Nickel drives bacterial community diversity in the rhizosphere of the hyperaccumulator *Alyssum murale*

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A B S T R A C T

Ultradomic soils display high concentrations of nickel and a number of nutrient deficiencies. Nickel-hyperaccumulator plants, such as *Alyssum murale*, have evolved in these environments and developed specific metal homeostasis, showing concentrations of nickel (Ni) sometimes exceeding 1% in their aerial biomass. Rhizosphere bacterial communities associated with Ni-hyperaccumulator plants can differ from those of non-accumulating plants growing on the same site. Among the edaphic factors that could influence the phylogenetic structure of the bacterial communities, altitude and metal-bioavailability such as Ni in particular, could be significant. Our objectives were to understand the specific changes in the structure of the *A. murale* rhizosphere bacterial community that occurred across two gradients: elevation and Ni geochemistry, using a high-throughput sequencing technique (454-pyrosequencing). In this study, *Chloroflexi* was the major phylum present, with 53–77% of relative abundance. Moreover, we found that the higher the soil’s chemically-available Ni contents, the higher was the relative abundance of *Proteobacteria* (particularly *Alphaproteobacteria*) and *Actinobacteria*. In contrast, the abundance of *Chloroflexi* decreased with increasing levels of available Ni. Our results demonstrate that the chemical-availability of Ni in the studied soil drives the bacterial community diversity in the rhizosphere of *A. murale*, regardless of elevation gradient and other soil physicochemical parameters.

1. Introduction

Soil is the physical, chemical and biological support of primary production of terrestrial ecosystems and therefore plays a key role in terrestrial biogeochemical cycles. The rhizosphere—i.e. the soil volume that is directly influenced by living roots—is the most intense biological reactor of terrestrial ecosystems. The rhizosphere is a unique interface: roots exert different actions on the external environment and create physicochemical conditions that are different from those of non-rhizospheric soil, i.e. by providing C substrate to microbes, as the result of their rhizodeposition (Benizri et al., 2007). Thus, rhizosphere soil hosts more than one billion bacteria per gram of soil, making this compartment the mostly colonized in comparison with bulk soil (Pankhurst et al., 1996). Rhizosphere bacteria provide fundamental ecological functions thereby contributing to ecosystem services such as soil fertility, biological regulation or water purification, which are essential for the primary production of both agricultural and contaminated soils, as well as those soils naturally rich in trace elements (TE).

The development of methods using plants, namely phytoremediation, is of been of increasing interest over recent decades (Cunningham and Ow, 1996; Bani et al., 2015). Phytoremediation entails cultivating hyperaccumulator plants in areas which are either polluted or naturally rich in TE. These plants extract metals from the soil and transport them to their aerial parts, where they are accumulated. The term ‘hyperaccumulator’ defines those plants able to accumulate metals in their tissues at concentrations at least...
100 times above the normal and at values greater than 1000 mg kg\(^{-1}\) (0.1%) (Brooks et al., 1977; Nedelkoska and Doran, 2000). Hyperaccumulator plants are taxonomically well-represented in the plant kingdom (Sheoran et al., 2009). Among them, *Abyssum murale* Waldst. & Kit, a Brassicaceae, has attracted a growing interest over the last decade (Bani et al., 2007; Rue et al., 2015; Durand et al., 2016) and much experimental evidence clearly shows that this species could be used in 'agromining'. Agromining is a non-destructive approach to the recovery of high value metals from metal-enriched soils and ores and could provide multiple ecosystem services, such as provisioning services (e.g. metal, fuel-biomass) and supporting services (e.g. amelioration over time, of the fertility of ultramafic soils). In this context, the use of the nickel-hyperaccumulator *A. murale* has been proven to be economically feasible in Europe (Albania), based on successful field experiments (Bani et al., 2007, 2015; Van der Ent et al., 2013, 2015; Saad et al., 2016). This hyperaccumulator plant is commonly found on ultramafic soils and can grow with an altitudinal extension ranging from sea level to 2000 m.a.s.l. Altitudinal gradients, recognized as useful "natural experiments" since they are characterized by dramatic changes in climate and abiotic characteristics, are determinants of the structure of soil microbial communities (Lauber et al., 2009; Andrew et al., 2012; Stomeo et al., 2012; Siles et al., 2017).

