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Decrease due to pollution in the rhizosphere microbial diversity can be amended by supplementation from adapted plants of another species

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Manipulating the rhizosphere microbiome to enhance plant stress tolerance is an environmentally friendly technology and a renewable resource to restore degraded environments. Here we suggest a sustainable bioremediation strategy on the example of Stebnyk mine tailings storage. We consider *Salicornia europaea* rhizosphere community, and the ability of the phytoremediation plant *Salix viminalis* to recruit its beneficial microbiome to mediate the pollution stress at the Stebnyk mine tailings storage. The tailings contain large amounts of brine salts and heavy metals that contaminate the ground water and surrounding areas, changing soil biogeochemistry and causing increased erosion. The species richness of the endophytic bacterial community of *S. viminalis* roots was assessed based on observed OTUs, Shannon-InvSimpson, and evenness index. Our results obtained using the plant-based enrichment strategy show that biodiversity was decreased across the contamination zones and that *S. europaea* supplementation significantly increased the species richness. Our results also indicate that the number of dominating bacteria was not changed across zones in both *S. europaea*-treated and untreated bacterial populations, and that the decrease in richness was mainly caused by the low abundant bacterial OTUs. The importance of selecting the bioremediation strains that are likely to harbor a reservoir of genetic traits that aid in bioremediation function from the target environment is discussed

Plant–soil–microbe interactions play an important role in plant community performance since microbial abundance and composition largely determine the health and productivity of any soil[1–3]. Plant growth-promoting microorganisms form beneficial relationships with plants, and their interactions evoke local and systemic responses in plants through several molecular and cellular mechanisms upon perception of environmental signals. This enables plants to cope with stresses such as temperature extremes, drought, flooding, salinity, and heavy metal pollutants[1,4]. Hence, the ability to manage the soil microbiome under pollution stress is likely to be the key to efficient bioremediation[5]. Since rhizobacteria were first described[6], single microbial isolates as well as microbial consortia have been used over decades for inoculation to influence the host plants to remediate various stress situations[7–12]. Environmental remediation using microbial inocula has several advantages over artificial chemical treatments[13,14]. As in natural conditions 95% of microbes are bacteria, plant growth promoting rhizobacteria (PGPR) have a special potential for bioremediation[15,16]. The rhizosphere microbiome is an environmentally friendly renewable resource, and activation of the resource restores soil and plant health[3,17,18]. At the same time there are general challenges that hinder the breakthrough of microbial inocula into commerce; in particular, inconsistent colonization and inefficiency of field application[1,4,7,19–25]. Historically PGPR strains have been selected based on their biochemical properties[1,7]. It is however highly important to consider microbial communities from the indigenous communities in a holistic manner as rhizosphere biodiversity and community composition determine ecosystem multifunctionality eventually leading to sustainable bioremediation strategies[1,4,7,26,27]. Here we considered the principle that while the bacterial rhizosphere microbiome is rather

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dynamic and unstable, there are endophytic communities that present relatively stable communities[1,4]. The other principle we considered is host-mediated microbiome selection (HMMS): a plant-based enrichment strategy that focuses on the host organism's intrinsic ability to recruit its own beneficial microbiome[28,29]. The microbiome can then be characterized by the high throughput metabarcoding and metagenomic approaches.[1,7,19–25].

One of the examples where bioremediation is greatly needed is a tailings storage on the north-eastern outskirts of the Stebnyk (Lviv region, Ukraine), which contains 22 million tons of waste including clay material, undissolved salts, brine with a high content of sodium chloride, heavy metals (HM) and salts[30]. The waste causes salinization of groundwater, reservoirs, and surrounding areas[30]. The negative effects of salt pollution include changes in soil biogeochemistry and composition, and characteristics such as decreased aeration and decreased soil permeability with increased erosion. Currently developed hydro-technical technologies at the tailing storage have to be followed by carefully planned bioremediation strategies[31]. Here we considered the potential of the basket willow (*Salix viminalis*) for the tailing storage bioremediation.

