RESEARCH LETTER

Changes in soil *Acidobacteria* communities after 2,4, 6-trinitrotoluene contamination

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Abstract

Despite their widespread occurrence in soils, the ecology of *Acidobacteria* and their response to environmental perturbations due to human activities remain very poorly documented. This study was aimed at assessing changes in the diversity and abundance of *Acidobacteria* in soils contaminated with 2,4,6-trinitrotoluene (TNT) compared with nonpolluted soils. The analysis of *Acidobacteria* communities at two sites with long-term and short-term contamination revealed that TNT has a drastic impact on the relative abundance of *Acidobacteria* in soil bacterial 16S rRNA gene libraries. The disappearance of most *Acidobacteria* from these soils was concomitant with a shift in *Acidobacteria* community composition and a loss of diversity, although the extent of diversity erosion depended on the sampling site.

Introduction

Since the late 1990s, molecular ecological surveys based on 16S rRNA gene sequences have revealed the widespread occurrence of the phylum *Acidobacteria* in a variety of ecosystems (Hugenholtz *et al.*, 1998; Barns *et al.*, 1999) and especially in soils, where it represents the second most abundant phylum after the *Proteobacteria* (Janssen, 2006). Despite recent successful attempts to cultivate some *Acidobacteria* (Janssen *et al.*, 2002; Sait *et al.*, 2002; Joseph *et al.*, 2003; Stevenson *et al.*, 2004; Davis *et al.*, 2005), their ecology and physiology remain very poorly characterized, and available information is derived mostly from 16S/23S rRNA gene surveys. Moreover, although knowledge on their phylogenetic breadth is continuously expanding with the accumulation of environmental data and the discovery of new subgroups (Zimmermann *et al.*, 2005; Barns *et al.*, 2007), the census of *Acidobacteria* is far from complete (Schloss & Handelsman, 2004).

In this context, information on the response of *Acidobacteria* to anthropogenic environmental perturbations remains

fragmented. Several reports detail how soil microbial communities, including *Acidobacteria*, respond to agroecosystem and forest management practices (Nüsslein & Tiedje, 1999; Chow *et al.*, 2002; Buckley & Schmidt, 2003; Sun *et al.*, 2004), but there are few and often divergent data regarding the effect of pollutants on the phylum. *Acidobacteria* are probably sensitive to various pollutants, as their presence is rarely mentioned among the dominant microbial taxa of contaminated sites. In some cases, their disappearance due to contamination has been directly observed, for example, in a soil treated for 10 years with phenylurea herbicides (El Fantroussi *et al.*, 1999) or at a hydrocarbon-contaminated site in Antarctica (Saul *et al.*, 2005). Conversely, *Acidobacteria* were reported to represent a significant proportion of the bacteria in benthic microbial mats exposed to petroleum compounds (Abed *et al.*, 2002), in parathion/methyl parathion- or PCB-contaminated soils (Nogales *et al.*, 1999; Debarati *et al.*, 2006), in radionuclide and metal-contaminated subsurface sediments (Barns *et al.*, 2007) and in metal-contaminated soils (Ellis *et al.*, 2003; Gremion *et al.*, 2003). Clearly, additional studies are necessary to get a more complete picture of how *Acidobacteria* respond to diverse environmental pollutants.

Our objective was to investigate the effect of 2,4,6-trinitrotoluene (TNT) on the abundance and diversity of *Acidobacteria* in soils. This nitroaromatic explosive and its reduced byproducts are highly stable, poorly soluble in water and toxic against many higher organisms. More recently, TNT has been shown to affect a variable extent total soil microbial communities as well, resulting in dominant populations of *Pseudomonadaceae* and *Xanthomonadaceae* (e.g. George *et al.*, 2008). However, these studies were based on cultivation or fingerprinting techniques with general bacterial primers and they did not allow a thorough investigation of total species richness in the soils analyzed. Our strategy here was to focus solely on the phylum *Acidobacteria* and to analyze its response to TNT contamination at two sites (Bourges, France, and El Gordo, Spain) with strongly contrasting pH (alkaline vs. mildly acidic soils), levels of TNT contamination (high vs. low TNT concentration), and history of exposure (long-term vs. short-term contamination). To our knowledge, this study represents one of the first investigations of the fate of *Acidobacteria* in human-impacted environments.

