Root exudate compounds change the bacterial community in bulk soil

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Abstract

The soil bacteria are diverse in nature both physiologically and phylogenetically with spatial variations within the soil microenvironments. Plant roots secrete organic substances called root exudates which benefit bacteria able to incorporate these. Subsequently, as the root grows, it changes the organic carbon status of adjacent bulk soil, stimulating growth of some of the resident bacteria. This growth induces a shift in the soil bacterial community and causes modifications in its metabolic activities. This nutrient infusion could also activate resting structures such as endospores to grow. We asked how the bulk soil microbial community responds when encountering root exudates and hypothesized that bacteria able to grow rapidly would become predominant upon introduction of root exudates. We added synthetic root exudate cocktail (Dietz et al. 2020) to the bulk soil from a wheat field on day 0 and day 1. We determined the aerobic culturable count on R2A, and *Bacillus cereus sensu lato* on Mannitol Egg Yolk Polymyxin agar, and bacterial community composition by sequencing the V3-V4 region of the 16S rRNA genes on days 0, 1, 2, 3, 4, 6, 8, 10, 12 and 14 of incubation. Alpha diversity (Shannon) decreased and recovered partially, indicating a shift in species evenness while the Chao1 index remained the same, indicating constant species richness. Beta diversity shifted substantially over time. Rare fast-growing genera like *Paenarthrobacter* and *Pseudarthrobacter* increased upon REC addition, while slow growing genera like *Bradyrhizobium* were constant over time. Some key genera like *Stenotrophobacter* responded only after ceasing of REC addition. Certain fast-growing genera like *Bacillus* did not increase in population density. Collectively, these results indicate that the bulk soil community shifted significantly when exposed to REC, and after termination of REC, continued to undergo shifts. This presents the root environment with diverse bacteria known to benefit growth, such as *Paenarthrobacter* and rhizobia.

Keywords: Rhizosphere, bacterial diversity, rare biosphere, root exudate, conditionally rare taxa

1. Introduction

Soil bacterial communities are diverse not only phylogenetically (Rosselló-Mora and Amann 2001) but also physiologically and in their responses to the environment (Hayat et al. 2010; Ernakovich and Wallenstein 2015). They perform diverse metabolic processes and collectively utilize a wide range of nutrients for growth, and energy production (Schloter et al. 2000; Torsvik and Øvreås 2002). Generation times across bacterial taxa vary widely from 20 minutes to days. Alphaproteobacteria on average grow slowly while Gammaproteobacteria grow significantly faster in the presence of sufficient organic carbon. This indicates conservation of growth physiology within higher order taxa (Kurm et al. 2017). The concentration of soluble organic carbon in bulk soil is limited, yet the chemical composition is diverse (Lu et al. 2020). Introduction of organic carbon into bulk soil is mostly limited to events such as introduction of litter by soil fauna (Bais et al. 2006; Liebeke et al. 2009). In contrast, the rhizosphere receives substantial organic carbon through root exudation, supporting nutritional needs of proximal bacteria (Bais et al. 2006; Sasse et al. 2018). Actively growing plants exude 11 to 40% of photosynthesis-derived carbon into the rhizosphere and establish nutrient hot-spots (Lynch and Whipps 1990; Badri and Vivanco 2009; Pausch and Kuzyakov 2018). The introduced carbohydrates, amino acids and organic and fatty acids are driving force for the growth of microorganisms in the rhizosphere (Bowen and Rovira 1999; Lugtenberg and Dekkers 1999; Sasse et al. 2018). Bulk soil microorganisms in the path of growing roots are immersed in nutrient change, and motile soil bacteria from the surrounding are attracted towards these roots. Several studies have revealed that exudates induce changes in soil bacterial communities and alter their functional activities (Jacoby and Kopriva 2019; Vives-Peris et al. 2020; Dhungana et al. 2023; Sandhu et al. 2023). The increased availability of organic carbon causes microbial communities in the rhizosphere to be

different than in bulk soil (Baudoin et al. 2003). Thus, root exudates play a major role in shaping the microbial community in the rhizosphere (Narula et al. 2012).

