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Evidence for shifts in the structure and abundance of the microbial community in a long-term PCB-contaminated soil under bioremediation

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ABSTRACT

Although the impact of bioremediation of PCB-contaminated sites on the indigenous microbial community is a key question for soil restoration, it remains poorly understood. Therefore, a small-scale bioremediation assay made of (a) a biostimulation treatment with carvone, soya lecithin and xylose and (b) two bioaugmentation treatments, one with a TSZ7 mixed culture and another with a *Rhodococcus* sp. Z6 pure strain was set up. Changes in the structure of the global soil microbial community and in the abundances of different taxonomic phyla were monitored using ribosomal intergenic spacer analysis (RISA) and real-time PCR. After an 18-month treatment, the structure of the bacterial community in the bioremediated soils was significantly different from that of the native soil. The shift observed in the bacterial community structure using RISA analysis was in accordance with the monitored changes in the abundances of 11 targeted phyla and classes. *Actinobacteria, Bacteriodetes* and α- and γ-*Proteobacteria* were more abundant under all three bioremediation treatments, with *Actinobacteria* representing the dominant phylum. Altogether, our results indicate that bioremediation of PCB-contaminated soil induces significant changes in the structure and abundance of the total microbial community, which must be addressed to implement bioremediation practices in order to restore soil functions.

1. Introduction

Polychlorinated biphenyls (PCBs) comprise a group of particularly persistent pollutants. They are considered as one of the most widely distributed class of chlorinated chemicals in the food chains, released into the environment by inappropriate use, improper disposal or accidental leakages [1,2]. Even though their production was banned more than 40 years ago they are still found in different compartments of the environment, causing serious threats to human health. In addition, by polluting the soil ecological system, they can seriously affect the capacity of the soil to perform its primary functions [3,4]. The physicochemical properties of PCBs make their biodegradation a wide-scale challenge. As a consequence, an important number of studies have focused on PCB degradation, and various microbial strains able to transform PCBs have been isolated [1,5–9]. Different studies have aimed at using microorganisms for removing PCBs from contaminated sites, which is considered as a potentially simple, economically and environmentally friendly bioremediation strategy [6,10,11]. Up to now, their focus has been set on: (i) the efficiency of the bioremediation process by monitoring PCB-disappearance, (ii) the description of active PCB-degrading bacterial populations by using cultivation-based approaches and, more recently, (iii) the monitoring of the PCB-degrading ability of the soil microflora by determining the occurrence of *bph* catabolic genes in the soil [12–18]. Undoubtedly microorganisms play fundamental roles in different soil ecosystemic services such as nutrient cycling, filtering, organic matter decomposition and climate regulation [19–21]. In this context, estimating the impact of bioremediation on the structure, composition and abundance of the soil microbial communities arises as a major issue [22–25].

However, although the effect of PCB-contamination on the soil microbial community has been investigated repeatedly [26–30], the impact of PCB-contaminated soil bioremediation on the microbial community has scarcely been monitored. The former studies, in which short-term, artificially contaminated soil was used, provide evidence that bioaugmentation of soils with PCB-degraders, biostimulation by the addition of inducers and rhizoremediation can lead to changes in the bacterial community structure [18,31–34]. However, to improve bioremediation practices, it is important to understand how the microbial community responds to bioremediation in less artificial systems such as long-term PCB-contaminated sites.

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In order to assess the impact of bioremediation on the soil microbial community, a small-scale bioremediation assay was designed to bioremediate the soil of a transformer station contaminated with PCBs since the 1991 Balkanian war events [35]. A previous study at the site had shown that biostimulation as well as bioaugmentation approaches resulted in the (i) degradation of 40% of the PCBs from the soil within a 1-year period and (ii) modification in the structure and the abundance of the functional PCB-degrading community [35]. The objective of this work was to monitor the response of the total bacterial community to three different bioremediation treatments in this PCB-contaminated soil. For this purpose, the structure of the bacterial community was monitored by ribosomal intergenic spacer analysis (RISA) while the abundances of 10 bacterial phyla and classes as well as of the total bacteria and crenarchaea were quantified by real-time PCR (qPCR) [36–38] after an 18-month bioremediation period.

