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RESEARCH ARTICLE

Plant genetics and site properties influenced the diversity of seed endophytic bacterial communities of *Odontarrhena* species from serpentine soil of Albania

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Abstract

Aims Seed endophytic bacteria (SEB) are able to improve plant growth and to protect them against abiotic or biotic stresses. This work aimed to characterize the seed endophytic bacterial communities associated with different species of the nickel hyperaccumulator *Odontarrhena*, which is adapted to extreme environments such as serpentine soils. Moreover, this work also aimed to study any potential congruency between SEB community diversity and plant phylogeny.

Methods Endophytic bacterial communities were characterized for seeds from 9 *Odontarrhena* populations, using high throughput sequencing. The plant genomes and environmental properties of the sites had previously been described.

Results and discussion All *Odontarrhena* populations shared more than 95% of their OTUs and metabarcoding revealed a large SEB core microbiome. The plant species was more determinant than the site in explaining the dissimilarities between SEB communities. Nonetheless, both site and *Odontarrhena* species factors were significant diversity drivers of the SEB communities and the best explanatory factor was the interaction between them. When focusing only on plant populations, some OTUs were over- or under-represented in the *O. chalcidica* SEB communities in comparison with the SEB communities of the 4 other *Odontarrhena* species. With the current genetic markers, the cophylogenetic analysis revealed a non-significant coherence of phylogenies between seed microbiota and corresponding host plants. The OTUs based prediction of metabolic functions, is a first step that would potentially allow the power of the microbiome to be harnessed, thereby improving hyperaccumulator production in an agromining context.

Keywords Bacterial diversity · *Odontarrhena* sp. · Endophyte · High throughput sequencing · Seed · Co-evolution

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Introduction

Over recent decades, nickel-hyperaccumulators have attracted a great deal of interest for both scientific research and practical applications. Of particular notes are those of phytoremediation and agromining, which together constitute a non-destructive approach to the recovery of high value metals from polluted or naturally metal-enriched soils (Bani et al. 2007, 2015; Barbaroux et al. 2011; van der Ent et al. 2013; Nkrumah et al. 2016; Lopez et al. 2017; Pardo et al. 2018). Plants belonging to the *Brassicaceae* family, such as species of the genus *Odontarrhena* (syn. *Alyssum pro parte*), are nickel-hyperaccumulators which have been extensively studied in Europe both as models of plant adaptation to atypical and inhospitable soils and as tools for agromining techniques (Cecchi et al. 2013; Lopez et al. 2017, 2019; Saad et al. 2021, 2018a, b, c;). Members of the *Odontarrhena* genus from serpentine outcrops are distributed in the Euro-Mediterranean region and their greatest diversity is found in Albanian territory (Chardot et al. 2007; Cecchi et al. 2010; Bettarini et al. 2019). A recent systematic revision of this genus (Cecchi et al. 2018) points to the existence of seven taxa in Albania, of which six Ni-hyperaccumulators are restricted to serpentine soils (except for *O. chalcidica*, facultative serpentinophyte). These serpentine soils are the most exploited for agromining and present unique geochemical characteristics, such as high levels of nickel (Ni), cobalt (Co) and chromium (Cr), low levels of macronutrients (N, P, K and Ca) and a high Mg/Ca ratio (Saad et al. 2018a, b, c).

Odontarrhena spp. (formerly genus *Alyssum* section *Odontarrhena*, Španiel et al. 2015), and especially *O. chalcidica,* can grow in altitudes ranging from sea level to 2000 m above sea level and these species are all typical xerophytes restricted to dry and open habitats. They are well known to tolerate and accumulate large amounts of Ni in their above ground tissues and this is interpreted as a defensive strategy against natural enemies due to toxic or repellent effects of the metal (Palomino et al. 2007). The *Odontarrhena* genus includes 87 species (Spaniel et al. 2015; updated version available on line at http://www.alysseae.sav.sk/) and the Balkan region is a major diversity center for *Odontarrhena*, with 15 to 25 species (Cecchi et al. 2010). Among them, *O. chalcidica, O. rigida, O moravensis, O. smolikana,* and *O. decipiens* have recently been described regarding their carpological features, ecological traits, flowering and fruiting times and distribution patterns (Cecchi et al. 2018). Their study underlined that *O. chalcidica* is widely distributed throughout Albania and able to accumulate the highest concentrations of Ni in its leaves (between 4.6 to 23 g kg^{-1} DW).

Over recent decades, plant endophytic bacteria have received much attention from researchers (Azevedo 2000), because some of them are able to stimulate the growth of their hosts and to protect them against stresses, such as pathogen infections, drought or high concentrations of trace elements (Sturz and Nowak 2000; Card et al. 2015). Among these microorganisms called 'plant growth promoting endophytes' (PGPE), some have the capacity to tolerate or resist to high metal concentrations and exhibit similar properties to those described for plant growth promoting rhizobacteria (PGPR) (Hardoim et al. 2008; Ullah et al. 2015). Many of these properties are associated with the secondary metabolism of the microorganisms, rather than with their central metabolic pathways. These activities included ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, production of indole-3-acetic acid, synthesis of siderophores, and phosphorus solubilization. It has already been shown that phytoextraction by plants exposed to metals may benefit from such PGPE inoculations (Ma et al. 2015). More recently, Durand et al. (2021), studying endophytic bacterial communities of the Ni, Zn and Cd hyperaccumulator *Noccaea caerulescens* across 14 sites in France, showed that some of the most frequently occurring OTUs found in the seed endophytic bacterial (SEB) communities could be potential PGPEs.

PGPEs and their plant growth promotion mechanisms have recently been reviewed in seeds, (Shahzad et al. 2018). A seed endosphere is a particularly specific habitat for microorganisms, since the dispersal of seeds as well as their transition to seedlings are the most critical stages of the plant life cycle (Nelson 2017). Moreover, seeds are of particular interest as they may transmit endophytes vertically from generation to generation (Durand et al. 2022). Due to the long-term interaction between the plant and the systemic endophytes, the seed-borne microorganisms must display a great physiological adaptation capacity to the conditions encountered during seed germination (Barret et al. 2015). Some of those SEBs may

have a competitive advantage for the colonization of the plant endosphere in the early stages of the plant's life. They may also express more of their beneficial properties from the rhizosphere for example, than other bacteria, (Johnston-Monje et al. 2016). Due to these combined properties, the seed endosphere may be a microbial habitat exerting a strong selective pressure that is capable of selecting non-pathogenic endophytes or mutualists. This would therefore enable vertically transmitted endophytes to co-evolve with their host plant and share a symbiotic relationship with it across generations (Mostert et al. 2000). The holobiont and hologenome concepts have become widely used by the scientific community to refer to entities that can be formed with symbiotic microbes, including those which affect the holobiont's phenotype and have coevolved with the host, those which affect the holobiont's phenotype, but have not co-evolved with the host, and those which do not affect the holobiont's phenotype at all (Theis et al. 2016). In this conceptual framework, the holobiont can act as a unit of selection, since combined genomes could influence the host phenotype on which selection may operate (O'brien et al. 2019). SEB communities have beneficial roles, such as the facilitation of seed germination by protecting them from predation and attack by pathogens, or leading to a reduction in abiotic stresses (Abdullaeva et al. 2021) as well as promoting plant growth (Card et al. 2015). However, to date, SEB communities have been little studied, particularly in the case of hyperaccumulating plants. Recently, using 16S rRNA metabarcoding, Durand et al. (2021) characterized SEB communities from 14 *Noccaea caerulescens* populations. Previously, Gonneau et al. (2017) carried out a genotyping using a combination of new chloroplast and nuclear neutral markers on those plant populations. A strong genetic structure was detected, allowing the definition of three genetic subunits, which refer to three groups of Western Europe plant populations, based on geographic proximity, shared haplotype and membership of a single gene pool. In the study of Durand et al. (2021) the factor that best explained dissimilarities between SEB communities of the 14 *Noccaea caerulescens* populations was the plant's genetic subunit. Moreover, this study revealed a large SEB core microbiome shared by all *Noccaea caerulescens* populations studied.

Although hyperaccumulating plants from the *Odontarrhena* genus have been abundantly studied in their environments for the ability to extract and accumulate metals and for their potential in agromining, little is known about the structure and composition of their SEB communities. In this study, the diversity and structure of SEB communities of 9 populations of *Odontarrhena* species, found in serpentine soils from Albania, were characterized using Illumina high throughput sequencing. We hypothesized first that these populations of *Odontarrhena* may harbor similar SEB microbiomes despite the differences in the physicochemical parameters of the soils where these species grew and secondly that there is a potential congruency between SEB community diversity and the plant phylogeny.

