Importance of microbial communities at the root-soil interface for extracellular polymeric substances and soil aggregation in semiarid grasslands

Betterman, A.; Zethof, J.H.T; Babin, D.; Cammeraat, E.L.H.; Solé-Benet, A.; Lázaro, R.; Luna, L.; Nesme, J.; Sørensen, S.J.; Kalbitz, K.; Smalla, K.; Vogel, C.

DOI
10.1016/j.soilbio.2021.108301

Publication date
2021

Document Version
Final published version

Published in
Soil Biology and Biochemistry

Soil Biology and Biochemistry 159 (2021) 108301

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Importance of microbial communities at the root-soil interface for extracellular polymeric substances and soil aggregation in semiarid grasslands

Antje Bettermann [a,1], Jeroen H.T. Zethof [b,1], Doreen Babin [a], Erik L.H. Cammeraat [c], Albert Solé-Benet [d], Roberto Lázaro [d], Lourdes Luna [d], Joseph Nesme [e], Søren J. Sørensen [e], Karsten Kalbitz [b], Kornelia Smalla [a], Cordula Vogel [b,*]

[a] Julius Kühn Institute (JKI) - Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11/12, Braunschweig, Germany
[b] Institute of Soil Science and Site Ecology, Technische Universität Dresden, Pienner Str. 19, Tharandt, Germany
[c] Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Science Park 904, Amsterdam, the Netherlands
[d] Estación Experimental de Zonas Áridas (EEZA-CSIC), Ctra. de Sacramento s/n, Almería, Spain
[e] Section of Microbiology, Department of Biology, University of Copenhagen, Universitetsparken 15, Bygning 1, Copenhagen, Denmark

ARTICLE INFO

Keywords:
High-throughput amplicon sequencing
Carbonate
Rhizosphere
Rhizoplane

ABSTRACT

In the past years, extracellular polymeric substances (EPS) produced by soil microorganisms received an increasing interest, as they not only protect microbes against environmental stresses, but seem to play a pivotal role in soil structure formation as well. Within soils, root deposits provide an important source of easily accessible energy and nutrients, stimulating microbial growth to produce EPS. Especially under semiarid climates, where a full vegetation cover cannot be sustained, large gradients in living conditions for microbes can be found between the root-soil interface and barren intercanopy spaces.

In this study, we aimed to elucidate the plant-specific effects on microbes, EPS production and soil aggregation. At two sites in southern Spain, differing in carbonate and graphite content, legume shrubs of *Anthyllis cytisoides* and grass tussocks of *Macrochloa tenacissima* were selected. Soil samples were taken in the adjacent bare interspace, under the canopy and of the rhizosphere. From these samples the microbial community (here bacteria and archaea), EPS(-saccharide) content and soil aggregation (<1 mm) were analysed. DNA extracted from the microbial cells detached from the surface of the sampled roots (rhizoplane), was subjected to 16S rRNA gene amplicon sequencing.

The rhizoplane microbial communities differed strongly between plant species and sites, whereby site was the most important factor shaping the communities. The plant species effect on microbial communities diminished strongly with distance to the root surface. At the carbonate-poor Rambla Honda site (site 1), plant species-specific effects were observed in the rhizoplane and rhizosphere, whereas in the carbonate-rich Alboloduy site (site 2) almost no plant species-specific effects were found at the genus level. The larger heterogeneity in microbial communities at site 1 was reflected in EPS-saccharide contents and subsequent soil aggregation, while no difference in soil aggregation was found at site 2. Both parameters increased strongest in the *Anthyllis cytisoides* rhizosphere at site 1.

Despite the lack of a strong gradient with distance from the root at the carbonate-rich site 2, microbial taxa were found by network analysis that positively correlated to EPS-saccharide contents and/or soil aggregation. The relationship between the identified taxa and EPS and/or aggregation relationships were clearest at the root-soil interface, while several other taxa were found to be widely occurring in the other soil compartments too.

In conclusion, we found in all compartments potential EPS producers, which could have influenced soil aggregation. Nevertheless, microbes with higher relative abundance in the rhizoplane were linked to higher EPS contents, especially in conjunction with legume shrubs, and subsequently related to soil aggregation. The spatial

* Corresponding author.
E-mail address: cordula.vogel@tu-dresden.de (C. Vogel).
[1] A. Bettermann and J.H.T. Zethof contributed equally to this work as first authors.

https://doi.org/10.1016/j.soilbio.2021.108301
Received 29 January 2021; Received in revised form 3 May 2021; Accepted 8 May 2021
Available online 13 May 2021

extent of the root effect was only governed by carbonate contents, as higher carbonate content diminished the observed root effects on the microbial community and subsequent soil aggregation.

1. Introduction

Plants are important drivers of spatial variability in soil microbial communities as they create plant species-specific microbial communities in the rhizosphere via rhizodeposits (Berg and Smalla, 2009; Zhalnina et al., 2018). It is indeed not only the plant species that shapes the soil microbial community, but the physical and chemical composition of the soil are important drivers as well (Berg and Smalla, 2009; Rousk et al., 2009; Schreiter et al., 2014; Fierer, 2017). Bulgarelli et al. (2012) showed, for instance, that despite 40% of the root-inhabited microbiota were similar between the *Arabidopsis thaliana* roots, the influence of soil types was more dominating. It is often recognized that local differences in microbial community composition result from nutrient availability and other environmental conditions like soil moisture contents (Fierer, 2017). In this regard, extracellular polymeric substances (EPS) might play a role as they prevent disconnection of the microbes from their nutrient sources (Schimel, 2018), whereby many bacterial isolates from soil have the potential to synthesize EPS (Flemming and Wuertz, 2019). EPS consist mainly of polysaccharides, structural proteins and enzymes, but also contain other biopolymers like nucleic acids (More et al., 2014; Flemming et al., 2016). The EPS matrix allows microbial cells to adhere to each other and attach to surfaces (Flemming and Wingender, 2010). At the same time EPS form bridges between soil particles, thereby changing the soil structure and potentially improving soil structural stability (Costa et al., 2018). A few studies attempted to create direct links between soil aggregate stabilization and the presence of certain bacterial strains (i.e. Caesar-TonThat et al., 2007; Davinic et al., 2012). Innovative and reliable approaches to obtain a better mechanistic understanding of this relationship are still lacking, especially as soil aggregates form heterogeneous habitats for soil bacteria themselves (Ebrahimi and Or, 2015; Tecon and Or, 2017), thereby influencing the overall microbial community composition.

Tisdall and Oades (1982) recognized the important role of microbial saccharides in soil aggregate formation and stabilization when they proposed the hierarchical aggregation model. A range of laboratory studies confirmed that EPS, adsorbed to mineral surfaces, take part in soil structure formation (i.e. Vardharajula and SkZ, 2015; Crouzet et al., 2019). However, natural systems have received only little attention (Chenu and Cosentino, 2011; Blankinship et al., 2016; Lehmann et al., 2017). As a result of recent advances in extraction methods for soils, the EPS constituents can be quantified and their role under field conditions studied (More et al., 2014; Redmile-Gordon et al., 2014; Bérard et al., 2020; Zethof et al., 2020). This allowed us in a previous study to show the importance of carbonates in microaggregate stability governed by EPS (Zethof et al., 2020). However, still little is known about the importance of these bonds for aggregate formation in the presence of other factors such as plant roots.

Under semiarid climates, landscapes are dominated by sparse vegetation cover growing in a banded or patchy pattern. This leaves the soil very vulnerable to erosion during seasonal rainfall. The role of individual plants in the protection of soil against degradation by their root systems and canopy cover are well understood and described (i.e. Morgan, 2005; Sandercock et al., 2017). Plant-derived rhizodeposits are quickly utilized by the soil microbial community, indicating the importance of roots for metabolic activity of soil microorganisms, which they (partly) use for EPS production (Oburger and Jones, 2018). This process takes mainly place at the root-soil interface, but the further spatial extent of plant influence on the microbial community in the surrounding soil is often overlooked (Veen et al., 2019), especially as plant canopies still provide an additional organic matter input and influence microclimatic conditions as compared to the surrounding bare soil (Goberna et al., 2007; Fierer, 2017; Tecon and Or, 2017).

In a previous study we showed that EPS-saccharide contents in semiarid soils around the legume shrub *Anthyllis cytisoides* L and grass species *Macrochloa tenacissima* (L) Kunth were shaped by microbial community composition and indirectly by organic matter availability (Zethof et al., 2020). Especially in a carbonate-rich soil a significant role of EPS in microaggregation was shown (Zethof et al., 2020). The parent material was the most important driver for microbial community compositions, rather than the studied plant species. To disentangle the effect of the plant species, in the present study rhizoplane and rhizosphere samples were analysed, as the plant-soil interface is known to be most dynamic and largest plant species-specific influences can be expected (Balbín-Suárez et al., 2020). The influence of these two plant species on their root-associated bacterial and archaeal communities (further on microbial communities) and subsequent EPS production has so far been unexplored.

In the current study, we aimed to obtain further insights into the role of soil microbial communities, associated to *Anthyllis cytisoides* and *Macrochloa tenacissima*, for the EPS contents and their influence on soil aggregation. We hypothesized that 1) the largest influence on microbial community composition of the studied plant species, i.e. the legume shrub *Anthyllis cytisoides* and the grass species *Macrochloa tenacissima*, can be expected in the rhizoplane, followed by the rhizosphere, as concentration of plant-specific rhizodeposits is highest in the rhizoplane. Therefore, plant species-specific communities are expected to be identified in the rhizoplane. 2) Differences in root systems and canopy shape/extent will have an important effect on the spatial extent of the plant influence, as the dense fibrous root system of *Macrochloa* will likely have a larger impact on the soil under the canopy compared to the taproot system of *Anthyllis*. Added to this, parent material has an important influence on shaping these root effects, since larger amounts of carbonates present might buffer rhizosphere effects. 3) Rhizodeposits will induce microbial growth and EPS production among some microbial taxa, resulting in increased EPS contents of the soil closest to the root. Differences in relative abundances of certain taxa might explain variances in EPS contents and subsequent soil aggregation.

2. Material and methods

2.1. Site description

Two study sites were previously selected in Almería province, southeastern Spain, based on their topography and the dominance of both *Macrochloa tenacissima* and *Anthyllis cytisoides* plant species (Zethof et al., 2020). The slope of the Rambla Honda site (37°7′45″ N, 2°22′30″ W), further on referred to as site 1, facing the southeast is characterized by shallow soils developed in mica-schist with graphite and garnets crossed by many quartz veins (Puigdefábregas et al., 1996). Also, the south-facing slope of the Alboloduy site (37°4′9″ N, 2°36′43″ W), further on referred to as site 2, is dominated by shallow soils developed in mica-schist, dominated by feldspar and chlorites, but without graphite. Soils at site 2 tend to be richer in inorganic C content, although relatively large spatial differences exist at the site 1. Both sites are influenced by dust deposition originating from local sources, i.e. marl Miocene sediments, and from North Africa as qualitatively shown by Queralt-Mitjans et al. (1993).

The climate in the area is semiarid Mediterranean, characterized by a dry period from May until September and an average annual temperature of 17.8 °C, as measured close by the town Tabernas. The annual precipitation of almost 300 mm falls mainly in autumn and winter and high intensity storms are less frequent than in other Mediterranean more

humid areas (Lázaro et al., 2001).

2.2. Sampling

During a sampling campaign at the end of April 2018, at the same five mid-slope plots as set out by Zethof et al. (2020), an *Anthyllis cytisoides* shrub and a *Macrochloa tenacissima* grass tussock were selected for sampling, resulting in a replication of five subplots per plant species/site combination. Due to limited rhizosphere material of the *Anthyllis* plants, not all measurements could be performed on all five replicates, resulting in a replication of four at those parameters indicated. First, a composite soil sample was taken by placing a 250 cm^3 sampling ring (8 cm diameter, 5 cm height) at both sides of the plant (Fig. 1), 70 cm from the stem, further on referred to as bare soil. Samples were taken at a 5–15 cm depth, to resemble the minimum rooting depth, and gently mixed to create a composite sample. After clipping of plants and undergrowth and removal of the loose (organic) material, a 30 cm circle around the stem of the plant was cut and the plant was excavated. The loose soil in this excavated clod was defined as plant affected soil. Roots were removed by gently breaking apart the clod and soil adhering to the root, i.e. rhizosphere, was mechanically sampled by gently brushing the roots. Brushed root fragments and the soil samples were stored cool during transportation. Finally, from these root fragments, total community DNA was extracted from the rhizoplane, i.e. external root surface (Estermann and McLaren, 1961), as described in the next section. Sample material destined for DNA and EPS extraction was sieved to 2 mm in the field, whereby soil aggregates were mechanically broken. In summary, four compartments, i.e. rhizoplane, rhizosphere, plant affected soil and bare soil, of each plant/site combination were sampled with a replication of five.