Despite the long history of interest in ultramafic flora and metal hyperaccumulator plants, the attention of microbiologists towards bacteria from ultramafic and metal-polluted soils is more recent, with the relevant exception of Lipman (1926). However, it is essential to better understand the close correlations between rhizosphere microorganisms, host plants and surrounding soil, as well as the genetic diversity of rhizosphere bacterial communities in these ultramafic areas. Indeed, it is known that the rhizosphere bacterial community can, for instance, in the case of the Ni-hyperaccumulator *A. murale*, promote plant development on contaminated or naturally metaliferous soils (Reeves and Adiguzel, 2008; Durand et al., 2016). Moreover, it has been shown that microbial communities in such soils influence the mobility and availability of pollutants to plants (Sessitsch et al., 2013; Cabello-Conejo et al., 2014). Thus, despite the recent interest paid to the study of these microbial communities in naturally metal-rich soils, few studies have focused on the characterization of the community structure based on taxonomic marker genes of rhizosphere bacterial communities of Ni-hyperaccumulator plants (Kumar et al., 2009; Mengoni et al., 2010).

The specific aim of the current work was to investigate the genetic diversity of bacterial communities present in the rhizosphere of the Ni-hyperaccumulator plant *A. murale* growing on ultramafic soils, by using tag-encoded pyrosequencing of the 16S rRNA. To our knowledge, this study is the first aimed at characterizing the microbiome of *A. murale* rhizosphere using 454-pyrosequencing of the 16S rRNA gene approach and investigating the potential influence of edaphic factors (i.e. altitude gradient and Ni availability).

## 2. Materials and methods

### 2.1. Site description and sampling

Rhizosphere samples of *A. murale*, from ultramafic areas, were collected in the Northern Pindus Mountains (Greece) from 5 sites across an elevation gradient (from 860 to 1837 m) (site I: Trigona, N 39°40’15” E 21°25’19.1”, 860 m; site II: Perivoli, N 39°59’6” E 21°0’7.24”, 1216 m; site III: Vououssa, N 39°52’6.6” E 21°2’59.2”, 1560 m; site IV: Katara Pass, N 39°47’45.9” E 21°13’44.3”, 1690 m; site V: Valia Kalda, N 39°54’31.7” E 21°0’7.25’4”, 1837 m) in summer 2014. Indeed, this area is known to present soils derived from ultramafic rocks (Chardot et al., 2005; Reeves et al., 2009). Whatever the altitude gradient, *A. murale* was able to grow and despite this altitude gradient, the plants were all harvested at the flowering stage. At each site, three samples of rhizosphere soil of *A. murale* were taken (5 plots, 3 replicates per plot). Rhizosphere soil was defined as the soil attached to roots after gentle shaking by hand. All samples were brought back to the laboratory as soon as possible and were stored at \(4^\circ\text{C}\) until processed not longer than 4 days. The soil was sieved at 2 mm and thoroughly homogenized; one portion was air-dried for soil property analysis and the other stored at \(-20^\circ\text{C}\) for DNA extraction.

The physico-chemical characteristics of the rhizosphere soils were determined by the INRA Laboratory of Soil Analyses, Arras (France), using samples that had previously been air-dried and sieved following French standardized methods (AFNOR, 2004).