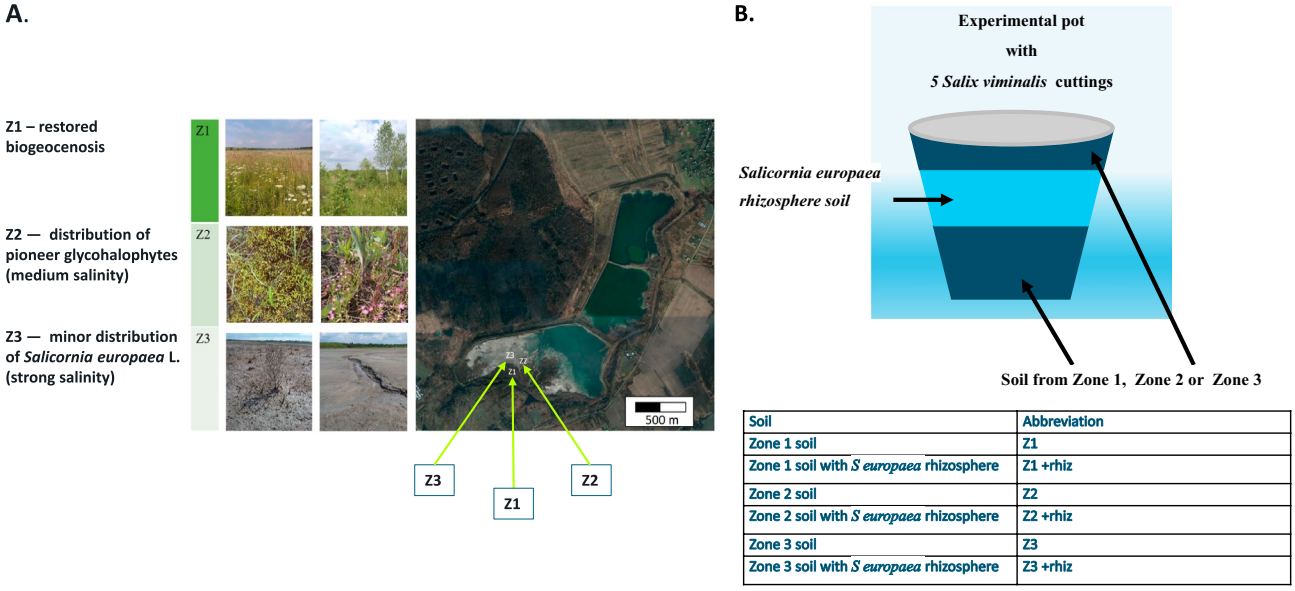
It has been suggested earlier that for biotechnologically effective strain selection harsh habitats should be preferred, since microorganisms from extreme environments have developed biological adaptations and strategies to manage stress situations[4,32] The bacterial ability to pick up genetic information in the form of horizontal gene transfer, plasmids or other mobile elements is mostly linked to bacteria from harsh environments[4]. This is used here as a third principle finding the effective bacterial consortia for the site bioremediation. The rhizosphere of *Salicornia europaea* at Stebnyk was applied as a potential genetic resource for biodiversity enhancement. The accessory genome can be shared among bacteria in an environment that favors them but can be absent in the 'same' bacterial species growing elsewhere[4,32]. The *S. europaea* plants were grown under the salt and HM pollution stress over four decades. Former research shows that *Salicornia* species may provide a microbiome that effectively attenuates saline stress[33,34].

Here we hypothesized that bacteria that have been living under HM and salt pollution stress over a long period of time have gained the potential to mitigate plant pollution stress. Hence as a first step we determined the pattern of endophytic bacterial colonization of *S. viminalis* across the Stebnyk contamination gradient. We predicted that *S. europaea* treatments would increase the abundance and species richness of the endophytic community. We tested these hypotheses by conducting DNA metabarcoding along replicated pollution zones.

Material and methods

Sampling and experimental design

In total, thirty-six (36) individual cuttings were examined at a gradient of Stebnyk mine tailing representing three contamination levels earlier described[30,35] (Fig. 1A,B). In this project the investigations were carried out in soils of three regions that were weakly, moderately and heavily contaminated (Zone 1, 2 and 3 respectively) (Fig. 1A,B[30,35]). Samples were taken at a depth of 0–25 cm at places of strong (49° 18′ 45.2″ N 23° 34′ 05.5″ E; 49° 18′ 45.4″ N 23° 34′ 03.4″ E; 49° 18′ 45.3″ N 23° 34′ 04.5″ E) and medium salinity (49° 18′ 45.0″ N 23° 34′ 07.7″ E; 49° 18′ 43.8″ N 23° 34′ 07.8″ E; 49° 18′ 43.3″ N 23° 34′ 07.4″ E), as well as in places of restored biogeocenosis (49° 18′ 39.8″ N 23° 33′ 59.3″ E; 49° 18′ 40.0″ N 23° 34′ 00.7″ E; 49° 18′ 41.1″ N 23° 33′ 57.7″ E), which was determined visually at the place of plant growth. To explore the diversity, samples were taken from six different sites at each of the three regions and used as growth substrate for willow (*Salix viminalis* L.) cuttings. The cuttings were left to grow in pots with the soils sampled at the three regions as well as with soil samples that were enriched with the rhizosphere soil from the *S. europaea* rhizosphere.