Materials and methods

Sampling and seed surface sterilization

Collection of soil and plant materials was performed during field trips to Albania in 2016 and 2017 (Bettarini et al. 2019). In total, seeds from 9 populations of plants belonging to the genus *Odontarrhena* were recovered from serpentine soils across the whole Albanian territory (Table 1). The term 'population' in this work signifies those plants belonging to each of the five species of *Odontarrhena* (*O. chalcidica*, *O. rigida*, *O. moravensis*, *O. smolikana*, and *O. decipiens*) recovered from a given sampling area (a site) and makes no assumption about the way individual plants breed.

Altitude (m) and concentrations of pseudo-total major (Fe, Mg, Ca and K) and minor (Ni, Mn, Co and Cr) elements (g kg^{-1}) in the bulk soils had previously been determined for each population from each site (Bettarini et al. 2019) (Table 2). Concentrations of major (Fe, Mg, Ca and K) and minor (Ni, Mn, Co and Cr) elements (mmol kg^{-1}) in the aerial plant parts had also previously been determined by Bettarini et al. (2019) (Table 3).

For each population, closed siliques containing seeds were sampled from a small area (around 400 m^2) from an assemblage of different individual plants (around 5 adult plants) of the population in order to obtain a seed stock. Siliques from each population were opened in the laboratory and undamaged seeds were selected for the next steps. Each seed sample corresponded to 50 mg of seeds (around 70 seeds)

Table 1 *Odontarrhena* population and sites from which seeds were collected. Sites are all located on serpentine soils from Albania

Population ID	Plant species	Sites where seeds were recovered	Latitude N Longitude E
Oc.Sc	*O. chalcidica*	Scutari (aka Shkodër)	42°03′08.60″ 19°32′01.03″
Oc.Sh	*O. chalcidica*	Shishice (in Elbasan)	41°05′54.28″ 20°08′46.13″
Oc.Dr	*O. chalcidica*	Drenovë (in Korçë)	40°34′22.43″ 20°48′23.45″
Oc.Po	*O. chalcidica*	Pogradeč (in Korçë)	40°54′49.86″ 20°38′15.97″
Oc.Pr	*O. chalcidica*	Prrenjas (on Mt. Shebenik)	41°09′14.08″ 20°32′18.60″
Or.Pr	*O. rigida*	Prrenjas (on Mt. Shebenik)	41°09′14.08″ 20°32′18.60″
Om.Vo	*O. moravensis*	Voskopoje (in Korçë)	40°36′01.08″ 20°35′40.27″
Os.Qa	*O. smolikana*	Qafë Shtamë (in Kruje)	41°31′31.94″ 19°54′07.49″
Od.Qa	*O. decipiens*	Qafë Shtamë (in Kruje)	41°31′11.42″ 19°52′51.85″

Table 2 Altitude (m), pH and pseudo-total concentrations (g kg^{-1}) of elements in the soils (Bettarini et al. 2019)

Population ID	Altitude	pH	Fe	Mg	Ca	K	Ni	Mn	Co	Cr
Oc.Sc	18	6.9	159	66	6	0.50	4.8	2.0	0.310	3.70
Oc.Sh	186	6.3	113	86	1.7	1.30	2.9	1.3	0.086	0.75
Oc.Dr	1121	7.2	97	96	7.0	0.80	2.5	1.7	0.118	0.88
Oc.Po	793	6.7	95	100	6.0	2.40	3.0	2.2	0.210	0.90
Oc.Pr	1248	6.8	80	142	5.0	0.70	3.5	1.9	0.220	0.74
Or.Pr	1248	6.8	80	142	5.0	0.70	3.5	1.9	0.220	0.74
Om.Vo	1428	6.5	85	97	13.0	1.90	1.4	1.2	0.150	1.30
Os.Qa	1155	6.9	123	131	13.0	0.69	3.2	1.8	0.190	1.20
Od.Qa	1004	6.7	111	145	17.0	0.70	3.9	1.2	0.150	2.75

Table 3 Concentrations (mmol kg^{-1}) of elements in the aerial parts of the plants (Bettarini et al. 2019)

Population ID	Fe	Mg	Ca	K	Ni	Mn	Co	Cr
Oc.Sc	5.09	119	573	169	224.87	1.02	0.356	0.673
Oc.Sh	16.81	333	486	215	258.94	1.29	0.373	0.192
Oc.Dr	13.74	530	401	176	327.09	4.15	0.596	0.212
Oc.Po	13.15	317	549	118	328.79	1.77	0.942	0.635
Oc.Pr	22.85	251	980	342	126.06	0.801	1.001	0.115
Or.Pr	13.74	530	401	176	327.09	4.15	0.596	0.212
Om.Vo	22.06	173	970	228	199.32	1.51	0.374	0.096
Os.Qa	6.33	428	636	153	139.69	1.29	1.7	0.115
Od.Qa	7.56	99	718	228	248.72	1.91	0.509	0.462

and four independent replicates were made for each plant population. Seed surface sterilization was carried out as follows: seeds were immersed for 30 s in an NaClO 2.6% solution supplemented with Triton 100×0.1%, then washed for 30 s with EtOH (97%) and rinsed three times for 30 s with sterile deionized water. Seed sterilization was confirmed by running a PCR test on the final rinse water (Sánchez-López et al. 2018). The PCR was designed to amplify the bacterial 16S rRNA gene using the following primers: 27f (5′- AGA GTT TGA TCA TGG CTC A -3′) and 1492r (5′- TAC GGT TAC CTT GTT ACG ACT T -3′) (Eurofins Genomics, Paris, France) and by using the thermoscientific DreamTaqTM Green PCR Master Mix (2X) kit (Thermo Fisher Scientific, Carlsbad, California). For each PCR mix, 25 µL of Dream Taq Green master mix were used, each universal primer was adjusted to 0.5 µM, 5 µL of the final rinse water were added and final volume was adjusted to 50 µL with nuclease free water. DNA amplification was

carried out in a thermocycler (Mastercycler gradient, Eppendorf, Hamburg, Germany) under the following conditions: 95 °C, for 2 min, 30 cycles 95 °C 30 s, 53 °C 30 s, and 72 °C 1 min, with an additional 10 min at 72 °C. Bacterial DNA from a previously isolated strain was used as a positive control. Finally, if no DNA was detected after PCR, the seed surface was considered to have been sterilized effectively.

Seed DNA extraction, targeted DNA amplification and sequencing

Immediately after surface sterilization, each seed sample was dried at ambient temperature in sterile conditions under a laminar flow hood and was ground under sterile conditions into a homogenous powder with a Mixer Mill for 30 s at 30 Hz (model MM400; Retsch Inc., Newtown, Pennsylvania, USA). Total DNA was extracted with a modified hexadecyltrimethylammonium bromide (CTAB) chloroform alcohol protocol (Healey et al. 2014). Briefly, the extraction protocol required 1 h at 65 °C with multiple agitations in the CTAB buffer (CTAB 2% (w:v), EDTA 20 mM, TrisHCl 100 mM, and NaCl 1.2 M), a heat shock (−80 °C to 65 °C), and enzymatic digestions with proteinase K (around 0.2 mg. mL^{-1} in a reaction), α-amylase from *Aspergillus oryzae* (around 3 $U.mL^{-1}$ in a reaction) and RNAse A (around 0.1 $mg.mL^{-1}$ in a reaction). The DNA precipitation was obtained firstly with isopropanol (at ambient temperature, 15 min) and next with ethanol 70% (at 4 °C, 30 min). A purification step was added using a QIAquick® PCR Purification Kit (Qiagen, Germany). The quantity and quality of the purified DNA were assessed using electrophoresis migration on a 1% agarose gel and with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, made in USA). The PCR targeted the V5-V6 hypervariable regions of the 16S rRNA gene with chloroplast DNA excluding 799f (5′—AACMGGATTAGATACC CKG—3′) and 1115r (5′- AGG GTT GCG CTC GTT G—3′) primers, resulting in an amplicon of a small size (~316 bp), appropriate for Illumina sequencing (Kembel et al. 2014). The resulting amplicons were purified with AMpure magnetic beads (Agencourt) and pooled in equimolar concentrations, prior to sequencing performed with an Illumina MiSeq platform (ADNid, France). These libraries were mixed with Illumina-generated PhiX control libraries (5%) and sequenced with the MiSeq Reagent Kit V3–600 cycles (Illumina Inc., San Diego, USA).