Soil moisture content was determined by heating subsamples to 105 \(^\circ\text{C}\) until a constant weight was achieved. Available elements in soil samples were extracted with a DTPA–TEA solution (0.005 M diethylene trimine pentaacetic acid, 0.01 M calcium chloride dihydrate, 0.1 M triethanolamine, \(pH\) 7.3) according to Lindsay and Norvell (1978) and concentrations in solutions were measured with an Inductively Coupled Plasma Atomic Emission Spectrometer (ICP–AES, Spectro-3000, Varian). Soil samples (5g and subsamples) were digested using freshly prepared Aqua Regia (6 mL 37% hydrochloric acid and 2 mL 70% nitric acid per sample) for a 2-h program and diluted with distilled water to 50 mL before ICP–AES analysis of pseudo-total elements. Soil \(pH\) was measured using a pH meter in a soil/water solution mixture (soil/water ratio: 1/5).

### 2.2. Soil DNA extraction and tag-encoded pyrosequencing of bacterial communities

Total genomic DNA was extracted from 0.5 g soil samples using a Fast DNA\textsuperscript{®} SPIN Kit for Soil (MP Biomedicals) according to the manufacturer’s instructions. The concentration and quality (ratio of A260/A280) of the DNA were determined with a spectrophotometer (SmartSpec\textsuperscript{TM} Plus, Bio-Rad). For the 15 soil samples (5 sites, 3 samples per site), barcoded amplicon sequencing was performed by Mr DNA (Shallowater, TX, USA), using the primer set 799F-1394R (chosen because it does not amplify non-target chloroplast sequences) targeting the V5–V6 region of the 16S rRNA gene (Santhanan et al., 2014). Briefly, a single-step 30 cycle PCR using HotStarTag Plus Master Mix Kit (Qiagen, USA) was used under the following conditions: 94 \(^\circ\text{C}\) for 3 min, followed by 28 cycles of 94 \(^\circ\text{C}\) for 30 s; 53 \(^\circ\text{C}\) for 40 s and 72 \(^\circ\text{C}\) for 1 min; after which a final elongation step at 72 \(^\circ\text{C}\) for 5 min was performed. Following PCR, all amplicon products from different samples were mixed in equal concentrations (40 ng mL\(^{-1}\)) and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Sequencing was performed on a Roche 454 following the manufacturer’s guidelines.

### 2.3. Processing of pyrosequencing data

All sequence analyses were conducted using the QIME pipeline (Caporaso et al., 2010). In brief, sequences were first selected according to the following criteria: (i) \(>200\) nucleotides in length, (ii) a perfect match to the primers and the barcodes and (iii) no ambiguous base allowed. After denoising, sequences of chloroplast or mitochondrial origin were removed from the bacterial dataset using the software Metaxa (Bengtsson et al., 2011). The software V-Xtractor (Hartmann et al., 2010) was then used to extract the V5-V6 region of the bacterial 16S rRNA gene. Bacterial sequences were then binned into OTUs, using a 97% identity threshold and the most abundant sequence from each OTU was
selected as a representative sequence for that OTU. Taxonomy was assigned to OTUs, by using the Basic Local Alignment Search Tool (BLAST) for each representative sequence against the Greengenes 13.8 reference database for bacteria (97% similarity) and the final nucleotide sequences obtained were deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) under accession numbers KX966532.1 — KX967491.1.

### 3. Results

#### 3.1. Soil properties

The reported characteristics were typical of ultramafic soils (Table 1) i.e. low concentrations of Ca, K, and P, elevated Ni, Mn, Mg and Fe. The pseudo-total Ni reached 2320 mg kg\(^{-1}\) in soil I. The Mg:Ca ratio was high (6.2—19.8), and the ratio of exchangeable Mg:Ca ranged from 0.28 to 1.30. The pseudo-total K concentration in these soils (ranging from 0.3 to 0.8%) was very low, as was exchangeable K (18.20—27.23 cmol\(^+\) kg\(^{-1}\)). Olsen-extractable P was also quite low (0.014—0.040 g kg\(^{-1}\)). Soil pH ranged from 6.28 to 7.03. The range of available Ni concentrations in the rhizosphere soils sampled was not correlated with the elevation gradient.

