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Abstract Phytoextraction represents an innovative approach in the management of nickel (Ni) rich soils whether natural (ultramafic) or anthropogenic (contaminated sites). However, its success depends both on the production of a high plant biomass and the ability of plants to accumulate metals. The application of nitrogen (N) fertilizer can improve the biological and chemical soil fertility and thus agricultural yields. Moreover, soil microorganisms play a key role by influencing nutrient flows, which are the main limiting factors of plant growth in degraded soils. In this work, we investigated the effects of two levels of both Ni and mineral N soil applications on the microbial activities and Ni phytoextraction efficiency by *Alyssum murale* growing in a pot experiment during 5 months. Plant growth, nutrients and Ni uptake, soil microbial populations and their enzymatic activities involved in the biogeochemical cycles of nitrogen, phosphorus, carbon and sulfur (urease, alkaline phosphatase, β -glucosidase and arylsulfatase, respectively) were determined. The results showed that plant dry mass was unsurprisingly not affected when the soil Ni concentration was increased. However, it led to an increase of the amount of Ni extracted per pot. A negative effect of Ni addition was observed on both total bacteria and urease activity, without any effect on other enzymes. On the contrary, N fertilizer played a significant positive role by promoting both plant growth and Ni phytoextraction, partly as a result of the stimulation and flourishing of bacterial populations.

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Introduction

The presence of heavy metals at concentrations higher than that of geochemical background levels has become an increasingly serious concern, posing risks to the environment and human health (Biney et al. 1994). One of these elements, nickel (Ni), can be present in soils in concentrations ranging from 2000 to 10,000 $\mu\text{g g}^{-1}$, the highest levels being encountered on ultramafic rocks such as serpentine, characterized by naturally high Ni concentrations (Chaney et al. 2008; van der Ent et al. 2013).

Soil metal contamination led to the development of many clean-up technologies. Among them, the most commonly used are physicochemical techniques which are generally expensive and may have deleterious effects on the physical, chemical and biological soils properties (Ma et al. 2009; Glick 2010). For these reasons, the remediation of most contaminated sites by these techniques has not evolved rapidly (Cunningham and Ow 1996). In recent years, phytoremediation techniques have been developed (Chaney et al. 2007, 2008). Their most striking feature is that they are acceptable among the public, relatively inexpensive and that they offer an opportunity to improve the biological and physicochemical properties of soils (Pulford and Watson 2003). Phytoremediation is based on the principle of combining the use of plants and their rhizosphere associated microorganisms to reduce the toxicity of pollutants such as trace elements (Khan 2005). Among the various techniques, phytoextraction represents an innovative method to remediate contaminated or naturally metal-rich sites. This technique relies on the absorption of metals by a hyperaccumulator plant, and their translocation into the harvestable aerial parts of the plant (Lasat 2002; Bani et al. 2010). However, the main limiting factors the phytoextraction efficiency could be the

low biomass production of hyperaccumulator plants due to the generally limited soil fertility (low nitrogen, potassium and phosphorus nutrient status) (Bani et al. 2007; Boyd and Jaffré 2009; Tumi et al. 2012). Consequently, many studies aimed to improve the plant biomass production by supplying either organic or inorganic amendments to soils (Bani et al. 2015). Indeed, over the past decade, several studies have highlighted that addition of mineral NPK amendments increased crop yields, which could reach at least 20 t ha⁻¹ of dry biomass and 400 kg ha⁻¹ of extracted Ni on serpentine soils (Chaney et al. 2007). Concerning organic amendments, such as compost or manure, they are known to improve fertility as well as the physical properties of soils (Vangronsveld et al. 2009). In addition, manure can increase metal mobility due to its high content of soluble and reactive organic matter, which can complex the metals (Tandy et al. 2009; Alvarez-López et al. 2016). Manure application increases hyperaccumulator plant growth and Ni phytoextraction yield but can lead to the dilution of Ni in shoots depending on the level of addition (Alvarez-López et al. 2016).