2.3. Analysis

2.3.1. Extraction and purification of total community DNA

Following the instructions of the manufacturer, a FastDNA®SPIN Kit for Soil (MP Biomedicals, Santa Ana, California, USA) was used for total community DNA extraction from 0.5 g (wet weight) of every soil sample. DNA from rhizoplane was extracted from cells detached from the roots by thoroughly shaking 0.5 g of roots per sample with 4.5 ml 0.85% NaCl and centrifugation (10 min at 7000×*g*) after removal of the root material. Cell lysis was done by two cycles of bead-beating (30 s at 5.5 m s^{-1}) with a FastPrep™ FP120 (Qbiogene, Inc., Carlsbad, California, USA). The resulting DNA solutions were purified using the GENECLEAN®SPIN Kit (MP Biomedicals, Santa Ana, California, USA) according to the instruction manual. Quality of the resulting total community DNA was confirmed by gel electrophoresis (0.8% agarose). Therefore, agarose gels were stained with ethidium bromide (0.005%) and photographed under UV light (Intas Gel Jet Imager 2004; Intas, Göttingen, Germany).

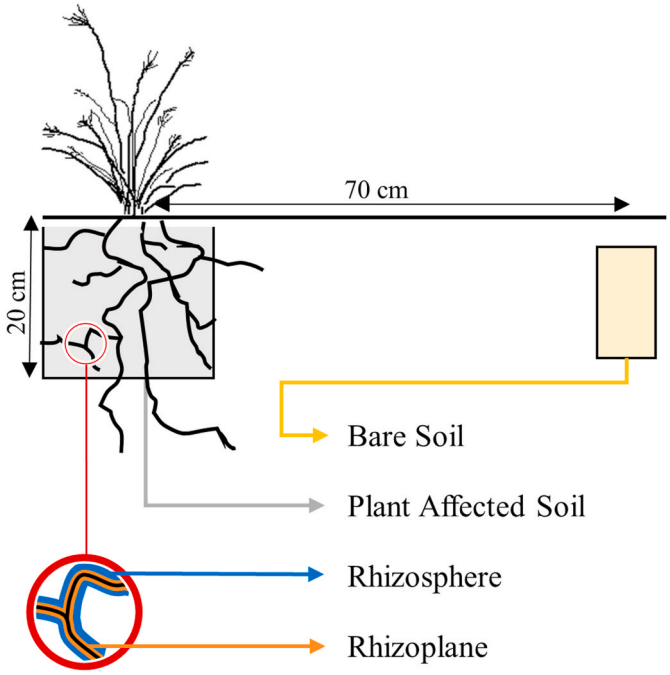

Fig. 1. Sampling approach whereby the bare soil, plant affected soil, rhizosphere and rhizoplane were sampled as described in Section 2.2.

2.3.2. Quantification of bacterial 16S rRNA gene fragments by quantitative real-time PCR (qPCR)

To assess ribosomal RNA operon copy numbers, a qPCR targeting the 16S rRNA gene was conducted as described by Vogel et al. (2014) using a CFX96 Real-Time System (Biorad, München, Germany) with a reaction volume of 50 µl. Description of primers and TaqMan probe specific for the domain Bacteria is available in Suzuki et al. (2000). The $\log_{10}$ copy number was calculated per g of soil dry weight or root fresh weight in case of the rhizoplane samples, respectively. Soil moisture content was determined by drying a subsample at 105 °C for 24 h.

2.3.3. Illumina sequencing of microbial 16S rRNA gene amplicons

Two PCR steps were performed to prepare sequencing libraries targeting the variable V3 and V4 regions of the 16S rRNA gene. For the first PCR, primers Uni341F (5′-CCTAYGGGRBGCASCAG-3′) and Uni806R (5′-GGACTACHVGGGTWTCTAAT), originally published by Yu et al. (2005) and modified as described in Caporaso et al. (2011) and Sundberg et al. (2013), were used to amplify both archaeal and bacterial 16S rRNA genes. In the second PCR, the primers included Illumina-specific sequencing adapters and a unique combination of index for each sample. PCR conditions, reagents and material were described in detail by Zethof et al. (2020). Amplicon sequencing was performed on an Illumina MiSeq platform using Reagent Kit v2 [2 × 300 cycles] (Illumina Inc., San Diego, California, USA).

Trimming of raw sequence reads, amplicon sequence variants (ASVs) identification and taxonomic annotation were done according to Zethof et al. (2020). Raw amplicon data are available at NCBI Sequence Read Archive (SRA, https://www.ncbi.nlm.nih.gov/) under accession number PRJNA689290.

Representative ASVs were classified using SILVArel132 database (Quast et al., 2013). After removal of sequences from the data set that were affiliated to Cyanobacteria/chloroplasts or mitochondria or were unclassified at domain level, a total number of 34,162 ASVs remained. On average, 40,949 quality-filtered sequences were obtained per sample.

2.3.4. EPS extraction and quantification

From field moist samples of the rhizosphere, plant affected and bare soil, EPS were extracted following the protocol of Redmile-Gordon et al. (2014), using 1 g cation-exchange resin (Sigma-Aldrich/DOWEX, Saint Louis, Missouri, USA, PN 91973) per 2.5 g soil material (dry weight equivalent). Before analysis, extracts were filtered through a 0.45 µm syringe filter (PET-45/25 Chromafil, Macherey-Nagel, Düren, Germany). Total saccharide content of the extracted EPS (EPS-saccharide) was quantified after Dubois et al. (1956) using D(+)-Glucose (Roth, Karlsruhe, Germany, PN X997) as a standard.

2.3.5. Soil carbon and nitrogen content

Soil samples (<2 mm) were ground by a table top grinder (Retsch MM200, Haan, Germany) in order to achieve homogenization. Inorganic carbon (C) was determined by measuring total C before and after acid treatment with excess of HCl, i.e. inorganic C = total C_{before} – total C_{after}, using an elemental CN analyser (Vario EL, Elementar, Langenselbold, Germany). The same device was used to measure total N of the soil samples. As the samples from site 1 contained graphitic C from the

parent material, graphitic C was quantified by the smart combustion method (Zethof et al., 2019) using a soli-TOC cube (Elementar, Langenselbold, Germany). Graphitic C was used to estimate the non-geogenic organic C, further on referred to as organic C. In summary: organic C = (total C-inorganic C) - graphitic C.

2.3.6. Soil aggregation

Soil samples were dried for 48 h at 40 °C and sieved through 1 mm. Slow wetting with controlled tension as a pre-treatment was chosen as it represents wetting by infiltrating rainfall and is a preferred method to distinguish differences in unstable soils (Le Bissonnais, 1996). Therefore, 0.3 g sample material (<1 mm) was slow-wetted for 20 min at pF 0.7, before the sample was gently added to a beaker with 500 ml deionized water. Using a Mastersizer 3000, equipped with a Hydro EV dispersion unit (Malvern Panalytical Ltd, Malvern, UK), particle size distribution was measured and the mean weight diameter calculated ($MWD_{slow.}$). The inline sonicator of the Hydro EV dispersion unit was used to apply energy (13 J ml^{-1}) and destroy aggregates, while consecutive measurements were made till no change in particle size distribution was observed. The sample was retrieved and 75 ml dispersant added (Calgon: 33 g l^{-1} $(NaPO_3)_6$ and 7 g l^{-1} Na_2CO_3). After overnight shaking on an end-to-end shaker (90 rpm), the particle size distribution was measured once again and the mean weight diameter was calculated ($MWD_{disp.}$). The difference between $MWD_{disp.}$ and $MWD_{slow.}$, i.e. $MWD_{diff.}$, was used as an indicator of soil aggregation.

2.4. Data analysis and statistics

To evaluate differences in relative abundances of microbial taxa, microbial alpha-diversity metrics (see below) and soil parameters, linear mixed effect modelling was utilized. This approach accounts for the hierarchical sampling design with samples being nested in subplots, i.e. four compartments sampled around the same plant per replicate plot, thereby violating the assumptions of independency and homogeneity. Following the procedure described by Zuur et al. (2009), using the software R (R Core Team, 2020) and the package *nlme* (Pinheiro et al., 2020), the effect of compartment (i.e. rhizoplane, rhizosphere, etc.), parent material from site 1 or site 2 or plant species (*Anthyllis cytisoides* or *Macrochloa tenacissima*) and their combined effects were analysed. The compartments, plant species and sites were taken as fixed effects and a random intercept for subplots was included. The model was then developed further by inclusion of random slopes or multiple residual variances for sites, plant species and/or compartments, to test for possible significant improvement. Residual plots were inspected visually to ensure that the best fitted model, according to the likelihood ratio test, fulfilled the assumptions of homoscedasticity and normality. If this was not the case, a model with lower quality indices was taken of which the residuals showed at least homoscedasticity. The resulting final model was fitted by using a restricted maximum-likelihood estimator, and the fixed effects were tested using ANOVA. If one of the fixed effects or combinations of the fixed effects showed significant differences, these were examined by *lsmeans* (Russell, 2016) to detect significant differences between the levels of the respective factors. Detailed model statistics are given in Supplementary Tables 1 and 2.

The calculation of alpha-diversity indices (Shannon, Pielou, Chao-1, species richness) from the ASV data set was done based on a 100 times randomly subsampled data set (n = 8532, representing the sample with the lowest number of reads) by using the packages *vegan* (Oksanen et al., 2019), *questionr* (Barnier et al., 2018) and *agricolae* (De Mendiburu, 2019) in R.

The detection of genera that differed significantly in relative abundance between sites, plant species and/or distance to the roots, i.e. responder analysis, was performed in R with the packages *phyloseq* (McMurdie and Holmes, 2013) and *edgeR* (Robinson et al., 2010). Data was normalized according to *edgeR* developer recommendations using likelihood ratio tests under negative binominal distribution and generalized linear models. ASVs belonging to the same genus, or else with higher closest taxonomic identification, were clustered together.

A Principal Coordinates Analysis (PCoA) was performed based on Bray-Curtis dissimilarities calculated for the ASV data set transformed to relative abundances using the packages *phyloseq* (McMurdie and Holmes, 2013). PERMANOVA with 9999 permutations (based on Bray-Curtis dissimilarity of the relative abundances), using the function *adonis* from the *vegan* package (Oksanen et al., 2019), was conducted to assess significant differences in the beta-diversity among the various combinations of compartments, plant species and sites. Further analysis of the influence of soil properties on the microbial community composition was performed by a partial Canonical Correspondence Analysis (pCCA) on the relative abundances from the same *vegan* package, partialling out variances between subplots. Rhizoplane samples could not be included in the pCCA as by definition the rhizoplane compartment does not contain soil and therefore no soil properties were available.

To examine the potential influence of the microbial community on the soil EPS-saccharide content and/or aggregation, the relative abundance of ASVs (with a minimum occurrence in four samples) was tested for a significant positive correlation with EPS-saccharide content and $MWD_{diff.}$ for each plant species/site combination. ASVs that were significantly correlated to at least one of the parameters in one of the plant species/site combinations, based on the partial Spearman's rank correlation (partialling out a nested effect from subplot) of the *ppcor* package (Kim, 2015), were included for a network analysis. From the selected ASVs, a co-occurrence network was constructed to explore the potential interactions between ASVs on soil aggregate formation. Pairwise partial Spearman's rank correlations were calculated with the *ppcor* package and only positive correlations with a cut-off at Spearman's $\rho = 0.6$ were used for the network construction. The network was visualized using the *igraph* package (Csardi and Nepusz, 2006).

Overall, we assumed a significant difference or effect in case the *p*-value was below the 0.05 level.

3. Results

3.1. Influence of site and plant species on the microbial communities in the rhizoplanes

In the PCoA a clear differentiation of the microbial community composition could be observed for the two plant species and sites, except

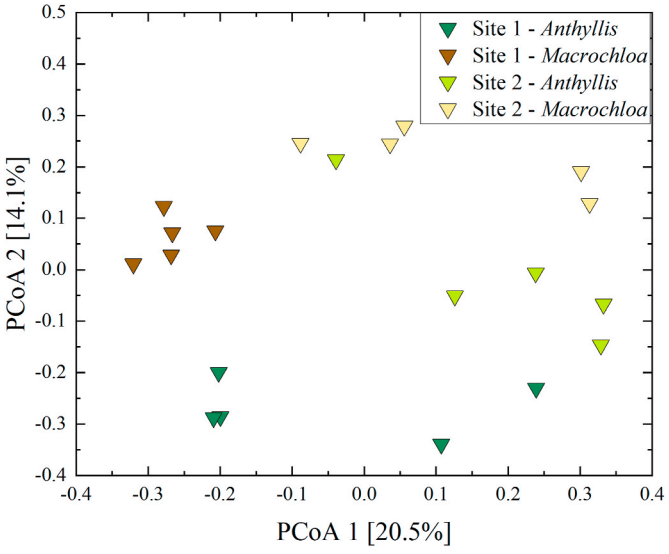

Fig. 2. Principal Coordinates Analysis (PCoA), based on Bray-Curtis dissimilarity of the microbial community composition, of the rhizoplanes based on 16S rRNA gene amplicon sequence variants (ASVs).

for one sample of the *Anthyllis* rhizoplane at site 2 (Fig. 2). The strongest explanatory axis differentiated the samples mainly over the two sites, whereas the second axis showed a strong differentiation between the two plant species. The effect was confirmed by PERMANOVA results (Supplementary Table 3), revealing that the factor site ($R^2 = 0.155$) had the strongest influence on the microbial community composition followed by plant species ($R^2 = 0.114$), whereby both were significant ($p < 0.001$).