Soil	Abbreviation
Zone 1 soil	Z1
Zone 1 soil with *S europaea* rhizosphere	Z1 +rhiz
Zone 2 soil	Z2
Zone 2 soil with *S europaea* rhizosphere	Z2 +rhiz
Zone 3 soil	Z3
Zone 3 soil with *S europaea* rhizosphere	Z3 +rhiz

Figure 1. (**A**) Sampling locations on the Stebnyk tailings storage: Zone 1 (Z1), Zone 2 (Z2) and Zone 3 (Z3). (**B**) Experimental design combining soils from zone 1, zone 2, and zone 3 with *Salicornia europaea* rhizosphere soil (for soil and pollution details see "Material and methods" and[30,35]).

The rhizosphere microbiome of *S. europaea* L. was collected on Stebnyk tailing under field conditions and transferred to sterile bags, which were transported to the laboratory. All soils were stored at 4 °C until potting the next day.

The rooted cuttings of *S. viminalis* were planted in pots according to the following scheme: 1. 5 cuttings in each pot that contained 2.5–3 kg of tailing's soil (Z1, Z2, Z3) (Fig. 1B). 2. 5 cuttings in each pot that contained 2.5–3 kg of tailing's soil on the bottom and upper layers, and a middle layer of potting soil with native bacteria *Salicornia europaea* L. (Z1 + rhiz, Z2 + rhiz, Z3 + rhiz) (Fig. 1B).

As a positive control for willow cuttings, 5 rooted cuttings were grown using a sand culture. After three months of growth the willow cuttings were harvested, and DNA extracted from the below-ground part of the plants.

DNA extraction and PCR amplification

DNA was extracted from the roots grown in the pots with soil from the zones 1, 2 and 3 (Z1, *Z2 and Z3)* and from the ones where the growth substrate contained soil from zones enriched with the *S. europaea* rhizosphere soil (Z1 + rhiz, Z2 + rhiz and Z3 + rhiz s).

Microbial DNA was obtained from 200 mg of *S. viminalis* roots using the Nucleo Spin® Soil kit (Macherey–Nagel, Germany). Quantitative determination of the concentration (ng/µl) and purity of DNA (A260/280) was performed spectrophotometrically using a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific). DNA concentration was determined fluorometrically using Qubit 2.0 (Invitrogen). The quality of the DNA was checked via 1% agarose gel electrophoresis. DNA samples were stored at − 20 °C for further analysis.

Prokaryotic 16S rRNA gene fragments were amplified by a two-step PCR procedure where the first step consisted of 2.5 ng extracted DNA, 2× Phusion PCR Mastermix (Thermo Scientific, Waltham, MA, US) and 10 µM of the primers pro341F/pro805R in 15 µl reactions. Two independent PCRs were run under the following conditions: 3 min at 98 °C, followed by 25 cycles of 98 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s and a final extension step of 10 min at 72 °C. The PCR products were then pooled and checked by 1% agarose gel electrophoresis. A single 30 µl reaction was performed for the second PCR, using 2 µM of primers with Nextera adaptor and index sequences, and 3 µl of the pooled PCR product from the first PCR. Conditions were the same as the first step, except for an annealing temperature of 55 °C and an extension time of 45 s, with 8 cycles. The final PCR products were purified using an E.Z.N.A.® Cycle-Pure Kit (Omega Bio-tek, Georgia) following the manufacturer's instructions. The amplicon size was checked by gel electrophoresis and the quality control was performed on a BioAnalyzer (Agilent, Santa Clara, CA, US). After quantification using a Qubit fluorometer (Invitrogen, Carlsbad, CA, US), libraries were created. Sequencing was performed on an Illumina MiSeq instrument using the 2,250 bp chemistry.