Moreover, the effectiveness of hyperaccumulation depends on the interaction of the roots with the rhizosphere microorganisms (Abou-Shanab et al. 2003). The rhizosphere, compared to bulk soil, is characterized by higher concentrations of nutrients and labile organic carbon (Duineveld et al. 2001). Consequently, it represents a unique ‘hot spot’ in terms of microbial ecology because soil microorganisms are stimulated by root activity (Hinsinger et al. 2006). Indeed, their localization, in this particular environment, allows microorganisms to benefit from the rhizodeposits (root exudates) which provide them nutrients and energy and eventually help to reinforce the growth of the hyperaccumulator plant by various mechanisms (Ma et al. 2009; Durand et al. 2016). Consequently, the structure and the diversity of the rhizosphere microbial community, as shown by numerous studies, are affected by the cultivated plant species due to the secretion of various root exudates (Smalla et al. 2001; Baudoin et al. 2003). Conversely, soil microorganisms drive, via their enzyme activities, the cycling of all major elements. These microbial enzymes play an important role at the soil level since they are involved in the biogeochemical cycles of carbon, nitrogen, sulfur, phosphorus and other nutrients (Caldwell 2005; Kibblewhite et al. 2008). This cycling affects the structure and functions of soil ecosystem as well as the ability of soils to provide ecosystem services. Soil enzyme activities have been reported to provide a unique integrative biochemical assessment of soil functions and conditions (Naseby and Lynch 2002) and to be useful indicators of soil functional diversity (Bending et al. 2004; Sowerby et al. 2005; Epelde et al. 2009). These activities of soil microorganisms are influenced by various abiotic parameters (e.g. soil type, nutrient status, pH, texture, organic matter content, moisture) and their interactions on the one

hand, and by plant factors (e.g. plant species, age, root zone) on the other hand (Baudoin et al. 2003).

However, among abiotic factors, the presence of heavy metals in soils also modulates the microbial activities and their interactions (Krumins et al. 2015). The interaction of metals with native soil communities and their activities is an important area of research as scientists strive to understand the effects of metal pollution or enrichment on soil properties and microbial activities (Hagmann et al. 2015; Lopez et al. 2017). Depending on the study, the effects of trace elements on rhizospheric enzyme activities are complex and contrasting effects can be observed (Egamberdieva et al. 2011). However, some soil microorganisms, including bacteria, archaea and fungi, are capable of withstanding high concentrations of metals (do Nascimento and Xing 2006).

Our experiment studied the effect of N additions on the cultivation of a Ni hyperaccumulator plant, *Alyssum murale* (Waldst. & Kit—Brassicaceae), with the aim of remediating a soil artificially contaminated with two different concentrations of Ni (40 and 80 mg kg⁻¹). In this regard, we wish to investigate the effect of mineral nitrogen fertilization on (1) plant biomass and phytoextraction efficiency, and (2) on the soil microbial properties, particularly the numbers of the cultivable bacterial communities and the associated microbial enzymatic activities.

Methods

Soil sampling

The 0–30 cm depth topsoil was collected from a natural site in Lebanon (33°11′34.59″N, 35°24′34.92″E) and then sieved to 5 mm. Preliminary analyses such as soil texture (SOP METH004.00), total calcium carbonate (NF ISO 10,693), pH (NF ISO 10390), electrical conductivity (AFNOR X 31-113) and organic material (ASTM D 2974) were performed (Table 1). The soil is clayey with an alkaline pH but a low percentage of total carbonate. The electrical conductivity is low. This soil, as predicted, has a low percentage of organic matter because it was selected from an uncultivated area and devoid of plant residues.

