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Tomato-Associated Archaea Show a Cultivar-Specific Rhizosphere Effect but an Unspecific Transmission by Seeds

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ABSTRACT

Archaea have recently been identified as substantial members of the plant microbiome. As for other microorganisms, the rhizosphere is a favorable habitat for archaea; however, less is known about their community assembly, composition, or origin. Therefore, we analyzed archaeal communities in the rhizosphere of tomato plants (*Solanum lycopersicum* 'Moneymaker' and 'Hildares F1') nurtured in two different soil types. In complementary experiments, archaeal communities were assessed in two generations of tomato seeds. The abundance of the archaea was significantly different for each plant genotype and habitat. In the rhizosphere of Moneymaker, the archaeal abundance was 10-fold higher than in Hildares F1, whereas the decrease in archaeal abundance from seeds of the first generation to the second was much higher by 10^4-fold in the same cultivar. Overall, the archaeal community in tomato was dominated by *Thaumarchaeota* and *Euryarchaeota*. The core community in tomato consisted of species assigned to the Soil Crenarchaeotic Group (*Thaumarchaeota*; 60.7%), *Methanosarcina* (*Euryarchaeota*; 12.6%), *Methanoculleus* (*Euryarchaeota*; 3.4%), and unassigned archaeal species (7.2%). Differences in abundance, diversity, and composition between cultivars were so distinct that they masked any effect determined by the different composition of soil. In seeds, archaeal abundance and diversity was comparably low and the composition showed random patterns; no indications of a plant-mediated vertical transmission were found. We assume that archaea represent only bystander microorganisms in seeds, while their cultivar-specific enrichment in the rhizosphere suggests a role in functioning of the plant holobiont.

Keywords: archaea, endophytes, plant microbiome, rhizosphere and phyllosphere, seed microbiome, soil ecology, *Solanaceae*

The plant microbiome was identified as a key for the next green revolution towards sustainable agriculture (Bender et al. 2016). At present, the focus of plant microbiome research is mainly on bacteria and fungi, whereas archaea are often overseen. So far, archaea have been found as part of numerous microbiomes, adapted to a great variety of conditions, colonizing soil, plants and animals, humans, and especially ruminants and termites (Moissl-Eichinger et al. 2018). In soils, both archaeal abundance and community structure can differ, as they are mainly shaped by the soil type and layer (Azziz et al. 2016; Chen et al. 2010). In plants, including domesticated plants such as arugula, olive trees, and maize, archaea represent a stable component of the microbiome (Hardoim et al. 2015). They have been found at high abundances in the rhizosphere and endosphere, mostly in nutrient-rich hotspots like rotten roots, and in lower abundances in the phyllosphere (Chelius and Triplett 2001; Müller et al. 2015; Taffner et al. 2019). Several abiotic factors, such as climate, pH, and accessibility to nutrients, but also biotic factors, such as plant genotype, development stage, and competition with bacteria and fungi, have been found to influence the archaeal fraction of the plant microbiome, reshaping community structure and abundance (Bengtson et al. 2012; Edwards et al. 2018; Nicol et al. 2008). Metagenomic analyses revealed the potential of Archaea to interact with the plant holobiont by three different modes of action: (i) competition and support (syntrophic interaction) with bacteria and fungi, (ii) nutrient supply for plants, and (iii) plant growth promotion through auxin biosynthesis (Song et al. 2018; Stams and Plugge 2009; Taffner et al. 2018, 2019). Some archaeal functions are prevalent in distinct plant microhabitats, which is also reflected by differences in archaeal community structure and abundance, e.g., archaea were shown to be enriched in the rhizosphere, but less is known about their assembly and transmission (Taffner et al. 2018).

Domesticated plants in particular harbor specific conditions for microorganisms due to intensive long-standing breeding, which may affect the microbiome assembly and the interaction with the host

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(Pérez-Jaramillo et al. 2016). To unveil the composition and structure of plant archaeal communities, tomato (*Solanum lycopersicum* L.) was selected as a model crop. Tomato plants represent the most important vegetable with a total yield of up to 182 million tons per year (FAOSTAT 2019). Together with other vegetables, tomatoes represent a significant part of a healthy diet linked to a reduced risk of heart disease and stroke, lower blood pressure, cancer prevention, and other numerous beneficial effects for human health (He et al. 2006). To date, the production and processing is commonly associated with conspicuous losses that reach up to 45% (FAO 2015). Soilborne pathogens, e.g., fungi from the genera *Fusarium*, *Rhizoctonia*, and *Verticillium*, are among the major microbiological threats for this crop that significantly limit its yields (Oerke 2006). For both its relevance and the problems connected with tomato production, the microbiome of the tomato plant has been widely characterized with several studies focusing on the below-ground plant compartments in correlation with its resistance to biotic and abiotic stresses (Liu et al. 2017; Upreti and Thomas 2015; Yan et al. 2003). A recent study by Bergna et al. (2018) identified tomato seeds as a key compartment for the vertical transmission of beneficial bacteria representing a significant portion of the plant microbiome in early developmental stages.

As of today, less is known about archaea in tomato plants apart from the strong impact of root exudates that enrich archaea in this habitat (Simon et al. 2005). Therefore, we studied the archaeal community in tomato plants to identify (i) if factors such as plant genotype and soil type shape the community structure of plant-associated archaea, and (ii) if archaeal taxa are transmitted from one generation to the next. The experimental treatment factors that were included in the study were two soil types, two tomato cultivars, and three different plant sample types. In analogy to the findings for bacterial communities (Bergna et al. 2018), we wanted to assess if and to what extent archaea are transmitted to the offspring, where they potentially support germination and plant development. To achieve this, we combined quantitative polymerase chain reaction (qPCR) and next-generation sequencing to quantify and describe the archaeal community of the tomato plant with a focus on the rhizosphere, two generations of seeds, and the soil in which the plants were grown.