Processing of the reads, clustering and taxonomic identification

16S rRNA analysis of MiSeq sequencing data was performed essentially as described in mothur MiSeq SOP[36]. Paired end reads were merged with make.contigs command using parameters "align = needleman, match = 1, mismatch = − 1, gapopen = − 2, gapextend = − 1". Mean alignment length was 466 bases, 97.5% sequences had 0 ambiguous bases. Merged reads were trimmed using screen.seqs command with "maxlength = 464, maxambig = 0" parameters. Duplicate sequences were removed using unique.seqs command and aligned to custom silva.v3v4.align reference alignment using align.seqs command. Custom 16S rRNA v3v4 region reference alignment was generated using *L. acidophilus* 16S rRNA gene sequence (EF533992.1) and SILVA seed reference file (silva.seed_v123.align, https://mothur.s3.us-east-2.amazonaws.com/wiki/silva.seed_v123.tgz). Briefly, the *L. acidophilus* 16S rRNA sequence covering the region amplified by pro341F/pro805R oligos was aligned to the SILVA reference file with mothur align.seqs command to obtain coordinates for trimming the SILVA reference. Then, the SILVA reference was trimmed to the v3v4 region using start and end coordinates 6388 and 25,316, respectively (465 bases), with the mothur pcr.seqs command. After alignment to reference, poorly aligned sequences and sequences with long homopolymer stretches were removed using the screen.seqs command with parameters start = 1, end = 18,928, maxhomop = 8. Overhangs on either side of the v3v4 region were removed using the filter.seqs command with parameters vertical = "yes" and trump = ".". Almost identical sequences with maximum 4 differences were merged using the pre.cluster command with the diffs = 4 parameter. Chimeric sequences were identified and removed from further analysis using the chimera.vsearch and the remove.seqs command. Retained sequences were classified taxonomically using the classify.seqs command with reference = "trainset16_022016.rdp.fasta" and taxonomy = "trainset16_022016.rdp.tax", where v16 RDP reference files were obtained from mothur (https://mothur.org/wiki/rdp_reference_files/). Sequences classified as belonging to taxa "Chloroplast-Mitochondria-unknown-Archaea-Eukaryota" were removed from further analysis. Sequences were clustered to OTU-s using the cluster.split command with parameters taxlevel = 4 and cutoff = 0.03. OTU counts were obtained using command make.shared, and the consensus taxonomy of each OTU was obtained using the classify.otu command. The mothurs' make.biom command was used with inputs from make.shared and classify.otu to generate a biom v1.0 file for downstream analyses.

Statistical analysis

For Bayesian modelling, we used the R libraries rstan vers. 2.21.3 (Stan Development Team 2020) and brms vers. 2.16.1[37]. Models were specified using extended R lme4 formula syntax as implemented in the R brms package[37]. We used weak priors to fit models. We ran minimally 2000 iterations and four chains to fit models. When suggested by brms, Stan NUTS control parameter adapt_delta was increased to 0.95–0.99 and max tree depth to 12–15. Hypotheses were tested using the alpha = 0.05 level, specifying the Bayesian confidence interval (credible interval), containing 1 − alpha = 0.95 (95%) of the posterior values, to determine the presence of an effect.

Ethics statement

The sampling, material identification and deposition was performed complying with national and international guidelines and legislation of the Department of Plant Physiology and Ecology of Ivan Franko National University of Lviv and ChML West-Plast (certifcate of GOE "Lvivstandardmetrologiya" No. PL 04/11 from April 25, 2018), Truskavets.

Results

Thirty-six (36) *S. viminalis* root samples were studied representing the 1,455,675 (after rarefaction 245,448) reads, comprising 26,611 unique OTUs (after rarefaction 8255). A total of 12,133 (after rarefaction 4209) and 19,731 (after rarefaction 5917) OTUs were analyzed from the *S. viminalis* roots grown in the unsupplemented soils from Z1–Z3 and the soils from Z1–Z3 supplemented with *S. europaea* rhizosphere, respectively. The lowest number of OTUs was found at Z3 (3537, after rarefaction 1311) and the highest number was at Z1 supplemented with *S. europaea* rhizosphere (9423, after rarefaction 3690). The roots from all three zones had their characteristic community after rarefied data and only 323 and 341OTUs were present at all three zones presenting the cuttings grown with and without *S. europaea* rhizosphere, respectively. This amounts to 7.6%, 95% CI (Bayesian credible interval) [6.8%, 8.4%] and 5.8%, 95% CI [5.2%, 6.4%] of the total number of OTUs, respectively (Fig. 2A,B). Analysis suggests that the plants grown in soils supplemented with *S. europaea* rhizosphere had 1.9 (95% CI [0.9, 2.8]) percentage points fewer OTUs common to all three zones than plants grown in unsupplemented soils, the probability of a non-zero effect was nearly 1. Accordingly, there were more unique OTUs in samples supplemented with *S. europaea* rhizosphere than in unsupplemented samples (Fig. 2C). Analysis of pairs of unsupplemented versus *S. europaea* rhizosphere-supplemented OTU sets at three zones showed that there was a higher proportion of unique OTUs relative to common OTUs in cuttings grown in soils supplemented with *S. europaea* rhizosphere from Z1 and Z3 (Fig. 2D,F,G). Whereas, in Z2 there were less unique OTUs relative to