In the rhizoplane samples the mean $\log_{10}$ bacterial 16S rRNA gene copy numbers ranged between 8.7 and 9.2 per gram root, with no differences observed for sites or plant species (Supplementary Fig. 1). Estimated (Chao) and observed species richness was highest in the rhizoplane of *Macrochloa* grown at site 1, but not statistically significant (Supplementary Fig. 2). Species diversity (expressed by Shannon index) and evenness (Pielou's index) were both significantly higher at site 2 than at site 1, whereas no significant differences were found between plant species (Supplementary Table 5).

More genera with significantly different relative abundances between sites were found in the rhizoplane of *Anthyllis* than of *Macrochloa.* Responders to the plant species in the rhizoplane were only detected at site 1 (Table 1). The responders were often affiliated to Actinobacteria and Alpha-/Gammaproteobacteria. Most of the enriched ASVs could not be affiliated to a distinct genus and were classified only down to family, order or class level (Table 1). Remarkable were the sequences with closest affiliation to *Micrococcaceae* and *Bacillaceae*, which both had a relative abundance higher than 10% on average in the rhizoplanes at site 2, while their relative abundance was 2.9% and 3.7% at site 1, respectively. Unidentified representatives from the family *Bacillaceae* were significantly more abundant in the rhizoplane of *Anthyllis* than of *Macrochloa* at site 1 (4.5% vs 2.8%). Unclassified ASVs belonging to Saccharimonadales (Patescibacteria) had a relative abundance of 12.2% in the *Macrochloa* rhizoplane of site 1, whereas in the other three rhizoplanes these ASVs had only a relative abundance of ~2.5%. Unidentified representatives of the family *Pseudomonadaceae* (Gammaproteobacteria) were strongly associated to *Anthyllis* rhizoplanes at both sites, although a high variability between replicate plants was observed (Table 1). The genus *Xanthomonas* (Gammaproteobacteria) seemed to be typical of the *Anthyllis* rhizoplane independent of site (0.5 ± 0.7%), as it was not found in any *Macrochloa* rhizoplane samples.

3.2. Changes in microbial communities over relative distance to the root surface

A PCoA of the microbial community composition in all samples indicated strong differences between the rhizoplane and the other compartments (Fig. 3a). Additionally, the larger scattering of rhizoplane samples indicated a higher heterogeneity among the samples, as compared to other compartments. In accordance with the PERMANOVA (Supplementary Table 4), the second axis of the PCoA on all samples indicated strong differences between sites, highlighting the importance of parent material not only for rhizoplane but also for the whole soil microbial community composition. The overall significant difference (p

Table 1
Relative abundances (%) ± standard deviation of microbial genera (>0.5%) that significantly differed, according to a false discovery rate of <0.05, in the rhizoplane between *Macrochloa* (Macr) and *Anthyllis* (Anth) depending on the site or between site 1 (S1) and site 2 (S2) depending on the plant species, respectively, as indicated by an X in the corresponding columns. If sequences could not be reliably classified at genus level, their closest taxonomic identification is indicated. No responders were found between *Anthyllis* and *Macrochloa* at site 2 that had relative abundances higher than 0.5%.

S1-Anth vs S2-Anth	S1-Macr vs S2-Macr	S1-Anth vs S1-Macr	Phylum	Genus/Closest taxonomic identification	Site 1		Site 2	
					Anthyllis	*Macrochloa*	*Anthyllis*	*Macrochloa*
x		x	Acidobacteria	Acidobacteria subgroup 6	0.5 ± 0.5	2.8 ± 1.1	0.6 ± 0.7	1.5 ± 1.1
x			Actinobacteria	*Rubrobacter*	0.3 ± 0.2	0.9 ± 0.3	0.7 ± 0.6	0.8 ± 0.5
x	x		Actinobacteria	*Geodermatophilaceae*	0.3 ± 0.3	0.2 ± 0.1	0.5 ± 0.4	0.3 ± 0.3
x			Actinobacteria	*Micrococcaceae*	2.9 ± 2.1	2.9 ± 2.7	11.0 ± 3.3	28.3 ± 15.7
x	x	x	Actinobacteria	*Mycobacteriaceae*	1.6 ± 0.6	1.0 ± 0.4	1.4 ± 0.5	0.5 ± 0.2
x			Actinobacteria	*Nocardioidaceae*	1.5 ± 0.6	1.5 ± 0.5	2.3 ± 1.2	1.5 ± 0.3
x	x		Actinobacteria	*Promicromonosporaceae*	1.4 ± 1.3	1.4 ± 1.2	3.1 ± 1.5	1.7 ± 0.3
x		x	Actinobacteria	*Pseudonocardiaceae*	2.9 ± 1.5	2.4 ± 0.9	1.8 ± 1.0	0.8 ± 0.6
x			Actinobacteria	*Rubrobacteriaceae*	0.4 ± 0.4	1.2 ± 0.5	1.2 ± 1.2	1.3 ± 0.8
x			Actinobacteria	*Solirubrobacteraceae*	1.4 ± 1.1	2.3 ± 0.6	1.5 ± 1.2	1.7 ± 1.1
x	x		Actinobacteria	*Streptomycetaceae*	2.3 ± 1.3	3.4 ± 1.1	2.1 ± 0.9	1.3 ± 0.8
x		x	Actinobacteria	*Thermomonosporaceae*	0.6 ± 0.6	0.2 ± 0.2	0.1 ± 0.1	0.0 ± 0.1
x		x	Actinobacteria	*Gaiellales*	0.0 ± 0.0	0.5 ± 0.5	0.4 ± 0.6	0.7 ± 0.6
x			Bacteroidetes	*Olivibacter*	1.2 ± 2.9	0.0 ± 0.1	0.1 ± 0.2	0.1 ± 0.2
		x	Chloroflexi	*Family A4b*	0.1 ± 0.0	0.6 ± 0.4	0.1 ± 0.1	0.4 ± 0.2
x			Chloroflexi	*Order SAR202 clade*	0.0 ± 0.0	0.2 ± 0.2	0.6 ± 0.8	0.6 ± 0.3
x		x	Chloroflexi	*Class Chloroflexi KD4 96*	0.1 ± 0.1	0.6 ± 0.3	0.2 ± 0.2	0.4 ± 0.4
x	x		Firmicutes	*Bacillus*	0.5 ± 0.4	2.1 ± 2.3	1.0 ± 0.5	0.8 ± 0.3
x			Firmicutes	*Paenibacillus*	0.2 ± 0.3	0.3 ± 0.3	0.4 ± 0.5	0.6 ± 0.6
x		x	Firmicutes	*Bacillaceae*	4.5 ± 7.2	2.8 ± 1.4	16.5 ± 11.0	11.3 ± 5.9
x		x	Gemmatimonadetes	*Gemmatimonadaceae*	0.7 ± 0.8	2.5 ± 1.0	1.7 ± 1.4	2.6 ± 1.4
x			Alphaproteobacteria	*Ensifer*	0.8 ± 1.1	0.2 ± 0.2	1.2 ± 0.7	0.5 ± 0.5
x			Alphaproteobacteria	*Inquilinus*	1.0 ± 0.7	0.3 ± 0.2	0.5 ± 0.3	0.2 ± 0.1
x		x	Alphaproteobacteria	*Caulobacteraceae*	0.9 ± 0.9	0.3 ± 0.2	1.2 ± 1.4	0.3 ± 0.3
x	x		Alphaproteobacteria	*Reyranellaceae*	0.5 ± 0.3	0.7 ± 0.2	0.3 ± 0.2	0.3 ± 0.2
x	x	x	Alphaproteobacteria	*Rhizobiaceae*	5.6 ± 6.8	3.7 ± 1.1	8.8 ± 4.5	4.0 ± 1.9
x	x		Alphaproteobacteria	*Sphingomonadaceae*	3.2 ± 2.6	5.9 ± 1.1	2.9 ± 2.5	3.4 ± 1.3
x			Alphaproteobacteria	*Xanthobacteraceae*	2.8 ± 1.6	4.0 ± 1.0	2.1 ± 1.3	3.0 ± 2.0
x		x	Alphaproteobacteria	Rhizobiales	0.1 ± 0.1	0.7 ± 0.3	0.1 ± 0.1	0.2 ± 0.2
x		x	Gammaproteobacteria	*Xanthomonas*	0.5 ± 0.5	0.0 ± 0.0	0.5 ± 1.0	0.0 ± 0.0
x		x	Gammaproteobacteria	*Burkholderiaceae*	6.0 ± 10.9	1.2 ± 0.4	1.9 ± 2.0	0.9 ± 0.8
x			Gammaproteobacteria	*Pseudomonadaceae*	6.8 ± 9.6	0.4 ± 0.3	7.0 ± 6.1	2.0 ± 2.5
x	x		Gammaproteobacteria	*Xanthomonadaceae*	1.4 ± 1.4	1.0 ± 0.4	0.4 ± 0.2	0.6 ± 0.4
x	x		Patescibacteria	Saccharimonadales	2.4 ± 2.5	12.2 ± 6.3	2.9 ± 2.4	2.2 ± 0.8

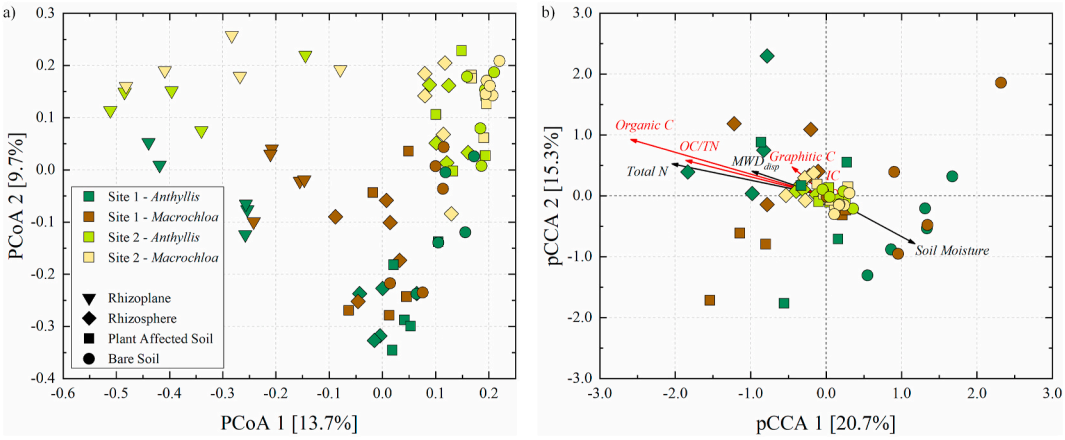

Fig. 3. Principal Coordinates Analysis (PCoA), based on Bray-Curtis dissimilarity of the microbial community composition, of all samples, based on 16S rRNA gene amplicon sequence variants (ASVs) (a). First and second axes of the partial Canonical Correspondence Analysis (pCCA) based on relative abundances from the microbial community data (b). Note that rhizoplane samples are not included in the pCCA, as no data is available for these samples. Red arrows are the significant and the black arrows non-significant explanatory variables, indicating their direction of increase (scaled for plotting). Percentages indicate the proportion of variance explained by the respective axis. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

< 0.001) between compartments was mainly driven by the difference between rhizoplanes and bare soils, while the other compartments were not significantly different from each other. Additionally, at site 2, excluding the rhizoplane, there was no significant difference between *Anthyllis* and *Macrochloa* plant species.

Changes in the microbial community composition with distance to the root surface were larger for site 1 compared to site 2, as the site 2 samples tended to cluster to the centre of the plot as indicated by the pCCA analysis (Fig. 3b). Here, subplot and thereby site effects were removed and an overall trend between the microbial communities of the soil compartments rhizosphere, plant affected and bare soil became obvious. Differences between sampling plots accounted for 38.5% of the variance whereas the soil parameters explained 12.2%. Of the included soil parameters (Supplementary Table 6), graphitic C, inorganic C, organic C contents and the ratio between organic C/total N (OC/TN-ratio) contributed significantly to the differences of microbial communities between the compartments. Furthermore, samples were differentiated on the first axis (pCCA1) mainly between the bare soil and the plant affected soil/rhizosphere, while on the second axis (pCCA2) a small differentiation between the rhizosphere and plant affected soil for site 1 can be recognized. This is in line with the observation that organic C, inorganic C and OC/TN were significantly higher in the rhizosphere, compared to the plant affected and bare soil compartments (Supplementary Table 7). Especially organic C differed significantly between the compartments at site 1, but at site 2 only between rhizosphere and bare soil (Supplementary Table 6). In the case of site 1, graphitic C seemed to play a role in differentiation of microbial communities as well (Fig. 3b). It should be noted that the soil at site 2 did not contain graphitic C.

In the next two subchapters the sites are treated separately, to disentangle the plant species-specific effect on the microbial community and to go beyond the differences induced by parent material.