2. Materials and methods

2.1. Bioremediation assay design

The soil used here was composed of 51.4% clay, 30.6% silt, 18.0% sand, 39.0 g kg^{-1} of organic matter, 22.6 g kg^{-1} of organic carbon, 2.1 g kg^{-1} of total nitrogen, with a C/N ratio of 11 and a pH value of 7.2. It was contaminated with up to 32 μg of PCB per gram of soil [35]. A large amount of soil (approx. 500 kg) was excavated with a shovel from a 10 m × 4 m plot (down to a 30-cm depth) alongside a transformer station damaged in 1991 during a war event (Zadar, Croatia). The soil was prepared for the assay by repeated sieving on a 4-mm mesh and manual mixing. The soil was placed in three plastic containers (0.84 × 0.41 × 0.16 m, approximately 90 kg per container). Each container was divided into two compartments, and submitted to three different bioremediation treatments: (a) bioaugmentation with a mixed culture (BAM, inoculation with TSZ7 culture along with mineral medium containing xylose (1 g l^{-1}) as a supplemental carbon source, carvone (100 mg l^{-1}) as an inducer of the PCB-degrading pathway and soya lecithin (5 g l^{-1}) as a surfactant to enhance PCB bioavailability), (b) bioaugmentation with a pure strain culture (BAP, inoculation with *Rhodococcus* sp. Z6 strain along with mineral medium containing xylose, carvone and soya lecithin) and (c) biostimulation (BS, treatment with a mineral medium containing xylose, carvone and soya lecithin). Inoculums were prepared by growing cultures at 30 °C under agitation in a phosphate-buffered mineral salt medium supplemented with biphenyl. The amendments were supplied every two weeks over an 18-month period. A detailed experimental design is described in [35], together with detailed instructions about how to prepare the inoculating cultures. GC–MS analysis revealed that by the end of the 18-month-long bioremediation period approximately 40% of total PCBs, representing mainly tri- and tetra-chlorinated congeners, had been degraded from the contaminated soil with all three bioremediation treatments. The monitoring of physicochemical parameters suggested continuous pH and no extreme changes in the soil moisture throughout the bioremediation treatment.

2.2. Extraction and purification of total DNA from the soil

At the end of the 18-month-long incubation period soil samples were collected from the three containers ($n = 3$ per treatment, $n_{tot} = 9$). A composite soil sample (approximately 1 kg) made of 5 random samplings (0–10-cm depth layer) was collected on the contaminated site, subsampled. and used as control soil ($n = 3$). Samples were kept at −20 °C until use ($n_{tot} = 12$). Total DNA was extracted following ISO 11063 [39] and soil DNA extracts were then purified using polyvinyl polypyrrolidone (PVPP) and Sepharose 4B spin columns (Sigma–Aldrich, USA) and the Geneclean Turbo Kit purification kit (Qbiogen, France) according to [35]. The integrity of soil DNA was checked by electrophoresis (1% agarose gel) and its amount was estimated using a BioPhotometer at 260 nm (Eppendorf, Germany).

2.3. Inhibition test

The absence of inhibitors in our soil extracts was verified for all samples by mixing a known amount of the plasmid pGEM-T Easy Vector (Promega, France) with the soil DNA extracts or water before running a qPCR with plasmid-specific T7 and SP6 primers as previously described [40]. The measured cycle threshold (Ct) values obtained for the different DNA extracts and for the water controls were not significantly different, indicating that no inhibition occurred.

2.4. Ribosomal intergenic spacer analysis

The global structure of the soil bacterial community was investigated using Ribosomal Intergenic Spacer Analysis (RISA). The 16S-23S intergenic spacer of the bacterial ribosomal operon was amplified from 25 ng of the DNA template using universal primers 38_r and 72_f [41]. PCRs were carried out in a PTC-200 gradient cycler (MJ Research, USA) under the following conditions: 5 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 2 min at 72 °C, followed by a 15 min cycle at 72 °C. The resulting amplicons were quantified on native agarose gels. For each sample approximately 100 ng of amplicon were loaded on a 6% acrylamide gel (16 h, 8 mA). The gels were then stained with SYBR green II (Molecular Probes, Netherlands) and scanned with a Storm 960 Phosphor Imager (Molecular Dynamisc, Sunnyvale, CA, USA).