MATERIALS AND METHODS

Experimental design. Surface-sterilized seeds (first generation) of tomato plants (*S. lycopersicum*) of cultivars Moneymaker (Austrosaat AG, Austria) and Hildares F1 (Hild Samen GmbH, Germany) were sown in 8 liter pots (one seed per pot). The two soil mixtures employed for the study were formulated mixing 10 parts of sterile quartz sand with (i) one part of commercial loamy soil (pH = 6.8, KCl = 1.6 g/liter, n = 100 mg/liter, P_2O_5 = 80 mg/liter, K_2O = 800 mg/liter, and Mg = 120 mg/liter; Ökohum GmbH, Herbertingen, Switzerland) later termed as loamy soil or with (ii) one part diluvial sand (Rühlmann and Ruppel 2005) later termed as sandy soil. The high proportion of sterile quartz sand was implemented in order to ensure comparable growth conditions for the plants. The seedlings were watered and fertilized once a week with 100 ml of nutrient solution per plant as described in the previous study of Bergna et al. (2018). A total of 100 planted pots (50 with each soil type) were kept in a nonacclimated greenhouse (approximately 24/20°C day/night temperature) of the Graz Botanical Garden (Graz, Austria) together with unplanted pots containing only the soil mixture. The pots were randomly distributed in the greenhouse; during harvest, tomato plants from four different pots and the same treatment were combined into one sample, resulting in five composite samples of each sample type. For the second generation seeds, only four composite samples could be obtained from the cultivar Moneymaker due to the number of collected seeds. Each composite sample was used for total community DNA extraction and subsequent analyses. For the cultivar Hildares F1, an insufficient number of samples was obtained at the end of the experiment, thus the second generation of seeds was not assessed.

Sample collection. At the late flowering stage of the tomato plants (85 days post planting), soil and plant specimens were collected followed by a second sampling at the ripening of fruits of Moneymaker plants. Soil samples were collected from the central section of the pots containing the soil-sand mixture by only removing the top layer of soil (2 to 3 cm) with sterile tools. Rhizospheric soil was obtained by shaking the root compartment and by collecting the material that was adhering to the roots in sterile bags. Commercial seeds from the same production batches that were used for planting the two tomato cultivars were used as first generation seed samples. At ripening of tomato fruits, the seeds of the second generation were collected from 10 Moneymaker plants and cleaned from leftover fruit tissues using sterile tools.

Samples were processed using a modified protocol presented by Bragina et al. (2012) following sample processing as described in the work of Bergna et al. (2018). Briefly, collected specimens of both soil and rhizosphere were suspended in 0.85% sodium chloride solution (NaCl) and shaken for 30 min. After this first step, the homogenate was transferred to 2-ml reaction tubes and the microorganism-containing pellets were obtained by centrifugation (20 min at 16,750 × g) and stored at –70°C. Seeds (I and II generation) of Moneymaker and Hildares F1 were washed in sterile water, divided in plastic vials (20 seeds per vial) with 4 ml of 0.85% NaCl, and gently shaken for 4 h. The seeds were then homogenized with mortar and pestle and suspended in 0.85% NaCl. After centrifugation (20 min at 16,750 × g), microorganism-containing pellets were stored at –70°C.

DNA extraction and generation of 16S rRNA gene fragment amplicons. The aforementioned pellets were used for the total community DNA isolations. DNA was isolated with the FastDNA SPIN Kit for Soil and the FastPrep Instrument (MP Biomedicals, Santa Ana, CA, U.S.A.) according to the manufacturer's protocol. Extracted community DNA samples were used for PCR-based barcoding. In order to strictly amplify the archaeal 16S rRNA gene alone, we performed a nested PCR using the archaea-specific primers 344f and 915r in the first PCR and modified primer pair S-D-Arch-0349-a-S-17/S-D-Arch-0519-a-A-16 (here 349f/519r [Klindworth et al. 2013]) with an additional 10-bp primer-pad (TATGGTAATT/AGTCAGCCAG) and linker (GT/GG) in the subsequent PCR, as previously described by protocols of the Earth Microbiome Project (Walters et al. 2016). Afterwards, the Golay barcodes were annealed in a third PCR. The PCR reactions were conducted as previously described (Taffner et al. 2019). All PCR reactions were conducted as triplicates, purified with the Wizard SV Gel and PCR cleanup system (Promega, Madison, WI, U.S.A.), and pooled in equimolar concentrations prior to sequencing. The Sequencing was then conducted using an Illumina HiSeq Personal Sequencer (GATC Biotech AG, Konstanz, Germany).

Quantitative real-time PCR with archaea-specific primers. The quantification of archaea in soil, rhizosphere, and two generations of seeds was conducted with primer pairs 344aF and 517uR (Probst et al. 2013). For the qPCR, 1 µl of extracted DNA was used in each 10-µl reaction mix. The reaction mix contained 5 µl of KAPA SYBR Green 2× MM Mix (Bio-Rad, Hercules, CA, U.S.A.), 0.5 µl of forward and reverse primers (344aF and 517uR) at a concentration of 10 pmol/µl, and 3 µl of PCR grade water. As a standard, 16S rRNA gene standards from *Haloferax denitrificans* were used. PCR amplifications were conducted in triplicates using a Rotor-Gene 6000 series (Corbett Research) thermal cycler with the

following program settings: 95°C/5 min, 95°C/15 s, annealing 60°C/30 s, extension 72°C/30 s; amplification steps were repeated 39 times. Final elongation was done from 72 to 96°C. Statistical analysis of the abundance measures was conducted using the Kruskal-Wallis test followed by pairwise Mann-Whitney *U* test. A Benjamini-Hochberg adjustment of the *P* value was performed to adjust for false discovery rate errors.