Plant cultivation

A column system (Polyvinyl chloride cylindrical tubes of 30 cm height and 15 cm diameter) was prepared for culturing. These columns were filled with 5 kg of soil (dry weight basis). The soil was artificially enriched with nickel sulfate (NiSO₄·6H₂O) to reach two different soil bulk concentrations: 40 and 80 mg Ni kg⁻¹ dry soil (Ni40 and Ni80 treatments, respectively). These concentrations were chosen to guarantee both a limited

Table 1 Physicochemical characteristics of the natural soil used in the experiments

Parameter	Unit	Value
Sand	%	23.4
Silt	%	15.3
Clay	%	61.2
Total carbonate	%	0.30
Electrical conductivity	mS cm ⁻¹	0.72
pH		7.96
Organic matter	%	0.70

toxicity to the soil microbial community and a sufficient Ni availability for phytoextraction. That is why, values were selected in the concentration range (0 to 90 mg Ni kg⁻¹ supplied as NiSO₄) studied by Saad et al. (2016) to evaluate the nitrogen fixation and growth of *Lens culinaris* var. *Beluga* (Fabaceae), France as affected by nickel availability. The soil was thoroughly homogenized and incubated at room temperature for 15 days after a 70% water retention capacity adjustment.

Seeds of *A. murale* Waldst. & Kit were harvested from a natural ultramafic site near Trigona (Thessaly, Greece) in August 2014. One seed per tube was sown (1 cm deep). The plants were cultivated in a growth chamber over a period of 5 months under controlled conditions: 16 h photoperiod, 22 °C/18 °C day/night temperature, 70% of relative humidity and a photosynthetic photon flux density of 350 µmol m⁻² s⁻¹. The tubes were watered with distilled water every 3 days up to 70% of soil water holding capacity. Two weeks after sowing, the solution of NH₄-NO₃ was surface applied to half of the columns. Each column received 0.3 g of NH₄-NO₃ dissolved in 50 mL distilled water (corresponding to 60 kg N ha⁻¹). So, the combination of these two factors makes it possible to obtain four treatments: non-fertilized (Ni40-NF and Ni80-NF) and N fertilized (Ni40-F and Ni80-F) Ni contaminated soils. The experiment had a factorial randomized complete block design with three replicates of the four treatments.

Plant harvest and analysis

At the end of the experiment, plants were collected. Fresh shoots were carefully washed with deionized water and then dried in the oven at 70 °C for 72 h to allow dry weight measurement. The samples were ground and homogenized. For metal analysis, a subsample (0.5 g) of plant tissue was acid-digested at 95 °C in 2.5 ml of concentrated HNO₃ and 5 ml of H₂O₂ (30%). The final solutions were filtered at 0.45 µm and diluted with deionized water to reach 25 ml. Metal concentrations in the solution were measured with an inductively coupled plasma mass spectrometry (ICP-MS; Thermo Fisher Scientific X Series 2, USA). Control material from *Noccaea caerulescens* (J.Presl & C.Presl—Brassicaceae) with known compositions (internal analyses carried out by INRA-USRAVE, Villenave d'Ornon, France), as

well as a certified solution (EU-H₄, SCP Science, Courtaboeuf, France) were included in all analyses for quantitative verification of the results.

Bacterial densities

At harvest, rhizosphere soil, considered as the soil strongly adhering to the roots and collected within the space exploited by the roots was collected (García et al. 2005). The soil particles loosely bound on the roots were shaken off and discarded. The remaining soil still attached to the roots (hence forth referred to as the rhizosphere soil) was swept off with a brush and collected. Care was taken to avoid cross contamination between different samples. A sample of 10 g of fresh rhizosphere soil was dropped into a flask containing 100 ml of NaCl buffer 9‰ (0.15 mol l⁻¹). All the flasks were then placed on an orbital shaker (17 rpm) for 30 min. Then, a 1-ml aliquot per each sample was transferred into a sterile test tube previously filled with 9 ml NaCl. Microbial suspensions were serially diluted and the dilutions (10⁻²–10⁻⁷) were used for the determination of the number of cultivable bacteria (i.e., number of colony-forming units (CFU)), by spread-plating them onto nutrient agar medium (NA) (M001 from Himedia—India) supplemented with amphotericin B solution to avoid any fungal strain development (0.25 µg of amphotericin B per ml). The agar plates were then incubated in the dark at 28 °C for 10 days. The moisture content of the soil collected as “rhizosphere soil” using a brush was measured before making the dilutions with NaCl solutions. The number of the cultivable bacteria was expressed as CFU g⁻¹ of soil dry weight.