Most soil occurs distal to roots and resident bacteria must possess mechanisms to persist in these nutrient-limited conditions. Successful population maintenance in low nutrient bulk soil requires an emphasis on long term survival over cell division (Roszak and Colwell 1987). Approaches include production of protective structures and tighter packaging of the cell components with reduced metabolic activity (Xu et al. 2022). The most effective survival mechanism entails formation of endospores that are highly resistant to heat and chemical attack (Hariram and Labbé 2015; Basta and Annamaraju 2020; Schreiber and Ackermann 2020). Endospore forming bacterial populations can occur as a mix of vegetative cells and resting spores, so that some members of the population can benefit whether conditions become harsh or growth supporting, the so-called bet-hedging strategy (Veening et al. 2008; Carey and Goulian 2019; Sandhu et al. 2021). Here individual cells take the risk of either being prepared for altered future conditions, or die. This diversification in phenotype of isogenic populations is termed phenotypic heterogeneity (Schreiber and Ackermann 2020). Bacteria able to change their phenotype in response to altered environmental conditions are able to thrive under divers consitions, termed phenotypic plasticity (Sandhu et al. 2023). The stark differences in available organic carbon between bulk soil and rhizosphere would benefit bacteria with a higher degree of phenotypic plasticity.

The specific composition of the bulk soil microbiota shifts after exposure to growing roots (Steinauer et al. 2023). The proportions of beneficial versus harmfull bacteria in this shifted community is important for plant performance (Saeed et al. 2021). Bacterial taxa able to take up nutrients and grow rapidly are not expected to thrive in bulk soil but are expected to increase in

number following the influx of root exudates, while slow growing taxa would require more time to take advantage of the situation. This suggests that certain species in bulk soil can increase in abundance upon exposure to root exudates, while others would lose dominance (Goldfarb et al. 2011). Copiotrophs are considered to adapted to the rich carbon source environments as their growth and reproducibility is high, so they need excessive nutrients (Roller and Schmidt 2015). As an example, the saprophytic endospore formers such as *Bacillus, Priestia* and *Paenibacillus* are copiotrophs known for rapid growth when provided with ample organic carbon (Kulkova et al. 2023). These copiotrophs have lower carbon use efficiency (CUE) than oligotrophs (Saifuddin et al. 2019), but more responsive to nutrient availability, as they use a wider range of metabolites. Some of the first colonies to appear after plating soil suspensions on rich media are *Bacillus cereus* sensu lato (Vilain et al. 2006). Exposure of endospores in bulk soil to root exudate should, therefore, lead to germination and rapid growth. The soil microbial community is, therefore, expected to change when growing roots change the carbon status of a specific location, with rapid growers becoming dominant (Zhalnina et al. 2018).

We asked how the bulk soil microbial community responds when encountered by root exudates. We hypothesized that bacteria able to grow rapidly would become predominant upon introduction of root exudates. To determine the response of bulk soil bacteria to root exudates, we supplemented bulk wheat field soil with a synthetic root exudate cocktail (Dietz et al. 2020) . We tracked the aerobic culturable, and endospore counts, the copiotrophic *B. cereus sensu lato*, and overall bacterial community using the 16S rRNA gene pool. Our data reveal that most of the fast-growing bacteria responded by increasing in proportion of the community, like *Rhizobium, Paenarthrobacter* and *Pseudarthrobacter*, while slower growing taxa such as *Bradyrhizobium* became less dominant. Intriguingly, endospore-forming aerobes like *Bacillus* responded by

germinating but then the spore count increased while the overall count remained constant, indicatimg sporulation.

Materials and Methods:

2.1. Soil microcosms

Soil was collected from a wheat field before harvest, located at 44.18N 96.46W. To obtain bulk soil, 40g of soil was passed through a sieve with 2mm size to remove roots. Sieved soil samples (40g) were placed into each of three sterile empty petri dishes (10 cm diameter). Synthetic root exudate cocktail (REC, 4 ml) was added to each of the three reps at time zero and after 24h (Fig. 1). Thereafter, sterile deionized water was supplied daily to keep soil moist for the duration of experiment (day 3-day 13). To differentiate the effect of water alone, we set up parallel soil micocosms supplemented with either REC or water. Synthetic rood exudate cocktail was prepared based on grass exudate composition (Dietz et al. 2020), and contained 0.5g each of arabinose, fructose, glucose, sucrose and xylose, 0.4g each of fumaric acid, lactic acid, malic acid, oxalic acid, succinic acid and tartaric acid, and 0.25g each of alanine, glutamic acid, glycine, methionine, leucine, proline, serine, threonine, tryptophan and valine added to 200 mL high purity water (Dietz et al. 2020). The REC solution containing 37g per L organic carbon was sterilized by filtration using a 0.2 μm pore size bottle top filter (Fisher brand FB12566510, Pittsburgh, USA).