Data analysis of 16S rRNA gene amplicons for determination of archaeal community structure. The 16S rRNA gene fragment paired-end sequences were joined (SeqPrep) and the barcodes were extracted with the "extract_barcodes.py" script in the Qiime1 environment (Quantitative Insights into Microbial Ecology, version 1.9.0) (Caporaso et al. 2010). Demultiplexing of the library was conducted with the "demux emp-paired" plugin integrated within Qiime2 environment (2019.4 release). Sequences were then denoised, dereplicated and clustered using the DADA2 ("dada2 denoise-paired") plugin. Chimeras were identified with the "vsearch uchime-denovo" plugin and subsequently filtered from the representative sequences. The features' taxonomy assignment was conducted using a fitted classifier (Scikit-learn) (Pedregosa et al. 2011) and the Silva 16S (349af-519ar 99 otusversion 128) archaeal database with the plugin "feature-classifier-classify-sklearn" and the recommended parameters; 0.7 is the default confidence threshold for limiting taxonomic depth. Unassigned and non-archaeal feature contaminants were filtered from the resulting feature table. A graphical rendering of the archaeal community structure at class level was produced using the open-source software Circos (Krzywinski et al. 2009). In order to display a more reliable differential abundance among samples, the number of reads of each sample has been normalized with the gene copy number obtained with the qPCR. By doing so, we coupled the quantification accuracy of the qPCR with the capacity of amplicon sequencing to describe complex microbial communities. Alpha diversity distances were calculated and rendered at feature level in the Phyloseq package (McMurdie and Holmes 2013) within R environment using Observed Species, Chao1, Shannon, and inverse Simpson measures. The Phyloseq package (McMurdie and Holmes 2013) was also employed for generating the principal coordinate analysis plot with Bray Curtis. Statistical analysis to determine the significance of differences between samples was performed using the package vegan v. 2.5.5 (Oksanen et al. 2019) with the Adonis test (999 permutations). In order to visualize the archaeal distribution among the habitats of the tomato plant Cytoscape 3.3.0 software was used (Shannon et al. 2003). The 16S rRNA gene fragment amplicon library was deposited in the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena) under accession number PRJEB34577.

RESULTS

Quantification of archaeal population density in tomato plants. Using a qPCR approach with specific archaeal primers, targeting the 16S ribosomal RNA gene, we quantified the archaeal population of two tomato cultivars (Moneymaker and Hildares F1) grown in two soil mixtures. Archaeal rRNA gene abundances spanned between 1.83×10^4 and 1.84×10^9 copies per ng of extracted DNA (Fig. 1). The highest archaeal abundance was found in the rhizosphere of Moneymaker plants. Irrespective of the soil mixture in which these plants were grown, archaea were significantly ($P < 0.05$) more abundant in the rhizosphere of this cultivar than in the rhizosphere of Hildares F1, showing 10-fold higher archaeal abundances. The composition and texture of the two soil mixtures did not result in significantly different archaeal abundance ($P > 0.05$) in loamy and sandy soil mixtures. However, there was a distinct difference in the number of archaeal gene copies among the two analyzed generations of Moneymaker seeds. We found the latter (second generation) characterized by a 10^4-fold lower abundance of archaea with 1.83×10^4 to 3.12×10^4 16S rRNA copies per ng of extracted DNA. In contrast, the archaeal gene copy numbers in the seeds of the first generation were higher with 3.01×10^8 16S rRNA gene copies per ng of extracted DNA, but with a high standard deviation. Further statistical analysis with the Kruskal-Wallis test confirmed the significance of the differences between the two seed generations ($P < 0.05$).

Structure of tomato-associated archaeal communities and diversity analyses. High throughput sequencing of 16S rRNA gene fragments obtained from two soil mixtures together with the rhizosphere and seeds (first and second generation) of two tomato cultivars (Moneymaker and Hildares F1) yielded in a total of 748,221 high quality archaeal reads that have been clustered in a total of 1,133 distinct features. The composition of archaeal communities in tomato was dominated by the phyla *Thaumarchaeota* and *Euryarchaeota* (Fig. 2). In all analyzed microenvironments, members of these two phyla accounted for more than 80% of the whole community. More precisely, *Thaumarchaeota* consistently accounted for more than 60% of the archaeal community and lower abundances of this phylum have been found in the Hildares F1 seeds used for generating the plants (first generation) as well as in the loamy soil. The low abundance of *Thaumarchaeota* (46%) in the Hildares F1 seeds was accompanied by a high abundance of *Euryarchaeota* (34%) and unassigned reads (20%). The archaeal community composition of the commercial loamy soil mixture represented a rather equal distribution with *Euryarchaeota* representing 49% of the community and *Thaumarchaeota* 48%. At the same time, among the two soil types a substantial difference in the abundance of these phyla was observed. While *Thaumarchaeota* represented 79% of the archaeal community in sandy soil, in loamy soil their abundance was only of 51%. The abundance of *Euryarchaeota*, on the other hand, varied between 11% in sandy soil to 48% in loamy soil.