Microbial enzymatic activities

Urease activity was measured according to the method of Tabatabai and Bremner (1972). Arylsulfatase, β -glucosidase and alkaline phosphatase activities were determined using 5 mM pNP-sulfate, 5 mM pNP- β -D-glucopyranoside and 5 mM pNP-phosphate, respectively (Sigma-Aldrich, France) according to the modified protocol of Deng et al. (2012). Substrates of arylsulfatase and β -glucosidase were prepared in sodium acetate buffer (0.5 M, pH 5.8) whilst substrate of alkaline phosphatase was prepared in Tris HCl buffer (0.1 M, pH 8). Four grams of fresh soil were incubated with 0.5 ml toluene for 10 min before adding 50 ml of distilled water. The mix was then homogenized with a polytron for 25 s. Four hundred µl were immediately sampled and put in a 2 ml microtube then 1 ml of the adequate substrate was added. Samples were incubated at 37 °C for 2 h. The reaction was stopped by adding 250 µl of 1 M NaOH solution. The blanks were realized by adding the substrate after the addition of the NaOH. Microtubes were then centrifuged for 2 min at 13,000g and 200 µl were transferred on a microplate in

order to read absorbance at 400 nm on a microplate reader (Biotek Instruments, Synergy HT, USA). In order to calculate the enzymatic activities, readings were compared to a p-Nitrophenol standard curve.

Statistical analysis

All statistical analyses were performed using R v3.1.1 with the lme, ade4 and FactomineR packages. When necessary, data were log-transformed in order to improve normality and variance homogeneity of residues. The relationships between variables were then assessed using principal component analysis (PCA) and correlation tests were performed using the Spearman method. Analyses of variance for plant and soil parameters were analyzed by a two-way ANOVA with fixed effect (type I *F* statistics). Post hoc tests were performed using the Tukey method.

Results

Plant growth, nutritional status and Ni uptake

Five months after sowing, the effects of both Ni contamination and N fertilizer on *A. murale* growth and Ni uptake by this hyperaccumulator plant were measured. *A. murale* exceeded the Ni hyperaccumulation threshold

value of 1000 mg kg⁻¹ whatever the Ni addition level. However, there was no significant effect of the soil Ni concentration on *A. murale* shoots dry weight (Table 2). Conversely, a positive effect of N application was observed as the shoot dry weight was significantly increased by, respectively 23 and 29% in Ni40 and Ni80 treatments after the addition of N fertilizer (Fig. 1a).

With regard to plant Ni concentration in shoot tissues, analysis of variance highlighted a significant effect of both Ni and N treatments as well as a significant interaction between treatments (Table 2). Indeed, the increase of Ni concentration in soil from 40 to 80 mg kg⁻¹ led to a significant increase of the shoot Ni concentration by a factor of 2.6 in the fertilized plants only (Fig. 1b). Similar results were obtained concerning the quantity of Ni extracted per pot (Fig. 1c, Table 2). A significant increase by 60% was observed in Ni80-F compared to Ni40-F.

Likewise, N application significantly increased Ni concentration in the shoots by, respectively 80 and 158% in Ni40 and Ni80 treatments (Fig. 1b). In addition, the amount of extracted Ni was significantly higher for the fertilized treatments (12 and 32 mg Ni pot⁻¹, respectively for Ni40-F and Ni80-F) than that of unfertilized (9 and 16 mg pot⁻¹, respectively for Ni40-NF and Ni80-NF) (Fig. 1c).