3.2.1. *Differences and drivers of the microbial communities at site 1*

With the exception of the rhizoplane, no distinct clusters could be observed between the different compartments and/or plant species of site 1 in the PCoA plot (Fig. 3a). On the phylum level, some differences between the compartments and/or plant species became apparent (Fig. 4a). For instance, *Macrochloa* significantly enriched Patescibacteria, whereas *Anthyllis* contained a significantly higher proportion of Gammaproteobacteria, although this effect was only observed in the rhizoplanes. In the other compartments, no clear plant species-dependent difference at the phylum level was found except a significantly higher relative abundance of Patescibacteria in the rhizosphere of *Macrochloa* compared to plant affected and bare soil. Overall, the relative abundance of Alpha- and Gammaproteobacteria increased towards the root surface, whereas Deltaproteobacteria, Verrucomicrobia and Chloroflexi showed the opposite trend, i.e. decreased in relative

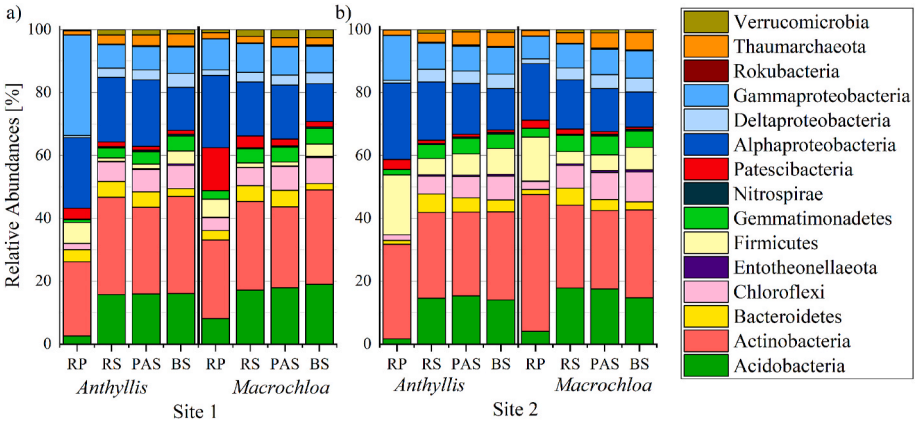

Fig. 4. Relative abundances of the identified phyla and proteobacterial classes. Boxplots per phylum/proteobacterial class can be found in the Supplementary Fig. 6. RP – Rhizoplane; RS – Rhizosphere; PAS - Plant affected soil; BS – Bare soil.

abundance towards the root surface.

Examining the genera with a relative abundance >0.5%, 66 genera (or closest taxonomic identification) were found to respond significantly to at least one of the compartments, independent of plant species (Table 2). Most responders were found between compartments closest and most distant to the root (i.e. rhizoplane vs bare soil, rhizosphere vs bare soil). Following the differences in relative abundance of Acidobacteria between the rhizoplane and other compartments (Fig. 4a), all responder genera were less abundant in the rhizoplane compared to bare soil. Especially unidentified representative(s) of Acidobacteria subgroup 6 had a relative abundance of 7.3% in bare soil, which was 5-fold higher in the bare soil than in the rhizoplane. This subgroup was significantly more abundant in the *Macrochloa* than in the *Anthyllis* rhizosphere (Supplementary Table 8). Furthermore, unidentified representatives of the families *Bacillaceae* (Firmicutes) and *Burkholderiaceae* (Gammaproteobacteria) had a more than 40-fold higher relative abundance in the rhizoplane compared to the plant affected soil (Table 2). Both were significantly more abundant in the rhizoplane of *Anthyllis* than of *Macrochloa* (Supplementary Table 8). Unidentified representatives of *Xanthomonadaceae* (Gammaproteobacteria) were significantly more abundant in the rhizoplane compared to the other compartments (Table 2). A genus of this family, *Xanthomonas*, was only found in *Anthyllis* rhizoplanes (Supplementary Table 8).

3.2.2. Differences and drivers of the microbial communities at site 2

Besides the rhizoplane, no distinctive clusters could be observed in the PCoA plot of soil microbial communities at site 2, although rhizosphere samples tended to form almost an own cluster (Fig. 3a). According to the PERMANOVA, the microbial community composition in the bare soil was significantly different from the other compartments, but no significant difference between plant species was found. On the class/phylum level, Gammaproteobacteria had a significantly higher relative abundance in the rhizoplane of *Anthyllis* compared to the other compartments (Fig. 4b). Patescibacteria were significantly more abundant in the rhizoplane of *Macrochloa* compared to the plant affected and bare soils. Although the Gemmatimonadetes phylum was more abundant in *Macrochloa* rhizoplanes compared to *Anthyllis*, its relative abundance in the rhizoplane was significantly lower compared to the other compartments. Independent of plant species, Alpha- and Gammaproteobacteria were more abundant closer to the root surface, while Deltaproteobacteria showed an opposite trend. Additionally, Thaumarchaeota and Chloroflexi showed a significant increase with distance to the root, independent of plant species. Although Bacteroidetes showed a decrease in relative abundance over distance from the root, their abundance in the rhizoplane was significantly lower than in the rhizosphere, independent of plant species.

Comparing the different compartments, most responders were found between the rhizoplane and plant affected or bare soil (Table 3). Most remarkable are the unidentified representatives of the family *Bacillaceae* (Firmicutes), which had a relative abundance of 13.9% in the rhizoplane, 2-fold higher compared to the other compartments. Furthermore, the family *Pseudomonadaceae* (Gammaproteobacteria) had a 40-fold higher relative abundance in the rhizoplane compared to the other compartments. Only two genera were significantly different between the two plant species. Namely, unclassified representatives of families *Spirosomaceae* (Bacteroidetes) and *Cytophagaceae* (Bacteroidetes) were mainly present in the rhizosphere of *Anthyllis*, but with a relative abundance of $0.2 \pm 0.2\%$ and $0.1 \pm 0.1\%$, respectively (data not shown).

3.3. Interlinking EPS-saccharide contents, soil aggregation and the relative abundance of specific microbial genera

EPS-saccharide contents were significantly higher in the rhizosphere compared to the bare soil, while the contents in the rhizosphere were almost significantly higher than in the plant affected soil of *Anthyllis* ($p = 0.053$), independent of site (Fig. 5a, Supplementary Table 7). Furthermore, the EPS-saccharide contents in plant affected soil of *Macrochloa* were site-independently significantly higher than in the bare soil ($p = 0.025$). Furthermore, a significant difference in MWD_{diff}, indicator for soil aggregation, between compartments was found (Supplementary Table 7). Especially at site 1, soil aggregation was significantly higher in the rhizosphere compared to both plant affected and bare soil, independent of plant species. Soil aggregation in the plant affected soil was almost significantly higher than in the bare soil ($p = 0.059$) in Rambla Honda. Although aggregation in the bare soil was slightly lower than in the plant affected soil and rhizosphere at site 2 (Fig. 5b), no significant differences were found. The linear mixed effect modelling revealed that increasing EPS-saccharide contents at site 1 explained increases in $MWD_{diff.}$, independent of plant species, but not for site 2 (Fig. 5c).

From the 34,162 ASVs identified in this study, 3626 ASVs were found in four or more samples and therefore were tested if they were correlated to EPS-saccharide contents and/or MWD_{diff}. 333 of them were significantly positively correlated to either EPS-saccharide contents or MWD_{diff}, whereby 36 ASVs correlated to both parameters in one of the plant species/site combinations, taking all compartments into account. From these 333 ASVs, 138 were found to be co-occurring, i.e. the relative abundances were correlated with at least one other ASV. The largest hub-species, i.e. the ASV with most co-occurrences, was an ASV affiliated to the family *Azospirillaceae* (No. 47 in Supplementary Fig. 5a) and co-occurred with 16 other ASVs. Although ASVs found in the same compartment and correlating to the same parameters tended to cluster, some interesting co-occurrences were observed (Supplementary Fig. 5). For instance, an ASV with closest identification as *Ensifer meliloti* (No. 46), associated mainly to the rhizoplane of *Anthyllis* independent of site, was found to co-occur with a *Sphingomonadaceae* ASV (No. 78 in Supplementary Fig. 5). While ASV No. 46 (*Ensifer meliloti*) was positively correlated to MWD_{diff}, ASV No. 78 (*Sphingomonadaceae)* correlated to EPS-saccharide contents. Furthermore, six out of eight ASVs of the family *Pseudonocardiaceae* (ASVs No. 17, 29–34, 42) were correlating to MWD_{diff}. These *Pseudonocardiaceae* ASVs were co-occurring with multiple other ASVs, often associated with ASVs identified as *Beijerinckiaceae* (No. 48–60), forming several hub-species in subsequent clusters. An exception was ASV No. 33 with closest identification as *Pseudonocardiaceae*, which formed a hub-species in a cluster of ASVs correlating with both EPS-saccharide and MWD_{diff}. The ASVs in this cluster had their highest relative abundances in *Anthyllis* rhizoplanes, independent of site. An ASV that was identified as *Inquilinus limosus* (ASV No. 84) was part of this cluster.

ASV No. 8 (Acidobacteria subgroup 6) and ASV No. 12 (*Acidobacterium* sp. *Ac 12 G8*) were the hub-species of a sub-cluster without a strong affiliation to roots, plant species or sites, but correlated to EPS-saccharide and/or MWD_{diff}. Among them was an ASV (No. 17) belonging to the genus *Crossiella*, which occurred in all compartments, except the rhizoplane (Supplementary Fig. 5).

4. Discussion

4.1. How does the rhizoplane microbial community differ between Macrochloa tenacissima and Anthyllis cytisoides?

By high-throughput sequencing of 16S rRNA genes, we studied the microbiota attached to the rhizoplane, in order to investigate the driving effects of the plant species. According to the PERMANOVA, parent material was the strongest driver of microbial community composition followed by the plant species in the rhizoplane compartment (Supplementary Table 3). It is well known that individual plant species recruit and form their own root microbiome, whereby physical and chemical compositions of the soil are important drivers (Berg and Smalla, 2009; Fierer, 2017; Schreiter et al., 2014). For instance, the sequences with closest identification to the *Micrococcaceae* (Actinobacteria) and *Bacillaceae* (Firmicutes) families had each a four-fold higher relative

Table 2
Relative abundances (%) ± standard deviation of microbial genera (>0.5%) that significantly differed, i.e. false discovery rate of <0.05, between the compartments of site 1 as indicated in the corresponding column with an X. Averages are of both plant species combined, whereby significantly differing genera between plant species can be found in Supplementary Table 8. RP – Rhizoplane; RS – Rhizosphere; PAS – Plant affected soil; BS – Bare soil.