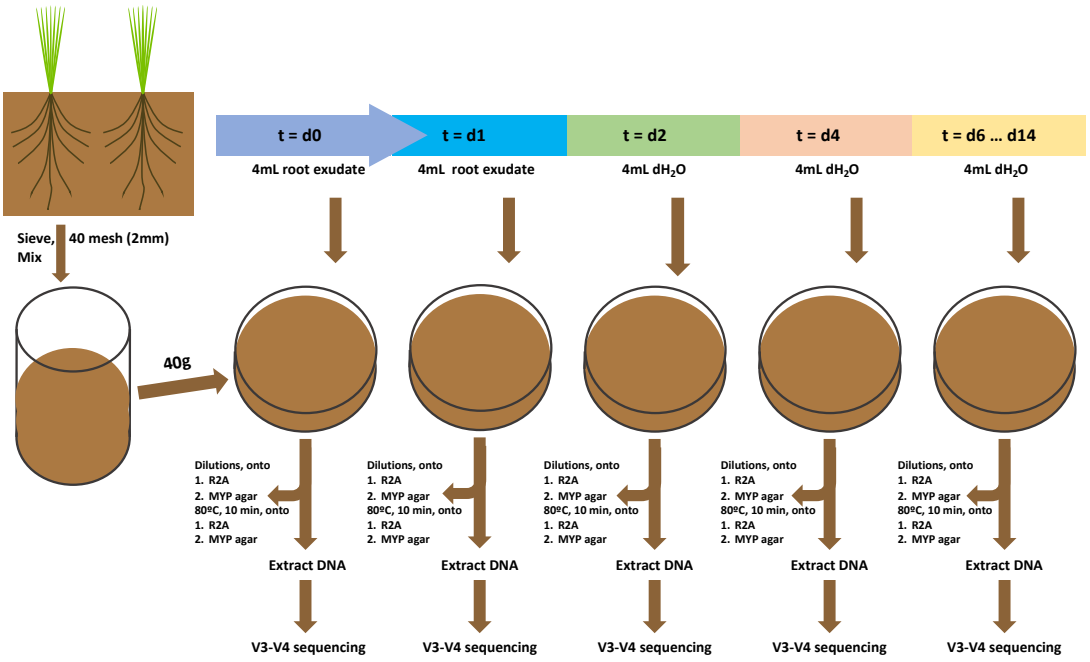

Fig. 1. Diagrammatic overview of sample treatment and processing.

2.2. Enumeration of culturable vegetative aerobes and endospores

For sampling, the soil in petri dishes was mixed usig a sterile spatula, and 1g of soil removed. Soil samples (1 g) were suspended in 9 mL sterile high purity water and mixed by vortexing for 5-10s 3 times. For the culturable count, 100 µL volumes were spread onto R2A (Research Products International Cat R24200, Mt Prospect, USA) and Mannitol Egg Yolk Polymyxin (MYP) agar (Oxoid catalog CM0929, Basingstoke, UK) supplemented with 10000 units of polymyxin per L (Oxoid catalog SR0099E, Basingstoke, UK) and sterile egg yolk emulsion in water. For endospore counts, the same dilutions were incubated at 80 °C for 10 min, and 100 µL aliquots plated on R2A and MYP agars. Each dilution was plated on three plates, and plates were incubated aerobically for 24h at 28 °C when colonies were counted. The only medium selective for mmebrs of *Bacillus* uses mannitol, lecithin and polymyxin, although there have been multiple variations. These media

are selective for *B. cereus sensu lato*. MYP is a selective and differential medium for enumeration of *B. cereus sensu lato*. *B. cereus* gives very large pink colonies as it does not ferment mannitol, and is surrounded by a ring or halo due to hydrolysis of egg yolk lecithin by excreted lecithinase (Peng et al. 2001). To determine whether *B. cereus sensu lato* occurring in the soil used can grow using the REC applied, selected pink colonies on MYP agar were subcultured for purity. Cell suspensions were prepared in sterile water, inoculated into diluted REC (5mL REC and 45mL sterile water) at A_{600} =0.01, and growth at 30 ℃ measured by absorbance.