Regarding the nutritional status of the plants at the end of the experiment (K, P, S and Ca content; Fig. 2), no significant difference was observed whatever the soil

Table 2 Analyses of variance for plant parameters accounted for Ni and nitrogen treatments and the interaction between both

Source	df	SDB	K	P	S	Ca	Ni	QNi
Nickel	1	0.14	0.34	0.28	0.02	0.04	10.3*	14.3**
Nitrogen	1	135***	3.42	0.44	0.02	0.27	54.9***	99.1***
Nickel x Nitrogen	1	1.8	0.6	0.42	0.01	2.89	7.27*	11.9**
Residuals	8							

The table shows test statistics with F values. Significant level: **P* < 0.05; ***P* < 0.01; ****P* < 0.001
SDB shoot dry biomass, K, P, S, Ca, Ni plant element concentration, QNi quantity of Ni extracted

Fig. 1 Variation of **a** dry shoot weight, **b** Ni concentration, and **c** QNi (quantity of Ni) in *Alyssum murale* shoots according to the soil Ni concentration (Ni40 and Ni80) and the N supply

(F fertilized, NF unfertilized). Results are represented by means with their standard error. Different letters indicate statistical difference (*P* < 0.05, Tukey test, *n* = 3)

Fig. 2 Variation of **a** potassium, **b** phosphorus, **c** sulfur and **d** calcium concentrations in *Alyssum murale* shoots according to the soil Ni concentration (Ni40 and Ni80) and the N supply

(F fertilized, NF unfertilized). Results are represented by means with their standard error. Different letters indicate statistical difference ($P < 0.05$, Tukey test, $n = 3$)

Ni concentration or the N supply (Table 2)., it was observed a global tendency of a greater element content for the fertilized treatments in comparison with those unfertilized (Electronic supplementary material (ESM), Table S1).

Rhizosphere properties

pH of the rhizosphere soil

The analysis of rhizosphere soil revealed a small significant decrease of soil pH by 0.3 unit in average with response to added Ni concentration without any significant effect of N application (Fig. 3, Table 3).

Soil bacterial densities

The effect of soil Ni concentration and N supply on the bacterial densities is presented in Fig. 4. Variance analysis revealed a significant effect of both Ni and N treatments as well as an interaction effect between

Fig. 3 Variation of rhizosphere soil pH according to the soil Ni concentration (Ni40 and Ni80) and the N supply (F fertilized, NF unfertilized). Results are represented by means with their standard error. Different letters indicate statistical difference ($P < 0.05$, Tukey test, $n = 3$)

Table 3 Analyses of variance for soil parameters accounted for Ni and N treatments and the interaction between both

Source	df	BacTot	URE	AP	AS	β GLU	pH
Nickel	1	168***	23.4**	0.75	0.09	0.26	25.6***
Nitrogen	1	320***	42.4***	0.64	0.00	0.03	0.40
Nickel x Nitrogen	1	6.01*	0.26	1.37	0.60	0.06	0.40
Residuals	8						

The table shows test statistics with F values. Significant level: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

BacTot total cultivable bacteria, *URE* urease activity, *AP* alkaline phosphatase activity, *AS* arylsulfatase activity, *β GLU* betaglucosidase activity

Fig. 4 Number of total cultivable bacteria according to the soil Ni concentration (Ni40 and Ni80) and the N supply (*F* fertilized, *NF* unfertilized). Results are represented by means with their standard error. Different letters indicate statistical difference ($P < 0.05$, Tukey test, $n = 3$)

treatments (Table 3). Increasing soil Ni concentration from 40 to 80 mg kg⁻¹ significantly decreased the numbers of total bacteria by 18 and 16%, respectively in the fertilized and unfertilized soils. A greater bacterial density was noticed in N-fertilized treatments, as shown by the significant increase of 32 and 29% respectively in Ni40-F and Ni80-F treated soils.