2.5. Real-time PCR quantification (qPCR)

The abundances of the total bacteria and crenarchaea were quantified by real time PCR (qPCR) as previously described. Taxa-specific 16S rRNA primers were used for quantification of the *Actinobacteria, Acidobacteria,* α-*Proteobacteria*, β-*Proteobacteria*, γ-*Proteobacteria*, *Bacteriodetes*, *Firmicutes*, *Gemmatimonadetes*, *Verrucomicrobia, Planctomycetes* and Archaea – *Crenarchaeota* by real-time PCR [36,37,42–44]. qPCR assays were conducted on an ABI 7900 HT Real-time PCR System (Applied Biosystems, USA) in 15 μl final volume containing SYBR green PCR Master Mix (Absolute QPCR SYBR Green Rox Abgene, France), 250 ng of T4 gp32 (Qbiogene, France), 1 μM of each primer and 2 ng of template DNA. For each 16S rRNA target, a standard curve was established using serial dilutions of linearized plasmid pGEM-T (10^2 to 10^7 copies) containing cloned 16S rRNA. No-template controls (NTC, $n = 2$) were also included in all the assays. Melting curves were generated after amplification in order to check the specificity of the assays.

2.6. Statistical analysis

The significance of differences between the data obtained with the three different bioremediation treatments was tested using XLStat 2009 (Addinsoft, Brooklyn, USA). Based on the normality test showing that our data were not following a normal distribution, we chose to analyse our results with the non-parametric Kruskal–Wallis test ($p < 0.05$). According to the test requirements, all data points were independent from each other and sample sizes were equal with three data points analysed for each treatment.

RISA fingerprints were analysed using PrepRISA [45] and ADE-4 package [46]. Using PrepRISA, the data from the 1D-Scan (ScienceTec, France) were converted into a matrix summarizing the

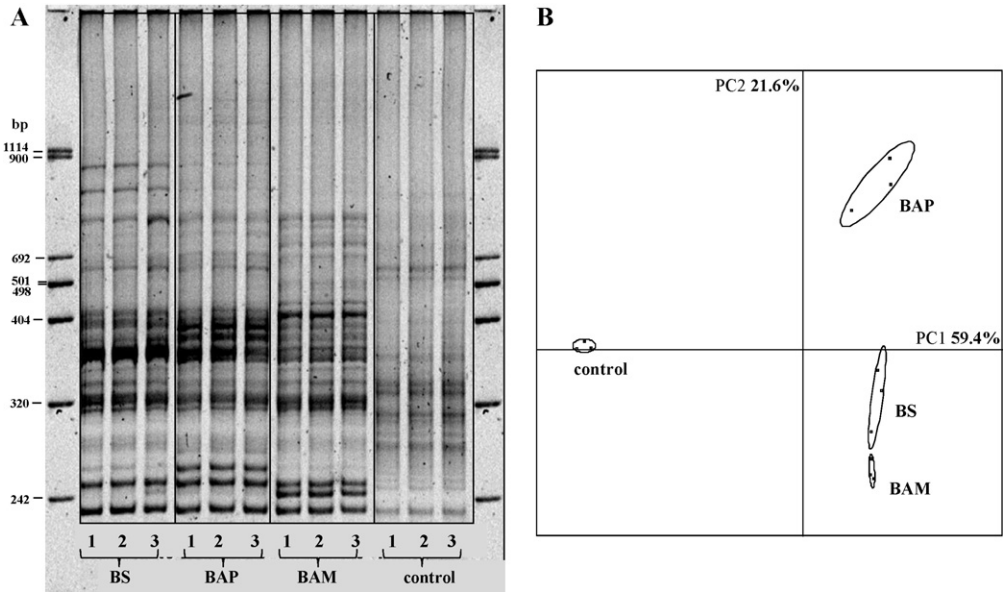

Fig. 1. RISA fingerprint (6%, acrylamid stained with SYBR green II) obtained from DNA extracted directly from soils under bioremediation treatments (BS, BAP and BAM) and control soil ($n = 3$ for each soil, $n_{tot} = 12$). The size of molecular marker BVIII 19–1114 bp (Roche, USA) is shown on the right side of the gel (A). Principal component analysis performed from RISA fingerprints obtained from DNA extracted from soils under bioremediation treatments (BS, BAP and BAM) and control soil ($n = 3$ for each soil, $n_{tot} = 12$) (B). *BAM*: bioaugmentation with mixed culture TSZ7, xylose, carvone and soya lecithin; *BAP*: bioaugmentation with *Rhodococcus* sp. Z6, xylose, carvone and soya lecithin; *BS*: biostimulation with xylose, carvone and soya lecithin.

bands' presence (i.e. peaks) and intensity (i.e. peak heights). Then, using ADE-4, principal component analysis (PCA) on the covariance matrix was performed. This method provided an ordination of bacterial communities and of the encoded bands, which were plotted in two dimensions based on the scores in the first principal components. Three data points were analysed for each treatment ($n_{tot} = 12$, per marker tested).