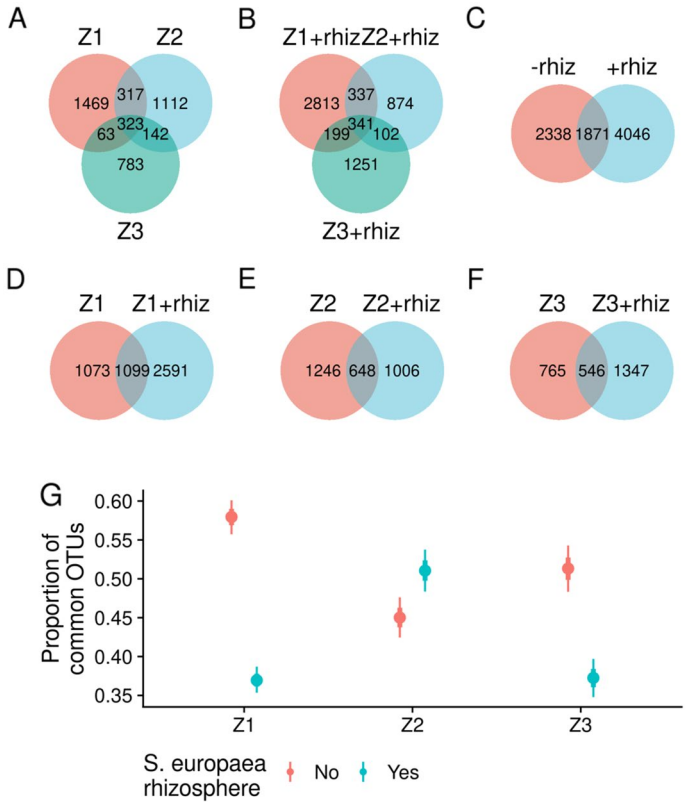


Figure 2. Relationships between OTU sets identified from *S. viminalis* roots grown in unsupplemented soils or in *S. europaea* rhizosphere-supplemented (rhiz) soils from three contamination zones. (**A**) OTU sets from *S. viminalis* cuttings grown in unsupplemented soil samples from three zones. (**B**) OTU sets from *S. viminalis* roots grown in *S. europaea* rhizosphere-supplemented soil from three zones. (**C**) Relationship between OTU sets from *S. viminalis* cuttings grown in unsupplemented vs *S. europaea* rhizosphere -supplemented soils. (**D**–**F**) Pairwise OTU sets of *S. viminalis* plants grown in unsupplemented or in *S. europaea* rhizosphere-supplemented soils from Z1 (**D**), Z2 (**E**), and Z3 (**F**). (**G**) Proportion of common OTUs relative to total from *S. viminalis* cuttings grown in *S. europaea* rhizosphere-supplemented vs. unsupplemented soil from three zones. Points denote the best fit of the aggregated binomial model [common|trials(total) ~ rhiz + zone + rhiz:zone]. Thick and thin lines denote 67% and 95% credible intervals, respectively. For OTU set comparisons, individual replicates were first rarefied and then merged by treatment groups.

common OTUs in plants grown in soils supplemented with *S. europaea* rhizosphere. (Fig. 2E,G). The number of OTUs common between plants grown either in *S. europaea* rhizosphere supplemented or unsupplemented soils decreased with increasing contamination from Z1 to Z3 (Fig. 2D–F). 203 OTUs out of 8255, comprising 2.5%, 95% CI [2.1%, 2.8%] of total OTUs, were present in plants from all conditions.

The observed OTU richness and Shannon diversity index of bacterial communities in *S. viminalis* plants grown in unsupplemented soil were decreased in Z3 versus Z1 (effect sizes − 413 OTUs, 95% CI [33, − 857], probability of effect size less than zero 0.975 and for Shannon index − 1.1, 95% CI [− 0.46, − 1.81], probability of effect size less than zero 0.999) and Z3 versus Z2 (effect size − 454 OTUs, 95% CI [− 18, − 955], probability of effect size less than zero 0.977 and for Shannon index − 1.0, 95% CI [− 0.32, − 1.72], probability of effect size less than zero 0.996) (Fig. 3A,B). Whereas, there was no decrease in the inverse Simpson index across contamination zones in *S. viminalis* plants grown in unsupplemented soil (Fig. 3C). *S. europaea* rhizosphere treatments increased the observed OTU richness in Z1 and Z3, and the Shannon diversity index in Z3 (Fig. 3A,B).

Beta diversity is illustrated in an NMDS plot based on Bray–Curtis distances of all samples (Fig. 4A). The samples clustered according to the pollution level along the NMDS1 axis with the more polluted clustering together in association with positive NMDS scores. The NMDS plots also confirmed that communities from *Salicornia* rhizosphere-treated plants were clustering together with untreated communities from their zone of origin. There is some apparent overlap of some Z1 samples with Z2. The community dispersion was not significantly affected by the contamination zone or *Salicornia* rhizosphere treatment (Fig. 4B).