Soil enzymatic activities

Figure 5 presents the values of the selected enzymes chosen for their role in the biogeochemical cycle of C, N, P and S. Variance analysis showed a significant effect of both Ni and N treatments without significant interaction (Table 3). Increasing soil Ni concentration from 40 to 80 mg kg⁻¹ significantly decreased soil urease activity by an average of 22%. Conversely, the addition of mineral nitrogen led to a significant increase in urease activity by an average of 30%. As a consequence, N fertilization alleviated the negative effect of increased soil Ni concentration as shown by the higher urease activity in Ni80-F compared to Ni40-NF (Electronic supplemen-

tary material (ESM), Table S2). Concerning alkaline phosphatase, β -glucosidase and arylsulfatase, enzymes belonging respectively to the P, C and S geochemical cycles, the results showed a non-significant variation of their concentrations regardless of the different Ni and N treatments. On average, the enzyme activity was respectively 1.15, 2.20 and 12.1 μ g p-nitrophenol g⁻¹ soil h⁻¹ for arylsulfatase, alkaline phosphatase and β -glucosidase for all treatments.

Relationships between plant and soil parameters

The correlations among soil and plant parameters are reported in Table 4. Shoot dry biomass (SDB) was strongly positively correlated with total bacteria and urease activity ($R = 0.73$) whereas plant K content was weakly positively correlated to total bacteria ($R = 0.52$). Apart the already mentioned correlations, it is to be noticed that SDB was also strongly positively correlated to plant Ni content ($R = 0.82$). Finally, regarding the relationships between soil parameters, two strong correlations were found, on one hand between total bacteria and urease activity ($R = 0.92$) and on the other hand between alkaline phosphatase and β -glucosidase activities ($R = 0.78$). A Principal Component Analysis (PCA) has been run with the data (Fig. S1). It confirmed that treatments separated in three statistically different groups along a gradient of both soil Ni concentration and N supply: Ni80-NF, Ni80-F/Ni40-NF and Ni40-F.

Discussion

Fertilisation effects on Ni phytoextraction

Concerning the variation of pH in the rhizosphere of *A. murale*, the addition of NiSO₄ to the soil induced a significant pH decrease. Similar results were observed by Saad et al. (2016) after increasing additions of NiSO₄ to an agricultural topsoil. This drop in pH is probably due to the complexation of Ni²⁺ to soil surface sites and to the exchange of Ni²⁺ with H⁺ from the soil exchange complex, both processes releasing surface H⁺ ions to soil solution. However, the reduction in pH did not af-

Fig. 5 Variation of **a** urease (URE), **b** alkaline phosphatase (AP), **c** β -glucosidase (β GLU) and **d** arylsulfatase (AS) concentrations according to the soil Ni concentration (Ni40 and Ni80) and the N

supply (F fertilized, NF unfertilized). Results are represented by means with their standard error. Different letters indicate statistical difference ($P < 0.05$, Tukey test, $n = 3$)

Table 4 Correlation matrix between plant and soil parameters

	Total cultivable bacteria (BacTot)	Urease activity (URE)	Alkaline phosphatase activity (AP)	Aryl-sulfatase activity (AS)	Beta-glucosidase activity (β GLU)	pH	Element concentration in shoots of <i>Alyssum murale</i>					Shoot dry biomass (SDB)
							K	P	S	Ca	Ni	
BacTot	1											
URE	0.92	1										
AP	-0.09	0.14	1									
AS	0.05	0.16	0.35	1								
β GLU	0.13	0.39	0.78	0.32	1							
pH	0.38	0.35	0.12	-0.20	0.01	1						
K	0.52	0.41	-0.34	0.08	-0.08	0.27	1					
P	0.29	0.21	-0.24	0.20	-0.14	0.13	<i>0.75</i>	1				
S	0.08	0.07	-0.51	0.07	-0.53	0.11	0.33	0.29	1			
Ca	0.18	0.22	0.10	-0.56	-0.03	0.18	-0.11	-0.34	0.16	1		
Ni	0.44	0.45	-0.20	-0.27	0.05	-0.35	0.38	0.16	0.01	0.34	1	
SDB	0.73	0.73	-0.16	0.02	0.05	-0.1	0.43	0.07	0.09	0.25	0.82	1

In bold and italic significant correlations at the respective 5 and 10% levels

fect Ni DTPA extractability nor phytoextraction. By contrast, N addition did not remarkably change the pH values.