#### 3.2. Rhizosphere bacterial diversity

A total of 46 260 bacterial sequences were obtained from the five sites analyzed with a mean of 3015 reads per sample (ranging from 1653 in the Site V to 5124 in the Site II). These sequences were clustered into 983 OTUs.

At the phylum level, there were 21 major bacterial taxa present within most sites. Across all studied sites, the most abundant bacterial groups, with a relative abundance greater than 1%, were Chloroflexi (63.5% ± 11.9%), Actinobacteria (15.8% ± 7.3%), Proteobacteria (8.2% ± 4.3%), TM7 (4.7% ± 3.0%), Bacteroidetes (2.9% ± 1.5%), Gemmatimonadetes (1.1% ± 0.8%) and Acidobacteria (1.2% ± 0.6%). The taxa exhibited different relative abundances among sites (Fig. 1). The major phylum within A. murale rhizosphere in ultramafic soils was Chloroflexi that was present with more than 50% of relative abundance, accounted around 66% of the whole bacterial population (Fig. 1). The major family in this phylum was Koul eotherixaceae with UG6 and Koul eotherixaceae (Fig. 2A) with a relative abundance of around 37%, that is to say 25% of the total bacterial population. The second best represented phylum was Actinobacteria (with a relative abundance of 5—24%) (Fig. 1). The undetermined genera UG26 of the Solirubrobacteriales order mainly represents this phylum, with a relative abundance of around 20% (Fig. 2B), far below Chloroflexi. The Proteobacteria phylum, which was around 8% of the total abundance (Fig. 1), was well represented with the families Hyphomicrobiaceae (from UG7 to Rhodoplanes) and Bradyrhizobiaceae (from UG6 to Bradyrhizobium) having abundance levels of around 15% and 14% respectively (Fig. 2C). These two families belong to the Alphaproteobacteria, which was

| Table 1
<p>| Chemical and physical properties of Alyssum murale rhizosphere soils. |</p>
<table>
<thead>
<tr>
<th>Sites</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevation (m a.s.l.)</td>
<td>860</td>
<td>1216</td>
<td>1560</td>
<td>1690</td>
<td>1837</td>
</tr>
<tr>
<td>Texture</td>
<td>Clay</td>
<td>Sandy loam</td>
<td>Silty loam</td>
<td>Silty loam</td>
<td>Silty clay</td>
</tr>
<tr>
<td>pH</td>
<td>7.03</td>
<td>6.67</td>
<td>6.69</td>
<td>6.84</td>
<td>6.28</td>
</tr>
<tr>
<td>Organic C (g kg(^{-1}))</td>
<td>62.9</td>
<td>109</td>
<td>37.8</td>
<td>24.9</td>
<td>141</td>
</tr>
<tr>
<td>Total N</td>
<td>4.42</td>
<td>5.45</td>
<td>2.05</td>
<td>2.08</td>
<td>2.59</td>
</tr>
<tr>
<td>Olsen P</td>
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<td>0.014</td>
<td>0.014</td>
<td>0.04</td>
<td>0.015</td>
</tr>
<tr>
<td>Pseudo-total major elements</td>
<td>Al (g kg(^{-1}))</td>
<td>12</td>
<td>16</td>
<td>23</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>2</td>
<td>11</td>
<td>8</td>
<td>8</td>
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<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>36</td>
<td>73</td>
<td>84</td>
<td>152</td>
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<td>1</td>
</tr>
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<td></td>
<td>Na</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pseudo-total trace elements</td>
<td>Ni (mg kg(^{-1}))</td>
<td>2320</td>
<td>1571</td>
<td>1414</td>
<td>1931</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>332</td>
<td>537</td>
<td>361</td>
<td>509</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>342</td>
<td>637</td>
<td>232</td>
<td>563</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>97</td>
<td>96</td>
<td>81</td>
<td>128</td>
</tr>
<tr>
<td>Available metal (extractable by DTPA)</td>
<td>Cu (mg kg(^{-1}))</td>
<td>0.109</td>
<td>0.15</td>
<td>0.156</td>
<td>1.022</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>67.95</td>
<td>100.71</td>
<td>160.81</td>
<td>61.35</td>
</tr>
<tr>
<td></td>
<td>Mn</td>
<td>1.81</td>
<td>41.9</td>
<td>20.53</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td>Ni</td>
<td>247.84</td>
<td>167.94</td>
<td>67.85</td>
<td>120.69</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>0.179</td>
<td>0.31</td>
<td>0.123</td>
<td>0.903</td>
</tr>
<tr>
<td>CEC total (cmol + kg(^{-1}))</td>
<td>64.72</td>
<td>66.63</td>
<td>79.71</td>
<td>90.83</td>
<td>65.95</td>
</tr>
<tr>
<td>Exchangeable cations</td>
<td>Ca</td>
<td>1.073</td>
<td>2.523</td>
<td>0.784</td>
<td>0.925</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>0.047</td>
<td>0.070</td>
<td>0.069</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>2.297</td>
<td>1.209</td>
<td>1.129</td>
<td>0.620</td>
</tr>
<tr>
<td></td>
<td>Mn</td>
<td>0.002</td>
<td>0.007</td>
<td>0.006</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Ni</td>
<td>0.015</td>
<td>0.005</td>
<td>0.008</td>
<td>0.006</td>
</tr>
</tbody>
</table>
well represented among Proteobacteria with an average of 60% for the 5 soils, while the Betaproteobacteria, Deltaproteobacteria and Gammaproteobacteria represent respectively only 16, 18 and 5% of the totality of the Proteobacteria.