Taxonomic analysis suggests a higher abundance of genera *Halomonas*, *Marinobacter*, *Idiomarina*, and *Marinimicrobium* in *S. viminalis* cuttings grown in Z3 soils (Fig. 5).

Discussion

Some of the challenges that plants face in climate change are drought, flooding, salinity and heavy metal pollutants. Plant breeding has been generating the breeding lines capable of dealing with the stress situations. Considering, however, the rapid nature of environmental changes, it is unsure if the changes in the plant genomes can outcompete the challenges. Unlike the genes and regulatory regions of the genome, microbial composition can be rapidly modified by environmental cues, and may thus represent a mechanism for rapid acclimation and adaptations of individuals to a changing environment. Recently, we proposed the microbiota-mediated acclimatisation concept, suggesting that changes in microbiota that originate from severe drought stress enable modern wheat cultivars to tolerate drought stress[4,11,12,38]. By a similar token here we hypothesise that the bacteria that have lived for long periods under HM and salt pollution stress have acquired the ability to tolerate the pollution, and are suitable for supporting bioremediation processes. While the Stebnyk tailings storage represents a threat to plant

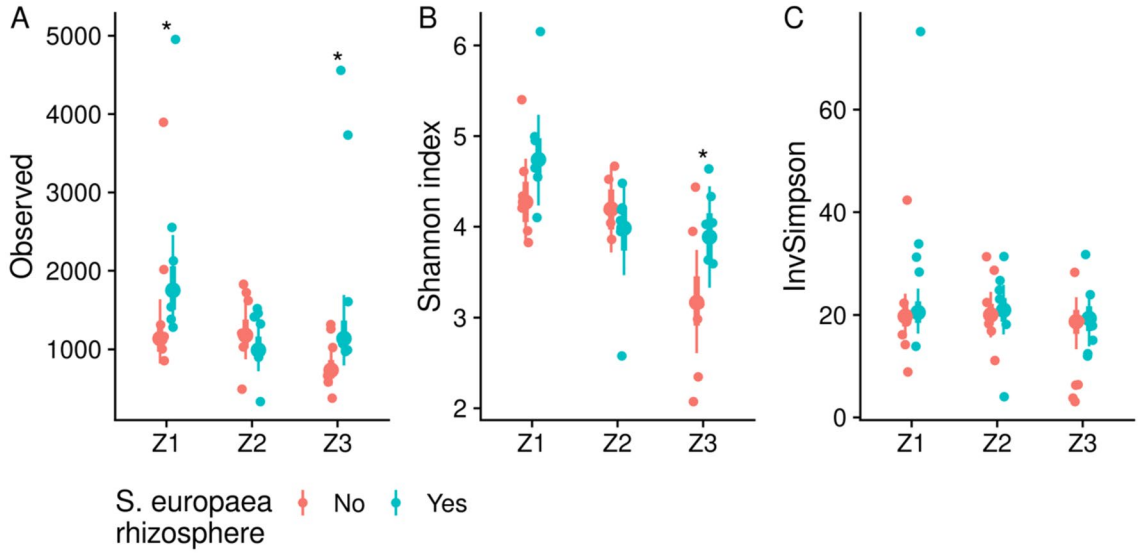


Figure 3. Alpha diversity measures of OTUs identified from *S. viminalis* roots grown in soils from three contamination zones with or without *S. europaea* rhizosphere supplementation. (**A**) Conditional effects of the contamination zone and *S. europaea* rhizosphere supplementation to the observed OTU richness estimated from negative binomial model, adjusted for sequencing library size. (**B**) Conditional effects of the contamination zone and *S. europaea* rhizosphere supplementation to the Shannon diversity index estimated from the robust linear model (Student's t likelihood), adjusted for sequencing library size. (**C**) Conditional effects of the contamination zone and *S. europaea* rhizosphere supplementation to the Inverse Simpson's diversity index estimated from the robust linear model, controlled for sequencing library size. Non-rarefied OUT sets were used for diversity index calculations. Large points denote the model's best fit. Thick- and thin lines denote 67% and 95% credible intervals, respectively. Small points denote individual observations, N = 6. Asterisks denote non-zero effect size in diversity measures (probability > 0.95) of *S. viminalis* cuttings grown in unsupplemented versus *S. europaea* rhizosphere-supplemented soils.