Bioinformatics and statistical treatment

The reads were assigned to each sample according to a unique barcode, and the following procedure was carried out using a Mothur v.1.40.5 pipeline (last update 06/19/2018) (Schloss et al. 2009). Briefly, all raw read pairs were merged at the overlapping region V5-V6 of 16S rRNA gene. Size and quality, as well as chimera, singleton and contaminant filters were applied. Non-discarded sequences (effective sequences) were then clustered into OTUs, defined at 97% sequence similarity. The 16S rRNA sequences retained were aligned with those present in the Silva database to remove non-16S rRNA sequences. Taxonomic assignments were made using a Bayesian approach (Wang et al. 2007) with the SILVA ribosomal RNA databases v1.3.8 (Dec 16, 2019) (Quast et al. 2013). The OTUs were derived using the Needleman distance and average neighbor clustering at a distance of 0.03. The following analyses were performed based on a dataset in which the number of reads per sample were rarefied to 3,646 with the Mothur 'sub.sample' function. OTU-based analysis of alpha diversity was performed using the following Mothur functions: 'sobs', 'chao', 'shannoneven', 'shannon', 'invsimpson', and 'coverage'. These estimates included: observed OTU richness, Chao estimation of OTU richness (Chao 1949), ACE (Abundance-based Coverage Estimator) estimation of OTU richness, Shannon diversity index, inverse Simpson diversity index, an evenness measurement based on the Shannon index and finally, coverage. The coverage calculator returned a Good's coverage OTU definition (Good 1953). Coverage was calculated using the following equation: $C=[1-(n/N)]*100$ (%), where 'n' is the number of sequences in each OTU that have been sampled once and 'N' the total number of sequences. For the following analyses, we used the R version 4.1.2 (latest update 01/11/2021) (R Core Team 2019). The 2-dimensional non-metric multi-dimensional scaling (NMDS) plots were calculated using the Bray Curtis method ($k=3$) based on the standardized (Wisconsin double) and square root transformation of OTU abundance, using the 'metaMDS' function of the vegan R package. Diversity and relative abundance (RA) of the most abundant OTUs

(cutoff 0.75% RA) of *Odontarrhena* seed bacterial communities were depicted using the 'riverplot' function of the riverplot R package.

These results were paired with a volcano plot using R and the 'gridExtra' and 'calibrate' packages, thereby showing the significantly over-represented OTUs (green) and significantly under-represented OTUs (red) in *O. chalcidica* seeds compared to the seeds of the 4 other *Odontarrhena* species. For each OTU, the ratio corresponded to the mean OTU RA in the SEB community of the 5 *O. chalcidica* populations divided by the mean OTU RA in the SEB community of the grouping of the 4 other *Odontarrhena* species. In order to explore relationships between community structure and soil properties, redundancy analyses (RDA) were performed using XLSTAT software (XLSTAT version Ecology 18.07, 243 http://www.xlstat. com). The correlogram was drawn using the 'cor' function of the 'corrplot' R package.

A Variation Partitioning Analysis (VPA) was performed to determine the effects of soil parameters, plant species, environmental factors (topography: slope and altitude) and interactions between these data on the structure of the bacterial communities. VPA was performed in R using the 'Vegan package'.

The metabolic functions of the OTUs were predicted using the T ax4Fun package (Aßhauer et al. 2015), which transforms the SILVA based OTUs into a taxonomic KEGG profile (Kyoto Encyclopedia of Genes and Genomes) organisms (fctProfiling = T), normalized by the 16S rRNA copy number (normCopyNo = T). Tukey HSD tests were performed on predictive functions to highlight significant differences between the different seed populations at *p-value* < 0.05.

Cophylogenic analysis

The cophylogeny of host plants and seed endosphere bacteria of the *Odontarrhena* originating from the whole Albanian territory, were calculated using paco (v0.3.1), an R package. Lines connecting dots on the phylogenies indicate plant–bacterial associations that were stronger than expected according to a host–bacteria co-evolution test (*p-value* < 0.05).

Bacterial abundances were pooled according to the plant populations reported in the UPGMA (Unweighted Pair Group Method with Arithmetic mean) clustering. Bacterial counts were summed and then normalized using relative abundance to account for the different sequencing depths produced by pooling. The sequences used for the plant phylogeny reconstruction were retrieved from a previous work focused on the phylogeny and the genetic structure of the Albanian *Odontarrhena* group (Coppi et al. 2020). Bray–Curtis distances were estimated using the 'vegdist' function of the vegan R package (version 2.5). Correlations between distance matrices were tested using the Mantel test implemented in the 'mantel.rtest' function of the ade4 R package (version 1.7). This function performs a random permutation test using Monte-Carlo randomizations (1′000 permutations in our case) and then assesses whether the correlation value obtained from the given matrices is different from random correlation values obtained by permuting observations. Comparison between trees was performed using the 'cophylo' function of the phytools R package (version 0.7).

Results

The seed endophytic bacterial community structure and composition

The SEB communities recovered from nine populations of *Odontarrhena* collected across Albania were characterized using Illumina MiSeq sequencing. Bioinformatic treatments allowed to obtain 131,566 effectives sequences for 36 samples (3,646 reads for each sample) that were clustered into 90 OTUs. The 'Good's coverage' index and the rarefaction curve analysis (data not shown) indicated that bacterial diversity was well represented.

Alpha diversity indices revealed that SEB communities have comparable richness and diversity, and no significant differences were detected (Table 4). The size of the communities was between 80 and 83 total OTUs. The richness evaluated with the Chao estimator varied between 93 (±6) and 123 (±8). The SEB communities showed a mean Shannon index of 3.55 (±0.06) and a mean inverse Simpson index of 17.22 (±0.85). In addition, a Venn diagram revealed that more than 95% of the OTUs were shared between the nine *Odontarrhena* SEB communities (data not shown).

Bacterial composition study (Fig. 1) of the samples revealed that *Proteobacteria* was the

Table 4 Diversity indices. All diversity statistics were calculated using an OTU threshold of ≥ 97% sequence similarity on randomly sub-sampled data at the lower sample size (3,646 reads). Richness was calculated using the Chao1 estimator. Diversity was estimated from the Shannon–Wiener (H), Inverse Simpson's (1/D), and Shannon Index Evenness (E) indices. Mean values and standard deviations (mean ± SD) are provided, n = 4

Population Id	Observed OTUs	Chao1 estimator	Shannon Index (H)	Inverse Simpson index (1/D)	Shannon Index Evenness (E)	Good's Coverage (%)
Oc.Sc	83 (± 1)	123 (± 8)	3.599 (± 0.011)	17.649 (± 0.144)	0.815 (± 0.002)	99
Oc.Sh	80 (± 1)	99 (± 5)	3.576 (± 0.010)	16.982 (± 0.096)	0.816 (± 0.002)	
Oc.Dr	82 (± 1)	108 (± 8)	3.600 (± 0.002)	17.942 (± 0.130)	0.818 (± 0.002)	
Oc.Po	83 (± 1)	106 (± 6)	3.567 (± 0.013)	17.093 (± 0.345)	0.807 (± 0.003)	
Oc.Pr	83 (± 1)	109 (± 5)	3.651(± 0.019)	19.020 (± 0.501)	0.827 (± 0.004)	
Or.Pr	83 (± 1)	101 (± 3)	3.512 (± 0.022)	16.850 (± 0.466)	0.796 (± 0.004)	
Om.Vo	81 (± 2)	94 (± 5)	3.510 (± 0.009)	16.697 (± 0.311)	0.800 (± 0.004)	
Os.Qa	82 (± 2)	120 (± 15)	3.488 (± 0.011)	16.452 (± 0.273)	0.791 (± 0.004)	
Od.Qa	80 (± 2)	93 (± 6)	3.467 (± 0.011)	16.333 (± 0.180)	0.792 (± 0.003)	