Materials and methods

Soil characteristics

Two contrasting series of soil samples were investigated. Their physico-chemical characteristics and their concentrations in TNT and TNT metabolites have been described in detail by George *et al.* (2008). The first series (samples 'TNT-F1' to 'TNT-F8', named 'KL2', 'KL1', 'F3', 'KX1', 'KF1', 'KF2', 'KF5' and 'KF6' in George *et al.*, 2008) was collected at a site in Bourges, France, that had been used for TNT destruction over the past 20 years. At the same site, uncontaminated soil samples (samples 'noTNT-F1' to 'noTNT-F4', named 'REF', 'KF4', 'F1' and 'F2' in George *et al.*, 2008) were collected as well. A second series of soil samples was provided by Fabricaciones Extremeñas S.L. from a site located at El Gordo, Cáceres, Spain, that had been artificially contaminated with TNT for 7 months. At this site, six soil samples were collected from TNT-contaminated plots (samples 'TNT-S1' to 'TNT-S6', named 'J29', 'IN', 'UEE', 'C15', 'J15' and 'C29' in George *et al.*, 2008) and one sample ('noTNT-S1', named 'OUT' in George *et al.*, 2008) from an uncontaminated plot. All samples were collected from the top 10-cm layer of soil, homogenized and sieved (2-mm mesh) before analysis. In a previous study (George *et al.*, 2008), the total and culturable bacterial communities of these soils were analyzed by PCR amplification of soil DNA (or plate-wash DNA for analysis of the culturable community) using general bacterial primers and denaturing gradient gel electrophoresis (DGGE).

DNA extraction

Soil DNA was extracted using the Ultra-Clean Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer's instructions. The quality and quantity of extracted DNA were evaluated on 1.5% agarose gels stained with ethidium bromide (EtBr). Quantification was performed using the IMAGE J software (available at http://rsb.info.nih.gov/ij/).

Construction of bacterial 16S rRNA gene libraries and calculation of the percentage of *Acidobacteria*

16S rRNA genes from soils 'noTNT-F1', 'noTNT-F2', 'noTNT-S1', 'TNT-F1', 'TNT-S1' and 'TNT-S2' were amplified using bacterial primers 11F (Kane *et al.*, 1993) and 1492R (Lane, 1991). The PCR mix (50 μL) contained 0.2 mM dNTPs, 0.25 μM of each primer, 10 μL of 5 × Green GoTaq Flexi Buffer (Promega, Madison, WI), 1.5 mM $MgCl_2$, 1.5 U of GoTaq DNA Polymerase (Promega) and *c.* 2–10 ng of target DNA. PCR was performed using a Biometra TGradient cycler (Biometra, Goettingen, Germany) under the following conditions: 94 °C for 4 min, 25 cycles (94 °C for 1 min, 52 °C for 1 min, 72 °C for 2 min) and a final extension at 72 °C for 10 min. PCR products were cloned into the pGem-T vector (Promega). The insert size was checked by PCR amplification with the M13F/M13R primer set following the protocol described above (with an annealing temperature of 55 °C), until 100 clones with the right insert size were obtained for each soil. A second PCR

round was performed with the *Acidobacteria*-specific primer ACID31F (Barns *et al.*, 1999) and primer 1492R. ACID31F is the only *Acidobacteria* phylum-specific primer published so far, although recent data suggest that it does not amplify some members of subdivisions 7 and 8 (Sait *et al.*, 2006) and the new subdivisions described by Barns *et al.* (2007). The number of *Acidobacteria*-positive inserts in 100 clones represented the percentage of *Acidobacteria* in the pool of 16S rRNA genes from the original soils.