At class level, both community-dominating phyla were mostly represented by a single class, the Soil Crenarchaeotic Group for *Thaumarchaeota* and *Methanomicrobia* for *Euryarchaeota*. Other *Euryarchaeota* classes, which were relevant for the archaeal

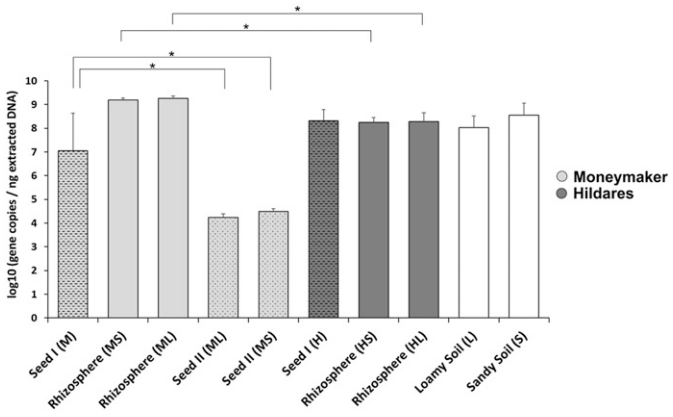

Fig. 1. Quantitative analysis of archaea in different samples based on qPCR approaches. Archaeal abundances were determined for seeds from the first (Seed I) and the second generation (Seed II), as well as the rhizosphere of cultivars Moneymaker (M) and Hildares F1 (H). Quantification of archaea was also conducted in loamy (L) and sandy soil (S). Significance was determined with the nonparametric Kruskal-Wallis test, followed by pairwise Mann-Whitney *U* tests. Significant measures are indicated with an asterisk, representing $P < 0.05$.

community, were identified as *Thermoplasmata* and *Methanobacteria* and found to be specific for the sandy soil (2 and 1%, respectively) while they accounted for up to 4 and 8% in the rhizosphere. A more detailed description of the archaeal community is provided in Supplementary Table S1.

Soil- and cultivar-driven variabilities. The diversity within archaeal communities was evaluated with metrics sensitive to dominant features (inverse Simpson's index), to rare features (Observed Species and Chao1), and incorporating both richness and evenness (Shannon). While alpha diversity values were not inferable for seed samples due to their reduced number of reads (low abundance across samples confirmed by qPCR results), the analysis showed a consistently higher diversity in the rhizosphere of Moneymaker plants regardless of the soil mixture employed (Fig. 3). The comparison of diversity levels of soil mixtures indicated that the sandy soil mixture has a higher archaeal diversity when compared with the commercial loam mixture. Interestingly, the rhizosphere of plants grown in these two substrates was not determined by the archaeal community characteristics of the soil. The rhizosphere of Moneymaker plants was found to harbor an increased diversity, which was threefold higher than soil and the Hildares F1 rhizosphere (observed and Chao1 indices).

