parameters are represented in bold and underlined.

tochrome P450″, "metabolism of xenobiotics *via* cytochrome P450″ and "polycyclic aromatic hydrocarbon degradation" (Fig. 6). Overall, two groups were clearly distinguished here: PPY and GCY Luvisols on the one hand and Pv, SER and TB Gleysols on the other hand. Except for the KEGG pathway "metabolism of xenobiotics *via* cytochrome P450″, PICRUSt values were significantly different between (i) PPY/GCY and (ii) Pv/SER/TB ($P < 0.05$, Kruskall-Wallis test with Conover Iman comparison). Concerning the KEGG pathway "metabolism of xenobiotics *via* cytochrome P450″, PPY was significantly different from Pv/SER/TB soils when GCY was significantly different from SER soil only ($P < 0.05$, Kruskall-Wallis test).

Thus, by functional inference, it appeared that the Luvisols (which presented a faster HMW PAHs degradation) contained a higher PAH degradation potential than the Gleysols (which presented a faster LMW PAHs degradation). As co-metabolism with several different micro-organisms is often necessary to degrade HMW PAHs, the degradation potential estimated by PICRUSt could be a quite good indicator of the degradation kinetics of HMW PAHs, but not necessarily for LMW PAHs which are more probably degraded through direct metabolism of few specific strains.

3.3. Links between initial bacterial community and PAH degradation

As discussed so far, PAH degradation seems to be related to soil biological parameters in the case of a recent contamination, and especially to the presence of some specific bacteria, especially belonging to the *Betaproteobacteria* class. The fact that soil physico-chemical parameters, and so PAHs bioaccessibility, is not a limiting factor in our study is partly due to the short contact time between spiked PAHs and the soils during our experiments (three months), which might result in a relatively good bioaccessibility of the PAHs [52]. In freshly contaminated soils, such as those that are chronically and diffusely contaminated, the bioaccessibility of PAHs, as well as OM content and nature, actually did not appear to be the main parameters limiting biodegradation [43,47].

The density of total cultivable bacteria was not correlated to PAH biodegradation, as shown using a PCoA analysis (Fig. 7). In contrast, a good correlation of LMW PAH degradation (%90) with (i) the amount of specific PHE degrading bacteria (MPN PHE) and (ii) the relative percentage of PHE degrading bacteria (see supplementary material SM3) among the total bacteria was clearly underlined here (Fig. 7). The bacteria involved in PHE degradation would then be, to a certain extent, the same (or at least co-occurring) as those involved in the degradation of FLT and PYR. The relative percentage of PHE degrading bacteria, or LMW PAH degrading bacteria in a larger extent, would reflect the competitiveness of these bacteria relative to the total microflora for a given soil, and thus their ability to adapt rapidly to a new addition of PAHs in the soil.

Soil pH also seemed to play a key role here. The soil pH was correlated to the degradation of LMW PAHs: the higher the pH was, the faster was the degradation rate (high V_o, low DT50 values). In contrast, an acidic pH correlated well with a higher degradation (%90) of HMW PAHs. A previous study [61] showed that neutral to slightly alkaline soil pH promotes bacterial growth in soils, while an acidic pH favors the growth of fungal species. On the other hand, Machin-Ramirez et al. (2010) showed that soil fungal communities are more capable of degrading BaP than bacterial communities [17]. Degradation of BaP, and HMW PAHs at large, could thus be favored in soils with a slightly acidic pH (PPY and GCY soils) by the coexistence of fungal and bacterial species (consortia), while a rather alkaline pH could favor the development of the bacterial communities responsible for the fast degradation of LMW PAHs. In this context, the results of the metagenomics inference by PICRUSt analysis were interesting. The potential for PAH degradation, including the potential metabolism *via* cytochrome P450 estimated by metagenomics inference, were found to be more abundant on acidic PPY and GCY soils. These soils were also those in which the higher HMW PAH degradation was observed. Finally, these results highlighted two points: (i) the degradation of LMW PAHs seems related to the abundance of specific bacteria able to quickly metabolize them and to the proportion of these bacteria compared to total soil bacteria, and (ii) HMW PAHs degradation seems related to the pH of the soil and to the potential metabolisms *via* cytochrome P450 as well as PAH degradation potential estimated by metagenomics inference.

4. Conclusions

This study showed the correlations between the degradation behavior of PAHs (LMW for PAHs ≤ 4 aromatic rings and HMW for PAHs ≥ 5 aromatic rings) in five freshly contaminated soils and the abundance of bacteria and the diversity parameters or PICRUSt inferences evaluated with data from total soil bacterial DNA sequencing. Although the role of bacteria in PAH degradation could not be directly established here, the presence of a large number of some OTUs (particularly from the *Betaproteobacteria* class) correlated to a good degradation of LMW PAHs, while the link with the HMW PAHs was more difficult to establish. The degradation of HMW PAHs seemed to require more complex mechanisms such as the actions of bacteria/fungi consortia, metabolisms implying cytochrome P450 or global PAH degradation potential as shown by metagenomic inference. It appeared that the bacteria involved in the degradation of LMW PAHs were also globally different from those involved in the degradation of HMW PAHs. The fate of PAHs after a recent contamination seemed to be essentially controlled by soil microbiology in the first months of the process.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2018.07.088.

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