DGGE analysis of *Acidobacteria* 16S rRNA genes

Acidobacteria 16S rRNA genes were amplified using primer ACID31F and the bacterial primer 518R (Muyzer *et al.*, 1993), with a GC-clamp (CGCCCGGGGCGCGCCCCGGG CGGGGCGGGGGCACGGGGGG) attached to the R primer ('518R-GC'). The PCR mix (50 μL) was the same as mentioned above, but with 0.8 μM of each primer, 2 mM $MgCl_2$, 2.5 U of GoTaq DNA Polymerase and *c.* 2–20 ng of target DNA. A touchdown PCR was performed under the following conditions: 94 °C for 6 min 30 s, 14 cycles [94 °C for 1 min, 65–52 °C for 1 min (decrease by 1 °C every cycle), 72 °C for 1 min], 20 cycles (94 °C for 1 min, 52 °C for 1 min, 72 °C for 1 min) and a final extension at 72 °C for 10 min. DGGE analysis was carried out with a DCode universal mutation detection system (Bio-Rad, Hercules, CA). PCR products were loaded onto 6% (w/v) polyacrylamide (37.5 : 1 acrylamide : bisacrylamide) gels with a linear denaturing gradient from 40% to 70% in 1 × TAE buffer [40 mM Tris base, 20 mM acetate, 1 mM EDTA (pH 8)]. The electrophoresis was run at 60 °C and 60 V for 17 h. The gel was stained with EtBr and photographed under a UV transilluminator. The DGGE gel was analyzed using the GELCOMPAR II software package (Applied Maths, Sint-Martens-Latem, Belgium). The DGGE gel was normalized and the DGGE patterns were compared using the Pearson product–moment correlation coefficient and the unweighted-pair group method with arithmetic averages clustering algorithm.

Construction of *Acidobacteria* 16S rRNA gene libraries

Acidobacteria 16S rRNA genes from soils 'noTNT-F1', 'TNT-F1', 'noTNT-S1', 'TNT-S1' and 'TNT-S2' were amplified using primers ACID31F and 1492R. The PCR mix (50 μL) contained 25 μL Red'y'Star Mix (Eurogentec, Seraing, Belgium), 1 μM of each primer, and *c.* 2–15 ng of target DNA. PCR reactions were performed under the following conditions: 95 °C for 10 min, 30 cycles (94 °C for 1 min, 52 °C for 1 min, 72 °C for 2 min) and a final extension at 72 °C for 10 min. PCR products were cloned into the pGem-T vector. The insert size was checked by PCR with the M13F/M13R primer set until *c.* 100 clones with the right insert size were obtained for each soil. For each 16S rRNA gene library of *c.* 100 clones, 690 bp were sequenced at the 5′ end of the amplicons with primers M13F and 907R (Lane *et al.*, 1985) at the University of Wisconsin-Madison Biotechnology Center using the Big Dye Terminator V1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). Our choice to sequence more partial-length 16S rRNA genes rather than fewer full-length ones was aimed at providing more statistical power to the test ∫-LIBSHUFF (Schloss *et al.*, 2004). The resulting *Acidobacteria* partial 16S rRNA gene sequences were used as input data for phylogenetic analyses and statistical tests.

Statistical analysis of *Acidobacteria* 16S rRNA gene libraries using DOTUR and ∫-LIBSHUFF

The composition of the *Acidobacteria* 16S rRNA gene libraries was compared using the default configuration of the program ∫-LIBSHUFF (Schloss *et al.*, 2004). Multiple pairwise comparisons of the libraries were implemented based on a single input file (distance matrix containing the five sequence libraries). Sequences were assigned to operational taxonomic units (OTUs) using the furthest neighbor algorithm and the default configuration of the program DOTUR (Schloss & Handelsman, 2005). A distance level of 0.03 was chosen for the comparison of richness and diversity between the different soil libraries assuming that it corresponded to the 'species' level. Rarefaction curves (i.e. number of OTUs as a function of sampling effort) were calculated with a 95% confidence interval for each library.