The bacterial diversity and richness of the different sites were calculated. Soils I and V had a greater diversity based on the Shannon index (7.29 ± 0.43 and 6.92 ± 1.06, respectively) than Soil III (6.05 ± 0.14). The soils II and IV showed intermediate Shannon index values (respectively, 6.84 ± 0.69 and 6.85 ± 0.59). Soil III had a lower richness based on the Chao1 index (348 ± 17) than the other four soils (nearly 370). The rarefaction curves (data not shown) indicated that all the samples studied tended to reach a saturation plateau. This means that the sequence coverage was still sufficient to capture the diversity of bacterial community.

A Canonical Correspondence Analysis (CCA) was performed to characterize the effect of environmental variables (soil properties and sites, Table 1) on the most abundant phyla (Fig. 3). The main plan explains 88.8% of the total variation. CCA linked Actinobacteria, Gemmatimonadetes, Acidobacteria, Bacteroidetes and Proteobacteria with the highest concentrations of available Ni and the higher values of exchangeable Ni (determined by Ni-CEC). These characteristics refer to Soils I and V (Ni.DTPA 250 and 300 mg kg⁻¹, respectively), although these two soils showed different pH values (7.03 and 6.28, respectively) and contrasting altitudes (860 and 1837 m, respectively). Conversely, in the Soil III (Ni.DTPA 7.03 and 6.28, respectively) and contrasting altitudes (860 and 1560 m, organic carbon 2 to 3 times lower than in Soils I and V) TM7 were predominant, as to a lesser extent, were Chloroflexi. Their presence was correlated with the lower contents of available Ni and higher Mn and K concentrations.

The influence of available Ni was evident at the phylum level, since the relative abundance of the three dominant bacterial phyla (Chloroflexi, Proteobacteria, Actinobacteria) changed in a concurring manner across the available Ni gradient (Fig. 4). The relative abundance of Proteobacteria and Actinobacteria increased with available Ni, whilst Chloroflexi showed the opposite pattern. These results suggest that locally available Ni is, directly or indirectly, a fundamental catalyst of soil bacterial community composition and diversity in the rhizosphere of A. murale, regardless of the elevation (climatic) gradient.