Responder from						Phyla	Genus/Closest taxonomic identification	Site 1			
RP vs RS	RP vs PAS	RS vs PAS	RP vs BS	RS vs BS	PAS vs BS			Rhizoplane	Rhizosphere	Plant affected soil	Bare soil
		x	x	x		Acidobacteria	*Bryobacter*	0.1 ± 0.1	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.2
	x	x	x	x		Acidobacteria	*Pyrinomonadaceae RB41*	0.0 ± 0.0	1.2 ± 0.5	1.1 ± 0.4	1.5 ± 0.7
x		x	x			Acidobacteria	*Blastocatellaceae*	1.5 ± 1.4	2.0 ± 0.6	2.4 ± 1.5	1.7 ± 0.8
	x	x	x	x		Acidobacteria	*Pyrinomonadaceae*	0.2 ± 0.3	1.9 ± 1.3	1.8 ± 0.7	2.0 ± 0.9
		x	x	x		Acidobacteria	*Solibacteraceae Subgroup 3*	0.5 ± 0.4	1.4 ± 0.7	1.3 ± 0.4	1.1 ± 0.4
x	x	x	x	x		Acidobacteria	*Holophagae subgroup 7*	0.1 ± 0.1	0.9 ± 0.4	1.0 ± 0.6	1.2 ± 0.6
		x	x	x		Acidobacteria	Order *uncultivated soil bacterium clone C112*	0.2 ± 0.1	0.6 ± 0.4	0.7 ± 0.5	0.5 ± 0.3
	x	x	x	x		Acidobacteria	Acidobacteria subgroup 6	1.7 ± 1.5	6.0 ± 1.7	6.5 ± 2.4	7.3 ± 1.8
	x		x	x		Actinobacteria	*Rubrobacter*	0.6 ± 0.4	1.9 ± 0.4	1.9 ± 0.7	2.8 ± 0.5
			x			Actinobacteria	*Solirubrobacter*	0.5 ± 0.3	0.4 ± 0.4	0.4 ± 0.2	0.3 ± 0.1
	x	x	x			Actinobacteria	*Geodermatophilaceae*	0.3 ± 0.2	1.5 ± 0.9	2.4 ± 1.8	1.2 ± 0.6
x	x	x	x	x		Actinobacteria	*Micrococcaceae*	3.2 ± 2.3	1.4 ± 0.6	1.5 ± 1.1	1.3 ± 0.6
		x				Actinobacteria	*Micromonosporaceae*	0.7 ± 0.3	1.5 ± 0.7	1.2 ± 0.9	0.9 ± 0.3
	x		x	x		Actinobacteria	*Mycobacteriaceae*	1.4 ± 0.7	1.1 ± 0.4	0.7 ± 0.5	0.6 ± 0.2
	x	x	x	x		Actinobacteria	*Nocardioidaceae*	1.6 ± 0.5	1.2 ± 0.8	1.1 ± 0.6	0.8 ± 0.2
	x	x	x	x		Actinobacteria	*Promicromonosporaceae*	1.5 ± 1.2	0.7 ± 0.7	0.4 ± 0.3	0.2 ± 0.1
x		x				Actinobacteria	*Pseudonocardiaceae*	2.9 ± 1.3	4.0 ± 0.7	2.0 ± 0.6	1.9 ± 0.5
	x		x			Actinobacteria	*Rubrobacteriaceae*	0.8 ± 0.6	2.2 ± 0.7	2.0 ± 0.8	3.4 ± 0.6
			x	x		Actinobacteria	*Solirubrobacteraceae*	2.0 ± 0.9	1.8 ± 0.6	1.9 ± 0.6	1.8 ± 0.8
			x			Actinobacteria	*Streptomycetaceae*	3.1 ± 1.2	2.7 ± 0.9	1.7 ± 0.9	1.7 ± 0.6
			x			Actinobacteria	*Streptosporangiaceae*	0.7 ± 1.0	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
x		x				Actinobacteria	*Thermomonosporaceae*	0.5 ± 0.5	0.6 ± 0.3	0.3 ± 0.2	0.3 ± 0.2
	x		x			Actinobacteria	Gaiellales	0.3 ± 0.4	0.8 ± 0.8	1.2 ± 0.9	2.0 ± 1.2
	x	x	x	x		Actinobacteria	Order IMCC26256	0.1 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.8 ± 0.3
		x				Actinobacteria	Microtrichales	0.2 ± 0.1	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.2
	x	x	x	x		Actinobacteria	Class 0319 7L14	0.1 ± 0.1	0.7 ± 0.6	0.7 ± 0.7	2.2 ± 1.2
	x	x	x	x		Actinobacteria	*Actinobacteria MB A2 108*	0.0 ± 0.0	0.2 ± 0.2	0.5 ± 0.5	0.8 ± 0.5
			x			Bacteroidetes	*Olivibacter*	0.7 ± 2.1	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		x		x		Bacteroidetes	*Chitinophagaceae*	1.1 ± 0.5	1.7 ± 0.6	1.8 ± 0.9	0.7 ± 0.4
	x	x	x	x		Bacteroidetes	*Hymenobacteraceae*	0.0 ± 0.0	0.4 ± 0.3	0.5 ± 0.4	0.4 ± 0.4
				x		Bacteroidetes	*Microscillaceae*	0.6 ± 0.4	0.9 ± 0.4	0.6 ± 0.2	0.2 ± 0.1
x		x		x		Chloroflexi	Family *AKYG1722*	0.2 ± 0.2	0.4 ± 0.2	0.5 ± 0.3	0.6 ± 0.6
			x			Chloroflexi	Family *JG30 KF CM45*	1.1 ± 0.9	0.9 ± 0.6	1.1 ± 0.6	0.4 ± 0.4
		x	x	x		Chloroflexi	*Roseiflexaceae*	0.1 ± 0.1	0.4 ± 0.2	0.3 ± 0.2	0.5 ± 0.3
x		x		x		Chloroflexi	Order S085	0.2 ± 0.2	0.3 ± 0.3	0.5 ± 0.3	0.5 ± 0.3
x		x	x		x	Chloroflexi	Order SAR202 clade	0.1 ± 0.2	0.2 ± 0.3	0.4 ± 0.3	1.0 ± 0.6
	x		x			Chloroflexi	Class Chloroflexi Gitt GS 136	0.1 ± 0.1	0.4 ± 0.2	0.5 ± 0.3	0.5 ± 0.2
	x	x	x	x		Chloroflexi	Class Chloroflexi KD4 96	0.3 ± 0.3	1.1 ± 0.3	1.4 ± 0.5	1.5 ± 0.6
x		x	x	x		Chloroflexi	Class Chloroflexi TK10	0.1 ± 0.1	0.3 ± 0.3	0.3 ± 0.2	0.6 ± 0.2
	x	x	x	x		Firmicutes	*Bacillus*	1.3 ± 1.7	0.3 ± 0.1	0.3 ± 0.2	0.6 ± 0.2
	x	x	x	x	x	Firmicutes	*Bacillaceae*	4.1 ± 5.1	0.8 ± 0.7	0.9 ± 0.9	2.9 ± 2.9
	x		x			Patescibacteria	*Saccharimonadaceae*	0.8 ± 0.9	0.1 ± 0.2	0.0 ± 0.1	0.0 ± 0.0
	x		x	x		Patescibacteria	Saccharimonadales	7.6 ± 6.6	2.5 ± 1.2	1.6 ± 0.9	1.3 ± 1.0
		x	x	x		Alphaproteobacteria	*Ensifer*	0.6 ± 0.8	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
			x	x		Alphaproteobacteria	*Inquilinus*	0.7 ± 0.6	0.2 ± 0.2	0.1 ± 0.0	0.0 ± 0.0
			x			Alphaproteobacteria	*Azospirillaceae*	0.1 ± 0.1	0.5 ± 0.3	0.7 ± 0.5	0.4 ± 0.3
		x		x		Alphaproteobacteria	*Caulobacteraceae*	0.7 ± 0.8	0.5 ± 0.3	0.4 ± 0.2	0.2 ± 0.1
		x		x		Alphaproteobacteria	*Devosiaceae*	1.5 ± 0.9	1.1 ± 0.3	1.1 ± 0.5	0.2 ± 0.1
x		x				Alphaproteobacteria	*Dongiaceae*	0.4 ± 0.4	0.5 ± 0.4	0.3 ± 0.2	0.2 ± 0.1
x	x		x			Alphaproteobacteria	*Reyranellaceae*	0.6 ± 0.3	0.3 ± 0.1	0.2 ± 0.2	0.2 ± 0.1
	x	x	x	x		Alphaproteobacteria	*Rhizobiaceae*	5.2 ± 4.8	1.0 ± 0.6	0.9 ± 0.8	0.2 ± 0.1

(*continued on next page*)

Table 2 (*continued*)

Responder from						Phyla	Genus/Closest taxonomic identification	Site 1			
RP vs RS	RP vs PAS	RS vs PAS	RP vs BS	RS vs BS	PAS vs BS			Rhizoplane	Rhizosphere	Plant affected soil	Bare soil
	x	x	x	x		Alphaproteobacteria	*Sphingomonadaceae*	4.9 ± 2.2	3.0 ± 0.7	3.0 ± 1.0	2.0 ± 0.9
	x		x			Alphaproteobacteria	*Xanthobacteraceae*	3.6 ± 1.3	3.5 ± 0.8	2.8 ± 1.1	3.0 ± 1.0
	x	x		x		Alphaproteobacteria	Rhizobiales	0.4 ± 0.3	0.8 ± 0.3	1.1 ± 0.5	0.8 ± 0.4
			x	x		Deltaproteobacteria	Family *bacteriap25*	0.4 ± 0.3	0.7 ± 0.3	0.8 ± 0.4	1.8 ± 0.9
			x			Deltaproteobacteria	Class Deltaproteobacteria	0.1 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	1.0 ± 0.3
x	x	x	x	x		Gammaproteobacteria	*Burkholderiaceae*	4.2 ± 8.0	1.1 ± 0.4	0.9 ± 0.5	0.6 ± 0.3
			x	x		Gammaproteobacteria	*Enterobacteriaceae*	5.4 ± 10.0	0.1 ± 0.1	0.1 ± 0.3	0.0 ± 0.0
		x	x	x		Gammaproteobacteria	*Nitrosomonadaceae*	0.3 ± 0.3	0.6 ± 0.2	0.6 ± 0.3	0.6 ± 0.2
	x	x	x	x		Gammaproteobacteria	*Pseudomonadaceae*	4.3 ± 7.6	0.1 ± 0.1	0.2 ± 0.4	0.0 ± 0.0
			x			Gammaproteobacteria	*Steroidobacteraceae*	1.7 ± 1.2	1.4 ± 0.6	1.0 ± 0.3	0.9 ± 0.4
	x	x	x	x		Gammaproteobacteria	Family *TRA3 20*	0.1 ± 0.1	1.3 ± 0.5	1.6 ± 0.3	1.9 ± 0.5
	x	x	x	x		Gammaproteobacteria	*Xanthomonadaceae*	1.3 ± 1.0	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
			x			Gemmatimonadetes	*Gemmatimonadaceae*	1.7 ± 1.2	3.2 ± 0.9	3.7 ± 1.5	4.0 ± 0.9
x	x	x		x		Verrucomicrobia	*Candidatus Udaeobacter*	0.0 ± 0.1	0.5 ± 0.3	0.7 ± 0.4	0.6 ± 0.5
x	x	x	x	x		Verrucomicrobia	*Chthoniobacteraceae*	0.1 ± 0.1	0.8 ± 0.3	1.0 ± 0.6	1.0 ± 0.9

abundance of more than 10% in the rhizoplane at site 2 than at site 1 (Table 1). Soil-dwelling strains of both families have been shown to tolerate extreme desiccation and were previously associated to desert shrubs (Jorquera et al., 2012; Lennon et al., 2012). Especially members of *Micrococcaceae* are often found in saline and alkaline soils (Dastager et al., 2014), explaining their higher relative abundances in the more alkaline soil of site 2.

Nevertheless, plant species had still an important and significant effect on the microbial community in rhizoplanes, which is not explained by differences in parent material (Supplementary Table 3). In line with the observation that Gammaproteobacteria were more abundant in *Anthyllis* rhizoplane as compared to *Macrochloa* (Fig. 4), the genus *Xanthomonas* (Gammaproteobacteria) was only found in *Anthyllis* rhizoplanes independent of site. Sequences with closest identification as *Pseudomonadaceae* and *Burkholderiaceae* (Gammaproteobacteria) were also more abundant in the *Anthyllis* rhizoplane (Table 1). Sequences from these families have recently been identified as keystone taxa in a revegetation project of mine tailings (Sun et al., 2020). Strains of both families are known to play a role in promoting plant growth (Lucy et al., 2004), whereas members of the *Burkholderiaceae* were also strongly associated with CO_2 fixation mechanisms (Sun et al., 2020). From the *Pseudomonadaceae* several isolates have been found to promote the growth of another *Anthyllis* plant species in mine soils, by solubilizing phosphorus and producing auxins and siderophores (Soussou et al., 2017).

Not surprisingly, sequences belonging to the *Rhizobiaceae* (Alphaproteobacteria) were more abundant in the rhizoplane of the legume *Anthyllis* compared to *Macrochloa*, including the genus *Ensifer* (previously known as *Sinorhizobium*) that in our study only consisted of one ASV identified at species level as *Ensifer meliloti* (Table 1). *Ensifer meliloti* is a well-studied N-fixing endosymbiont (Finan et al., 2001) and was reported, for example, as root nodule colonizer of the drought-tolerant shrub *Genista saharae* in the Algerian Sahara (Chaïch et al., 2017). Nevertheless, some other Rhizobiales tended to be more abundant in the *Macrochloa* rhizoplane, like sequences of the *Xanthobacteraceae* and other unidentified representatives of the order *Rhizobiales* (Table 1). Especially the unidentified representatives of *Xanthobacteraceae* are of interest, as this family is often associated with wet soils and sediments contrarily to the arid and well-drained soils of our study sites (Oren, 2014).

Most rhizoplane responders were enriched by *Anthyllis* roots (Table 1). The few genera with highest relative abundances in the *Macrochloa* rhizoplane compared to *Anthyllis* had even higher relative abundances in the other soil compartments, as will be discussed in more detail in the next section. This indicates that the microbial community in the *Macrochloa* rhizoplane is less shaped by the plant itself in comparison to *Anthyllis*. For instance, unidentified representatives of the *Gemmatimonadaceae* (Gemmatimonadetes) were about two-fold more abundant in the *Macrochloa* rhizoplanes compared to *Anthyllis*, but even more abundant in the other soil compartments (Table 2). Although the phylum *Gemmatimonadetes* is one of the major phyla found in soils, little is known due to a lack of cultured representatives (Hanada and Sekiguchi et al., 2014). *Gemmatimonadetes* seem to be predominant in arid environments, as their abundances were inversely correlated to soil moisture (DeBruyn et al., 2011; Crits-Christoph et al., 2013).

Overall, we identified several microbial genera that were mainly associated to the rhizoplane of *Anthyllis* plant species, independent of parent material. This showed that plant species have a very explicit effect on several taxa in the rhizoplane, which can be determinative for the overall soil functioning. Taxa rather related to the *Macrochloa* rhizoplane were found to be even more abundant in the other soil compartments, meaning that the fibrous roots system of *Macrochloa* has likely a lower influence than *Anthyllis* on the microbial community at the root-soil interface.

Table 3
Relative abundances (%) ± standard deviation of microbial genera (>0.5%) that significantly differed, i.e. false discovery rate of <0.05, between the compartments of site 2 as indicated in the corresponding column with an X. Averages are of both plant species combined. In all the studied compartments, no differences in microbial genera (>0.5%) between plant species were found. RP – Rhizoplane; RS – Rhizosphere; PAS – Plant affected soil; BS – Bare soil.