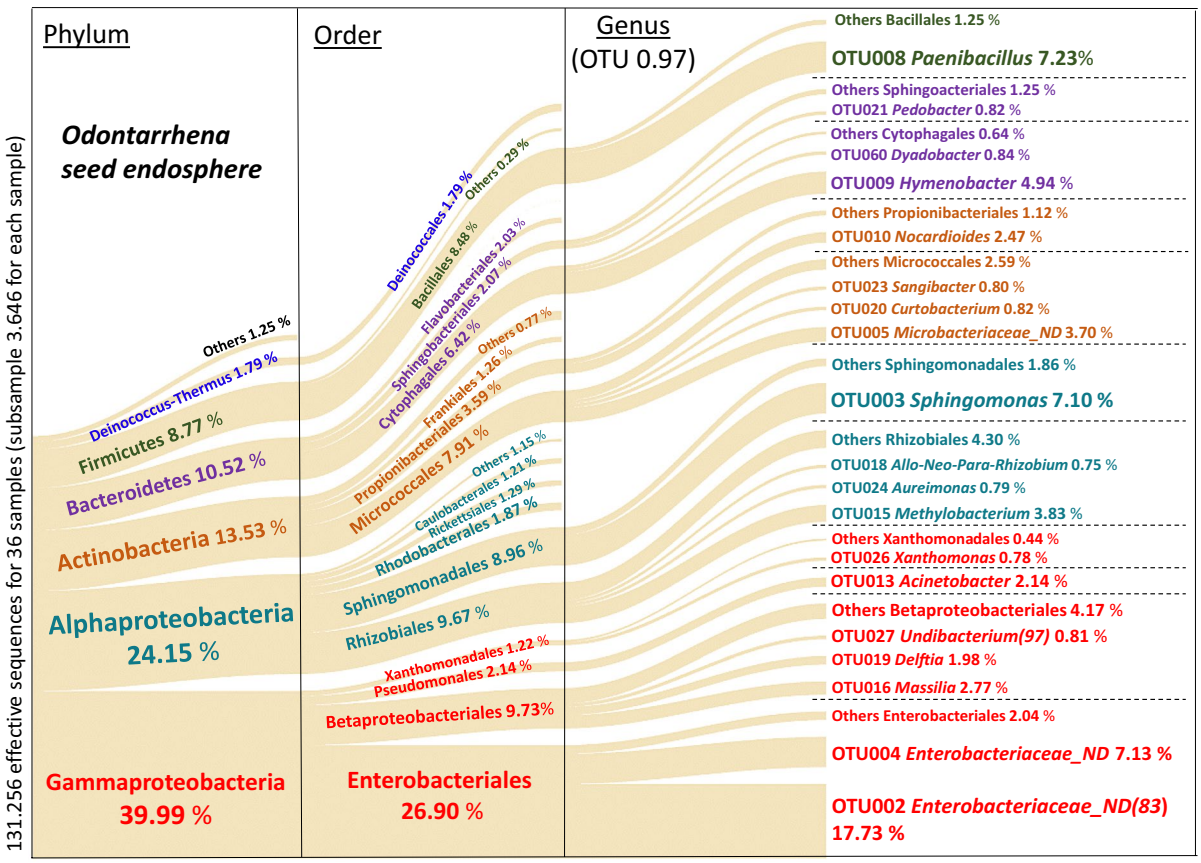

Fig. 1 Representation of the relative abundance of the most abundant endophytic taxa from the seeds of *Odontarrhena* samples. The rarest taxa were not shown and grouped together in 'Others'. The cut-off to classify an OTU as 'Others' was set at 1.5% relative abundance for phyla, 1% for orders, and 0.75% for genera. Color are as follows: *Gammaproteobacteria* (red), *Alphaproteobacteria* (light blue), *Actinobacteria* (yellow), *Bacteroidetes* (purple), *Firmicutes* (green), *Deinococcus-Thermus* (dark blue), and unassigned bacteria (black). For genera, the operational taxonomic unit (OTU) number was also provided

dominant phylum for *Odontarrhena* SEB communities and reached a mean relative abundance of 64.14%. Among the *Proteobacteria*, only *Gammaproteobacteria* (39.99%) and *Alphaproteobacteria* (4.15%) subphyla were found. The *Gammaproteobacteria* were essentially composed of OTUs belonging to the *Enterobacteriales* (relative abundance of 26.90%), *Betaproteobacteriales* (9.73%), *Pseudomonales* (2.14%), and *Xanthomonadales* (1.22%) orders. The *Alphaproteobacteria* were essentially composed by OTUs from *Rhizobiales* (9.67%) and *Sphingomonadales* (8.96%). *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Deinococcus-Thermus* phyla were also identified with relative abundances of 13.53, 10.52, 8.77 and 1.79%, respectively. The SEB communities also had OTUs belonging to *Actinobacteria* (13,53%) and assigned to the following orders: *Micrococcales* (7.91%), *Propionibacteriales* (3.59%), and *Frankiales* (1.26%). In the seeds studied, *Cytophagales* (6.42%), *Sphingobacteriales* (2.07%), and *Flavobacteriales* (2.03%) were the only *Bacteroidetes* orders found. The *Firmicutes* OTUs belonged essentially to *Bacillales* (8.48%), while the *Deinococcus-Thermus* OTUs tobelonged to *Deinococcus* (1.79%). The most abundant OTU was found among *Proteobacteria* OTU002 *Enterobacteriaceae*_ND(83) (17.73%). Three other OTUs were remarkably abundant in the community: the *Firmicutes* OTU008 *Paenibacillus* (7.23%), the *Proteobacteria* OTU004 *Enterobacteriaceae_ND* (7.13%), and the OTU003 *Sphingomonas* (7.10%). Combined, the four previously mentioned OTUs represented more than a third of the *Odontarrhena* SEB communities (39.19%).

A visualization of a global analysis using NMDS plots showed a clear clustering between another Ni hyperaccumulator *Noccaea caerulescens* (*Brassicacaea*) populations, previously studied (Durand et al. 2021) and SEB communities from the nine *Odontarrhena* populations (this study) (Fig. S1). At this scale, the distinction between the SEB communities of the various *Odontarrhena* species was unclear. An additional NMDS was done to compare only the five species (*O. chalcidica*, *O. rigida, O. moravensis, O. smolikana,* and *O. decipiens*). of the SEB communities from the nine *Odontarrhena* populations. The stress of the NMDS plot was 0.19, which could be considered as'satisfactory' representation of the dataset in a reduced dimension. In this plot, *O. chalcidica* populations formed a specific cluster, while the 4 other species (*O. rigida, O. moravensis, O. smolikana,* and *O. decipiens*) were clustered together (Fig. 2). To identify the major factors influencing bacterial

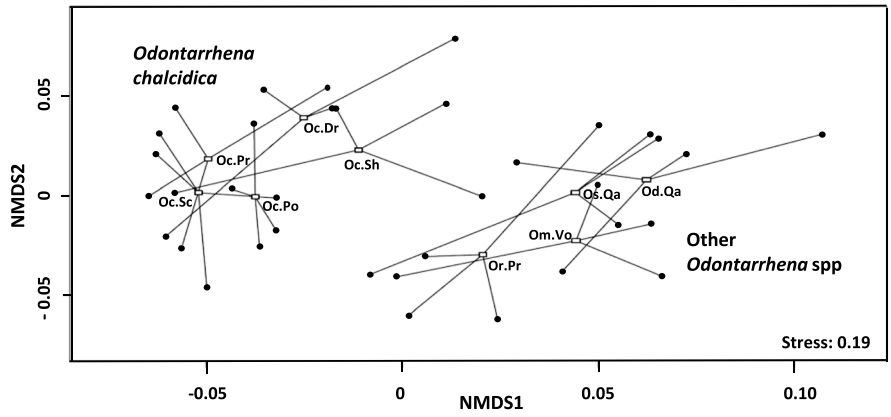

Fig. 2 Non-metric multidimensional scaling (NMDS) plot of endophytic bacterial communities associated with the seeds of the 9 *Ondotarrhena* populations using Bray–Curtis dissimilarity measurement. Each dot coordinate depends on the diversity the bacterial community described in each seed sample for a total of 36 samples. The dots are linked to the population ID of the plant population from which the seeds were recovered. Abbreviations of plant populations: Oc.Sc (*O. chalcidica* from Scutari), Oc.Sh (*O. chalcidica* from Shishice), Oc.Dr (*O. chalcidica* from Drenovë), Oc.Po (*O. chalcidica* from Pogradeč), Oc.Pr (*O. chalcidica* from Prrenjas), Or.Pr (*O. rigida* from Prrenjas), Om.Vo (*O. moravensis* from Voskopoje), Os.Qa (*O. smolikana* from Qafë Shtamë), and Oc.Sc (*O. decipiens* from Qafë Shtamë) (see Table 1). These population ID are shown as rectangle and are placed in the plan as barycenter of the linked dots. Stress of the representation in reduced dimensions is shown in the bottom right corner

diversity and community composition of the dataset, an analysis of similarities (ANOSIM) was performed to test the effects of the site where seeds were recovered; of the plant species, as well as of the interaction between these two factors (Table 5). This ANOSIM showed that all the models tested significantly explained the dissimilarities between the sample with *p-value* < 0.001. Dissimilarities in SEB communities due to the site or due to the plant species resulted in *R-values* of 0.54 and 0.63, respectively. However, the interaction between site and plant species showed a higher *R-value* of 0.71. The model that compared samples associated to *O. chalcidica* seeds *versus* samples of the 5 other species, gave the best explanation for the dissimilarities between SEB communities, with a *R-value* of 0.88.