3. Results

3.1. Global structure of microbial communities in soils under different bioremediation treatments

RISA, revealing the length polymorphism of the intergenic spacer of the bacterial 16S rRNA operon, was used to estimate the impact of bioremediation treatments (BAM, BAP, and BS) on the global structure of the soil microbial community. Visual examination revealed that RISA fingerprints, which revealed up to 20 major bands, were well replicated for each treatment, revealing that DNA extraction and PCR amplification from the different treatments were efficient and reproducible. Interestingly, the structure of the communities from BAM, BAP, BS and control soils differed as to their numbers (19, 18, 16 and 13 in BAM, BAP, BS and control, respectively) and as to the relative intensities of the detected bands (Fig. 1A). For comparison analysis, RISA fingerprints were digitized and further analysed by pairwise comparison using Principal Component Analysis (PCA) in order to ordinate the microbial communities on the plane defined by the first two principal components, in accordance to the bioremediation treatment applied (Fig. 1B). The first principal component (PC1) represented 59.4% of the variances in the data while the second principal component (PC2) represented 21.6%. Analysis of the factorial map revealed that ordination along PC1 allowed us to distinguish control from BS/BAM /BAP treatments. We could not differentiate between bioremediation treatments on PC1 but ordination on PC2 showed a clear separation of these three bioremediation treatments.

3.2. Abundance of the total bacterial community and 11 targeted phyla in our soils

The qPCR values were expressed as copy numbers per ng of soil DNA to minimize the possible bias related to the DNA extraction yield. All targeted 16S rRNA genes were successfully amplified from BAM, BAP, BS and from control soil with PCR efficiencies ranging between 81% and 100% except for the crenarchaea for which a lower efficiency of 64% was observed (Table 1).

Table 1
Primer pairs used for the qPCR assays to estimate the abundances of phyla and class-specific bacteria.

Target group	Primers	Amplicon size (bp)	Annealing T (°C)	PCR efficiency (%)	Reference
All groups	341F/534R	194	60	93	[43]
Acidobacteria	Acid31/Eub518	500	55	94	[36]
Actinobacteria	Actino235/Eub518	300	60	90	[36]
α-*Proteobacteria*	Eub338/Alf685	342	60	100	[36]
β-*Proteobacteria*	Eub338/Bet680	360	55	96	[36]
γ-*Proteobacteria*	Gamma395f/Gamma 871r	497	56	81	[42]
Bacteroidetes	Cbf319/Eub518	210	60	95	[36]
Firmicutes	Lgc353/Eub518	181	55	95	[36]
Gemmatimonadetes	Gem440/534R	461	55	96	[37]
Verrucomicrobia	Ver349/Eub518	186	60	87	[37]
Planctomycetes	Plancto352f/Plancto920r	565	60	81	[42]
Crenarchaea	771F/957R	228	55	64	[44]