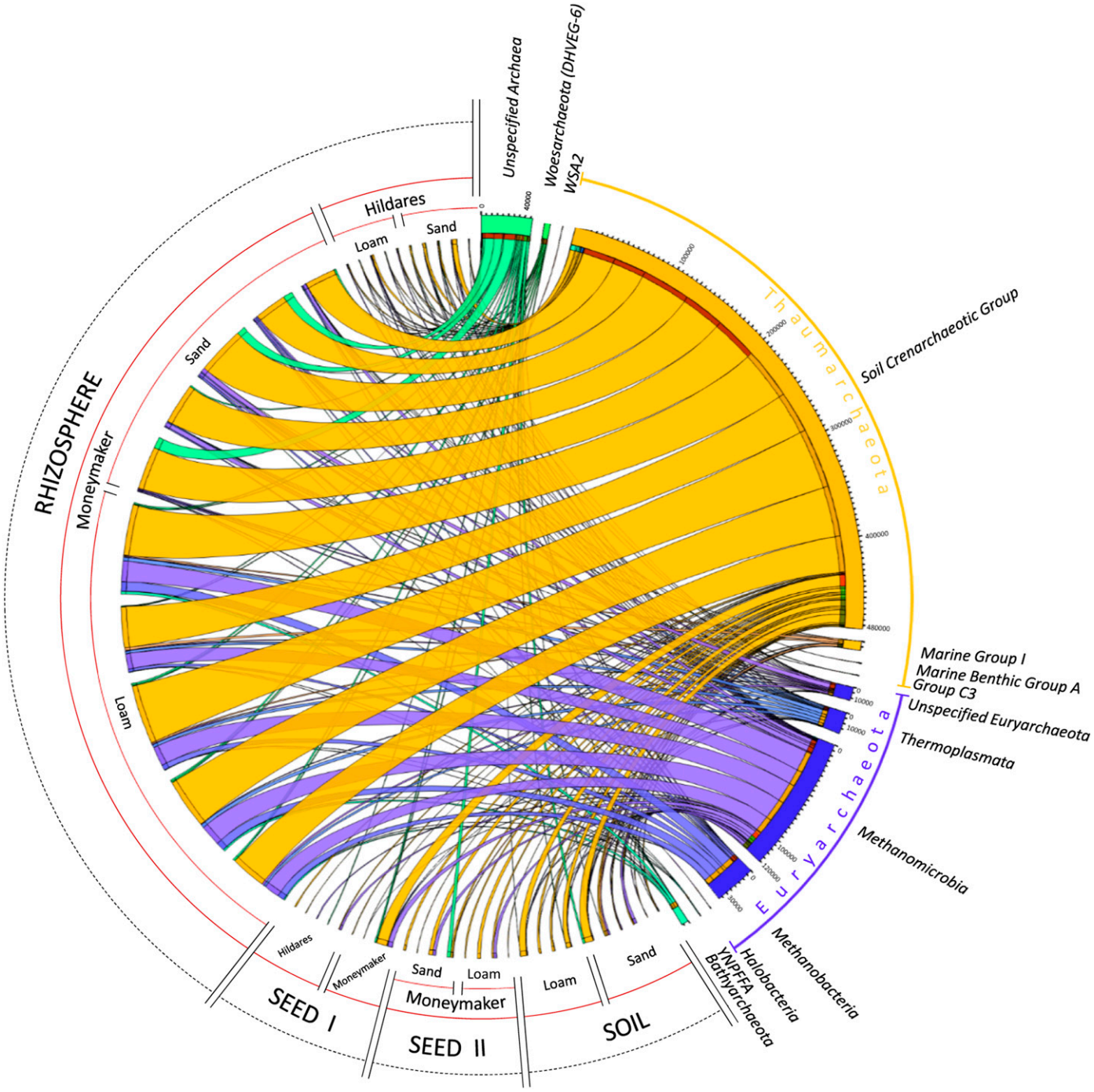

Fig. 2. Archaeal communities of two soil–sand mixtures, seeds, and the rhizosphere of two tomato plant cultivars (Moneymaker and Hildares F1). Total abundances of the respective archaeal populations were adjusted with a qPCR-based quantification. The graph was obtained using the open-source software Circos (http://circos.ca).

In order to analyze similarities and dissimilarities among the archaeal communities of different samples, the beta-diversity analysis has been graphically rendered with a principal coordinate analysis using the Bray Curtis distance (Fig. 4). Complementary to the alpha-diversity analysis, the beta-diversity analysis highlighted the unique composition of the rhizospheric communities of Moneymaker plants in this system. Rhizosphere archaeal communities of Moneymaker plants showed slightly different archaeal communities ($R^2 = 0.69006$, P (>F) = 0.01) among soil types. Regardless of the soil type, the archaeal community of this sample group showed significant differences to all other samples (Hildares F1 rhizosphere, first and second generation seeds, and both soil mixtures) ($R^2 = 0.49868$, P (>F) = 0.001). In parallel, no intersample similarities linked to the soil mixture were evident from the principal coordinate analysis.

Composition of the archaeal community associated with tomato plants. In the tomato endosphere, the archaeal core community consisted of species assigned to the Soil Crenarchaeotic Group (*Thaumarchaeota*; 60.7%), *Methanosarcina* (*Euryarchaeota*; 12.6%), *Methanoculleus* (*Euryarchaeota*; 3.4%), and unassigned archaeal species (7.2%), which were shared among all habitats, including the seeds (Fig. 5). Further, the seeds of Moneymaker harbored archaea of the genus '*Candidatus* Nitrososphaera' and several *Euryarchaeota* genera. Overall, a higher archaeal diversity was found associated to the cultivar Moneymaker, than in Hildares F1. In the rhizosphere of Moneymaker, several archaeal taxa were found to be exclusive; they were not detected in any other sample type or in samples derived from the Hildares F1 cultivar. Furthermore, the minor phylum of *Bathyarchaeota* was exclusively found associated to Moneymaker and loamy soil.

DISCUSSION

Habitat specificity and rhizosphere enrichment of archaeal communities in tomato plants. In the present study, the abundance of archaea across the tomato plant was found to be highly habitat-specific and indicated a strong rhizosphere effect. This is in line with previous findings for soil *Crenarchaeota*, which were shown to be enriched in the rhizosphere of tomato plants due to the presence of nutrient-rich root exudates (Simon et al. 2005). The rhizosphere is also a well-known hotspot of bacterial colonization; therefore, it is possible that the high archaeal abundance found in this habitat is