Phylogenetic composition of *Acidobacteria* 16S rRNA gene libraries

Acidobacteria 16S rRNA gene sequences available in the RDP II database in November 2006 were imported into the ARB database (2004 version) and integrated in the existing alignment using the automated aligning tool of the ARB software package (Ludwig *et al.*, 2004) completed by a manual alignment. The topology of the *Acidobacteria* subtree was optimized using the ARB-parsimony ('global optimization') tool. The significance of the tree topology was tested by applying Maximum Parsimony and Maximum Likelihood methods. To construct the tree, only sequence positions that shared identical nucleotides in at least 50% of the *Acidobacteria* sequences were used. The *Acidobacteria* partial 16S rRNA genes (690 bp) generated from this study ($n = 509$) were then aligned against all the 16S rRNA gene sequences available in the updated ARB database using the automated aligning tool with final alignments edited manually. First, these sequences were inserted into the consensus tree using an ARB parsimony tool that does not affect the initial tree topology. The resulting tree was pruned for clarity of the visual representation. Second, Neighbor Joining (NJ) distance matrices were generated using the ARB

program package with the Jukes–Cantor correction for multiple substitutions and no filter. These NJ distance matrices were built for each clone library individually and for the five libraries pooled together. They were used as input files for the statistical programs described above.

Nucleotide sequence accession numbers

Sequences have been deposited in the GenBank database under accession numbers EU122442–EU122950.

Results and discussion

Percentage of *Acidobacteria* in 16S rRNA gene clone libraries from uncontaminated and TNT-contaminated soils

16S rRNA genes were amplified from six soils (three uncontaminated soils, three TNT-contaminated soils) with general primers (11F-1492R), and the clone libraries (100 clones each) were screened in search of *Acidobacteria* with specific primer ACID31F and universal primer 1492R. The percentage of *Acidobacteria* 16S rRNA genes in the clone libraries ranged between 8% and 31% in uncontaminated soils (mean = 19%; SD = 11.6; $n = 3$), which is consistent with numbers cited in the literature for uncontaminated soils (Janssen, 2006), and was significantly lower in the TNT-contaminated soils (value $\leq 1\%$; mean = 0.33%; SD = 0.58; $n = 3$) (one-tailed t test, $P = 0.0260$) (Table 1). Moreover, in a previous study based on the same soils, total bacterial numbers (DAPI-stained cells per gram of soil) were shown to be about 6.5 times lower in the TNT-contaminated soils than in the uncontaminated ones (George *et al.*, 2008). Therefore, as the percentage of *Acidobacteria* is lower in 16S rRNA gene libraries from TNT-contaminated soils *and* these soils harbor lower total bacterial numbers, it could be inferred that soil *Acidobacteria* numbers are negatively affected by the presence of TNT, even when the pollutant is in low concentration as in soil 'TNT-S1'. Such results reinforce the assumption that *Acidobacteria* communities are mostly sensitive to soil pollution by xenobiotics.

Table 1. Percentage of *Acidobacteria* in 16S rRNA gene libraries (100 clones each) from three uncontaminated soils and three TNT-contaminated soils

Soil	Concentration of TNT [g TNT kg^{-1} (dry wt) of soil]	% of *Acidobacteria*
noTNT-F1	Uncontaminated	17
noTNT-F2	Uncontaminated	31
noTNT-S1	Uncontaminated	8
TNT-S1	0.044	1
TNT-S2	1.57	< 1 (ND)*
TNT-F1	23.0	< 1 (ND)*

*ND, no *Acidobacteria* detected among 100 sequences.

DGGE patterns of *Acidobacteria* 16S rRNA gene fragments in TNT-contaminated and uncontaminated soils