Responder from					Phylum	Genus/Closest taxonomic identification	Site 2			
RP vs RS	RP vs PAS	RS vs PAS	RP vs BS	PAS vs BS			Rhizoplane	Rhizosphere	Plant affected soil	Bare soil
			x		Actinobacteria	*Rubrobacteriaceae*	1.3 ± 1.0	3.2 ± 0.7	2.9 ± 0.6	4.6 ± 0.8
				x	Bacteroidetes	*Microscillaceae*	0.3 ± 0.3	1.1 ± 0.4	0.5 ± 0.1	0.6 ± 0.3
	x				Bacteroidetes	*Chitinophagaceae*	0.5 ± 0.4	2.2 ± 0.8	1.6 ± 0.8	1.1 ± 0.5
			x		Chloroflexi	Family *JG30 KF CM45*	0.4 ± 0.3	0.4 ± 0.2	0.5 ± 0.3	0.3 ± 0.2
x			x		Chloroflexi	Order SAR202 clade	0.6 ± 0.6	1.4 ± 0.4	2.0 ± 0.6	3.0 ± 0.9
x			x		Firmicutes	*Bacillus*	0.9 ± 0.4	0.7 ± 0.6	0.8 ± 0.5	1.3 ± 0.7
x	x		x		Firmicutes	*Bacillaceae*	13.9 ± 8.8	3.4 ± 2.3	4.5 ± 1.8	5.6 ± 2.1
	x	x	x		Alphaproteobacteria	*Devosiaceae*	2.1 ± 1.5	1.0 ± 0.5	0.9 ± 0.9	0.4 ± 0.2
	x	x			Alphaproteobacteria	*Rhizobiaceae*	6.4 ± 4.1	1.0 ± 0.3	0.7 ± 0.3	0.4 ± 0.2
			x		Deltaproteobacteria	Family *bacteriap25*	0.4 ± 0.4	1.4 ± 0.2	1.6 ± 0.3	2.3 ± 0.4
			x		Deltaproteobacteria	Class Deltaproteobacteria	0.3 ± 0.3	0.7 ± 0.2	1.0 ± 0.2	1.2 ± 0.2
	x	x			Gammaproteobacteria	*Pseudomonadaceae*	4.5 ± 5.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2
	x				Gammaproteobacteria	*Xanthomonadaceae*	0.5 ± 0.3	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
			x		Thaumarchaeota	*Nitrososphaeraceae*	1.7 ± 1.2	3.0 ± 1.2	4.3 ± 1.0	4.8 ± 1.0

4.2. How does the parent material influence the plant species-specific effect on the microbial community and its spatial extent?

The microbial community was most heavily influenced by parent material resulting in substantial differentiation in relative abundance of microbial genera between the two sites. Furthermore, the plant species-specific influence was also affected by parent material (Supplementary Table 4), as microbial communities associated with *Macrochloa* differed significantly between sites (excluding the rhizoplane). For example, sequences with closest identification to the order *Saccharimonadales* (Patescibacteria) had a five-fold and almost three-fold higher relative abundance in the rhizoplane and rhizosphere at site 1, respectively, compared to site 2 (Table 1 and Supplementary Table 8). The high carbonate content of the soil at site 2 has likely reduced the root effects as the higher buffering capacity of the soil reduces the acidification process by rhizodeposits (Hinsinger et al., 2002). Along this line, one could expect a larger spatial extent of the pH gradient from the root surface into the bare soil at site 1, resulting in a larger diversification of microbes (Rousk et al., 2010; Fierer et al., 2017). Subsequently, plant species-specific influences on the microbial communities were found in the rhizoplane and rhizosphere of site 1, but not at site 2 (Supplementary Table 8).

Although largest plant influences were observed in the rhizoplane (see also section 4.1), these diminished with increasing distance to the root. Likewise, several responders belonging to the orders Rhizobiales and Sphingomonadales (Alphaproteobacteria) were found to decrease in relative abundance with increasing distance from the root, independent of plant species (i.e. unidentified responders of *Devosiaceae* and *Rhizobiaceae*, Tables 2 and 3). Rhizobiales and Sphingomonadales include many N-fixing species (Tsoy et al., 2016), which is reflected in a significant higher total N content in the rhizosphere samples (Supplementary Table 6). Especially in the rhizosphere of *Anthyllis* at Rambla Honda, total N contents were significantly higher, coinciding with an almost two-fold higher relative abundance of sequences belonging to the *Rhizobiaceae* as compared to the *Macrochloa* rhizoplane at the same site (Supplementary Table 8). At the carbonate-rich site 2, there were no significant differences between the rhizospheres of the two plant species in the total N contents as well as for the orders Rhizobiales and Sphingomonadales. As discussed in the previous section, overall no differentially abundant genera were found between the rhizoplanes of *Anthyllis* and *Macrochloa* at the carbonate-rich site 2, highlighting a very weak plant species-specific effect. Subsequently, less responders at the genus level between the compartments, independent of plant species, were found for site 2 (Table 3).

Sequences belonging to the *Chitinophagaceae* (Bacteroidetes) were one of the few responders that were clearly more abundant in the plant affected soil in comparison to the other compartments at both sites (Tables 2 and 3). Several strains of this family are known for their ability to produce myxospores and degrade long chain polymers like chitin and cellulose (Glavina Del Rio et al., 2010; Proença et al., 2017). This indicates that plants impact the soil microbial communities not only via their root exudates, but also by creating different microclimatic conditions and providing litter via their canopy (Goberna et al., 2007; Veen et al., 2019). Between the compartments of site 1, OC/TN ratios significantly decreased with distance to the roots (Supplementary Table 6), potentially indicating a stronger change in substrate composition as compared to site 2. Furthermore, the unidentified responders of Acidobacteria subgroup 6 increased in relative abundance with decreasing OC/TN ratios at site 1 (Table 2). De Chaves et al. (2019) found that Acidobacteria subgroup 6 increased in relative abundance with the sugarcane *Saccharum* spp., but decreased in relative abundance upon addition of nitrogen fertilizers. This is only opposite to the inverse correlation between Acidobacteria subgroup 6 and OC/TN ratio in our study. It is important to note that the parent material of Rambla Honda contained geogenic N as well (Zethof et al., 2019), likely resulting in the low OC/TN ratio in the bare soil compartment, highlighting the

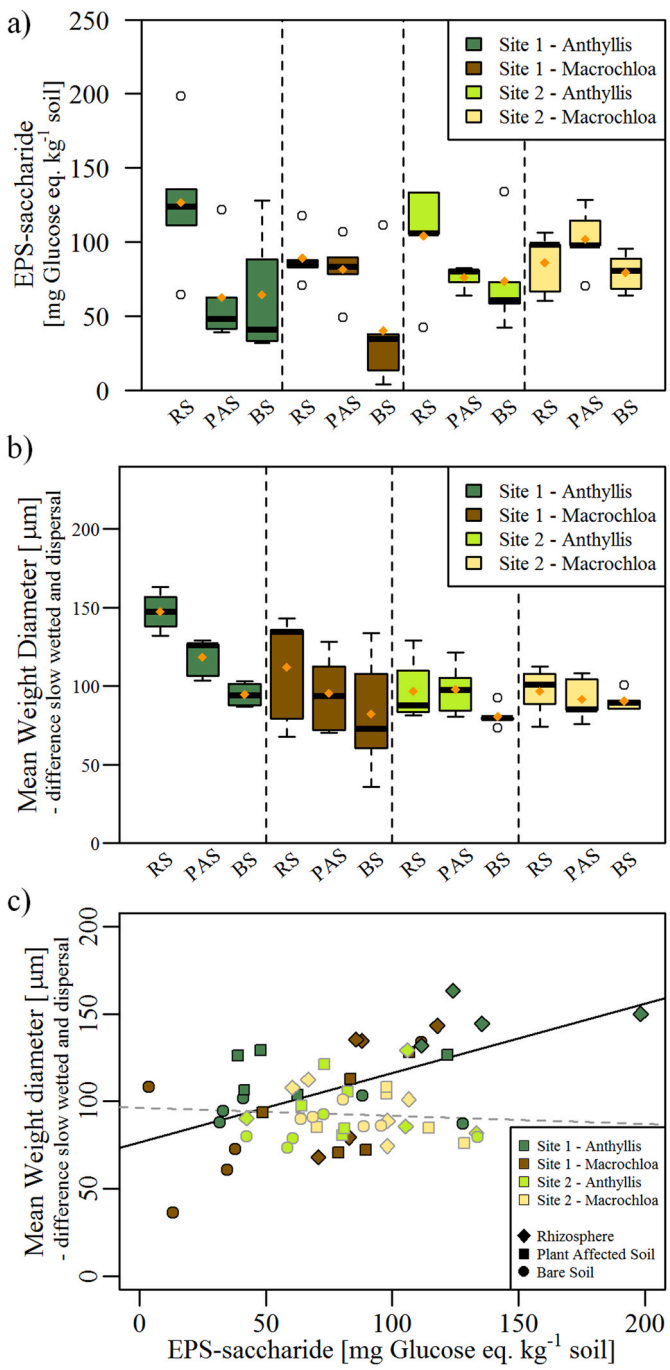

Fig. 5. EPS-saccharide content of the soil samples as measured in the Rhizosphere (RS), Plant Affected Soil (PAS) and Bare Soil (BS) (a). Change in Mean Weight Diameter of the soil samples (<1 mm) between slow wetting and dispersal with ultrasound and Calgon (b, $MWD_{diff.}$). Box-and-whisker diagrams showing the median (black line), 25th/75th percentile (respective upper/lower part box), min./max. (whiskers) and mean values (orange dot), with five replicates per box except for the rhizosphere of *Anthyllis* with four replicates per site. In figure (c) the linear trend between $MWD_{diff.}$ and EPS-saccharide is indicated, which was significant for site 1. The solid black line represents the linear relation for site 1 and the dashed grey line for site 2. See Supplementary Table 10 for subsequent regression statistics. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

importance of considering different forms of N when studying the effect on microbial community composition.

In summary, plant species-specific effects on the microbial communities observed in the rhizoplane diminished with distance to the root. At site 1, responders between the two plant species on the genus level were found in rhizoplane and rhizosphere, while this was not the case at the carbonate-rich site 2, hinting on a buffering effect by the carbonates. Not all compartment responders showed a strong root effect as, for example, sequences belonging to the *Chitinophagaceae* were more abundant under the plant canopies compared to the rhizoplane or bare soil compartments, independent of site and/or plant species.

4.3. Can soil aggregation be associated to certain ASVs via soil EPS-saccharide contents?

Similar to the microbial community, soil aggregation differed mainly between plant species and compartments at site 1 (Fig. 5b). Subsequently, a correlation between soil aggregation and EPS-saccharide contents was only found at site 1 (Fig. 5c). Note that in this study we defined soil aggregation as the change in mean weight diameter (MWD) between samples (<1 mm) treated by slow wetting and subsequent chemical and physical dispersion (i.e. MWD_{diff}). The mean weight diameter after aggregate dispersal (MWD_{disp}) correlated well with inorganic C (Supplementary Fig. 3b). This could indicate a strong stabilizing effect on soil aggregates by carbonate binding, which were not destroyed by the (non-acidic) dispersal treatment. Although it is well-known that carbonates have a cementing effect (i.e. Tisdall and Oades, 1982; Muneer and Oades, 1989; Totsche et al., 2018), this was previously linked to an interaction with EPS-saccharide contents for microaggregates (Zethof et al., 2020). Indications from field and laboratory experiments showed as well that EPS can facilitate the precipitation of carbonates (Ivanov and Chu, 2008; Ercole et al., 2012), potentially explaining the higher mean weight diameter after aggregate dispersal (MWD_{disp}) in the EPS-rich rhizosphere compartment (Supplementary Fig. 4b).

Despite the lack of a gradient in soil aggregation at site 2, we could identify several ASVs at both sites that were potentially involved in the formation of EPS and/or stabilization of soil aggregates, as these ASVs were found at site 1 as well (Supplementary Fig. 5b). For instance, a relative large cluster with ASV 84 (*Inquilinus limosus*), ASV 114 (*Burkholderiaceae*), ASVs 116 and 117 (*Pseudomonadaceae*), ASV 33 (*Pseudonocardiaceae*), and ASVs 70/72/73 (*Rhizobiaceae*) were mainly found in *Anthyllis* rhizoplane, independent of parent material, but correlated well to both EPS-saccharide contents and aggregation in the other compartments. As discussed in the previous section, *Rhizobiaceae* contain many N-fixing taxa that are often capable of producing EPS (Rodriguez-Navarro et al., 2007; Vuko et al., 2020). Moreover, *Inquilinus limosus* and species belonging to *Pseudomonadaceae* have been studied for their cooperation in sturdy biofilm formation in patients with cystic fibrosis (Herasimenka et al., 2007; Lopes et al., 2012, 2014), indicating that taxonomically related strains adapted to the soil environment have likely the capability to produce EPS as well (Lennon et al., 2012; Vuko et al., 2020).

Many ASVs were not part of the large clusters, but formed couples or small clusters. For instance, ASV 122 with closest identification as *Xanthomonadaceae* (Supplementary Fig. 5) was found in most soil samples, but with highest relative abundance in the rhizoplane, co-occurring with ASV 71 (*Rhizobiaceae*). Especially ASV 122 correlated to both EPS content and aggregation, fitting previous observations for *Xanthomonadaceae* in alfalfa related microaggregate stabilization by Caesar-Ton-That et al. (2007). Similarly, ASV 46 (*Ensifer meliloti*) correlated to soil aggregation in our study. Members of this N-fixing species have been well-studied for their ability to produce EPS on plant roots (Finan et al., 2001; Fraysse et al., 2003; Primo et al., 2020). As discussed in section 4.1, ASV 46 was clearly enriched by *Anthyllis* roots, independent of parent material.

Several ASVs belonging to the family *Pseudonocardiaceae* (No. 17, 29–34) were placed in key positions of different clusters in the co-occurrence network. Among them ASV 17 was identified as the genus *Crossiella*. This genus was previously found to be highly abundant in biofilms of multiple cave systems (Riquelme et al., 2015; Spilde et al., 2016), indicating a more generic role in biofilm formation on mineral surfaces in porous systems. ASV 17 (*Crossiella*) was in our case found in all compartments outside the rhizoplane, suggesting the potential role of non-rhizoplane associated bacteria for the overall EPS-saccharide contents in soil.