A correlation analysis was performed between the physicochemical variables selected by the envfit function of the Vegan package in R software (6 variables: Fe.DTPA, Ni.DTPA, pH, CEC.Ca, CEC.Ni and Organic.C) and the 113 bacterial families from the 3 major phyla of A. murale rhizosphere (36 Actinobacteria, 28 Chloroflexi and 49 Proteobacteria, respectively). Whatever the bacterial family, heatmaps showed 146 significant correlations between physicochemical variables and bacterial families with 79 positive and 67 negative correlations (Fig. 5). For the Chloroflexi phylum (Fig. 5A), there were in total 52 significant correlations with 30 positives and 22 negatives. In Chloroflexi phylum, the Kowalewskiaaceae family showed a relative abundance of around 37% in the 5 sites. The correlation between the relative abundance of Kowalewskiaaceae and NLDTPA, CEC.Ca and Organic.C was negative. For the Actinobacteria phylum (Fig. 5B), we observed in total 60 correlations: 28 positives and 32 negatives. The most represented family in Actinobacteria phylum belongs to the order of Solirubrobacterales and its relative abundance (around 20%) was negatively affected by Fe.DTPA and positively by CEC.Ca. Among the Actinobacteria phylum, the Gaiellaceae family (around 7% of representativeness) was weakly correlated to Organic.C and pH, but conversely showed a strongly positive correlation with Ni.DTPA and CEC.Ni. This last observation corroborated the fact that the relative abundance of the Actinobacteria phylum, to which this family belongs, increased with the Ni.DTPA concentrations present in soils (Fig. 4). Finally, there were 34 significant correlations for the Proteobacteria phylum (Fig. 5C) with 21 positive and 13 negatives correlations. At the family level, more than a third of these positive correlations depend on the CEC.Ni. In the Proteobacteria phylum, among the 13 families affected by CEC.Ni, 8 were positively correlated with CEC.Ni and among the 4 families affected by the Ni.DTPA, 3 were positively affected (Hyphomonadaceae, Enthococcaceae and Haliangiaceae). This may explain why the relative abundance of Proteobacteria increased with available Ni (Fig. 4).

4. Discussion

4.1. 16S rRNA gene amplicon sequencing

An increasing number of studies have attempted to characterize how microbial distribution patterns respond to environmental factors (Nielsen et al., 2010; Rousk et al., 2010). The conclusions of previous studies regarding ultramaecos ecosystems have been limited by the techniques used (Lenczewski et al., 2009; Bordez et al., 2016). The application of analyses based on 16S rRNA gene amplicon sequencing is routinely used today to analyze the microbial communities of various ecosystems, with wide application in the study of soils and recently ultramaecos ecosystems (Chodak et al., 2013; Goebelwshi et al., 2014; Yasir et al., 2015). However, to our knowledge, no study has ever investigated the bacterial diversity in the rhizosphere of a hyperaccumulator plant, such as
A. murale, growing on ultramafic soils. Indeed, next-generation sequencing (NGS) technologies, such as Roche/454, have been used to study the rhizobiome of Arabidopsis thaliana, Populus deltoides, Lactuca sativa and Zea mays, in TE-contaminated soils and recently in ultramafic soils (Bordez et al., 2016), but this approach has not yet been used to characterize the microbial community associated with the rhizosphere of Ni-hyperaccumulators. So, the application of the analyses based on 16S rRNA gene amplicon sequencing to ultramafic ecosystems was expected to greatly expand our knowledge of the microorganisms inhabiting these environments (von Wettberg and Wright, 2011; Bordez et al., 2016) and therefore appears to be an essential approach for investigating the diversity and ecology of the hyperaccumulator rhizobiome (Visioli et al., 2015).