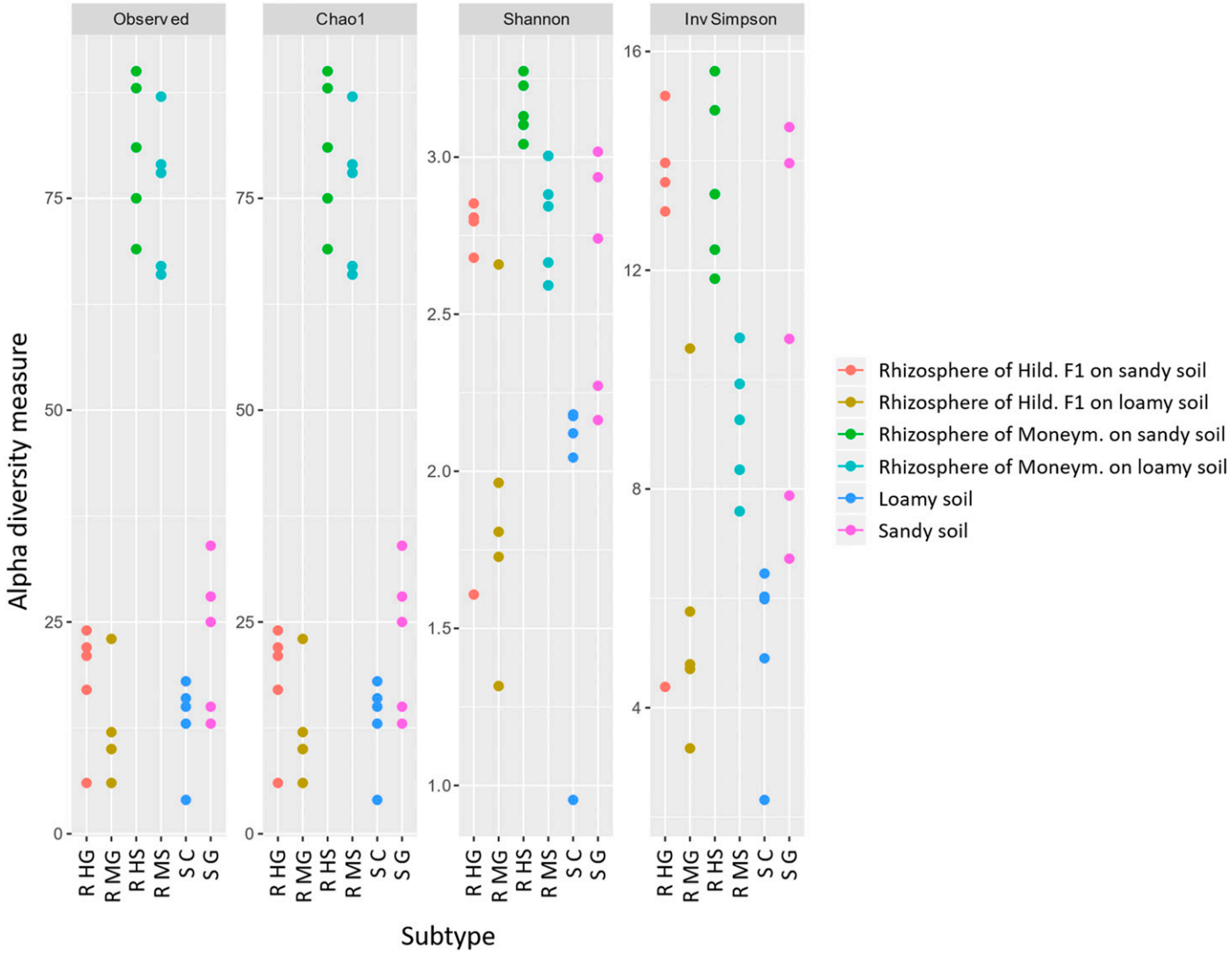

Fig. 3. Assessment of alpha diversity across the rhizosphere and soil samples. Four different diversity measures were used: observed species, Chao1, Shannon, and inverse Simpson. A combination of measures sensitive to rare features (observed species and Chao1) and to dominant features (inverse Simpson's index) was performed in order to provide a comprehensive assessment of bacterial diversity in the plant system.

connected to a specific bacterial community setup that favors archaeal colonization, e.g., by synergistic interactions or by the absence of competition and antagonistic interactions.

Soil quality shapes the archaeal community in bulk soil. The archaeal community in the two soil types selected for this study (loamy and sandy) showed differences in their diversity and in the abundance of specific dominant archaeal taxa. Our findings confirm that soil quality in the absence of plant exudates is an important determinant for the soil archaeal community structure (Chen et al. 2010; Di et al. 2010), and that it can favor archaeal taxa with specific characteristics (Azziz et al. 2016). The phylum *Thaumarchaeota* composed most of the archaeal community structure associated to the tomato plants in this study. In addition, *Thaumarchaeota* also showed increased abundance in sandy soil compared with loamy soil when bulk soils were compared. The second most abundant phylum was assigned to *Euryarchaeota*. This phylum consists for the most part of methanogens such as *Methanomicrobia*, which represented the most abundant taxa of *Euryarchaeota* found. These anaerobic methanogens are usually part of microbiomes of crops, such as maize or arugula, mainly located in anoxic niches in the rhizosphere of the plants (Chelius and Triplett 2001; Taffner et al. 2019).

The impact of soil type on the archaeal community in the rhizosphere. In contrast to the differences observed in bulk soils, we could not detect nor infer any significant soil type-related effect on the abundance of the archaeal population in the rhizosphere. On the one hand, it is possible that the absence of the effect might be due to the experimental design of this study; the substantial dilution of both loamy and sandy soils with sterile sand might have mitigated the effects of soil texture on the rhizosphere. On the other hand, a previous analysis with an identical experimental setup (Bergna et al. 2018) observed that the differences in the bacterial communities of these two specific soils were highly conserved for the bacterial community of the rhizosphere of tomato plants. This different sensitivity of archaea and bacteria to soil types is not new, but still not fully understood. While it is known that these microorganisms respond differently to soil depth, where the ratio of archaea to bacteria increases (Leininger et al. 2006), archaea inhabit a far more restricted ecological niche in soils compared with bacteria (Bates et al. 2011). In addition, a recent study defined the process of rhizosphere formation as a dynamic and almost bacterial-exclusive process, which does not include archaea until the last stages of plant development (Edwards et al. 2018). For these reasons, it is possible to hypothesize that, in contrast to what applies for bulk soil, archaeal rhizosphere communities are much less affected by soil quality compared with bacteria. Instead, archaea in the rhizosphere might be deeply influenced by the coexistence with bacterial communities and by the adjacent plant root system (Kang et al. 2019).