Because of the low percentage of *Acidobacteria* in TNT-contaminated soils, PCR amplification of *Acidobacteria* 16S rRNA genes before DGGE analysis was particularly challenging in these soils, and only nine out of 14 TNT-contaminated soils gave consistent PCR amplicons for subsequent DGGE analysis. DGGE fingerprints of *Acidobacteria* 16S rRNA genes amplified from five uncontaminated and nine TNT-contaminated soils revealed a high diversity of *Acidobacteria* in all soils (Fig. 1). Unlike most uncontaminated soils, the DGGE fingerprints of the TNT-contaminated soils from El Gordo ('TNT-S1' to 'TNT-S6') showed the existence of some predominant *Acidobacteria* species in these soils. Despite some variability in the soil physicochemical characteristics at each sampling site (George *et al.*, 2008), *Acidobacteria* DGGE patterns of El Gordo and Bourges formed two distinct clusters. One exception was soil 'noTNT-S1' (the uncontaminated soil from El Gordo), which clustered with the soils from Bourges. Generally speaking, cluster analysis suggested that the diversity of *Acidobacteria* depended both on the contamination status of the soil and on the sampling site. This was later confirmed by detailed phylogenetic analyses of *Acidobacteria* 16S rRNA gene fragment libraries from two uncontaminated and three TNT-contaminated soils.

Phylogenetic analysis of *Acidobacteria* 16S rRNA gene libraries from uncontaminated soils and TNT-contaminated soils

Among the two soil series from Bourges and El Gordo, five soils were chosen for a detailed analysis of their composition

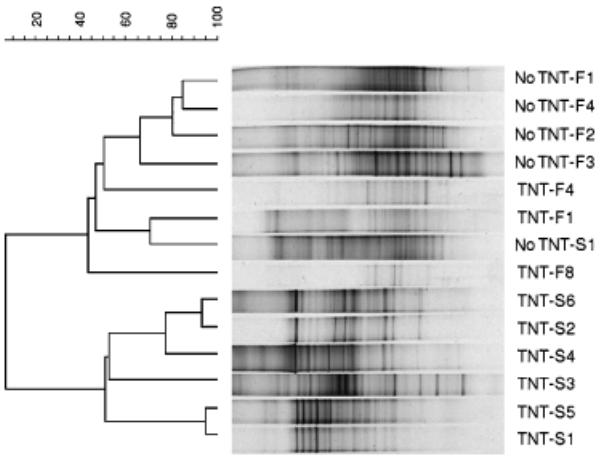

Fig. 1. Cluster analysis of DGGE fingerprints of *Acidobacteria* 16S rRNA gene fragments in uncontaminated and TNT-contaminated soils after PCR amplification with primers ACID31F and 518R-GC.

in *Acidobacteria* 16S rRNA genes: 'noTNT-F1' (uncontaminated soil from Bourges), 'TNT-F1' (TNT-contaminated soil from Bourges, 23.0 g TNT kg^{-1} of soil), 'noTNT-S1' (uncontaminated soil from El Gordo), 'TNT-S1' and 'TNT-S2' (TNT-contaminated soil from El Gordo, 0.044 and 1.57 g TNT kg^{-1} of soil, respectively). Clone libraries of *Acidobacteria* 16S rRNA genes were built from these soils and, respectively, 100, 95, 111, 102 and 100 clone inserts were partially sequenced (690 bp) and analyzed.

The great majority of sequences clustered into *Acidobacteria* subdivisions 1, 3, 4, 6 and to a minor extent in subdivision 5 (Fig. 2, Table 2). The two *Acidobacteria* clone libraries from the Bourges site contained sequences belonging mainly to subdivisions 4 and 6 (Table 2). The libraries from the mildly acidic site of El Gordo contained a moderate ('noTNT-S1') to a large ('TNT-S1' and 'TNT-S2') proportion of sequences belonging to subdivision 1, i.e. sequences that were barely present in the alkaline soils of Bourges (Table 2). These results confirm previous reports on the link between low pH and the presence of subdivision 1 *Acidobacteria* (Sait *et al.*, 2006; Eichorst *et al.*, 2007). The percentage of $OTU_{0.03}$ in the various subdivisions was slightly different from the distribution of sequences in the same subdivisions (Table 2). In soils 'noTNT-F1', 'TNT-F1' and 'noTNT-S1', the percentage of $OTU_{0.03}$ belonging to subdivision 4 was lower than the percentage of sequences belonging to subdivision 4, and the opposite trend was observed for subdivision 6. In other words, the number of sequences $>$ 97% similar was greater in subdivision 4 than in subdivision 6 in those soils. In all soils from El Gordo ('noTNT-S1', 'TNT-S1' and 'TNT-S2'), the percentage of $OTU_{0.03}$ belonging to subdivision 1 was lower than the percentage of sequences belonging to the same subdivision, revealing a higher redundancy of sequences in subdivision 1. Yet, it remained difficult to determine from these sole observations whether the structure and diversity of the five *Acidobacteria* libraries were statistically different or not. Therefore, we used the statistical programs ∫-LIBSHUFF and DOTUR (Schloss *et al.*, 2004; Schloss & Handelsman, 2005) to compare the libraries.