2.3. Culture independent community composition

For each DNA extraction, the soil in petri dishes was mixed usig a sterile spatula, and 1g of soil removed. DNA was extracted using the RNeasy PowerSoil Total RNA Kit (Qiagen Cat 12866-25, Hilden, Germany) and RNeasy PowerSoil DNA Elution Kit (Qiagen Cat 12867-25, Hilden, Germany). DNA was quantified fluorometrically using the Qubit™ dsDNA BR Assay Kit in a Qubit® 3.0 Fluorometer (Thermo Fisher). The extracts were preserved at −80 °C until further processing. The 16S rRNA gene amplicon pools were sequenced by Illumina using standard procedures. The V3-V4 regions of the bacterial 16S rRNA gene were PCR amplified using Illumina 16S Amplicon primers with overhang adaptors (forward: 5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG3′and reverse: 5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAC-TACHVGGGTATCTAATCC 3′). Sequencing libraries were prepared using the Nextera XT library preparation kit and dual indexing method (Illumina, Inc., San Diego, CA, USA) as per the manufacturer's protocol. After purification, quantification, and normalization, all 36 libraries were pooled and run on an Illumina Miseq platform using paired-end V2 chemistry. The raw data is

available on NCBI's Sequence Read Archive (SRA) database under SRA: SUB13879876; Bioproject: PRJNA1020191(https://www.ncbi.nlm.nih.gov/sra/PRJNA1020191 Released October 14, 2023).

2.4. Data analysis

Bacterial communities were analyzed using 16S rRNA sequences (V3-V4 region) in Quality Insights Into Microbial Ecology (QIIME version 2.2) (Bolyen et al. 2019). The cut of values for trimming were 25, 9 for left forward and left reverse respectively while 240 and 220 for forward trunc-length and reverse-trunc-length respectively. Quality filtering of demultiplexed reads was performed using demux plugin and denoised using DADA2 (Callahan et al. 2016). Mafft (Katoh et al. 2002) was used to align all sequences to construct the phylogeny with FastTree (Price et al. 2010). Normalization of reads was done by rarefying to 25000 reads which was enough to count the total diversity and taxonomy. The outputs table.qza, rootedtree.qza and taxonomy.qza were exported for analysis. Alpha diversity was reported using phylogenetic distance while beta diversity was performed using Unifrac. Beta diversity was compared by Principal Component Analysis using Bray-Curtis index. The visualization of data was done using MicrobiomeAnalyst (Lu et al. 2023) by default parameters except for the correlation network ($p<0.01$ using SECOM-2 and 100 permutations).

3. Results

3.1. Culturable count of the bacterial community

To follow population density upon availability of REC, we determined the CFU of aerobic heterotrophs, and counted colonies after 24h of incubation. The culturable count increased more than ten-fold within 24h of REC addition, and another ten-fold by 72 h (Fig. 2). This indicated that taxa known to grow rapidly increased in numbers when organic substances became available. To simulate decrease in exudation, we stopped adding REC, and substituted it with water. This was associated with no further increase in the number of rapidly growing taxa, but interestingly cell density remained constant (Fig. 2). We had planned to also count slower growing taxa through longer incubation, but *Bacillus mycoides* and other prolific spreaders obscured the many smaller colonies by the second day. Colony counting after longer incubation became unreliable, so, the data in Fig. 2 represent only the rapidly growing taxa that formed visible colonies within 24h.

To follow the response of aerobic endospore forming bacteria, we employed two approaches. Total aerobic heterotrophic endospores were enumerated by plating heat treated cell suspensions on R2A. Members of *B. cereus sensu lato* were enumerated using MYP agar with polymyxin B and lecithin. The rapidly growing heterotrophs in bulk soil were predominated by heat resistant spores. The d_0 R2A spore count was almost as high as the R2A total count. As spores generally germinate to form colonies on R2A, a substantial part of the total count entailed spores. Similarly, a large population of *B. cereus sensu lato* occurred in bulk soil as spores (Fig. 2). The introduction of REC was associated with an increase in total aerobic culturable over heat treated counts, indicating vegetative growth. Surprisingly, the endospore counts did not decrease within 24 h, indicating that endospores did not germinate. Despite further REC addition after 24h, the overall and MYP counts

did not increase further, while the endospore counts did increase. This indicated sporulation of newly grown cells despite availability of fresh nutrients. This pattern was observed in several repeats using other wheat field soil samples (data not shown). To determine whether the carbon sources in the REC could be utilized by *B. cereus sensu lato*, selected isolates were cultured in REC. The three isolates evaluated all grew in REC, demonstrating that they were able to utilize components of REC as carbon source (Fig. 2d). This suggested that growth and/or germination of the *Bacillus* in the soil was inhibited by factors in the soil.Collectively, these results indicate that the fast-growing heterotrophic community contains a substantial proportion of endospore forming bacteria, and a substantial subset of these occurs as endospores that did not germinate, even after introduction of REC.