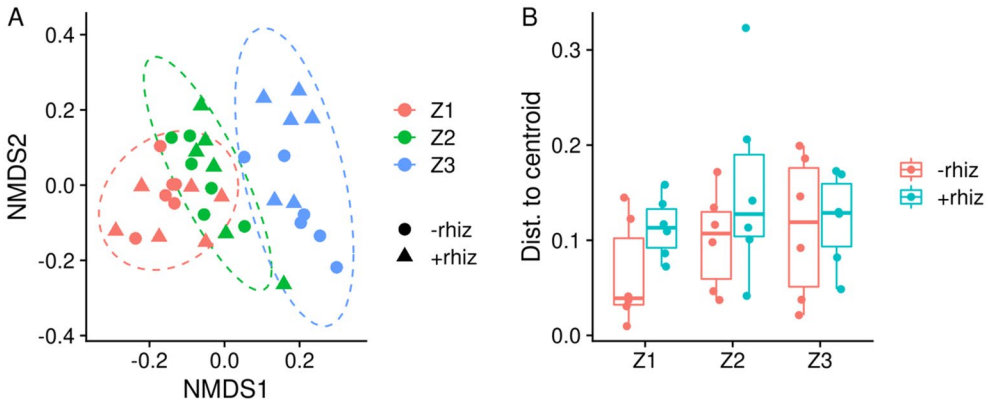


Figure 4. (**A**) Non-metric multidimensional scaling (NMDS) using Bray–Curtis distances. Color denotes the contamination zone. The point shape denotes *S. europae*a rhizosphere supplementation. Ellipses are 95% confidence regions of t distributions. (**B**) Distances to centroid calculated for NMDS Bray–Curtis distances.

and microbial communities putting ecosystem integrity at risk, the *S. europaea* plants grown under the pollution over four decades represent a reservoir of genetic traits to aid bioremediation of *S. viminalis*.

Our data representing the contamination gradient clearly showed that the alfa diversity of the bacterial community decreased, and the community composition changed, along the pollution gradient, suggesting a homogenization of the communities as the pollution appeared. The sequencing of the 16S rDNA library revealed the presence of a relatively rich endophytic community, indicated by the number of identified OTUs. The biodiversity and the richness of the bacterial endophytic community were assessed based on the observed number of OTUs, Shannon-, and InvSimpson index. Modelling showed that the observed number of OTUs decreased from zone 1 to zone 3. *S. europaea* supplementation significantly increased the number of OTUs in zones 1 and 3. The biodiversity was significantly decreased across the contamination in un-supplemented plants as indicated by the Shannon index. *S. europaea* supplementation lessened the decline based on observed OTUs and the Shannon index. The inverse Simpson index was not significantly affected by the *S. europaea* treatments in any of the zones. This suggests that the number of dominating OTUs is not changing across zones in both *S. europaea*-treated, and untreated, bacterial populations, whereas the decrease in richness is mainly caused by low-abundance OTUs.

While the pattern of Z1 and Z2 communities was relatively homogeneous, considerably larger changes in the community were observed in zone 3. *Marinobacterium, Idiomarina, Marinamicrobium,* and *Halomonas* were sparsely represented in communities Z1 and Z2 but were abundant in Z3. Many of the OTUs associated with the above-mentioned taxa have been frequently described as linked with *Marinobacterium, Idiomarina, Marinimicrobium,* and *Halomonas* representatives have earlier been chosen as inoculants to support growth under HM salt conditions[39,40]. The rhizoremediation principle is that the plant roots colonize in the contaminated soil and associate with the subset of microorganisms present in the soil that help to tolerate the pollution stress. It may also involve metabolization of the pollutant. The endophytes are capable of degrading various organic pollutants and accelerating the extraction of toxic metals[13,14]. The bacterial metabolic pathways can synthesize natural chelators that improve the metallic availability for plant uptake and degradation[41–44]. However, it is important to understand that we can only speculate about the ecological role of the detected taxa given the fluid nature of bacterial taxa[4,45] and even more so because the information on the taxa is based on what has previously been described in other systems. The basic biodiversity principle is that different organisms enhance the ecosystem functions under stress conditions. The observed increase in species richness can differentially influence the functioning of the ecosystem. If all species contribute approximately equally to the functioning of the ecosystem, the species effect may be additively decelerating if some of the species are to some extent functionally redundant. If the pool of species contains few species that can mediate handling pollutants efficiently, the effect will also be decelerating.

Any effective plant beneficial microbial application requires effective colonization. Although several endophytes have shown bioremediation activities, microorganisms under field conditions face competition with a myriad of microbes naturally adapted to the environment. Hence, on the example of Stebnyk tailing we present a sustainable bioremediation strategy using plant-based enrichment. We identified native endophytic strains of *S. viminalis (the bioremediation species)* and *S. europaea (local species adapted to pollution)* that would be capable of colonizing and have potential efficiently supporting phyto-remediation at the Stebnyk mine. How the microbial isolates behave under specific environmental conditions and what are the most influential consortia needs further metagenomic study in order to predict functional traits under the conditions.