Table 5 ANalysis Of SIMilarities (ANOSIM) is similar to an ANOVA hypothesis test, but uses a dissimilarity matrix (OTU Table) as input instead of raw data. It shows the p-value (i.e. significance levels of clustering) and R-value (i.e. the level of dissimilarities between clusters where 1 is maximum dissimilarity). This ANOSIM was performed on three factors: site where seed were recovered, *Odontarrhena* species, and plant population. We also compared SEB communities dissimilarities from the groups '*O. chalcidica* populations' (Oc.Sc (*O. chalcidica* from Scutari), Oc.Sh (*O. chalcidica* from Shishice), Oc.Dr (*O. chalcidica* from Drenovë), Oc.Po (*O. chalcidica* from Pogradeč), Oc.Pr (*O. chalcidica* from Prrenjas)) *versus* 'all other *Odontharrena* populations' (Or.Pr (*O. rigida* from Prrenjas), Om.Vo (*O. moravensis* from Voskopoje), Os.Qa (*O. smolikana* from Qafë Shtamë), and Oc.Sc (*O. decipiens* from Qafë Shtamë))

Factors and interactions between factors	R-value	*p*-value
Site where seeds were recovered	0.54	1.10^{-3}
Odontarrhena species	0.63	1.10^{-3}
Plant population (site:species)	0.71	1.10^{-3}
'*O. chalcidica* populations' *vs* 'all other *Odontharrena* populations'	0.88	1.10^{-3}

A volcano plot has been drawn (Fig. 3) to visualize OTUs which were over- or under-represented in the SEB community of *O. chalcidica* compared to the 4 other *Odontarrhena* species (*O. rigida, O. moravensis, O. smolikana*, and *O. decipiens*). This volcano plot revealed that among the OTUs that composed the SEB communities, 4 OTUs were significantly under-represented (*p*-value < 0.05 and decrease of RA > 25%) and 16 which were significantly over-represented (*p*-value < 0.05 and increase of RA > 25%) in *O. chalcidica* communities, when compared to *Odontarrhena* from the other species studied. Three OTUs were highly significantly under-represented in *O. chalcidica* SEB community: OTU009 *Hymenobacter* (ratio = 0.57 and RA = 3.71%), OTU016 *Massilia* (ratio = 0.42 and RA = 1.72%) and OTU015

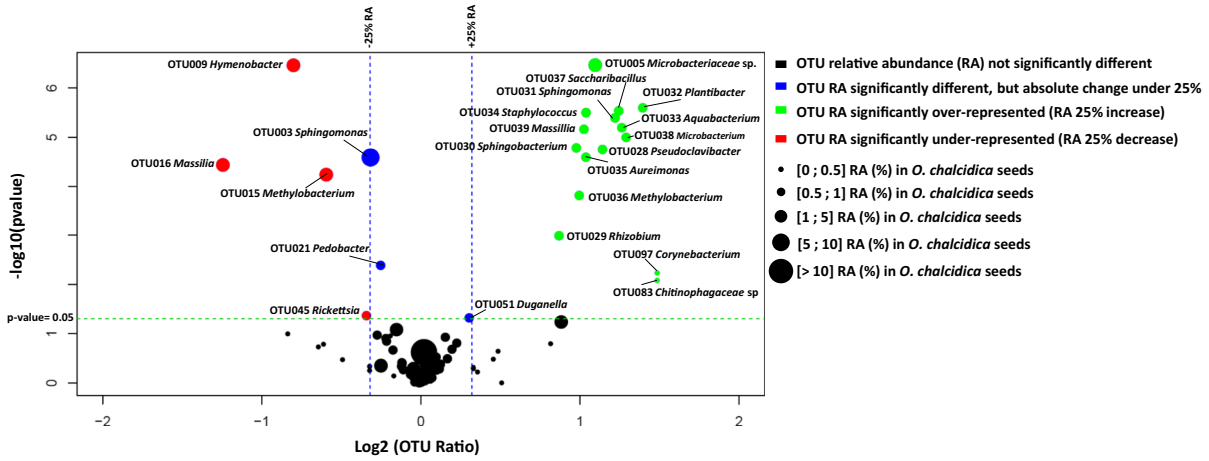

Fig. 3 Volcano plot showing the significantly over-represented OTUs (green) and significantly under-represented OTUs (red) in the seeds of the five *O. chalcidica* populations (Oc.Sc (*O. chalcidica* from Scutari), Oc.Sh (*O. chalcidica* from Shishice), Oc.Dr (*O. chalcidica* from Drenovë), Oc.Po (*O. chalcidica* from Pogradeč), Oc.Pr (*O. chalcidica* from Prrenjas)) compared to the seeds recovered from the population of the 4 other *Odontarrhena* species (Or.Pr (*O. rigida* from Prrenjas), Om.Vo (*O. moravensis* from Voskopoje), Os.Qa (*O. smolikana* from Qafë Shtamë), and Oc.Sc (*O. decipiens* from Qafë Shtamë)). For each OTU, the ratio corresponded to the mean OTU RA in the SEB community of the 5 *O. chalcidica* populations divided by the mean OTU RA in the SEB community of the grouping of the 4 other *Odontarrhena* species

Methylobacterium (ratio = 0.66 and RA = 3.12%). Two OTUs of the SEB community of *O. chalcidica* have a significantly differing RA in comparison with those of the 4 other *Odontarrhena* species, although this RA decrease was slight. For example, this was the case of the OTU003 *Sphingomonas* (ratio = 0.81 and RA = 6.40%). Among the 16 over-represented OTUs, the most significant increases were observed for the most abundant OTUs. This was the case of the OTU005 *Microbacteriaceae* sp. (ratio = 2.14 and RA = 4.85%). In contrast, some OTUs were largely over-represented in the SEB community of *O. chalcidica*, but showed a low RA. These include OTU097 *Corynebacterium* (ratio = 2.8 and RA = 0.2%) and OTU083 *Chitinophagaceae* sp. (ratio = 2.8 and RA = 0.2%).

Metagenome prediction was applied to infer the metagenomic content of the seed endophyte bacterial communities of *Odontarrhena* species and to evaluate the functional potential of the bacterial community metagenomes from their 16S rRNA gene profiles. Based on the predicted metagenomes, 19 of the Level 2 KEGG Orthology (KO) groups were found with a relative abundance > 1% (Fig. 4). These KO belonged to the potential processes related with the cells, the environment, the genetic and the metabolism. The 22 other KO were grouped under the 'others < 1%' denomination. The functional profiles suggested that the overall functional structure of the SEB community was dominated by metabolism-related KEGG pathways, especially that of carbohydrates (pyruvates, amino and nucleotide sugars, glyoxylate and dicarboxylate metabolisms) and the ones of amino acids and nucleotides (alanine, aspartate and glutamate among others). The other dominant KO was membrane transport, related to the environmental information process, with the dominance of ABC transporters. Based on a Tukey HSD test (Supplementary Table 1), differences of potential functions between the SEB communities from the different plant populations could be underlined. Indeed, the major differences can be observed for the population of *O. rigida* from Prrenjas (Or.Pr) and *O. smolikana* from Qafë (Os.Qa). Predictive functions of genetic information processing

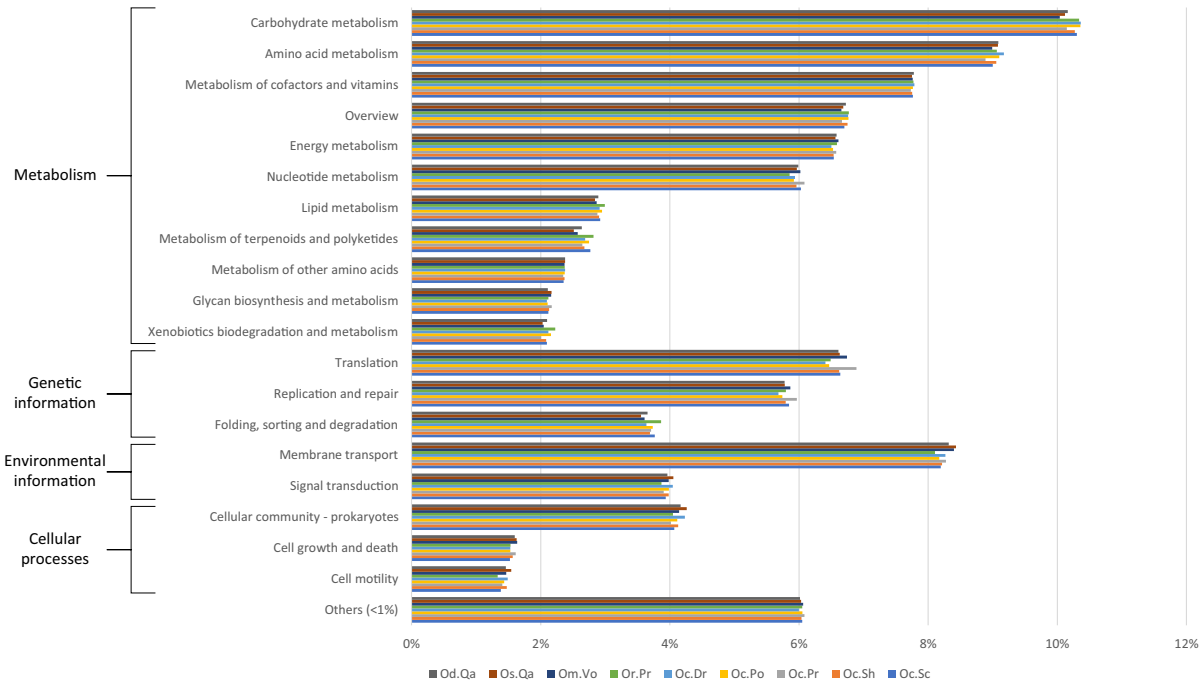

Fig. 4 Gene profiles predicted of bacterial community in seed endophyte of *Odontarrhena* species using Tax4Fun. Abbreviations of plant populations: Oc.Sc (*O. chalcidica* from Scutari), Oc.Sh (*O. chalcidica* from Shishice), Oc.Dr (*O. chalcidica* from Drenovë), Oc.Po (*O. chalcidica* from Pogradeč), Oc.Pr (*O. chalcidica* from Prrenjas), Or.Pr (*O. rigida* from Prrenjas), Om.Vo (*O. moravensis* from Voskopoje), Os.Qa (*O. smolikana* from Qafë Shtamë), and Oc.Sc (*O. decipiens* from Qafë Shtamë) (see Table 1)