The results obtained in this study indicated clearly that mineral N supply significantly improved *A. murale* growth under the different Ni additions tested (Ni40 and

Ni80). These results are in accordance with the positive effect of mineral fertilization on *A. murale* observed in several studies on plant biomass production (Bani et al. 2015) on the relief of stress conditions (Álvarez-López et al. 2016). Chaney et al. (2007) showed that *A. murale* crop yields could reach at least 20 tons of dry biomass per hectare (equivalent to 400 kg Ni ha⁻¹) after the application of mineral fertilizers (NPK), herbicide and the use of an appropriate irrigation management. Such results can be explained by the high needs of nitrogen by the hyperaccumulator plants (McNear et al. 2010; Liu et al. 2016; Kidd et al. 2017) and especially the species from the Brassicaceae family. The supply of N fertilizers improves plant dry weight but also the phytoextraction efficiency as shown for the Cd, Ni and Zn hyperaccumulator *Nocca caerulescens* (J.Presl & C.Presl—Brassicaceae) due to the acidification of soil (Sirguey et al. 2006) and the Ni hyperaccumulator *A. murale* (Bani et al. 2015). However, there are obvious differences reported in plant biomass and metal accumulation by *N. caerulescens* according to the form of N supplied, i.e. NH₄⁺ or NO₃⁻, the latter stimulating phytoextraction yield (Schwartz et al. 2003; Monsanto et al. 2008). In our case, we supplied both forms as ammonium nitrate so we did not provoke such strong effects. Moreover, the results from this study confirmed the absence of a Ni dilution phenomenon in the aerial parts of *A. murale* when plant biomass increased. Nickel concentrations in plant tissues increased with biomass yield. However, the increase of soil Ni concentration had no significant effect on the production of plant dry biomass.

Soil microbial activities

The soil microbial community represents a sensitive bioindicator of the environmental alteration (Sinsabaugh et al. 2008). Therefore, any changes in soil properties resulting either from natural or anthropogenic activities (like adding fertilizer or heavy metals to the soil) could affect the functioning and composition of soil microorganisms and, as a consequence, those of plants. Our study highlighted that increasing Ni concentrations in the soil significantly reduced bacterial density as shown for other metals in several studies (Vásquez-Murrieta et al. 2006; Sobolev and Begonia 2008; Li et al. 2009; Klimek 2012). However, in our case if the increase in Ni bioavailability was significant, the final level was quite limited compared to strongly polluted soils or most ultramafic soils (Benizri and Kidd 2018).

β -glucosidase, urease, arylsulfatase and alkaline phosphatase activities, representative of C, N, S and P biogeochemical cycles, respectively, were determined in the rhizosphere soil in response to increasing soil Ni concentrations and mineral N supply. Heavy metals contamination could lower soil enzyme activities due to protein denaturation, forming a complex with the substrate, interacting with the residues involved in enzyme functions, or by reacting with the enzyme-substrate

complex (Hemida et al. 1997). Concerning the activity of urease, our study revealed a decrease with higher Ni concentrations in soil as shown previously (Pattnaik and Equeenuddin 2016). More precisely, Xiao et al. (2017) indicated that urease activity was negatively correlated to the bioavailability of heavy metals in soils which is consistent with our findings as soils were artificially contaminated with highly available Ni salts.

