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Diversity of root-associated microbial populations of *Tamarix parviflora* cultivated under various conditions

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ABSTRACT

Soil microbial communities are shaped by many different biotic and abiotic factors, whose effects, however, are not fully understood. In this study, our objective was to analyze microbial communities in the root zone of *Tamarix parviflora* grown in pristine, nutrient-rich or legacy contaminated, nutrient-poor soil and how these communities change in response to increased soil salinity (a possible stress factor) and soil inoculation with allochthonous bacteria. Our results reveal significant differences in microbial community structure between horticultural and contaminated soils. When controlling for the effect of soil, microbial community structure was significantly affected by salinization, however inoculation with allochthonous bacteria did not significantly change the total community. The genera *Arthrobacter*, *Bacillus*, *Burkholderia*, *Dyella* and *Ktedonobacter*, among others, were significantly enriched in the contaminated root zone, whereas populations in the root zone of horticultural soil were found to be more diverse. In this type of soil, representatives of genera such as *Flavobacterium*, *Ignavibacterium*, *Sediminibacterium*, *Terrimonas*, *Chitinophaga* and *Hydrotalea* were significantly enriched. Regardless of soil type, members of genera *Bradyrhizobium*, *Parachlamydia*, *Dongia*, *Vampirovibrio*, *Flavisolibacter* and *Rhodomicrobium* were enriched in non-salinized soil, while the genus *Acidothermus* was enriched in salinized soil. Phospholipid-derived fatty acid (PLFA) analysis showed higher bacterial and fungal biomass in horticultural soil as compared to contaminated soil, demonstrating that soil nutrition is one of the most important factors with respect to microbial richness.

1. Introduction

Plant-microbe interactions primarily occur in the rhizosphere, a unique environment immediately surrounding the roots (Kowalchuk et al., 2002; Smalla et al., 2001). Plant roots release large quantities of chemically diverse exudates, which stimulate microbial activity in the immediate vicinity of the roots (Dennis et al., 2010) and influence the physicochemical properties of the surrounding soil (Rambelli, 1973). Rhizosphere microorganisms, in turn, affect root development and plant growth (Hrynkiewicz and Baum, 2012). Apart from plant exudates, which exert selective pressure on microbial communities in the rhizosphere, other abiotic factors, such as weather conditions, aeration, soil pollutants and soil characteristics themselves (salinity, water content, pH), influence the composition of these communities (Ibekwe et al., 2010; Marschner et al., 2001; Pennanen et al., 1999; Sutton et al., 2013). Analysis of how soils and plants affect the structure of plant-associated microbial communities is essential for an understanding of microbial responses to different environmental conditions and of microbial roles in ecosystem functioning (Berg and Smalla, 2009).

Many studies have focused on whether and how plant species, different fertilization methods, nutrient availability and soil type influence microbial community structure (Berg and Smalla, 2009; Marschner et al., 2004; O'Donnell et al., 2001; Prober et al., 2015; Singh et al., 2007; Sun et al., 2014). However, considerably less information is available on how plant-associated bacterial communities differ across various stress conditions, such as increased salinity, how distribution/dispersal of root-associated microorganisms may constrain community structure and whether, for example, inoculation of contaminated soil with a pollutant-degrading strain results in ecologically significant changes in community structure.

Salt cedars (*Tamarix* spp.), which are flowering plants native to Eurasia and North Africa, are intentionally spread to other continents

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Table 1
Experimental settings used in this study.

Sample*	Soil type	Salinization	Bioaugmentation
CN0	Contaminated soil	No	No
CN1	Contaminated soil	No	Yes
CS0	Contaminated soil	Yes	No
CS1	Contaminated soil	Yes	Yes
HN0	Horticultural soil	No	No
HN1	Horticultural soil	No	Yes
HS0	Horticultural soil	Yes	No
HS1	Horticultural soil	Yes	Yes

* H – horticultural soil, C – contaminated soil, S – salinized soil, N – non-salinized soil, 0 – non-inoculated soil, 1 – inoculated soil.

by anthropogenic means (Sher, 2013). The expansion of this genus is determined by its capacity to grow under a variety of environmental conditions and to successfully resist stress, including heat, drought, low soil nutrients and high soil salinity (Bianchi et al., 2010; Sher and Quigley, 2013; Zhang et al., 2002). This makes *Tamarix* plants suitable for phytoremediation (Kadukova et al., 2008; Santos et al., 2017) and wastewater treatment (Fountoulakis et al., 2017) purposes.

Given all the above, we hypothesized that differences in environmental conditions, including increased soil salinity and soil inoculation with allochthonous bacteria, significantly influence the composition of microbial communities present in the plant's root zone. Therefore, we conducted a microcosm-based study in order to analyze microbial communities in the root zone of *T. parviflora* grown in pristine, nutrient-rich or legacy contaminated, nutrient-poor soil and determine how these communities change in response to increased soil salinity (a possible stress factor) and soil inoculation with allochthonous bacteria. To do this, we used two complementary community approaches: 16S rRNA gene pyrosequencing, with its finer phylogenetic resolution but poor abundance measurement capacity, and whole-cell PLFA analysis, with its coarse-scale taxonomic resolution but stronger prediction capacity of the relative abundances of different detectable taxonomic groups.