4.2. Dominant phyla in A. murale rhizosphere

Our study revealed the presence of 21 phyla, among which 7 had a relative abundance greater than 1%. These were Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes, Proteobacteria and TM7. These genera have been found in both the rhizosphere and the endosphere of hyperaccumulators, regardless
of the specific metal composition of the soil (Visioli et al., 2015). The presence of the 7 major phyla observed in our study confirmed Janssen’s results (2006) which showed that, based on a study of the sequences of the 16S rRNA genes from 21 libraries, soil bacterial communities found in bulk soils were dominated by 9 major groups of the 7 major phyla observed in our study comprising 5% of the bacterial rRNA clones analyzed were those of the Chloroflexi phylum, geothermal soils, low-temperature meadow soils, sea and lake sediments, and hydrothermally active sediments (Yamada and Sekiguchi, 2009), thus suggesting that this phylum is abundant in extreme conditions (Boomer et al., 2002; Nübel et al., 2002). Ultrafast organisms, which are deficient in essential plant nutrients but enriched with heavy metals such as nickel, are considered as stressed environments due to their extreme mineral composition (Pal et al., 2005; Mengoni et al., 2010) for most plant species and for many microorganisms (Lipman, 1926). Among the Chloroflexi phylum, the Kouleothrixaceae family was the most represented. Chloroflexi have been characterized as bacteria specialized in polysaccharide degradation produced by other microorganisms and on decaying cells (Kragelund et al., 2007). It is known that (i) among rhizodeposits, mucilage, which improve root penetration into soil and play an important role in resistance to drought, are composed of polysaccharides and (ii) this mucilaginous layer has been frequently observed on the root surface of many plants and more particularly at the root tip (Nguyen, 2003).

In our study, the second most represented phylum was Actinobacteria, although far below Chloroflexi. The predominance of this phylum can be explained by the high adaptability of these Gram-positive bacteria to toxic concentrations of metals in soils. Nickel was investigated in particular for cellular adaptive responses in Actinobacteria (Schmidt et al., 2005) and it has been shown that their strong secondary metabolism enables them to cope with...
Fig. 5. Representation of significant correlation analysis between 6 physicochemical variables (Fe.DTPA, Ni.DTPA, pH, CEC.Ca, CEC.Ni and Organic.C) and bacterial families for the three major phyla Chloroflexi (A), Actinobacteria (B) and Proteobacteria (C). The y-axis depicts the 6 variables and the x-axis the 113 bacterial families. The heatmap shows significant correlation (p-value < 0.10) with blue squares for positive changes in relative abundance and red squares for negative changes. The intensity of color correlates with the magnitude of the correlation value. UF: Undetermined Family.
stress factors including toxic levels of heavy metals (So et al., 2001). Moreover, The abundance of *Actinobacteria* in ultramafic soil or polluted soils has already been detected by several authors (Abou-Shanab et al., 2010). These studies confirmed the high adaptability of these Gram-positive bacteria to toxic concentrations of metals (DeGrood et al., 2005). Indeed, at the phylum level, the relative abundance of *Actinobacteria* was positively correlated with soil available Ni regardless of the elevation (climatic) gradient. In addition, the *Gaiellaceae* family showed a strong positive correlation with Ni.DTPA and CEC.Ni. These observations confirmed the previous results underlined the adaptability of these bacteria to toxic concentrations of metals present in soils. Moreover Szoboszlay et al. (2016) highlighted the influence of flavonoid compounds (e.g. 7,4-dihydroxyflavone) on *Gaiellaceae* development and their interaction with plant hosts. Flavonoids play a major role in the hyperaccumulation process, by allowing the formation of a complex with metal for uptake and root-to-shoot transport (Barceló and Poschenrieder, 2003). We can hypothesize that flavonoids could be exuded by *A. murale* and could explain the presence of this family in the rhizosphere of this hyperaccumulator.

