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Effect of hyperaccumulating plant cover composition and rhizosphere-associated bacteria on the efficiency of nickel extraction from soil

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ABSTRACT

Most plant species selected as appropriate candidates for phytoextraction have been studied as monocultures. However, alternative cropping patterns which include rhizosphere microbial communities can significantly influence the extraction of metals, as well as soil protection and quality. Therefore, the objective of this work was to study the effect of species-rich vegetation cover, which consisted of three hyperaccumulator plant species, on the efficiency of nickel extraction from a naturally mineralized ultramafic soil. An experiment was set up with three hyperaccumulator species (*Leptoplax emarginata*, *Noccaea tymphaea* and *Alyssum murale*). Plants were cultivated separately (monospecific cover), or in combination (multispecies cover) in mesocosms under controlled conditions, on a nickel-rich ultramafic soil. Plants were grown for 92 days in controlled conditions. Each plant produced more biomass when grown in multispecies cover than alone. *Noccaea* and *Alyssum* showed the highest shoot Ni concentrations but *Alyssum* had by far the lowest shoot biomass. So, in this soil, *Noccaea* and *Leptoplax* have greater potential for hyperaccumulation than *Alyssum*. The amount of nickel accumulated in total biomass of *Noccaea* alone and of the multispecies cover was higher than that accumulated in either the monospecies *Leptoplax*or *Alyssum*. Furthermore, the highest values of microbial biomass were obtained with the multispecies cover and a consistent production of auxin compounds by bacterial communities was measured, which emphasized the role of rhizosphere bacteria. The bacterial genetic structure also depended on the plant covers. A combination of the three species (multispecies cover), could be a good strategy for phytoremediation.

1. Introduction

Worldwide contamination of soils with heavy metals causes a serious threat to the ecosystem and to human health. In recent decades, increasing concern about metal contamination and its toxicity to microorganisms, plants and animals has been reported (Kidd et al., 2009). Removal of metals from contaminated soils is particularly challenging because heavy metals are non-biodegradable (Garbisu and Alkorta, 2001) and can accumulate in plant tissues. Bioremediation based on microorganisms, plants or other biological systems provides a cost-effective and environmentally friendly method for metal clean-up (Chaney et al., 2007). Also, the phytomining of metals (i.e. metallurgical recovery of metal hyperaccumulated in plant biomass) has been considerably developed and is now ready for field application with nickel (Bani et al., 2007; Chaney et al., 2007; Tang et al., 2012). To increase phytomining yield, plant hormone external application has been tested directly on hyperaccumulator growth and metal uptake but it gives contradictory results (Cassina et al., 2011; Cabello-Conejo et al., 2013).

For nearly 10 years, the use of PGPR (plant growth-promoting rhizobacteria) in the remediation of soil inorganic pollutants has increased steadily (Lebeau et al., 2008; Glick, 2010). It has been shown that, in pollutant-hyperaccumulating plants, PGPR promotes their germination, increases the root biomass and also facilitates the survival of plants, despite the stress exerted by the pollutant. Increased exudation of 1-aminocyclopropane-1-carboxylate (ACC) together with a decrease in the synthesis of ethylene, known as a plant response to stress due to the pollutant, have also been observed (Glick, 2005). This results in a better plant

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development, although a bacterial growth was observed because ACC may act as a source of carbon (C) and nitrogen (N) for the rhizosphere microflora. In contrast, PGPR survival is hampered by nutrient-limiting conditions, unlike most polluted soils, underlining the important role of root exudates as a source of C and N for the microflora. Thus, many works have attempted to relate the association of different plants and the efficiency of inorganic pollutants extraction, with the hypothesis that these vegetation covers promote the development and the activities of some microorganisms, such as PGPR. Studies up to now, have mainly concerned crop associations. In particular, these studies have involved associations of tobacco in the presence of a legume such as clover (Liu et al., 2011), rice and maize in the presence of bean (Murakami and Ae, 2009), rape with alfalfa (Pan et al., 2008), co-cultures of various *Brassica* species (*B. junica*, *B. chinensis*) (Liu et al., 2007), or different tree species (Pulford and Watson, 2003). However, few studies have concerned the effect of the combination of metal hyperaccumulator plants with other species (Epelde et al., 2012). These experiments show that co-culture with non-hyperaccumulator plants could enhance the growth of the hyperaccumulator and increase the absorption of metals. Even fewer articles have concerned the effect of the combination of plants known to be hyperaccumulators (Whiting et al., 2001).

The objective of this work was to study the effect of species-rich vegetation cover, which only consisted of three hyperaccumulating plant species, on the efficiency of nickel extraction from a soil naturally rich in this metal. The effects on some soil physicochemical properties and on microbial communities colonizing the rhizosphere were also evaluated.

2. Materials and methods

2.1. Soil sampling and analysis

The soil used in this study had been collected from the top layers (A_1 and B_W horizons; 5–30 cm) of a natural forest ultramafic Hypermagnesic Hypereutric Cambisol (Vosges Mountains, eastern France, 07°06′42.2″ E, 48°11′03.7″ N).

The soil's physicochemical properties were determined by the Soil Analysis Laboratory of INRA (Arras, France), (Table 1). The soil material was a clay loam, with a pH of 5.83 and an organic matter content of 14.1%, C/N 13.4 and 416, 391 and 193 g kg^{-1} clay, silt and sand, respectively. Developed on a serpentinized harburgite, this soil was naturally rich in nickel (Ni) and the total Ni content was 1170 mg kg^{-1}.

2.2. Plant characteristics

Three *Brassica* sp., *Leptoplax emarginata* (Bois) O.E. Schulz, *Noccaea tymphaea* (Hausskn.) F. K. Mey. and *Alyssum murale* Waldst. & Kit were selected for this study based on their demonstrated ability to accumulate substantial amounts of nickel in shoots (Bani et al., 2009, 2014). At flowering stage (maximal Ni accumulation), *L. emarginata* exceeds one meter, *A. murale* exceeds 60 cm and *N. tymphaea* barely reaches 20 cm. Seeds were collected in Greece. *Nocceae* seeds were taken July 19, 2011 from the Katara Pass (1700 m) in Greece (39°47′765″ N, 21°13′739″ E). *Leptoplax* and *Alyssum* seeds were taken on July 20, 2011 and come from a site near the village of Trigona (830 m) in Greece (N 39°4′223″, E 21°15′869″); (see characteristics of the three species in Bani et al., 2009).

2.3. Experimental design

Three kilograms of soil (on a DW basis) were placed in parallelepiped pots 13 cm × 24 cm × 16 cm (l × L × h). The mesocosms were planted with species used either separately (monospecific

Table 1
Chemical and physical properties[a] of the soil used.

pH	Clay	Silt	Sand	Organic carbon	Total nitrogen	P_2O_5	C.E.C.	Ca	Mg	K	$Fe_{Oxalate}$	Fe_{BCD}	Total Cr	Total Cu	Total Ni	Total Zn	Total Co	Ni_{DTPA}	Fe_{DTPA}	Mn_{DTPA}
(water)	(g kg^{-1})						(cmol + kg^{-1})				(g kg^{-1})		(mg kg^{-1})							
5.83	416	391	193	81.7	6.11	0.015	30	3.1	25.8	0.27	21.3	60.4	2680	13.4	1170	216	133	62.5	331	10.7

[a] Analyzed by INRA, Arras, France.