2. Materials and methods

2.1. Plant and soil material

T. parviflora cuttings were taken from a fully grown tree in Central Bohemia, Czech Republic (50°11′28.9″N, 14°06′53.4″E). The cuttings were rooted in tap water for ~2 weeks and were then potted in 1.1 L plastic pots lined with aluminum foil and filled with ~1 L of either horticultural or legacy contaminated soil. The horticultural soil (Agro, Czech Republic) had the following characteristics according to the supplier's description: pH 5–7, organic matter content min. 50%, biologically available N 50–300 $mg{\cdot}L^{-1}$, P 35–130 $mg{\cdot}L^{-1}$, K 65–330 $mg{\cdot}L^{-1}$ and risk element concentrations below Czech Republic legal limits. The legacy contaminated soil, whose characteristics have been previously published (Pavlíková et al., 2007; Stella et al., 2015; Uhlík et al., 2012), and which was primarily contaminated by polychlorinated biphenyls (PCBs), was collected from a refuse dump in Lhenice, South Bohemia, Czech Republic.

The experiment had a 3 × 2 full factorial treatment design, with three replications and completely randomized treatment allocation. The factors tested (Table 1) included: (i) two soil types; (ii) artificial salinization; and (iii) soil inoculation. Soil salinization was carried out by mixing 9 L of soil with 36 g of NaCl dissolved in water followed by homogenization. Soil inoculation was performed at 14-day intervals with suspensions of allochthonous PCB-degrading bacterial strains *Pseudomonas alcaliphila* JAB1 (Rídl et al., 2018) and *Achromobacter xylosoxidans* S3 (Koubek et al., 2012). The bacterial suspensions for soil inoculation were prepared as follows: (i) both strains were cultivated in liquid mineral salt solution (LMSS) with 5 $mg{\cdot}L^{-1}$ biphenyl (Uhlík et al., 2012) for 48 h; (ii) excess biphenyl was removed using centrifugation (5000 × *g*, 5 min) and the cells were resuspended in LMSS; (iii) the suspensions were again centrifuged, the cells were resuspended in an exact amount of LMSS to obtain a suspension at a McFarland density of 1; and (iv) 10 mL of this suspension was applied to the soil. Each treatment was performed in triplicate. The plants were cultivated in a cultivation room at a stable temperature of ~25 °C for 16 months under a daily light/dark regime 16/8 h.

Upon completion of the cultivation phase (16 months), soil from the plant's root zone was harvested. The soil samples were subjected to the isolation of metagenomic DNA.

2.2. Metagenomic DNA isolation and community 16S rRNA gene amplification

The DNA from all soil samples was isolated using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, USA), with 300 mg of each sample homogenate being used as starting material. The 16S rRNA genes were amplified using universal primers 515-530f and 1068-1052r (Leewis et al., 2016), with regions V4–V6 of 16S rRNA genes being targeted in prokaryotes. KAPA HiFi HotStart ReadyMix (KAPA Biosystems, USA) was used for 16S rRNA gene amplification in a 15 µl PCR mixture containing 4.5 pmol of each primer (Generi Biotech, CZ) and 5–20 ng of template DNA. Amplification conditions were as follows: 95 °C for 5 min (initial denaturation) followed by 25–30 cycles at 98 °C for 20 s, primer hybridization at 50 °C for 15 s, extension at 72 °C for 40 s; and a final extension step at 72 °C for 5 min.

The PCR products were separated by agarose electrophoresis, the amplicons of desired size were excised from the gel and the DNA was extracted using the Zymoclean™ Gel DNA Recovery Kit (Zymo Research, USA). The extracted DNA was purified using the SPRIselect Reagent Kit (Beckman Coulter, USA) according to the manufacturer's recommendations.

Table 2
Average sums of PLFAs for total bacteria and their subgroups.

Sample*	Total bacteria	G−	G+	Actinobacteria	Fungi	Total microbial biomass
	[ppm]					
CN0	2.7 ± 0.3	0.6 ± 0.1	1.0 ± 0.1	0.17 ± 0.03	0.0 ± 0.0	2.7 ± 0.3
CN1	3.1 ± 0.6	0.7 ± 0.2	1.09 ± 0.03	0.19 ± 0.04	0.1 ± 0.1	3.2 ± 0.7
CS0	2.9 ± 0.3	0.6 ± 0.1	1.1 ± 0.1	0.19 ± 0.03	0.0 ± 0.0	2.9 ± 0.3
CS1	3.5 ± 0.9	0.9 ± 0.4	1.3 ± 0.3	0.2 ± 0.1	0.0 ± 0.0	3.5 ± 0.9
HN0	34.8 ± 2.8	16.1 ± 1.3	8.1 ± 0.7	1.1 ± 0.3	0.9 ± 0.3	35.7 ± 3.1
HN1	42.0 ± 7.3	19.2 ± 3.5	9.7 ± 2.0	1.4 ± 0.3	0.9 ± 0.2	42.9 ± 7.1
HS0	17.5 ± 7.7	7.1 ± 4.6	4.4 ± 0.8	0.3 ± 0.1	0.2 ± 0.1	17.7 ± 7.9
HS1	35.0 ± 12.4	18.5 ± 10.3	6.3 ± 0.7	1.1 ± 0.4	0.9 ± 0.3	35.9 ± 12.7

* H – horticultural soil, C – contaminated soil, S – salinized soil, N – non-salinized soil, 0 – non-inoculated soil, 1 – inoculated soil.