The *Proteobacteria* phylum, which is around 8% of the total content, was well represented by the *Hyphomicrobiaceae* and *Bradyrhizobiaceae* families, with abundance levels of around 15% and 14% respectively. The relatively low presence of *Proteobacteria* in ultramafic soils could be correlated to the fact that these bacteria are r-strategists, found in rich environments, and also known to be sensitive to toxic pollutants (Kunito et al., 2001). Indeed, Ellis et al. (2003) found in their study focusing on five different metal-contaminated soils that the *Gammaproteobacteria* (mainly *Pseudomonas* spp. and a *Xanthomonas* sp.) increased in relative abundance in the least-contaminated soil samples, while *Alphaproteobacteria* were absent. On the other hand, it has been shown that bacteria belonging to the alpha-subdivision of *Proteobacteria* might have selective advantages over other bacteria in soils with high metal amendments (Sandaa et al., 1999). A possible explanation might be an increase in numbers of transferable plasmids mediating metal resistance with increasing metal contamination and therefore the relative abundance of members of the alpha-subdivision of *Proteobacteria*, might be caused by an increase in one initially resistant population of a member of this subdivision. Another hypothetical explanation is the occurrence of plasmid-mediated transfer of heavy metal resistance within members of the alpha-subdivision of *Proteobacteria* (Sandaa et al., 1999). In our study, the genera belonging to the alpha-subdivision of *Proteobacteria* (Bradyrhizobium, Rhodoplanes and those belonging to Rhodospirillaceae and Sphingomonadaceae) showed a relative abundance among the most represented divisions, such as those belonging to the delta-subdivision of *Proteobacteria* (Myxococcales order). However, it is mainly those families that belong to the delta-subdivision of *Proteobacteria* which were positively correlated with CEC.Ni and Ni.DTPA. Nevertheless, Gołębiowski et al. (2014) have questioned the sensitivity of this phylum, as their study found a great abundance of *Proteobacteria* in multi-contaminated soils by metals such as Cr, Zn and Pb.

Siles et al. (2017) underlined in their study the effect of altitudinal gradient on structure, abundance and microbial activities. Indeed, they showed that the temperature, linked with elevation gradient, induce modifications in the microbial proliferation. In our study, whatever the major phylum considered, the elevational gradient showed no effect on bacterial diversity. These results were in accordance with those of Fierer et al. (2011); both at the whole community level and at the level of individual phyla, there was no significant influence of elevation on bacterial diversity.

5. Conclusion

Our observations that underlined the effect of available Ni on the diversity of bacterial communities also confirmed the results obtained with other pollutants, e.g. Hg (Müller et al., 2001) or Cr (Desai et al., 2009). These findings underscore the complexity of interactions between physicochemical parameters and soil bacterial diversity, because of the complexity of the action and interaction of many factors where diversity is concerned. Indeed, other factors were previously known to affect the composition of bacterial communities, such as pH (Lauber et al., 2005; Roux et al., 2010), nutrient availability, trace metal solubility (Müller et al., 2002), soil carbon (Assuming-Brempong et al., 2008) and nitrogen content (Fierer et al., 2007), or soil moisture and climate variations (Lauber et al., 2009). Conversely, in the rhizosphere of *A. murale* grown on ultramafic soils, we found no evidence for an elevational gradient in bacterial diversity as there was no significant relationship between elevation and the genetic structure of the bacterial communities.

The next step of this research will be to expand the observations to other ultramafic areas under other climatic conditions. In particular, ultramafic bedrock is widespread and extensive in tropical regions such as Cuba, New Caledonia or Indonesia (Van der Ent et al., 2013), e.g. in Sulawesi (about 15 400 km² of ultramafic outcrops) or in Northern Maluku (8000 km² of ultramafic outcrops). Then, it will be possible to verify the predominance of *Chloroflexi* in ultramafic soil bacterial communities. The role and significance of this phylum in such stressful ultramafic environments will have to be investigated and understood.

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