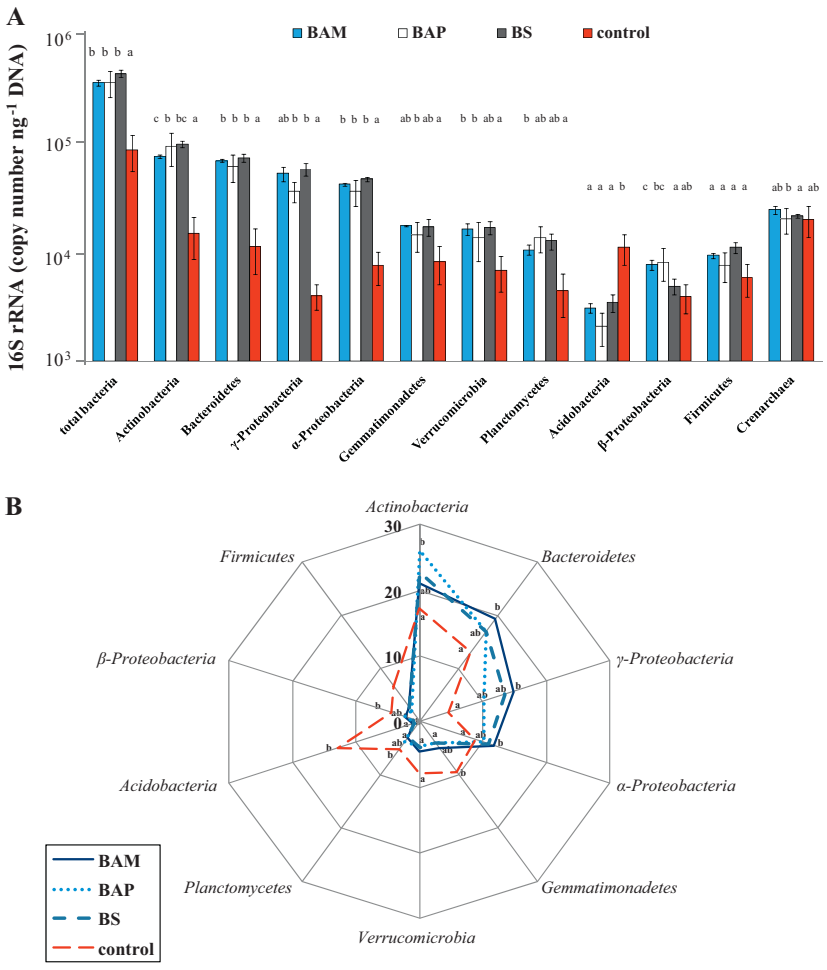

Fig. 2. 16S rRNA sequences copy number of total and targeted bacterial taxonomic groups (*Actinobacteria, Acidobacteria, α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, Bacteriodetes, Firmicutes, Gemmatimonadetes, Verrucomicrobia, and Planctomycetes*) and *Archaea* (*Crenarchaeota*) determined by qPCR in soils under different bioremediation treatments (BAM, BAP, and BS) and in control soil (A). Relative abundances of targeted bacterial taxonomic groups within the total bacterial community determined by qPCR in soils under different bioremediation treatments (BAM, BAP, and BS) and in control soil, presented in percentages (%) (B). BAM = mixed culture TSZ7 + xylose + carvone + soya lecithin; BAP = *Rhodococcus* sp. Z6 culture + xylose + carvone + soya lecithin; BS = xylose + carvone + soya lecithin. Error bars represent standard deviation of a mean value ($n = 3$ for each soil, $n_{tot} = 12$). Letters (a, b, c) assigned to each value represent groups appointed by the Kruskal–Wallis statistical analysis ($p < 0.05$). Values in the same group are not significantly different from each other.

No-template negative controls yielded negligible values in all qPCR assays.

The copy numbers of the total 16S rRNA genes ranged from 8.5×10^4 to 4.3×10^5 genes per ng of DNA extracted from the different soils, with a significantly lower abundance of the total bacteria in the control soil as compared to the bioremediated soils (Fig. 2A).

The abundances of the different bacterial taxa ranged from 3.4×10^3 to 8.5×10^4 16S rRNA gene copies per ng DNA. Lower abundances were observed in the control as compared to BAM, BAP and BS treatments for most taxa. However differences were significant only for the *Actinobacteria, Bacteriodetes* and *α-Proteobacteria*. On the contrary, a significantly higher abundance of *Acidobacteria* was found in the control soil compared to BAM, BAP and BS treatments while the abundance of the *Firmicutes* did not significantly differ between treatments (Fig. 2A).

For further insights into the composition of the bacterial community, we calculated the relative abundances of the different phyla and classes within the total bacterial community (Fig. 2B). We found that the ten targeted groups represented 79–90% of the abundance of the total microbial community, depending on the treatment. *Actinobacteria* and *Bacteroides* were the dominant phyla in bioremediated soils with relative abundances of 23% and 17% while they represented 17% and 13% of the community in the control soil, respectively. γ- and α-*Proteobacteria* were less abundant with an average 12% (bioremediated soils) and 7% (control soil) of the bacterial community. Other groups were less abundant and represented less than 5% of the total community. Interestingly, the abundance of those groups was higher in the control than in the bioremediated soil. This trend was particularly clear for *Acidobacteria* which represented less than 1% in bioremediated soils while it averaged 12% in the control soil.