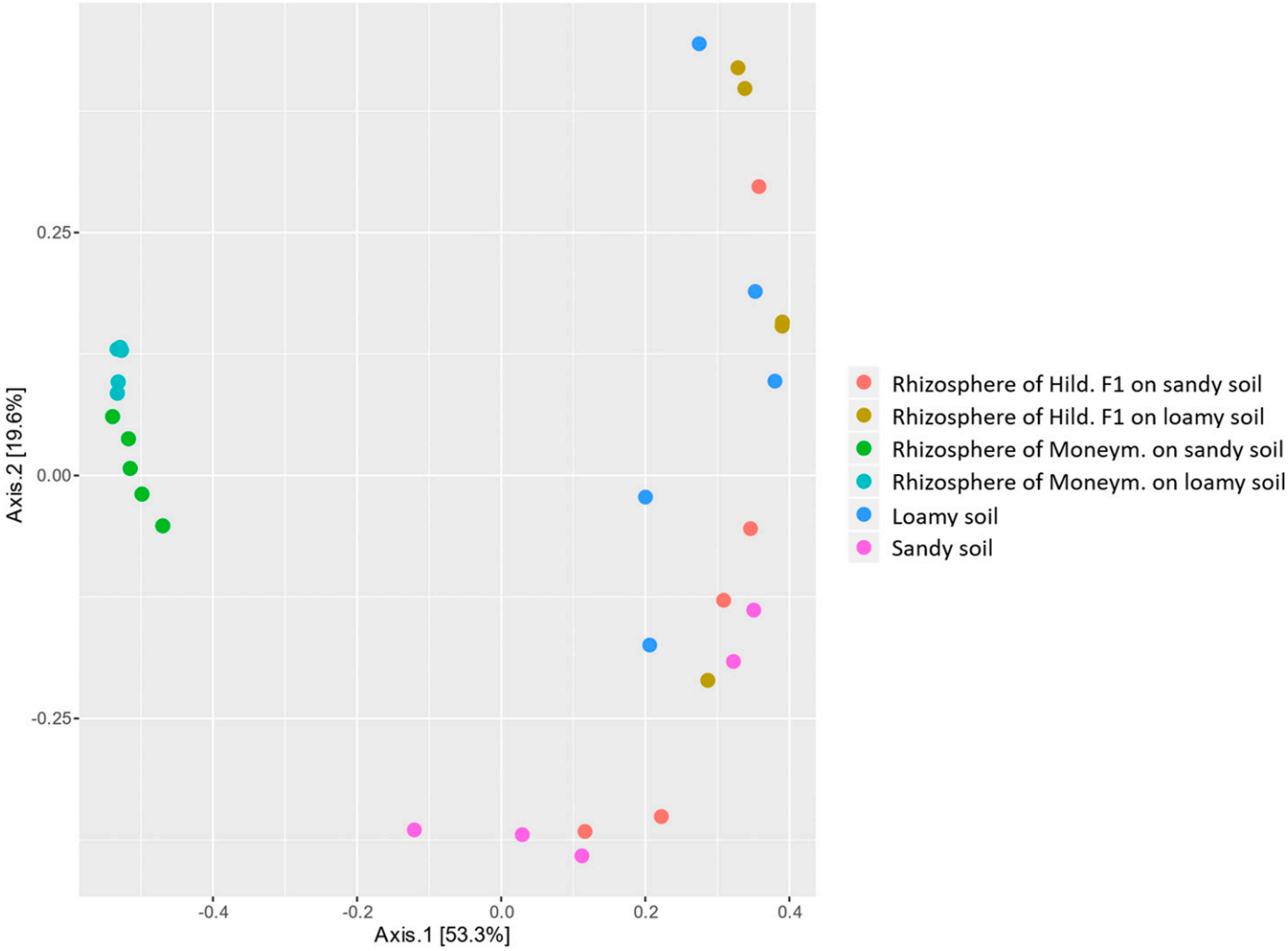

Fig. 4. Principal coordinate analysis plot calculated using Bray Curtis metrics plotting the similarities/dissimilarities among samples based on their archaeal community composition. Samples are colored according to the different microhabitats and the shape refers to the soil mixture employed.

The plant genotype is a main driver for archaeal community in the rhizosphere. The archaeal diversity and abundance observed in the rhizosphere of tomato plants was consistently higher compared with both bulk soils employed. This rhizosphere effect is likely to be connected with the production of root exudates that represent a constant source of nutrients (Mendes et al. 2011) that attracts and allows the colonization of archaea. Interestingly, the diversity shift in the rhizosphere was observed to be even clearer in Moneymaker plants that hosted a three times more diverse archaeal community than Hildares F1 plants (a generalized overview is presented in Figure 6). This is the first time that a plant genotype effect of this magnitude has been observed on archaeal communities in the rhizosphere of agricultural plants. So far, a similar plant genotype-driven effect has been reported only for archaeal methanogens in the rhizosphere of rice in an aquatic environment (Wu et al. 2009). This highly specific effect might not only be explained by the differences in the quality and quantity of root exudates, but also by different nutrient-uptake strategies of each plant cultivar (Grayston et al. 1997). It is in fact known that specific archaea, and notably AOA, highly accumulate in N-demanding plants (Thion et al. 2016). Further, another explanation of this effect can be found in the interdomain interactions that archaea can establish (Taffner et al. 2019). In this framework, the effects that different root exudates have on bacterial and fungal microorganisms were shown, such as modifying the presence of metabolites in the rhizosphere and the soil–plant interface. Similarly, archaeal abundance is known to correlate with mycorrhizal abundance (Grayston et al. 1997); for this reason it is valuable to also integrate fungal communities in the analysis of archaea–plant interactions.

The potential ecological role of archaea in tomato seeds. Recently, we described how the seed can represent an important vehicle for the vertical transmission of beneficial bacteria across generations (Bergna et al. 2018). Since archaea play relevant roles for plant nutrient cycling in the soil and the rhizosphere (Erkel et al. 2006; Leininger et al. 2006; Mendes et al. 2013), we initially hypothesized that archaea would have been conveyed by the plant to the seed where, as for bacteria (Bergna et al. 2018), they might support the germination and development of the offspring plant. The archaeal abundance assessed by qPCR in tomato seeds was in line with recent observations of Wassermann et al. (2019) in seeds of native alpine plants. Nevertheless, we found indications for an overestimated total number of archaea in distinct samples by implementing the qPCR-based quantification method and have therefore normalized gene copy numbers according to the recovered total community DNA concentrations. This facilitated a comparative assessment of different samples, but would require a targeted approach to determine the total number of archaea in tomato-associated microhabitats. Taxonomic analyses of the archaeal community of tomato seeds did not provide any evidence that could indicate a selection of archaea for the offspring plant. Archaea were previously defined as "late colonizers" of plants (Edwards et al. 2018) and thus their vertical transmission to new plant generations might not be essential. This is in contrast to bacteria, which are early colonizers and transmitted through seeds that represent the primary vehicle of beneficial microorganisms for the early stages of plant development. Therefore, we hypothesize that archaea, which appear to be nonessential for the first stages of plant development, are not found in the seed. Moreover, it is more likely that archaea might have developed as bystander microorganisms in seeds, possibly based on syntrophic relationships with bacteria (Morris et al. 2013). We have also observed a significant decrease of archaeal abundance between the two seed generations, which is most likely due to the growth conditions and the implemented soil types. Another possible explanation could be given by the use of commercial seeds for the first generation and harvested seeds for the second generation. Archaea might accumulate during commercial processing (e.g., washing or drying) or later during the storage. Further experiments are required to determine the factors shaping archaeal community structure and abundance in plant seeds.