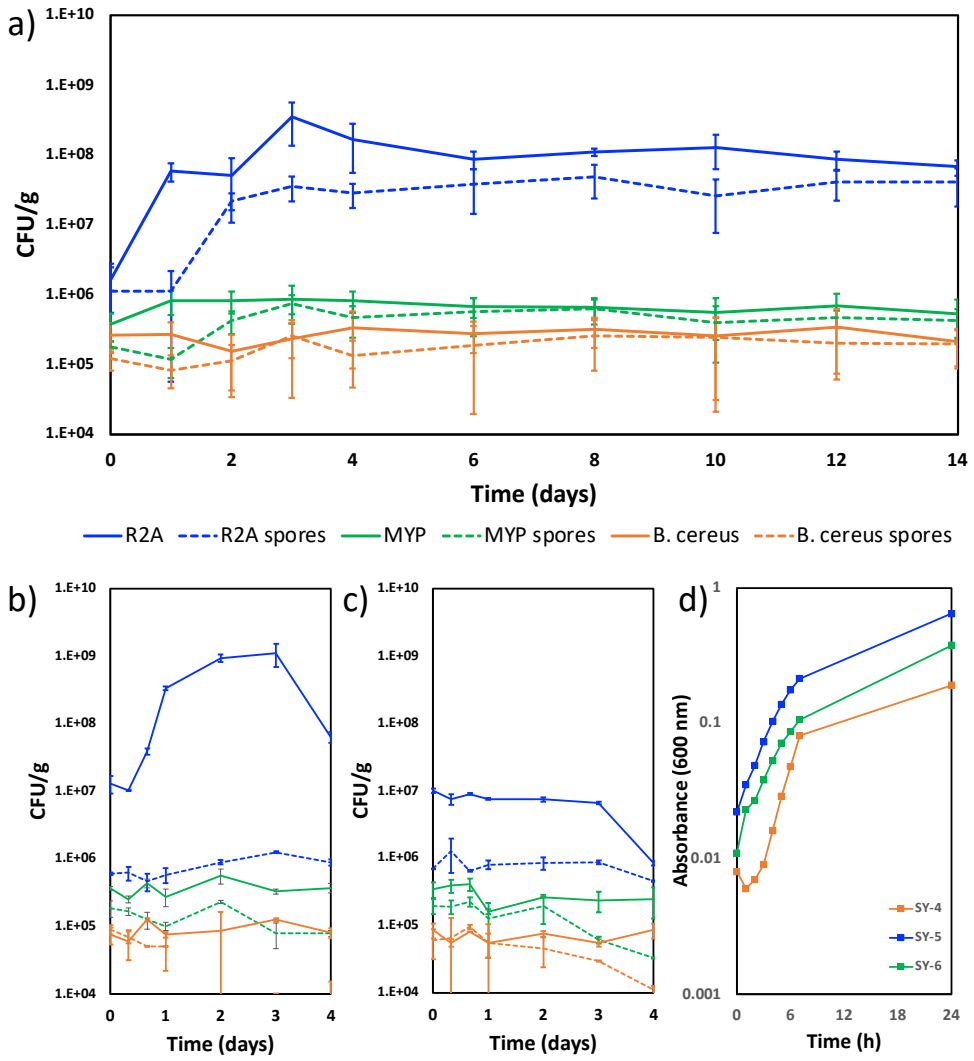

Fig. 2: Culturable counts of bulk soil samples amended with REC (a, b) or water (c). The aerobic heterotrophic count was determined by plating dilutions on R2A and incubating at 28ºC for 24h. The *B. cereus sensu lato* culturable count was determined by counting pink colonies surrounded by halos on MYP agar. Endospores were enumerated by incubating dilutions at 80ºC for 10 min before plating. Growth of three *B. cereus sensu lato* isolates in the REC cocktail liquid medium while shaking at 28°C was quantified by absorbance (d).

3.2. Shifts in community composition following addition of REC

To characterize the entire bacterial community, we analyzed the 16S rRNA gene pool by amplifying and sequencing its V3-V4 regions. The genus level alpha diversity by Shannon index decreased upon introduction of REC and again after the second addition (Fig. 3a). In contrast, the Chao1 index (Fig. 3b) did not decrease much, indicating no or little loss in rare taxa. Rather, some taxa increased in number driving down Shannon diversity. After termination of REC addition, richness (Chao1) increased only slightly while evenness (Shannon) increased somewhat. Yet Shannon diversity did not return to the original bulk soil level within the 14-day period. These results indicate partial rebounding of slower growing taxa while rapidly growing taxa did not further dominate the community.