4. Discussion

In the year 1991, a PCB-filled transformer station situated in Zadar (Croatia) was damaged during a military attack, and as a consequence the surrounding area was contaminated with substantial amounts of PCBs [47]. In order to restore this contaminated site a series of experiments were conducted. Among these, a small-scale assay was designed to test the efficiency of different bioremediation strategies on PCB degradation from the soil. A TSZ7 mixed bacterial culture and the *Rhodococcus* sp. Z6 strain, enriched on biphenyl from the contaminated soil, were used as seed cultures [48]. Our results suggest that all three bioremediation treatments were efficient, as they led to the degradation of up to 40% of PCBs from our contaminated soil over an 18-month period. To get a better

insight into the biological processes that accompany PCB degradation, we decided to study the structure, the composition and the abundance of the bacterial community in response to our bioremediation strategies of the site with molecular tools based on direct soil DNA extraction.

An insight into the structure of the bacterial community was achieved by RISA. The analysis revealed that after an 18-month-long treatment the bacterial communities in the BAM, BAP and BS bioremediation treatments were significantly different from that of the control soil. This indicated that bioremediation resulted in a shift in the structure of the total bacterial community, suggesting the development of communities in the bioremediated soils that differed from the original one from the contaminated site. Interestingly, we noticed that the PCB-contaminated site was characterized by a bacterial community made of fewer phylotypes (13 phylotypes, compressed between 250 and 340 bp), which is a typical feature of stress environments [49] and could result from PCB toxicity to living cells [50]. Conversely, bioremediated soils were characterized by an increase in the number of phylotypes (16, 18 and 19 phylotypes in BS, BAM and BAP, respectively, with sizes up to 880 bp), suggesting modifications in the soil bacterial community composition, with several dominating phylotypes.

All bioremediation treatments were biostimulated by carvone to induce the synthesis of the PCB-degrading enzyme system, and by soya lecithin as a surfactant to promote PCB bioavailability. Besides their expected effect, the amendments may also have been used as nutrient sources allowing the growth of opportunistic microbial populations [51]. Moreover, they may have had negative effects on some bacterial populations, such as suggested by some authors [52]. Consequently, they may lead to changes in the structure of the global microbial communities. As shown previously, the availability of carbon substrates in the highly oligotrophic soil environment can greatly modify the structure of microbial communities by favouring the growth of r-strategist microbial groups [33,53]. This could explain the shift in the structure of the bacterial community observed between control and bioremediated soils along the first principal component (first axis). However, the differentiation between biostimulated and bioaugmented soils on axis two of the PCA suggested that inoculation with PCB degraders also has an effect on the structure of the bacterial community. Even though the inoculated *Rhodococcus* strain can survive in the soil and be detected throughout the 18-month-long assay [35], it cannot fully explain the differences observed on the RISA profiles due to its low abundance (i.e. less than 1% of the total bacterial community). Nevertheless, it should be emphasized that the differences among all three bioremediated soils were small compared to those observed between control and bioremediated soils. Altogether, these results show that bioremediation treatments affect the structure of the soil bacterial community.

Interestingly, we observed that even if the different treatments led to the establishment of bacterial communities differing in their structure, their overall performance was similar with approximately 40% depletion of PCBs [35]. This might be explained by the fact that RISA only gave an insight into the abundant populations within the total bacterial community but not into the functional community responsible for PCB degradation. Indeed we previously showed that the PCB-degrading potential quantified by targeting *bphA* genes was increased in a similar way by the three bioremediation treatments [35]. Therefore the efficiency of the bioremediation treatment most likely resulted from the increase in the abundance of this functional community in combination with the increase in PCB bioavailability.

RISA was used to get a first insight into the impact of bioremediation on the structure of the total soil bacterial community. Although this approach was shown to be of interest to observe shifts in the bacterial community structure in response to different stresses, it does not allow us to monitor the changes in the taxonomic composition of the community. Therefore, the composition of the microbial communities was also studied by quantifying the relative abundances of the ten bacterial (*Actinobacteria, Acidobacteria, α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, Bacteriodetes, Firmicutes, Gemmatimonadetes, Verrucomicrobia, and Planctomycetes*) and one Archaea (*Crenarchaeota*) taxa. According to the meta-analysis of 32 16S rRNA gene libraries from a variety of soils [54] these groups represent up to 90% of the soil bacterial community. Our qPCR results showed that the bacterial communities responded similarly to biostimulation and bioaugmentation approaches, which is in accordance with our RISA findings.