Hub-species in a co-occurrence network are often assumed as keystone taxa for the microbial community (Layeghifard et al., 2017; Banerjee et al., 2018), although care should be taken as this does not necessarily prove a direct interaction between species. The large number of ASVs, 333 identified in this study, correlating with EPS-saccharides and/or soil aggregation, still indicate a clear potential of soil-dwelling microbes for improving soil aggregation via EPS production. Most of them were only identified to the family level, hampering the interpretation of the observed correlations (Supplementary Table 9). Therefore, further studies should involve microbial cultivation in order to test the EPS-production potential in soil environments. In summary, we could identify a range of ASVs that was either related to EPS-saccharide contents and/or soil aggregate stabilization, as measured by the change in mean weight diameter upon dispersal. A substantial part of the ASVs was correlated to EPS-saccharide contents and had their highest relative abundances in the vicinity of the plant roots. Plant effect on microbial community, EPS-saccharide contents and soil aggregation quickly diminished with relative distance to the root-soil interface, whereby the largest gradient was observed between the rhizosphere, plant affected and bare soil compartments of *Anthyllis*. The root effect was only governed by soil carbonate contents, since the observed root effects on the microbial community and subsequent soil aggregation diminished with higher carbonate contents. Many more potential EPS producers were found, which were not necessarily associated to the rhizoplane/-sphere compartments, highlighting the complex process in overall EPS formation in the soil environment.

Author contributions

Shared first authorship between AB and JZ, as AB processed, analysed and wrote parts of the paper, same as JZ. CV advised on the EPS and aggregation part. DB gave advice on ASV data processing/interpretation. EC assisted in sampling design and data interpretation. AS, RL and LL assisted in sampling design, provided information on the sites/ecosystems and helped interpreting the results. JN and SJS provided the Illumina sequencing and primary data processing and advice on further data analysis. KS and KK wrote the DFG proposal, supported with data interpretation and linking the work.

All co-authors critically reviewed the manuscript and gave advice for improvements.

Funding

This study was financially supported by the Deutsche Forschungsgemeinschaft (DFG), Bonn, Germany, under grants KA 1737/13–1 and SM 59/18–1 “Extracellular polymeric substances and aggregate stability - how microorganisms affect soil erosion by water”. DB was funded by the BMBF project DiControl (http://dicontrol.igzev.de/en/; research grant 031B0514C). AS, RL and LL were supported thanks to the Research Project ‘Biocrust Dynamics’ (DINCOS, CGL2016-78075-P), funded by the Spanish State Plan for Scientific and Technical Research and Innovation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Manuela Unger and Gisela Ciesielski for the laboratory assistance and Ilse-Marie Jungkurth for checking the language of the manuscript. Further we want to thank the Max Planck Institute for Biogeochemistry (Department Biogeochemical Processes, Jena) for the Graphitic C measurements.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2021.108301.

Data availability statement

Raw amplicon data were deposited at NCBI Sequence Read Archive (SRA, https://www.ncbi.nlm.nih.gov/) under accession number PRJNA689290. The underlying dataset with the individual soil properties is available via https://doi.org/10.25532/OPARA-114.

References

Balbín-Suárez, A., Lucas, M., Vetterlein, D., Sørensen, S.J., Winkelmann, T., Smalla, K., Jacquiod, S., 2020. Exploring microbial determinants of apple replant disease (ARD): a microhabitat approach under split-root design. FEMS Microbiology Ecology. https://doi.org/10.1093/femsec/fiaa211 fiaa211.

Banerjee, S., Schlaeppi, K., van der Heijden, M.G.A., 2018. Keystone taxa as drivers of microbiome structure and functioning. Nature Reviews Microbiology 16, 567–576. https://doi.org/10.1038/s41579-018-0024-1.

Barnier, J., Briatte, F., Larmarange, J., 2018. Questionr: functions to make surveys processing easier. R package version 0.7.0. https://CRAN.R-project.org/package=questionr. (Accessed 7 October 2020).

Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology 68 (1), 1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x.

Bérard, A., Clavel, T., Le Bourvellec, C., Davoine, A., Le Gall, S., Doussan, C., Bureau, S., 2020. Exopolysaccharides in the rhizosphere: a comparative study of extraction methods. Application to their quantification in Mediterranean soils. Soil Biology and Biochemistry 149, 107961. https://doi.org/10.1016/j.soilbio.2020.107961.

Blankinship, J.C., Fonte, S.J., Six, J., Schimel, J.P., 2016. Plant versus microbial controls on soil aggregate stability in a seasonally dry ecosystem. Geoderma 272, 39–50. https://doi.org/10.1016/j.geoderma.2016.03.008.

Bulgarelli, D., Rott, M., Schlaeppi, K., van Themaat, E.V.L., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F.O., Amann, R., Eickhorst, T., Schulze-Lefert, P., 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 488, 91–95. https://doi.org/10.1038/nature11336.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. In: Proceedings of the National Academy of Sciences of the United States of America, vol. 108, pp. 4516–4522. https://doi.org/10.1073/pnas.1000080107. Suppl 1, S.

Caesar-TonThat, T.C., Caesar, A.J., Gaskin, J.F., Sainju, U.M., Busscher, W.J., 2007. Taxonomic diversity of predominant culturable bacteria associated with microaggregates from two different agroecosystems and their ability to aggregate soil in vitro. Applied Soil Ecology 36 (1), 10–21. https://doi.org/10.1016/j.apsoil.2006.11.007.

Chaïch, K., Bekki, A., Bouras, N., Holtz, M.D., Soussou, S., Mauré, L., Brunel, B., de Lajudie, P., Cleyet-Marel, J.-C., 2017. Rhizobial diversity associated with the spontaneous legume Genista saharae in the northeastern Algerian Sahara. Symbiosis 71, 111–120. https://doi.org/10.1007/s13199-016-0414-y.

Chenu, C., Cosentino, D., 2011. Microbial regulation of soil structural dynamics. In: Ritz, K., Young, I. (Eds.), The Architecture and Biology of Soils: Life in Inner Space. CABI, pp. 37–70. https://doi.org/10.1079/9781845935320.0037, 37–70.

Costa, O.Y., Raaijmakers, J.M., Kuramae, E.E., 2018. Microbial extracellular polymeric substances: ecological function and impact on soil aggregation. Frontiers in Microbiology 9. https://doi.org/10.3389/fmicb.2018.01636.

Crits-Christoph, A., Robinson, C.K., Barnum, T., Fricke, W.F., Davila, A.F., Jedynak, B., McKay, C.P., DiRuggiero, J., 2013. Colonization patterns of soil microbial communities in the Atacama Desert. Microbiome 1, 28. https://doi.org/10.1186/2049-2618-1-28.

Crouzet, O., Consentino, L., Pétraud, J.-P., Marrauld, C., Aguer, J.-P., Bureau, S., Le Bourvellec, C., Touloumet, L., Bérard, A., 2019. Soil photosynthetic microbial communities mediate aggregate stability: influence of cropping systems and

