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Influence of new agromining cropping systems on soil bacterial diversity and the physico-chemical characteristics of an ultramafic soil

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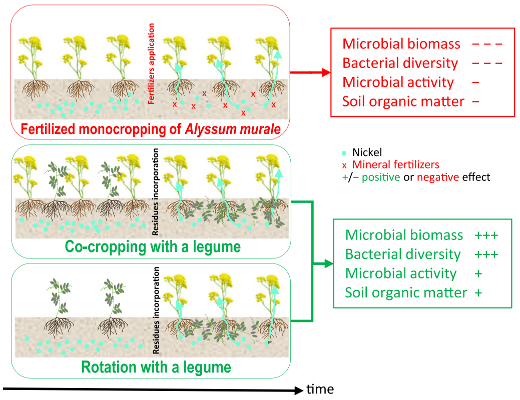
HIGHLIGHTS

- Plant diversity in agromining can modify the soil characteristics and processes.
- Microbial biomass and enzymatic activities were affected by the legume insertion.
- Soil bacterial diversity was increased with the legume insertion.
- Mineral fertilization diminished bacterial diversity.

GRAPHICAL ABSTRACT



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ABSTRACT

Most of the research dedicated to agromining has focused on cultivating a single hyperaccumulator plant, although plant diversity has been shown to positively modify soil characteristics. Hence, we compared the effect of cropping a nickel-hyperaccumulator *Alyssum murale* with a legume (*Vicia sativa*) to *A. murale*'s monoculture, on the bacterial diversity and physico-chemical characteristics of an ultramafic soil. A pot experiment with 5 replicates was conducted in controlled conditions for 11 months. The treatments studied were: co-cropping and rotation vs. mineral fertilization controls and bare soil. The introduction of legumes induced a clearly positive effect on the soil's microbial biomass carbon and nitrogen. Arylsulfatase and urease activities tended to be enhanced in the co-cropping and rotation treatments and to be lessened in the mineral fertilization treatments. However, β-glucosidase and phosphatase activities were seen to decrease when legumes were used. Our results showed that the rotation treatment induced a higher organic matter content than the fertilized control did. *Actinobacteria* was the most-represented bacterial phyla and had lower relative abundance in treatments associating legumes. Conversely, the relative abundance of *Acidobacteria* and *Gemmatimonadetes* phyla increased but not significantly in treatments with legumes. The relative abundance of *Chloroflexi* phylum was shown to be significantly higher for the fertilized rotation control. The relative abundance of β-*Proteobacteria* subphylum increased but not significantly in treatments with legumes. NMDS analysis showed a clear separation between planted treatments and bare soil and between co-cropping and rotation and fertilized controls. Shannon index

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showed reduction in microbial diversity that was mainly due to chemical inputs in the soil. This study showed that these new cropping systems influenced both the bacterial diversity and the physico-chemical characteristics of an ultramafic soil. In addition, this study provides evidence that mineral fertilization can negatively impact bacterial communities and some of their functions linked to biogeochemical cycles.

1. Introduction

Over the past decade, numerous studies have highlighted the need to optimize the phytomining process and have described a more integrated chain called agromining (van der Ent et al., 2013, 2015). Agromining in its first stages, used conventional crop management methods in order to increase plant biomass and thus the extraction of metals, such as nickel (Ni), for further hydrometallurgical recovery. The use of NPK mineral fertilizers, herbicide application and irrigation management have been successfully described in order to optimize agromining crop yields (Nkrumah et al., 2016). Consequently, the latter has been seen to reach 10–20 tons ha^{-1} of dry biomass with 100–400 kg Ni ha^{-1}, depending on local conditions (Chaney et al., 2007; Bani et al., 2015). However, there is a need to develop agromining in a more agro-ecological and environmentally-sound way. This has meant that organic amendments (such as compost or manure) have been studied as a substitute for mineral fertilization (Kidd et al., 2015; Nkrumah et al., 2016; Álvarez-López et al., 2016). The use of PGPR bacteria (Plant Growth Promoting Rhizobacteria) which on the one hand, favors the absorption of metals by plants and on the other hand, the growth of inoculated plants (Haslmayr et al., 2014), has also recently been introduced in agromining. Consequently, PGPR isolated from the native rhizosphere of *Alyssum serpyllifolium*, *Alyssum malacitanum* and *Alyssum murale* have been shown to significantly increase plant biomass and Ni uptake (Abou-Shanab et al., 2003, 2008; Rajkumar and Freitas, 2008; Becerra-Castro et al., 2013; Durand et al., 2016).

Besides using PGPR bacteria to optimize agromining, researchers have also been interested in studying plant covers. However, most studies dedicated to agromining have been based on the cultivation of a single hyperaccumulator plant that can accumulate, for example, >1000 mg Ni kg^{-1} in its aerial dry biomass (Baker and Brooks, 1989; Reeves et al., 1996). Conversely, only a few studies have investigated the impact on phytoextraction efficiency of associating different plants (Gove et al., 2002; Wu et al., 2007; Pan et al., 2008; Jiang et al., 2010; Epelde et al., 2012). In the case of the exclusive use of hyperaccumulator plant covers, the coexistence of several hyperaccumulators has been seen to improve the biomass production of each plant species, to promote the bioavailability of metals in the soil, as well as modifying the genetic and phenotypic structures of rhizosphere bacterial communities (Lucisine et al., 2014).