We observed that *Actinobacteria*, *Bacteriodetes* and *Acidobacteria* dominated in the control transformer station soil in which elevated concentrations of PCBs up to 32 $\mu g\, g^{-1}$ soil were found, while *Firmicutes* and *Planctomycetes* were lowly represented. Even though abundances of different taxa were shown to vary between different soils and are closely related to soil characteristics, these results are in an agreement with recent studies showing that these taxa were also dominant in other soils [38,54–56]. Interestingly, while *Gemmatimonadetes* are usually considered one of the less numerous taxa [54,56], they were quite abundant in our control soil in which they represented approximately 10% of the community. This suggests that this group has been favoured by exposure to PCB at the contaminated site but the effect of other environmental conditions at the PCB-contaminated site cannot be ruled out.

The decline in the soil PCB content observed at the end of the 18-month small-scale bioremediation assay suggests that bioremediation could have induced the shift observed in the community structure. This significant degradation of PCB can therefore be correlated with the selection of specific bacterial groups in soil under bioremediation. For example, the abundance of *Actinobacteria* and *Bacteroidetes* as well as α- and γ-*Proteobacteria* increased up to threefold as a result of the bioremediation treatment compared to the control soil. Even though changes in the microbial community structure during PCB degradation have seldom been monitored, a few studies record higher abundances of *Proteobacteria,* Gram-positive bacteria, and *Actinobacteria* along with PCB degradation [26,31,32]. This can be explained by the fact that these groups contain well-known PCB degraders (*Rhodococcus, Arthrobacter, Corynebacterium, Sphingomonas, Pseudomonas, Acinetobacter, etc.*). However, *Bacteroidetes* have not been known to respond to PCBs so far. Different studies correlate the predominance of *Bacteroidetes* with agricultural practices such as C amendments [54,55,57]. It can be hypothesized that the selection of these phyla might be due to the alteration of soil characteristics as a consequence of bioremediation treatments. Interestingly, our bioremediation treatments led to a decrease in the proportion of most of the targeted taxa, with the highest decrease observed for *Acidobacteria* (tenfold), which represented less than 1% of the overall community at the end of bioremediation. The microbial communities developed under our three different bioremediation treatments had similar structures when compared at higher taxonomical ranks. This could further be correlated with the similar PCB-degrading activity observed in all three bioremediated soils. Overall we found that the bioremediation treatments in our small-scale assay resulted in significant shifts in the soil microbial community at high taxonomical ranks, which might reflect the ecological coherence of the targeted phyla and classes [58].

Even though it is difficult to determine the functional role of the targeted taxa, the predominance of the *Actinobacteria* phylum in all three bioremediated soils (>20% of the total community) suggests that it might be of importance in the PCB degradation process. Indeed, the ability of bacterial populations belonging to the *Rhodococcus* genus to degrade xenobiotics is well documented, along with their ability to persist in soils even in starvation

conditions [59,60]. This hypothesis is further supported by the fact that *Rhodococcus*-like bacterial populations, belonging to the *Actinobacteria* phylum and harbouring *bphA* and *bphC* catabolic genes for PCB-degradation, were promoted in response to bioremediation treatments at the same site [35]. The addition of the particular inducer of PCB-catabolic enzymes [61], along with the presence of hydrophobic cell walls and production of surfactants may have led to the development of this *Rhodococcus* bacterial population which appears to be more competitive for PCBs. As reported by [33] the choice of particular amendments combined with habitat characteristics has a strong enrichment effect on certain bacterial populations, thereby affecting bacterial community composition along with the pattern of PCB degradation.

5. Conclusions

Our small-scale bioremediation assay revealed that bioremediation can significantly modify the structure of the total bacterial community as well as the abundance of the targeted bacterial taxa. Indeed, the microbial community at the contaminated transformer station site was significantly different from the ones present in the three bioremediation treatments. However, despite differences in the bioremediation strategy that was used (i.e. biostimulation or bioaugmentation), similar responses of the microbial community were observed. Bioremediation was also shown to stimulate the abundances of *Actinobacteria, Bacteroidetes* and α- and γ-*Proteobacteria* phyla but had a negative impact on the abundance of *Acidobacteria*. The predominance of the *Actinobacteria* phylum in bioremediated soils suggested their relevance in the PCB degradation process. Since microorganisms are key players in crucial soil functions, further research is required to assess whether the changes we observed in microbial community structures in response to bioremediation also altered the functioning of the bioremediated soil.

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