herbicide use in an agricultural soil. Frontiers in Microbiology 10, 1319. https://doi.org/10.3389/fmicb.2019.01319.
Csardi, G., Nepusz, T., 2006. The igraph software package for complex network research, 1695. InterJournal, Complex Systems. https://igraph.org.
Dastager, S.G., Krishnamurthi, S., Rameshkumar, N., Dharne, M., 2014. The family Micrococcaceae. In: Rosenberg, E., Delong, E., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes. Springer, Heidelberg, pp. 709–726. https://doi.org/10.1007/978-3-642-30138-4_168.
Davinic, M., Fultz, L.M., Acosta-Martinez, V., Calderón, F.J., Cox, S.B., Dowd, S.E., Allen, V.G., Zak, J.C., Moore-Kucera, J., 2012. Pyrosequencing and mid-infrared spectroscopy reveal distinct aggregate stratification of soil bacterial communities and organic matter composition. Soil Biology and Biochemistry 46, 63–72. https://doi.org/10.1016/j.soilbio.2011.11.012.
De Chaves, M.G., Silva, G.G.Z., Rossetto, R., Edwards, R.A., Tsai, S.M., Navarrete, A.A., 2019. Acidobacteria subgroups and their metabolic potential for carbon degradation in sugarcane soil amended with vinasse and nitrogen fertilizers. Frontiers in Microbiology 10, 1680. https://doi.org/10.3389/fmicb.2019.01680.
De Mendiburu, F., 2019. Agricolae: statistical procedures for agricultural research. R package version 1.3-1. Available online at: https://CRAN.R-project.org/package=agricolae. (Accessed 10 December 2020).
DeBruyn, J.M., Nixon, L.T., Fawaz, M.N., Johnson, A.M., Radosevich, M., 2011. Global biogeography and quantitative seasonal dynamics of Gemmatimonadetes in soil. Applied and Environmental Microbiology 77 (17), 6295–6300. https://doi.org/10.1128/AEM.05005-11.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.T., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28 (3), 350–356. https://doi.org/10.1021/ac60111a017.
Ebrahimi, A., Or, D., 2015. Hydration and diffusion processes shape microbial community organization and function in model soil aggregates. Water Resources Research 51 (12), 9804–9827. https://doi.org/10.1002/2015WR017565.
Ercole, C., Bozzelli, P., Altieri, F., Cacchio, P., Del Gallo, M., 2012. Calcium carbonate mineralization: involvement of extracellular polymeric materials isolated from calcifying bacteria. Microscopy and Microanalysis 18 (4), 829. https://doi.org/10.1017/S1431927612000426.
Estermann, E.F., McLaren, A.D., 1961. Contribution of rhizoplane organisms to the total capacity of plants to utilize organic nutrients. Plant and Soil 15, 243–260. https://doi.org/10.1007/BF01400458.
Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology 15, 579. https://doi.org/10.1038/nrmicro.2017.87.
Finan, T.M., Weidner, S., Wong, K., Buhrmester, J., Chain, P., Vorhölter, F.J., Hernandez-Lucas, I., Becker, A., Cowie, A., Gouzy, J., Golding, B., Pühler, A., 2001. The complete sequence of the 1,683-kb pSymB megaplasmid from the N2-fixing endosymbiont Sinorhizobium meliloti. Proceedings of the National Academy of Sciences of the United States of America 98 (17), 9889–9894. https://doi.org/10.1073/pnas.161294698. Aug 14.
Flemming, H., Wingender, J., 2010. The biofilm matrix. Nature Reviews Microbiology 8, 623–633. https://doi.org/10.1038/nrmicro2415.
Flemming, H.C., Neu, T.R., Wingender, J. (Eds.), 2016. The Perfect Slime: Microbial Extracellular Polymeric Substances (EPS). IWA publishing, London.
Flemming, H.C., Wuertz, S., 2019. Bacteria and archaea on Earth and their abundance in biofilms. Nature Reviews Microbiology 17, 247. https://doi.org/10.1038/s41579-019-0158-9.
Fraysse, N.F., Couderc, F., Poinsot, V., 2003. Surface polysaccharide involvement in establishing the Rhizobium-legume symbiosis. European Journal of Biochemistry 270, 1365–1380. https://doi.org/10.1046/j.1432-1033.2003.03492.x.
Glavina Del Rio, T., Abt, B., Spring, S., Lapidus, A., Nolan, M., Tice, H., Copeland, A., Cheng, J.F., Chen, F., Bruce, D., Goodwin, L., Pitluck, S., Ivanova, N., Mavromatis, K., Mikhailova, N., Pati, A., Chen, A., Palaniappan, K., Land, M., Hauser, L., Chang, Y.J., Jeffries, C.D., Chain, P., Saunders, E., Detter, J.C., Brettin, T., Rohde, M., Göker, M., Bristow, J., Eisen, J.A., Markowitz, V., Hugenholtz, P., Kyrpides, N.C., Klenk, H.P., Lucas, S., 2010. Complete genome sequence of Chitinophaga pinensis type strain (UQM 2034). Standards in genomic sciences 2 (1), 87–95. https://doi.org/10.4056/sigs.661199.
Goberna, M., Pascual, J.A., Garcia, C., Sánchez, J., 2007. Do plant clumps constitute microbial hotspots in semiarid Mediterranean patchy landscapes? Soil Biology and Biochemistry 39 (5), 1047–1054. https://doi.org/10.1016/j.soilbio.2006.11.015.
Hanada, S., Sekiguchi, Y., 2014. The phylum gemmatimonadetes. In: Rosenberg, E., Delong, E., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes. Springer, Heidelberg, pp. 677–681.
Herasimenka, Y., Cescutti, P., Impallomeni, G., Rizzo, R., 2007. Exopolysaccharides produced by Inquilinus limosus, a new pathogen of cystic fibrosis patients: novel structures with usual components. Carbohydrate Research 342 (16), 2404–2415. https://doi.org/10.1016/j.carres.2007.07.012.
Hinsinger, P., Plassard, C., Tang, C., Jaillard, B., 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant and Soil 248 (1–2), 43–59. https://doi.org/10.1023/A:1022371130939.
Ivanov, V., Chu, J., 2008. Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. Reviews in Environmental Science and Biotechnology 7 (2), 139–153. https://doi.org/10.1007/s11157-007-9126-3.
Jorquera, M.A., Shaharoona, B., Nadeem, S.M., de la Luz Mora, M., Crowley, D.E., 2012. Plant growth-promoting rhizobacteria associated with ancient clones of creosote bush (larrea tridentata). Microbial Ecology 64, 1008–1017. https://doi.org/10.1007/s00248-012-0071-5.
Kim, S., 2015. Ppcor: an R package for a fast calculation to semi-partial correlation coefficients. Communications for Statistical Applications and Methods 22 (6), 665–674. https://doi.org/10.5351/CSAM.2015.22.6.665.
Layeghifard, M., Hwang, D.M., Guttman, D.S., 2017. Disentangling interactions in the microbiome: a network perspective. Trends in Microbiology 25 (3), 217–228. https://doi.org/10.1016/j.tim.2016.11.008.
Lázaro, R., Rodrigo, F.S., Gutiérrez, L., Domingo, F., Puigdefábregas, J., 2001. Analysis of a 30-year rainfall record (1967–1997) in semi–arid SE Spain for implications on vegetation. Journal of Arid Environments 48 (3), 373–395. https://doi.org/10.1006/jare.2000.0755.
Le Bissonnais, Y.L., 1996. Aggregate stability and assessment of soil crustability and erodibility: I. Theory and methodology. European Journal of Soil Science 47, 425–437. https://doi.org/10.1111/j.13652389.1996.tb01843.x.
Lehmann, A., Zheng, W., Rillig, M.C., 2017. Soil biota contributions to soil aggregation. Nat. Ecol. Evol. 1, 1828. https://doi.org/10.1038/s41559-017-0344-y.
Lennon, J.T., Aanderud, Z.T., Lehmkuhl, B.K., Schoolmaster Jr., D.R., 2012. Mapping the niche space of soil microorganisms using taxonomy and traits. Ecology 93 (8), 1867–1879. https://doi.org/10.1890/11-1745.1.
Lopes, S.P., Ceri, H., Azevedo, N.F., Pereira, M.O., 2012. Antibiotic resistance of mixed biofilms in cystic fibrosis: impact of emerging microorganisms on treatment of infection. International Journal of Antimicrobial Agents 40 (3), 260–263. https://doi.org/10.1016/j.ijantimicag.2012.04.020.
Lopes, S.P., Azevedo, N.F., Pereira, M.O., 2014. Emergent bacteria in cystic fibrosis: in vitro biofilm formation and resilience under variable oxygen conditions. BioMed research international, 2014. https://doi.org/10.1155/2014/678301.
Lucy, M., Reed, E., Glick, B.R., 2004. Applications of free living plant growth-promoting rhizobacteria. Antonie van Leeuwenhoek 86 (1), 1–25. https://doi.org/10.1023/B:ANTO.0000024903.10757.6e.
McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS One 8, e61217. https://doi.org/10.1371/journal.pone.0061217.
More, T.T., Yadav, J.S.S., Yan, S., Tyagi, R.D., Surampalli, R.Y., 2014. Extracellular polymeric substances of bacteria and their potential environmental applications. Journal of Environmental Management 144, 1–25. https://doi.org/10.1016/j.jenvman.2014.05.010.
Morgan, R.P.C., 2005. Soil Erosion and Conservation, third ed. Blackwell, Malden, p. 304pp.
Muneer, M., Oades, J.M., 1989. The role of Ca-organic interactions in soil aggregate stability. III. Mechanisms and models. Soil Research 27 (2), 411–423. https://doi.org/10.1071/SR9890411.
Oburger, E., Jones, D.L., 2018. Sampling root exudates–mission impossible? Rhizosphere 6, 116–133. https://doi.org/10.1016/j.rhisph.2018.06.004.
Oksanen, J., Guillaume, Blanchet F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. vegan: community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan. (Accessed 7 October 2020).
Oren, A., 2014. The family Xanthobacteraceae. In: Rosenberg, E., Delong, E., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes. Springer, Heidelberg, pp. 709–726. https://doi.org/10.1007/978-3-642-30197-1_258.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2020. Nlme: linear and nonlinear mixed effects models. R package version 3.1-148. https://CRAN.R-project.org/package=nlme. (Accessed 7 October 2020).
Primo, E.D., Cossovich, S., Nievas, F., Bogino, P., Humm, E.A., Hirsch, A.M., Giordano, W., 2020. Exopolysaccharide production in Ensifer meliloti laboratory and native strains and their effects on alfalfa inoculation. Archives of Microbiology 202, 391–398. https://doi.org/10.1007/s00203-019-01756-3.
Proença, D.N., Whitman, W.B., Shapiro, N., Woyke, T., Kyrpides, N.C., Morais, P.V., 2017. Draft genome sequence of the cellulolytic endophyte Chitinophaga costaii A37T2T. Standards in Genomic Sciences 12, 53. https://doi.org/10.1186/s40793-017-0262-2.
Puigdefábregas, J., Alonso, J.M., Delgado, L., Domingo, F., Cueto, M., Gutiérrez, L., Lázaro, R., Nicolau, J.M., Sánchez, G., Solé, A., Vidal, S., 1996. The Rambla Honda field site: interactions of soil and vegetation along a catena in semi-arid southeast Spain. In: Brand, C.J., Thornes, J.B. (Eds.), Mediterrean Desertification and Land Use. John Wiley & Sons, Ltd, Chichester.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. In: Nucleic Acids Research, vol. 41, pp. D590–D596. https://doi.org/10.1093/nar/gks1219 (Database issue).
Queralt-Mitjans, I., Domingo, F., Sole-Benet, A., 1993. The influence of local sources on the mineral content of bulk deposition over an altitudinal gradient in the Filabres Range (SE Spain). Journal of Geophysical Research: Atmosphere 98 (D9), 16761–16768. https://doi.org/10.1029/93JD01281.
R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Redmile-Gordon, M.A., Brookes, P.C., Evershed, R.P., Goulding, K.W.T., Hirsch, P.R., 2014. Measuring the soil-microbial interface: extraction of extracellular polymeric substances (EPS) from soil biofilms. Soil Biology and Biochemistry 72, 163–171. https://doi.org/10.1016/j.soilbio.2014.01.025.
Riquelme, C., Rigal, F., Hathaway, J.J., Northup, D.E., Spilde, M.N., Borges, P.A., Gabriel, R., Amorim, I.R., Dapkevicius, M.D.L.N., 2015. Cave microbial community composition in oceanic islands: disentangling the effect of different colored mats in diversity patterns of Azorean lava caves. FEMS Microbiology Ecology 91 (12). https://doi.org/10.1093/femsec/fiv141.

Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140.

Rodríguez-Navarro, D.N., Dardanelli, M.S., Ruíz-Saínz, J.E., 2007. Attachment of bacteria to the roots of higher plants. FEMS Microbiology Letters 272 (2), 127–136. https://doi.org/10.1111/j.1574-6968.2007.00761.x.

Rousk, J., Bååth, E., Brookes, P., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal 4, 1340–1351. https://doi.org/10.1038/ismej.2010.58.

Russell, V.L., 2016. Least-squares means: the R package lsmeans. Journal of Statistical Software 69 (1), 1–33. https://doi.org/10.18637/jss.v069.i01.

Sandercock, P., Hooke, J., De Baets, S., Poesen, J., Meerkerk, A., van Wesemael, B., Cammeraat, L.H., 2017. Effectiveness of plants and vegetation in erosion control and restoration. In: Combating Desertification and Land Degradation. Springer, Cham, pp. 79–104. https://doi.org/10.1007/978-3-319-44451-2_4.

Schimel, J.P., 2018. Life in dry soils: effects of drought on soil microbial communities and processes. Annual Review of Ecology Evolution and Systematics 49, 409–432. https://doi.org/10.1146/annurev-ecolsys-110617-062614.

Schreiter, S., Ding, G.C., Heuer, H., Neumann, G., Sandmann, M., Grosch, R., Siegfried, K., Smalla, K., 2014. Effect of the soil type on the microbiome in the rhizosphere of field-grown lettuce. Frontiers in Microbiology 5, 144. https://doi.org/10.3389/fmicb.2014.00144.

Soussou, S., Brunel, B., Pervent, M., van Tuinen, D., Cleyet-Marel, J.-C., Ezékiel Baudoin, E., 2017. Rhizobacterial Pseudomonas spp. strains harbouring acdS gene could enhance metallicolous legume nodulation in Zn/Pb/Cd mine tailings. Water, Air, & Soil Pollution 228, 142. https://doi.org/10.1007/s11270-017-3309-5.

Spilde, M.N., Northup, D.E., Caimi, N.A., Boston, P.J., Stone, F.D., Smith, S., 2016. Microbial mats communities in Hawaiian lava caves. In: Proceedings 16th Int. Symposium Vucanospeleology 2016. http://www.cavepics.com/IVS17/SPILDE.pdf.

Sun, X., Xu, R., Dong, Y., Li, F., Tao, W., Kong, T., Zhang, M., Qiu, L., Wang, X., Sun, W., 2020. Investigation of the ecological roles of putative keystone taxa during tailing revegetation. Environmental Science & Technology 54 (18), 11258–11270. https://doi.org/10.1021/acs.est.0c03031.

Sundberg, C., Al-Soud, W.A., Larsson, M., Alm, E., Yekta, S.S., Svensson, B.H., Sørensen, S.J., Karlsson, A., 2013. 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. FEMS Microbiology Ecology 85 (3), 612–626. https://doi.org/10.1111/1574-6941.12148.

Suzuki, M.T., Taylor, L.T., DeLong, E.F., 2000. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5′-nuclease assays. Applied and Environmental Microbiology 66 (11), 4605–4614. https://doi.org/10.1128/aem.66.11.4605-4614.2000.

Tecon, R., Or, D., 2017. Biophysical processes supporting the diversity of microbial life in soil. FEMS Microbiology Reviews 41, 599–623. https://doi.org/10.1093/femsre/fux039.

Tisdall, J.M., Oades, J., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science 33, 141–163. https://doi.org/10.1111/j.1365-2389.1982.tb01755.x.

Totsche, K.U., Amelung, W., Gerzabek, M.H., Guggenberger, G., Klumpp, E., Knief, C., Knief, C., Lehndorff, E., Mikutta, R., Peth, S., Prechtel, A., Ray, N., Kögel-Knabner, I., 2018. Microaggregates in soils. Journal of Plant Nutrition and Soil Science 181, 104–136. https://doi.org/10.1002/jpln.201600451.

Tsoy, O.V., Ravcheev, D.A., Èuklina, J., Gelfand, M.S., 2016. Nitrogen fixation and molecular oxygen: comparative genomic reconstruction of transcription regulation in Alphaproteobacteria. Frontiers in Microbiology 7, 1343. https://doi.org/10.3389/fmicb.2016.01343.

Vardharajula, S., Ali, S.Z., 2015. The production of exopolysaccharide by Pseudomonas putida GAP-P45 under various abiotic stress conditions and its role in soil aggregation. Microbiology 84, 512–519. https://doi.org/10.1134/s0026261715040153.

Veen, C., Fry, E., ten Hooven, F., Kardol, P., Morrien, E., De Long, J.R., 2019. The role of plant litter in driving plant-soil feedbacks. Frontiers in Environmental Science 7, 168. https://doi.org/10.3389/fenvs.2019.00168.

Vogel, C., Babin, D., Pronk, G.J., Heister, K., Smalla, K., Kögel-Knabner, I., 2014. Establishment of macro-aggregates and organic matter turnover by microbial communities in long-term incubated artificial soils. Soil Biology and Biochemistry. https://doi.org/10.1016/j.soilbio.2014.07.012.

Vuko, M., Cania, B., Vogel, C., Kublik, S., Schloter, M., Schulz, S., 2020. Shifts in reclamation management strategies shape the role of exopolysaccharide and lipopolysaccharide-producing bacteria during soil formation. Microbial biotechnology 13 (2), 584–598. https://doi.org/10.1111/1751-7915.13532.

Yu, Y., Lee, C., Kim, J., Hwang, S., 2005. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnology and Bioengineering 89, 670–678. https://doi.org/10.1002/bit.20347.

Zhalnina, K., Louie, K.B., Hao, Z., Mansoori, N., da Rocha, U.N., Shi, S., Cho, H., Karaoz, U., Loqué, D., Bowen, B.P., Firestone, M.K., Northen, T.R., Brodie, E.L., 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nature microbiology 3 (4), 470–480. https://doi.org/10.1038/s41564-018-0129-3.

Zethof, J.H.T., Leue, M., Vogel, C., Stoner, S.W., Kalbitz, K., 2019. Identifying and quantifying geogenic organic carbon in soils – the case of graphite. Soils 5, 383–398. https://doi.org/10.5194/soil-5-383-2019.

Zethof, J.H.T., Bettermann, A., Vogel, C., Babin, D., Cammeraat, L.H., Solé-Benet, A., Lázaro, R., Luna, L., Nesme, J., Woche, S.K., Sørensen, S.J., Smalla, K., Kalbitz, K., 2020. Prokaryotic community composition and extracellular polymeric substances affect soil microaggregation in carbonate containing semiarid grasslands. Frontiers in Environmental Science 8, 51. https://doi.org/10.3389/fenvs.2020.00051.

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer Science & Business Media. https://doi.org/10.1007/978-0-387-87458-6.