Unassigned archaeal features in tomato plants. The bioinformatic reconstruction of the archaeal community associated with the plant habitats was performed using an up-to-date and

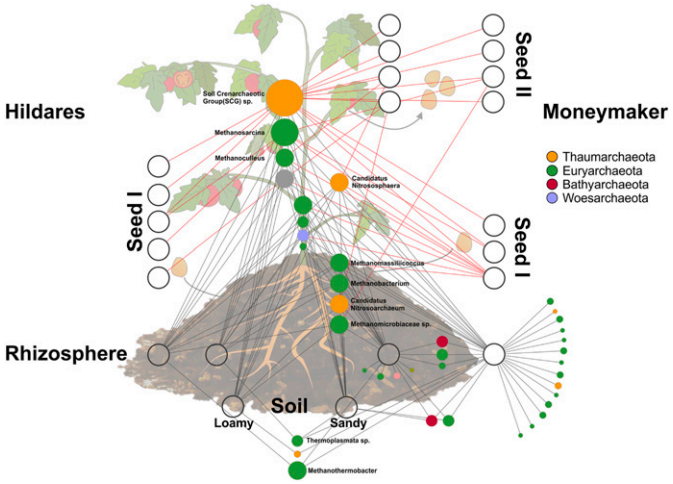

Fig. 5. Feature network of the archaeal communities at the genus level, based on 16S rRNA gene fragment analysis. Empty circles represent different sample types (soil, rhizosphere, as well as the first and second generation of seeds of tomato plant cultivars Moneymaker and Hildares F1) obtained from loamy and sandy soil. The colored circles represent archaeal taxa found in the respective sample types while different colors indicate distinct archaeal phyla: *Thaumarchaeota* in orange; *Euryarchaeota* in green; *Bathyarchaeota* in red; *Woesarchaeota* in purple; unassigned taxa are shown in gray. Red lines connecting the associated sample highlight taxa found in the seeds. The bubble size represents the relative abundance of the archaeal taxa throughout all habitats.

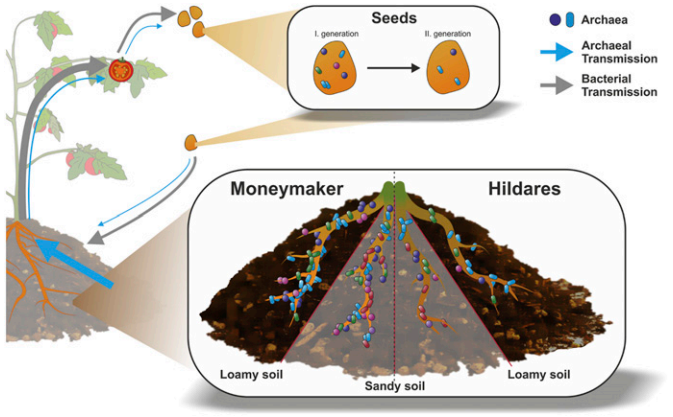

Fig. 6. Graphical illustration of the colonization and transmission of archaea in tomato plants. The rhizosphere of both analyzed tomato cultivars (Moneymaker and Hildares F1) in loamy and sandy soil, as well as the first and second generation of the seeds of Moneymaker are included in the model. Blue arrows highlight archaeal transmission between the different habitats. Gray arrows indicate the bacterial transmission as assessed in a previous study (Bergna et al. 2018). The arrow size indicates the relative proportion of transmitted microorganisms.

robust pipeline. This approach resulted in a well-defined archaeal community structure that was though not exempt from several taxonomically unassigned features. Features without taxonomical assignment represented 15% of the total features found in these habitats, but can represent up to 20% at class level in seed samples. This is a well-known limitation for the characterization of novel habitats, especially for archaea. In fact, the rather low frequency of archaeal community investigations resulted in the use of smaller and often incomplete taxonomy databases. The relatively low ratio of unassigned features of this analysis excludes the presence of significant PCR off-target effects or low read length. On the other hand, the high ratio of unassigned taxa in a low characterized habitat, such as the seed, indicates that this problem is seemingly due to still poorly defined reference databases that can be increased only with further investigation of the archaeal domain.

Conclusion. Archaea are numerically substantial components of the tomato microbiota with specific compositions in the rhizosphere and endosphere. The plant genotype (tomato cultivar) was identified as main factor influencing abundance and diversity, while the soil type did not notably affect archaeal communities. Our results show for the first time transmission of archaea from the parent to the offspring plant, but there is no indication for a targeted selection as shown for bacteria. In comparison with the seed, the rhizosphere showed cultivar-specific increased abundance and diversity of archaea indicating a role for the developed plant. The plant microbiome is known to change during its life cycle; accordingly, different members of the plant microbiome seem to have different abundances and functions. This has to be considered in management strategies developed for healthy plant microbiomes in sustainable agriculture.

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