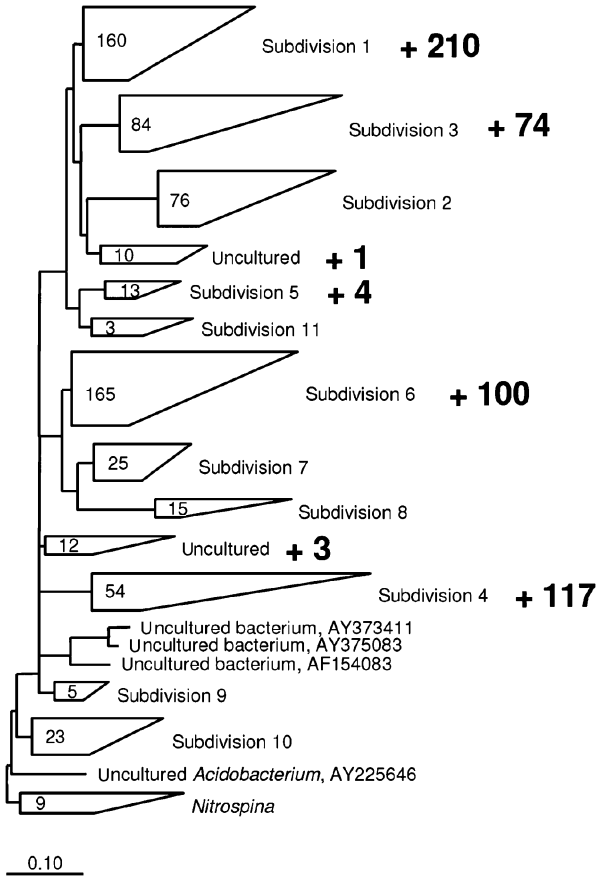

Fig. 2. Phylogenetic tree of the division *Acidobacteria*. This consensus tree was constructed using the ARB software package and a 16S rRNA gene sequence database containing all sequences included in the January 2004 ARB release, plus newly published *Acidobacteria* 16S rRNA gene sequences available in the RDP II database in November 2006. The tree was built with sequences $>$ 1400 bp. Their number in each subdivision is mentioned in the wedges. The depth of the wedge for each subdivision represents the branching depth of the sequences within the subdivision. The 509 *Acidobacteria* partial sequences from the present study were added (in bold, with a '+' sign) to the tree without changing its topology. *Nitrospina* was chosen as an outgroup. The scale bar indicates 0.10 changes per nucleotide.

Library structure

The composition in 16S rRNA genes of the five libraries was statistically compared using ∫-LIBSHUFF. For each pair of libraries, ∫-LIBSHUFF calculated P values < 0.001. After applying a Bonferroni correction (Sokal & Rohlf, 1995) to account for the increased probability of detecting a statistically significant difference by chance when making multiple comparisons, we could conclude with 95% confidence that the five *Acidobacteria* 16S rRNA gene libraries contained different taxonomic lineages. In particular, libraries from the two uncontaminated soils contained different taxonomic lineages when compared with each other, which revealed the effect of sampling location on the composition of *Acidobacteria* communities.