(folding, sorting and degradation) and metabolism (related to lipids, terpenoids, polyketides and xenobiotics) were significantly higher for Or.Pr SEB community, while functions from cellular processes (cell motility) and environment information processing (membrane transport and signal transduction) were higher for Os.Qa SEB.

Influence of soil physicochemical parameters and altitude on the seed endophytic bacterial communities

A correlogram was drawn to display correlations between the physicochemical variables and altitude (Table 2) and the mean relative abundance of the bacterial phyla of SEB communities from the 9 populations of *Odontarrhena* (Fig. 5). The analysis revealed 11 interactions with significant Spearman correlations (*p-value* < *0.05*). SEB communities were significantly enriched with the *Actinobacteria* phylum, when the pseudo-total Mn concentration increased (R = 0.50), while it was significantly depleted in *Actinobacteria,* when pseudo-total Ca concentration increased (R = − 0.88). In addition, the communities were significantly enriched with the *Bacteroidetes* phylum when pseudo-total Ni and Cr concentrations increased (R = 0.30 and 0.65, respectively), while *Bacteroidetes* depletion in the communities was correlated to pseudo-total K concentration (R = − 0.38). The relative abundance of *Patescibacteria* phylum was positively correlated to pseudo-total Co concentration (R = 0.60) and negatively correlated to pseudo-total Ca concentration (R = − 0.39). SEB communities were also significantly depleted in *Firmicutes* and *Gammaproteobacteria* when pseudo-total concentrations in Ca (R = − 0.68) and Mn (R = − 0.51) increased.

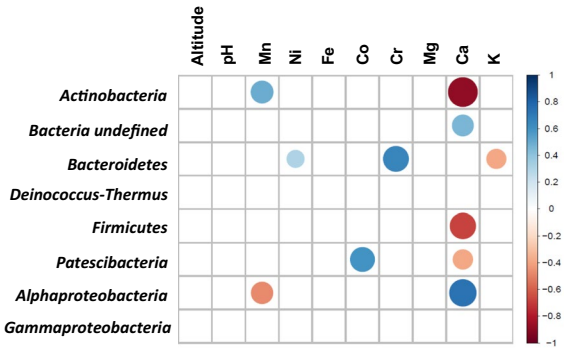

Fig. 5 Correlogram depicting Spearman correlations between relative abundances of *Odontarrhena* seed endophytic bacterial dominant phyla and soil physicochemical parameters and the altitude of the sites where seeds were collected. The size and color of the circle correspond to the magnitude of the correlation. Pseudo-total concentrations of elements were measured in bulk soils from the sites where the plants grew

Redundancy analysis (RDA) was performed between concentrations of elements in the aerial parts of the plants (Table 3), and the relative abundance of the major phyla of SEB communities from the 9 *Odontarrhena* populations (Fig. 6). The main plane (F1-F2) explained 92.60% of the total variability, thereby showing that the seed populations were clearly distinguishable in this analysis on the basis of plants' element concentrations and in the diversity of SEB communities. The F1 axis that explained 76.06% of the total variability discriminated the samples of SEB communities collected from *O. chalcidica* populations (right part of the main plane) from the SEB communities collected from *O. smolikana, O. decipiens* and *O. moravensis* populations (left part of the main plane). Graphical interpretation revealed that *O. chalcidica* populations were discriminated from other populations by higher concentrations of Ni in their aerial parts and the higher RA of *Actinobacteria* (Fig. 6). Nonetheless, although plants with higher Ni concentrations in shoots had the tendency to be enriched with *Actinobacteria*, statistical analysis revealed that no Spearman correlation was detected (p-*value*: 0.055, R_s-*value*: 0.499).

In addition, a Variation Partitioning Analysis (VPA) (Fig. 7) was performed to determine the effects of soil parameters, plant species, environmental factors (topography: slope and altitude) and interactions between these data on the structure of the bacterial communities, with the aim to determine which parameters were the most important drivers of the SEB microbiome. The VPA revealed that, considered alone, plant species explained the highest percentage of the variation observed, at 9%. Nonetheless, interaction between plant species and abiotic data (accounting for 21%) and interaction between plant species and topographic data (also accounting for 21%) appeared as better drivers of the SEB diversity. Finally, the most explanatory factors corresponded to the interaction between the three parameters considered.

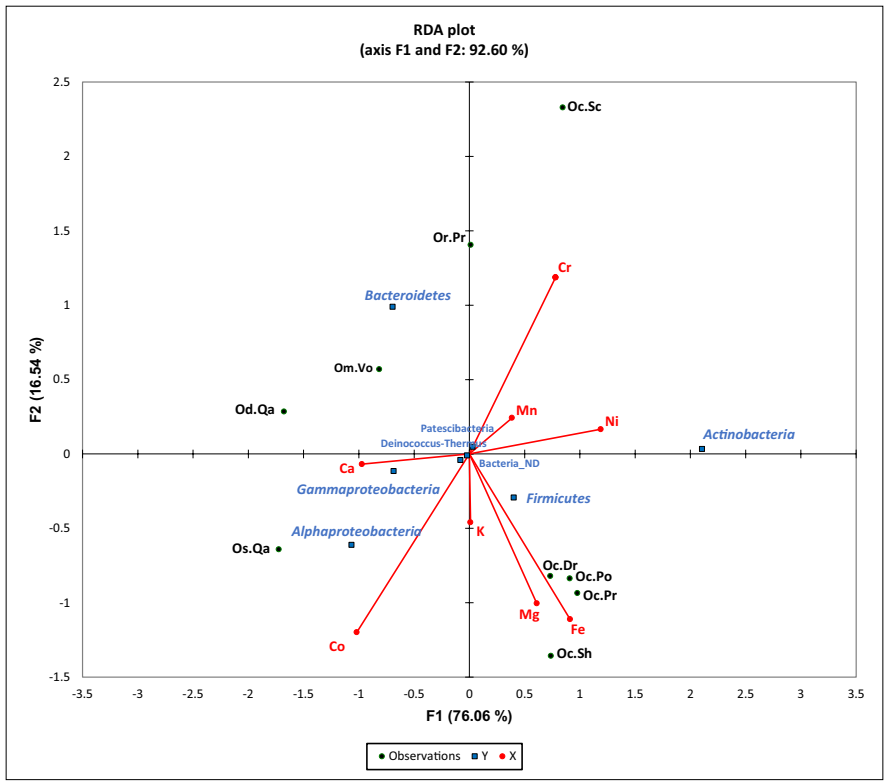

Fig. 6 Redundancy Analysis (RDA) performed between the soil concentrations of major (Fe, Mg, Ca and K) and minor (Ni, Mn, Co and Cr) elements, and the relative abundance of the major phyla of SEB communities from *Odontarrhena* populations. Dots are observations and correspond to the SEB communities from one population of *Odontarrhena.* Abbreviations of plant populations: Oc.Sc (*O. chalcidica* from Scutari), Oc.Sh (*O. chalcidica* from Shishice), Oc.Dr (*O. chalcidica* from Drenovë), Oc.Po (*O. chalcidica* from Pogradeč), Oc.Pr (*O. chalcidica* from Prrenjas), Or.Pr (*O. rigida* from Prrenjas), Om.Vo (*O. moravensis* from Voskopoje), Os.Qa (*O. smolikana* from Qafë Shtamë), and Oc.Sc (*O. decipiens* from Qafë Shtamë) (see Table 1)

Co-evolution analysis

Bacterial endophyte communities reported a distribution pattern clustered around plant species (*O. chalcidica versus* other species). However, plant population phylogeny did not show any clear clustering pattern of plant populations belonging to the same species (Fig. 8). Consequently, phylogenetic distance and bacterial diversity largely diverged as reported by the Mantel test on the two matrices (Fig. S2). This reflected the distribution reported in the NMDS analysis (Fig. 2), in which the *O. chalcidica* endophytic communities are tightly clustered together than the other plant species.