Many studies have assumed that differences in exudation patterns were partly responsible for diversity divergences of the soil microbial communities (Grayston and Campbell, 1996; Baudoin et al., 2001, 2002; Steinauer et al., 2016). These results have been attributed to the effect of exudate diversity and availability. Conversely, few studies have focused on the effect of multi-species vegetation covers on soil microbial diversity (Spehn et al., 2000; Zak et al., 2003; Innes et al., 2004; Benizri and Amiaud, 2005). These studies, focusing on agricultural cropping systems or on grasslands, have shown that increased plant diversity enhances soil microbial biomass, activity and diversity (Benizri and Amiaud, 2005; Chung et al., 2007; Tiemann et al., 2015). Indeed, diversified organic compounds (i.e. rhizodeposits), released from different plants growing together in multi-species vegetation cover, can change the rhizospheric environmental conditions and affect the abundance, functions and diversity of microbial communities (Berg and Smalla, 2009). It has been shown that microbial biomass is significantly correlated to plant diversity in experimental fields involving 1 to 16 plant species (Zak et al., 2003; Prober et al., 2015). Moreover, the association of different plant species allowed, on the one hand, an increase in the size of the microbial community by 15–20% when compared to a monoculture and on the other hand, a relative stimulation of certain functions of the associated rhizosphere's microbial community (Gao et al., 2010, 2012). Indeed, concerning microbial activities and functions, Gao et al. (2010, 2012) pointed out that plant diversity induces a complex microbial community and more diverse microbial functional groups. The activity of soil microorganisms was found to be dependent on the availability of different substrates in the soil (Burns et al., 2013) and consequently, a plant cover characterized by the presence of different plant species could both induce differences in a soil's chemical properties and changes in microbial activities and functions.

It has previously been shown by Saad et al. (2018) that new cropping systems associating a hyperaccumulator plant with a legume can increase Ni-phytoextraction and biomass production. In this study, the aim was to focus solely on the impact of these new agromining cropping systems on soil bacterial diversity and the physico-chemical characteristics of an ultramafic soil. To our knowledge, no studies have so far investigated the impact of cropping systems dedicated to agromining, which are based on cropping a hyperaccumulator plant with a non-accumulator (e.g. a legume), with the hypothesis that these mixed plant covers could have an impact on rhizosphere bacterial diversity, microbial functions and on soil physico-chemical parameters. The objective of this work was to study the effect of two plant species, a hyperaccumulator plant (*Alyssum murale*) and a legume (*Vicia sativa*) grown in co-cropping or rotation cultural systems dedicated to agromining, both on the diversity of bacterial communities and on the physico-chemical characteristics of a Ni-rich ultramafic soil.

2. Materials and methods

2.1. Soil characteristics and experimental design

A pot experiment, using the hyperaccumulator plant *Alyssum murale* (Waldst & Kit.) and the legume *Vicia sativa* var. Prontivesa, was conducted for 11 months in a growth chamber. *A. murale* seeds were harvested from a natural site near Trigona (39°47′17,5″N, 21°25′19,1″E, Greece) in August 2014, and *V. sativa* seeds were provided by Semillas Batlle (www.semillasbatlle.es, Spain). The ultramafic soil used for the experiment was collected from the topsoil of the region of Melide in Spain (42°49′54.5″N, 8°00′13.5″W, Agolada, Pontevedra, Spain), where field agromining experiments are currently been carried out. Soil physico-chemical properties were determined by the Soil Analysis Laboratory of INRA (Arras, France). This topsoil contained 17.5% clay, 30.6% silt and 51.9% sand, had a C/N ratio of 13.9, a Mg/Ca (exchangeable) ratio of 2.79 and an available phosphorus content (P-Olsen) of 23 mg kg^{-1}. Soil pH was 5.76 and the total and available nickel (Ni-DTPA) contents were 861 and 24.8 mg Ni kg^{-1} of dry soil, respectively.

The treatments were as follows: co-cropping CoC (Co-Cropping of the legume and *A. murale*, legume shoots were cut and buried in the soil after 3 months of co-culture), fertilized control of co-cropping FCon-CoC (*A. murale* alone with mineral fertilization; 120 kg N per hectare are added as NH_4NO_3 at the time of burying legume residues in the co-cropping treatment), non-fertilized control of co-cropping NFCon-CoC (*A. murale* alone without fertilization), rotation Ro (legume culture for 3 months followed, after burying legume shoots, by the culture of *A. murale*, this treatment being put in place three months prior to the Co-Cropping treatment), fertilized control of rotation FCon-Ro