Library diversity

DOTUR calculated 61, 51, 49, 24 and 16 $OTU_{0.03}$ ('species') in the 'noTNT-F1', 'TNT-F1', 'noTNT-S1', 'TNT-S1' and 'TNT-S2' soil libraries, respectively (Table 2). None of the rarefaction plots calculated by DOTUR leveled off, which suggested that only part of the *Acidobacteria* diversity had been captured in our clone libraries (graph available upon request). As the $OTU_{0.03}$ numbers mentioned above referred

Table 2. Size and composition of the five *Acidobacteria* 16S rRNA gene fragment libraries

Soil name	Library size (number of sequences)	Number of $OTU_{0.03}$	% of sequences belonging to subdivision							% of $OTU_{0.03}$ belonging to subdivision						
			1	2+ associated	3	4	5	6	Other	1	2+ associated	3	4	5	6	Other
Soils from Bourges, France																
noTNT-F1	100	61	4	0	16	36	2	39	3	3	0	18	26	2	46	5
TNT-F1	95	51	0	0	6	53	2	39	0	0	0	10	31	4	55	0
Soils from El Gordo, Spain																
noTNT-S1	102	49	22	0	31	23.5	0	23.5	0	16	0	31	18	0	35	0
TNT-S1	111	24	92	0	4	4	0	0	0	79	0	8	13	0	0	0
TNT-S2	101	16	81	1	16	2	0	0	0	38	6	44	12	0	0	0

to libraries whose size (i.e. number of sequences) was slightly different, we considered an identical sampling effort of 95 sequences to compare the libraries. The corresponding number of $OTU_{0.03}$ calculated using DOTUR was then 59, 51, 47, 22 and 15, respectively. Therefore, a loss of richness of 8 $OTU_{0.03}$ (13.5%) was observed at the French site and of 25 $OTU_{0.03}$ (53%) and 32 $OTU_{0.03}$ (68%) at the Spanish site between the uncontaminated and the TNT-contaminated soil libraries. A reduction in the number of *Acidobacteria* phylotypes has been reported in some uranium-contaminated soils as well (Barns *et al.*, 2007).

It is difficult to explain why the greatest decrease in *Acidobacteria* diversity was observed at the less contaminated site (El Gordo). A similar trend had been observed for the total soil bacterial communities of the two soil series using DGGE analysis (George *et al.*, 2008): the contaminated soils of El Gordo were exclusively dominated by *Gammaproteobacteria*, whereas various *Alpha-*, *Beta-* and *Gammaproteobacteria* were identified as dominant bacteria in the contaminated soils of Bourges (George *et al.*, 2008). It is probable that biotic factors like plant cover or total microbial community composition may influence the response of *Acidobacteria* to TNT pollution. Such factors have not been extensively characterized in this study, but we know that the soils from El Gordo are arable whereas the soils from Bourges have not been cultivated for decades. Differences in physicochemical parameters could explain as well the difference in diversity loss at the two sites. For example, soil pH was lower at El Gordo (5.2–6.5, vs. 7.2–8.4 at Bourges), and TNT has been reported to be more bioavailable and therefore more toxic toward invertebrates in more acidic soils (Schäfer, 2002). Finally, the greater carbon content at the French site (5–8% vs. 0.8% at the Spanish site) could have favored the maintenance of a more diverse *Acidobacteria* community following exposure to TNT, despite a dramatic reduction in *Acidobacteria* numbers. There is no published study so far on the cross-effect of soil carbon content on changes in microbial community diversity after exposure to TNT. However, Zhou *et al.* (2002) reported that carbon-rich soils preserve microbial diversity in the presence of high chromium contents. Likewise, upon artificial contamination of a mineral and an organo-mineral soil with benzene, Girvan *et al.* (2005) observed a large decrease in bacterial numbers in both soils but the maintenance of a greater diversity among the survivors in the organo-mineral soil.

Conclusion

The analysis of *Acidobacteria* communities at two different sites contaminated with the nitroaromatic TNT revealed the dramatic impact of this pollutant on the relative abundance of *Acidobacteria* in soil bacterial 16S rRNA gene libraries. Moreover, *Acidobacteria* clone libraries from TNT-contaminated soils were different and less diverse than those from uncontaminated soils, although the extent of diversity loss varied at the two sampling sites. On the whole, our results suggest that *Acidobacteria* are sensitive to TNT and can be considered a biomarker group of TNT pollution. We are currently investigating the response of *Acidobacteria* to other environmental pollutants.

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