Discussion

In this study, the diversity and structure of SEB communities of 9 populations of *Odontarrhena* species, found in serpentine soils from Albania, were evaluated by using Illumina high throughput sequencing. Based on taxonomical assignment of the OTUs, the composition of SEB communities was found to be very similar for all *Odontarrhena* populations with only marginal dissimilarities. However, focusing on those marginal dissimilarities between the SEB communities of nine *Odontarrhena* populations, the SEB communities associated with *O. chalcidica* were separated from the SEB communities of the four other

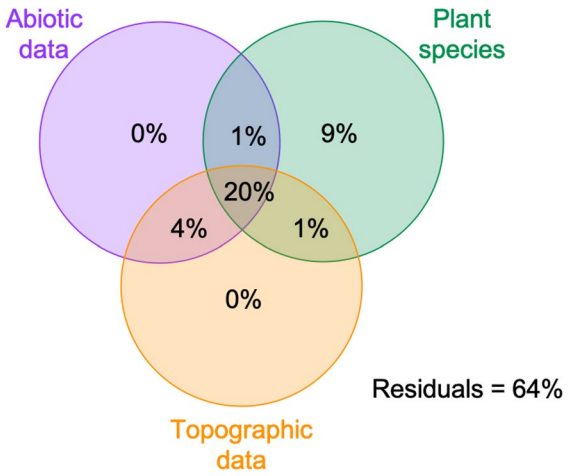

Fig. 7 Variation partitioning analysis (VPA) to determine the effects of soil characteristics, plant species, environments, and interactions between these parameters on the structure of the bacterial community. Circles without overlap showed the percentage of variation explained by each factor alone. The overlap region of two or three circles displayed the explanation of variation between two or three of these factors

species (*O. chalcidica, O. rigida, O moravensis, O. smolikana,* and *O. decipiens*), which were clustered together. Moreover, it appeared that among the explanatory factors of the observed dissimilarities, those relating to the plant phylogeny had a R-*value* in ANOSIM analysis higher than those relating to the sites. These results are in accordance with a previous work on the hyperaccumulator *Noccaea caerulescens* (Durand et al. 2021), in which these authors observed very low variation in alpha diversity and a large core SEB community. Indeed, 85% of OTUs were shared between the 14 populations studied, despite varying soil properties parameters.

A core SEB community of *Odontarrhena* despite heterogeneous sites and species

All *Odontarrhena* populations shared more than 95% of their OTUs (data not shown). Dissimilarities between the SEB communities of each of the nine populations of *Odontarrhena* species were far less than the dissimilarities in SEB communities of *Noccaea caerulescens* populations (Figs. S1 and 1). Furthermore, no significant differences were found for the alpha diversity indices

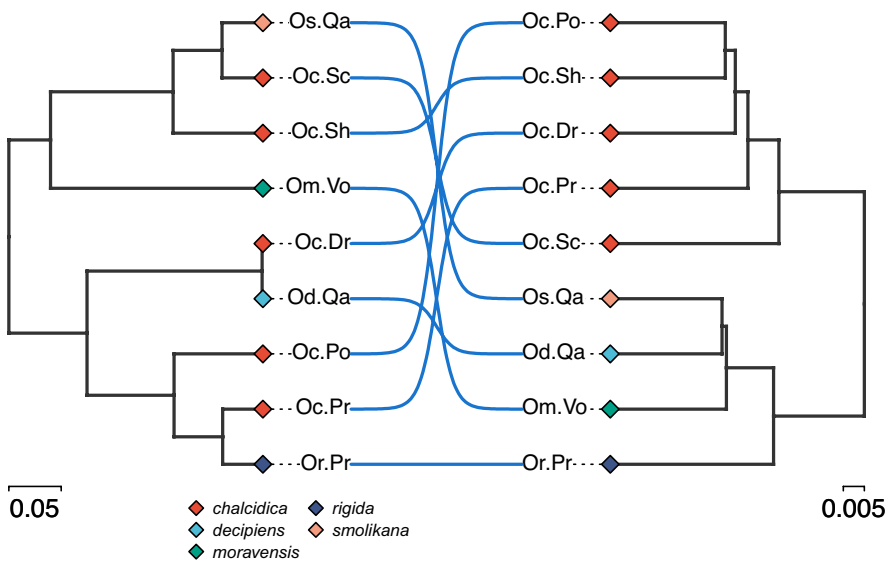

Fig. 8 Comparison of trees obtained with Bray–Curtis distance on pooled SEB communities at an OTU level (on the right) and UPGMA clustering of *Ondotarrhena* populations (on the left). Distance scales were reported on the bottom right (right tree) and bottom left (left tree) part of the plot. Tips are colored according to different *Ondotarrhena* species and samples from the same population are connected using blue lines. Abbreviations of plant populations: Oc.Sc (*O. chalcidica* from Scutari), Oc.Sh (*O. chalcidica* from Shishice), Oc.Dr (*O. chalcidica* from Drenovë), Oc.Po (*O. chalcidica* from Pogradeč), Oc.Pr (*O. chalcidica* from Prrenjas), Or.Pr (*O. rigida* from Prrenjas), Om.Vo (*O. moravensis* from Voskopoje), Os.Qa (*O. smolikana* from Qafë Shtamë), and Oc.Sc (*O. decipiens* from Qafë Shtamë) (see Table 1)

(OTU richness, Chao1 estimator, Shannon index, and inverse Simpson), when comparing the nine SEB communities (Table 4). The composition and structural similarities between SEB communities of the 9 *Odontarrhena* populations allowed a common core SEB microbiome to be described for all the *Odontarrhena* seeds (Fig. 1), despite the numerous differences in the sampling sites (Table 2). The main phyla and sub-phyla were: *Gammaproteobacteria* (39.99%) > *Alphaproteobacteria* (24.15%) > *Actinobacteria* (13.53%) > *Bacteroidetes* (10.52) > *Firmicutes* (8.77%) (Fig. 1). These results were in accordance with those of many previous studies, including those of Abdullaeva et al. (2021) when studying the seed-associated microbiome of four cereal crops along with their respective ancestors. They also agreed with those of Bziuk et al. (2021a, b) studying barley SEB communities. These phyla were also observed to be predominant in the rice seed microbiome (Raj et al. 2019; Matsumoto et al. 2021), in the wild cabbage seed microbiome (Tyc et al. 2020) and in the pumpkin seed microbiome (Adam et al. 2018). They were also confirmed by Barret et al. (2015), studying 28 plant genotypes mostly affiliated to *Brassicaceae*. These authors found that OTUs belonged mainly to the *Proteobacteria* phylum (13.1%, 5.8%, and 56.1% in the *Alpha-*, *Beta-* and *Gammaproteobacteria* subphyla, respectively), *Firmicutes* (11.3%), and *Actinobacteria* (9.1%). In the same way, Durand et al. (2021), studying SEB communities of 14 *Noccaea caerulescens* populations collected in France, showed the existence of a core microbiome composed of the main phyla and sub-phyla: *Gammaproteobacteria* (56.56%) > *Alphaproteobacteria* (32.23%) > *Actinobacteria* (7.93%) > *Firmicutes* (3.78%). These phyla were found in seeds of numerous plant species, including dicots and monocots (Bziuk et al. 2021b). It has been suggested that bacteria affiliated to these phyla could belong to a universal core seed microbiome. This core microbiome would be adapted to the seed endosphere, which could exert a filtering effect on the SEB communities regardless of the plant species, origin, physiology or metabolism. Moreover, studying rhizosphere bacterial communities of plants growing on contaminated or naturally metal-rich soils (Jeong-Myeong and Shim 2008; Lopez et al. 2019), or non-contaminated (Miethling et al. 2003), several authors showed that the phylum *Proteobacteria* generally was the dominant member of the rhizosphere microbiome (Uroz et al. 2010; Yang et al. 2017) followed by other phyla, such as *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes* (Prashar et al. 2014). *Acidobacteria* as well as *Bacteroidetes* and *Gammaproteobacteria* occurred in rhizospheric soils of *Odontarrhena chalcidica* (Saad et al. 2021). Our data revealed seeds poorly colonized by *Acidobacteria* and *Bacteroidetes*, while highly enriched with *Gammaproteobacteria*. Those similar enrichments patterns between the metal-rich rhizosphere and the seeds suggested a continuum between rhizosphere and seed microbial habitats, however with a seed filtering effect resulting in the variation in relative abundances of the dominant phyla.