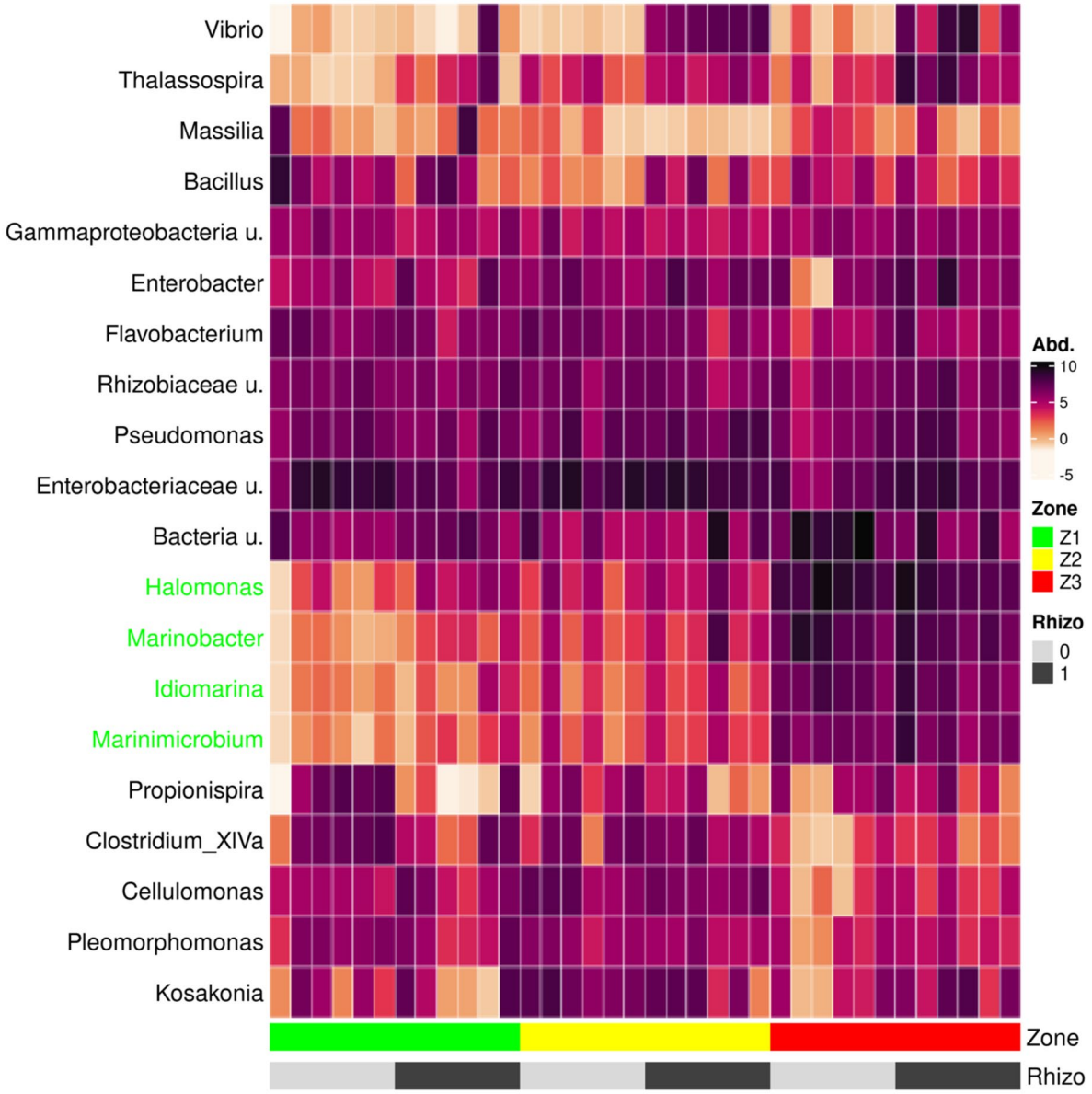


Figure 5. Relative abundance (Abd) of the top twenty genera. Samples were ordered by the contamination zone and *S. europae*a rhizosphere supplementation.

Data availability

The data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB73979 (https://www.ebi.ac.uk/ena/browser/view/PRJEB73979).

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Author contributions

ST conceived and designed the study; AF Stebnyk sampling, DNA extraction, PCR amplification, library preparation; TP processing the reads, taxonomic identification, statistical analysis; ST and TP wrote the manuscript; All authors contributed to the article and approved the submitted version.

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Competing interests

The authors declare no competing interests.

Additional information

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