Inversely to urease, our data showed non-significant variation of the three other soil enzyme activities (β -glucosidase, arylsulfatase and alkaline phosphatase) when the concentration of Ni in soil increased from 40 to 80 mg kg⁻¹ (Fig. 5). Similarly, Pessoa-Filho et al. (2015) found that enzyme activities related to C, P and S cycles were not affected by high Ni concentrations, where the enzymatic activities were compared on three ultramafic sites with different concentrations of DTPA-extractable Ni (5.20; 134 and 603 mg kg⁻¹). The changes in soil microbiological functioning observed in their study were more related to organic matter content than to Ni availability. If Epelde et al. (2009) confirmed that metal pollution did not cause a clear inhibition of soil enzyme activities, other studies have shown a negative effect of heavy metals on those activities with different patterns according to the study (see Kandeler et al. 2000; Renella et al. 2005).

Concerning the effect of N fertilizer, this study revealed that mineral N supply can reduce the detrimental effect of Ni on soil microbial activity. Indeed, N addition consistently increased the total cultivable bacteria and urease activity in the rhizosphere, whatever the Ni concentration applied to the soil. It is possible that the positive effect of N fertilization on the bacterial cells number and urease was indirect through a better growth of *A. murale* plants, which in turn provided higher carbon stocks to the rhizosphere microflora. Previous works again showed contradictory effects of N application on the microbial biomass and enzymatic activities. Similarly to our study, Jinghua et al. (2005) found that a certain level of N concentration would increase bacterial density. In fact, N addition would improve root development and exudation by alleviating soil N limitation and thus increasing microbial biomass (Zhang et al. 2017). An acceleration of urease activity has been also revealed by several studies after N addition on non-polluted soils (e.g. Guan et al. 2011); this could be attributed to the presence of different microorganism groups with contrasting responses to N addition.

In contrast to our finding, Geisseler and Scow (2014) have shown that N fertilization can significantly depress microbial biomass. This was also confirmed by a synthesis on 82 field studies revealing that N fertilization reduced the microbial biomass by an average of 15% (Treseder 2008). Also, Ajwa et al. (1999) found that N fertilization to the soil reduced the urease activity. In fact, N additions can repress the microbial production of urease in soils through the end products or by products formed from the microbial assimilation of excessive N (Saha et al. 2008).

Practical consequences for the agromining technology

These results confirmed that fertilization improves biomass yield without reducing the Ni concentration in plants. They also clearly show that continuous agromining of Ni from natural soils is expected to improve the soil microbial fertility (e.g. urease activity) over the long term as it will decrease Ni availability. Fertilization has also the effect of enhancing soil microbial activity and therefore of decreasing the toxicity effect of Ni on soil bacteria.

The rate of Ni removal after one crop was 6% for the Ni40 treatment and 8% for the Ni80 treatment with N fertilization. Assuming that one hectare contains 3200 tons of arable soil (0–20 cm), the Ni yield per ha was 8 kg ha⁻¹ for the N-fertilized Ni40 treatment and 20 kg ha⁻¹ for the N-fertilized Ni80 treatment with a 33 and 50%, respectively due to N fertilization compared to non-fertilized treatments. In terms of practical consequences, this means that fertilization of agromining plots is a necessary condition to improve phytoextraction yield but also to improve soil biological fertility (an ecosystem service).

Conclusion

This study has confirmed that nitrogen application may improve the phytoextraction efficiency of Ni by enhancing both biomass yield and Ni concentration in shoots. The measurement of total cultivable bacteria has also confirmed the toxic effect of Ni contamination on both soil microbes and urease activity. However, the supply of nitrogen fertilizer could alleviate the negative effect of Ni on soil microbial activities. In fact, N fertilizer has ameliorated both the bacterial community and the urease activity in the rhizosphere of *A. murale* independently of the soil Ni content. This resulted in a better plant growth and a higher accumulation of Ni in the aerial part and a subsequent alleviation of soil Ni toxicity for microbes.

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