Based on the predicted metagenomes using the Tax4Fun approach, genes belonging to metabolism were identified as the major gene families at the Level 2 KO groups. Our results, focusing on SEB communities, had confirmed studies where the metabolism-related functions, were also found in great abundance in the rhizospheres of hyperaccumulator plants (Lopez et al. 2019; Saad et al. 2021). In our study, this was particularly the case for SEB community of *O. rigida*. In contrast, SEB community of *O. smolikana* showed a higher significant percentage for genes related to environmental information pathways than the SEB from the other hyperaccumulator populations, especially for the membrane transport and signal transduction categories. In addition, a significant increase for the cellular process pathways (KEGG Level 2 pathways: cell motility) was also observed for this SEB community. These results could be explained by the fact that membrane transport and cell motility could permit the bacteria to interact with their surroundings and react to chemical contents and other signals in the seed endosphere (Somers et al. 2008). Membrane transporters play an important role in many different aspects of bacterial physiology, such as the extrusion of toxins and antimicrobial compounds (Davidson and Chen 2004; Lin et al. 2013). Biochemical analyses of the seed contents could allow to a better understanding of these mechanisms.

A lower influence of the soil compared to the plant species on the SEB communities

Focusing only on the nine *Odontarrhena* populations, the non-metric multidimensional scaling plot

for bacterial microbiota structure based on weighted Bray–Curtis distances (Fig. 2) showed that *O. chalcidica* populations were clearly clustered and separated from the other *Odontarrhena* populations. The ANOSIM revealed that the sites where seeds were collected partly explained the dissimilarities between the nine *Odontarrhena* SEB communities ($R = 0.54$, $p\text{-value} = 0.001$), while the interaction of '*O. chalcidica*' versus 'all other *Odontarrhena* species' studied was the best model for explaining dissimilarities between SEB communities ($R = 0.88$, $p\text{-value} = 0.001$) (Table 5). Nonetheless, both site and *Odontarrhena* species factors were significant drivers of the diversity of the SEB communities and the best explanatory factor was the interaction between them. A part of the SEB community structure may be explained by the properties of the site (Fig. 5), yet our results emphasized that the species of the plant, was the factor that explained a greater number of dissimilarities between SEB communities than the site properties or the topography taken in isolation (Fig. 7).

The low correlation between the soil characteristics and the SEB diversity was surprising when compared with previous works. Indeed, focusing on rhizospheric microbiomes, numerous studies have shown relations between microbes and environmental factors, such as soil texture (Sessitsch et al. 2001), pH (Fierer and Jackson 2006), nutrients and contaminants like heavy metals (Sandaa et al. 2001). However, several works focusing on SEB communities have highlighted the propensity of the bacterial community diversity to stay unchanged in a plant species, despite variations in soil physicochemical parameters (Johnston-Monje et al. 2014; Durand et al. 2021).

Among the OTUs which were significantly over-represented in the SEB community of *O. chalcidica* compared to the four other *Odontarrhena* species, we observed that OTU005 *Microbacteriaceae* sp. showed the highest ratio (2.14) and a RA of 4.85% (Fig. 3). The study by Borah et al. (2018) revealed that several *Microbacteriaceae* bacteria produced significant amounts of indole-acetic-acid, were able to solubilize high amounts of potassium and were also able to solubilize metal compounds such as Zn. They concluded that endophytic *Microbacteriaceae* bacteria exhibited multiple PGP activities. Moreover, it has been shown that isolates belonging to the *Microbacterium* are known to persist during periods of dryness (Goodfellow and Williams 1983), or exhibit high levels of desiccation tolerance and even enhance plant drought tolerance (Ali et al. 2014a, b; Lucas et al. 2014). This could explain why OTU005 *Microbacteriaceae* sp. was over-represented in the SEB communities of *O. chalcidica*, which is a plant clearly preferring disturbed habitats such as ruderal sites and dry pastures (Coppi et al. 2020).

We also detected several OTUs which were significantly over-represented in the SEB community of *O. chalcidica* when compared to the four other *Odontarrhena* species, but with a smaller RA (Fig. 3). This was the case for OTU097 *Corynebacterium* and OTU083 *Chitinophagaceae* sp.. Indeed, these bacteria were over-represented with a low RA (less than 0.5%), even if they had higher RA ratios than the other *Odontarrhena* species (2.8). *Corynebacterium* resides both in soils and plants (Collins et al. 2004). Indeed, this bacterium was found in the form of endophytic bacteria in maize plants, potato tubers, lemon roots (*Citrus jambhiri*) and beet roots(*Beta vulgaris*) (Chanway 1998). Moreover, as previously observed by Suhandono et al. (2016), *Corynebacterium* produced natural biopesticides to control some pathogens and were suspected of possessing an antipathogen mechanism. The OTU083 *Chitinophagaceae* sp. was a rare OTU (< 0.75%) that was classified in 'Other *Cytophagales*' in Fig. 1. Carrión et al. (2019) found that bacteria belonging to the *Chitinophagaceae* family were enriched within the plant endosphere and protected their plant host from pathogens. Furthermore, these endophytes showed enhanced enzyme activities associated with both fungal cell wall degradation as well as the biosynthesis of beneficial secondary metabolites. *O. chalcidica*, which is the species that grows on serpentine soils with the highest Ni concentrations, seemed to host bacteria which are more resistant to metals and exhibit more PGP properties than other plant species.

The OTU009 *Hymenobacter* were significantly under-represented in the SEB microbiome of *O. chalcidica* in comparison with the other *Odontarrhena* species (Fig. 3). The *Hymenobacter* genus was first described by Hirsch et al. (1998) and belongs to the *Hymenobacteraceae* family in the *Bacteroidetes* phylum (Munoz et al. 2016). These bacteria are oligotrophic and can be found in

various ecosystems and are known for their cold, aridity and UV radiation resistance (Koo et al. 2014). However, data on those bacteria in the endosphere of plant and especially in seeds, are scarce.

The OTU039 and the OTU016 were both assigned to *Massilia* genus, although the first was over-represented and the second under-represented in *O. chalcidica*. *Massilia (Betaproteobacteria*, *Oxalobacteraceae*) is a major group of rhizosphere and root colonizing bacteria of many plant species and these bacteria have been classified as copiotrophic (Ofek et al. 2012). They were retrieved from hyper-arid environments and from sites highly contaminated with heavy metal (Abou-Shanab et al. 2010; Kuffner et al. 2010) and had been previously isolated from the rhizosphere of the Ni-hyperaccumulator *Alyssum murale*. Moreover, some *Massilia* isolates exhibited in vitro attributes relating to plant growth promotion, including IAA production (Kuffner et al. 2010), siderophore production (Hrynkiewicz et al. 2010) and in vitro antagonism towards *Phytophthora infestans* (Weinert et al. 2010). As exudates of the four *Odontarrhena* species, *O. rigida*, *O. moravensis*, *O. smolikana* and *O. decipiens,* could be quantitatively and qualitatively different from those of *O. chalcidica,* a different selection process could have occurred, favoring specific OTUs in the SEB communities. Indeed, as *Massilia* sp. are classified as copiotrophic, certain OTUs belonging to this genus can be over- or under-represented in SEB communities, depending on the other bacteria present in the SEB communities of the different *Odontarrhena* species.

The cophylogenetic analysis revealed a non-significant coherence of phylogenies between seed microbiota at an OTU level or with that corresponding plant hosts. Consequently, this phylogenetic analysis did not suggest a plant–microbe co-adaptation related to the plant genotype since no effect of the *Odontarrhena* species on the endophytic bacterial community could be shown. It would seem that molecular markers used for plant phylogeny characterization did not discriminate clearly enough to discern plant populations at the species level. Moreover, Coppi et al. (2020) evidenced that a genetic admixing at the population and individual levels for *Odontarrhena* could be related to hybridization events among the Albanian taxa of *Odontarrhena*. This also explained why phylogenic analysis failed to show any clear population clustering patterns of plants of any given species.

Conclusion

Plant genetics influenced to a greater extent the SEB communities from the nine *Odontarrhena* populations collected on Albanian serpentine soils than sites properties. Nonetheless, the phylogenetic distance of the host plant and bacterial diversity largely diverged, which suggest that these two associated components have not co-evolved. Finally, both site properties and *Odontarrhena* species were significant drivers of the SEB community diversity and the interaction between them was the best explanatory factor.

We also observed a core microbial community shared by the *Odontarrhena* populations studied, and dominated by *Gammaproteobacteria* and *Alphaproteobacteria*. Indeed, the nine hyperaccumulator populations shared 95% of their OTUs. This taxonomical assignment of OTUs of this core microbial community combined with the OTUs based prediction of metabolic functions, is a first step that would potentially allow the power of the microbiome to be harnessed, thereby improving agromining.

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Declarations

This article does not contain any studies with human participants or animals performed by any of the authors.

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