Beta diversity was compared by Principal Component Analysis using the Bray-Curtis index at genus level (Fig. 3c). The bacterial communities in the three bulk soil samples were very similar. Upon introduction of REC the community composition shifted substantially. Importantly this shift was not identical but similar across the three reps. The second dose of REC added at 24h led to a further substantial shift along the first axis. Following termination of REC addition, and during 12 days of further incubation, the communities shifted less, primarily along the second axis. Differences continued to be seen among the three reps over time. A comparison of relative proportion of the fifty most dominant genera showed substantial differences across three reps of each time point as visualized in a heat map (Fig. 4). Relative abundance of each taxon per rep was presented as a cell colored according to the scale indicated. While three adjacent cells at d0 were generally similar in proportion (as shown by similar color of the cells), variation across three cells from d1 to d14 varied. This supported the variation in community progression over time. Collectively, these data indicate that the bulk soil community shifted substantially when exposed to REC, and then continued to undergo minor shifts in composition.

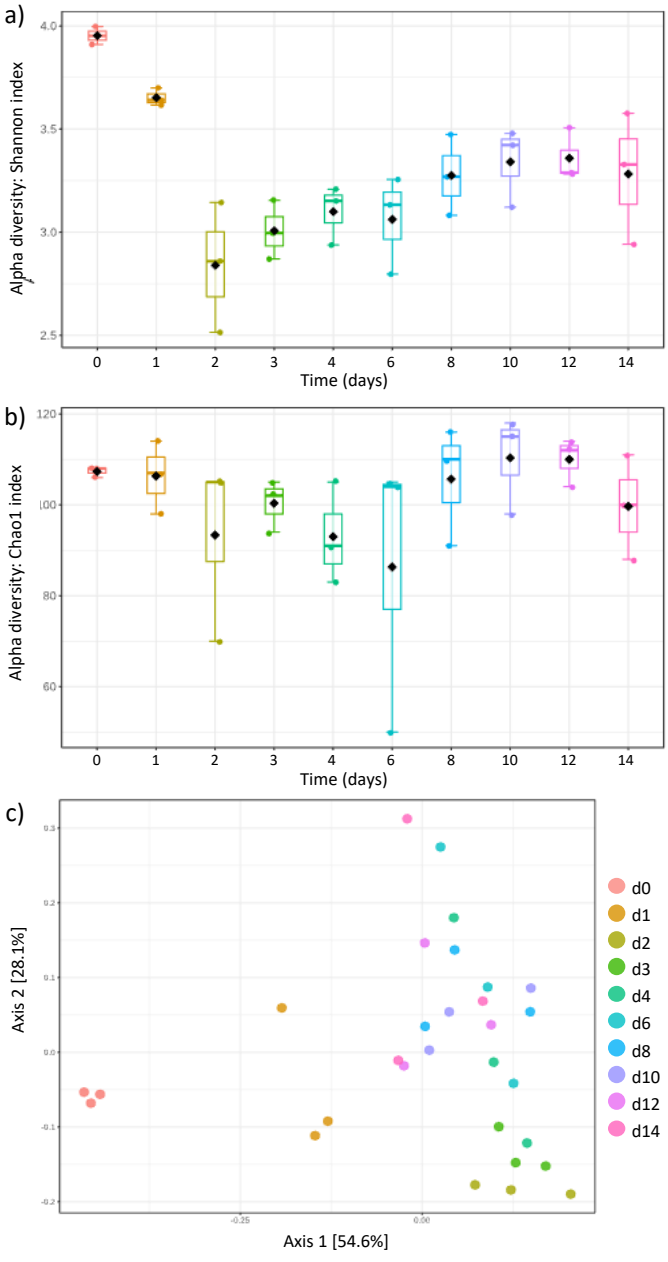
a)
Alpha diversity: Shannon index
4.0
3.5
3.0
2.5
0
1
2
3
4
6
8
10
12
14
Time (days)
b)
Alpha diversity: Chao1 index
120
100
80
60
0
1
2
3
4
6
8
10
12
14
Time (days)
c)
Axis 2 [28.1%]
Axis 1 [54.6%]
d0
d1
d2
d3
d4
d6
d8
d10
d12
d14

Fig. 3: Diversity of the bulk soil bacterial community when exposed to REC, showing Alpha diversity as determined using the Shannon (a) and CHAO1 indices (b), and Beta diversity compared by Principal Component Analysis using the Bray-Curtis index.

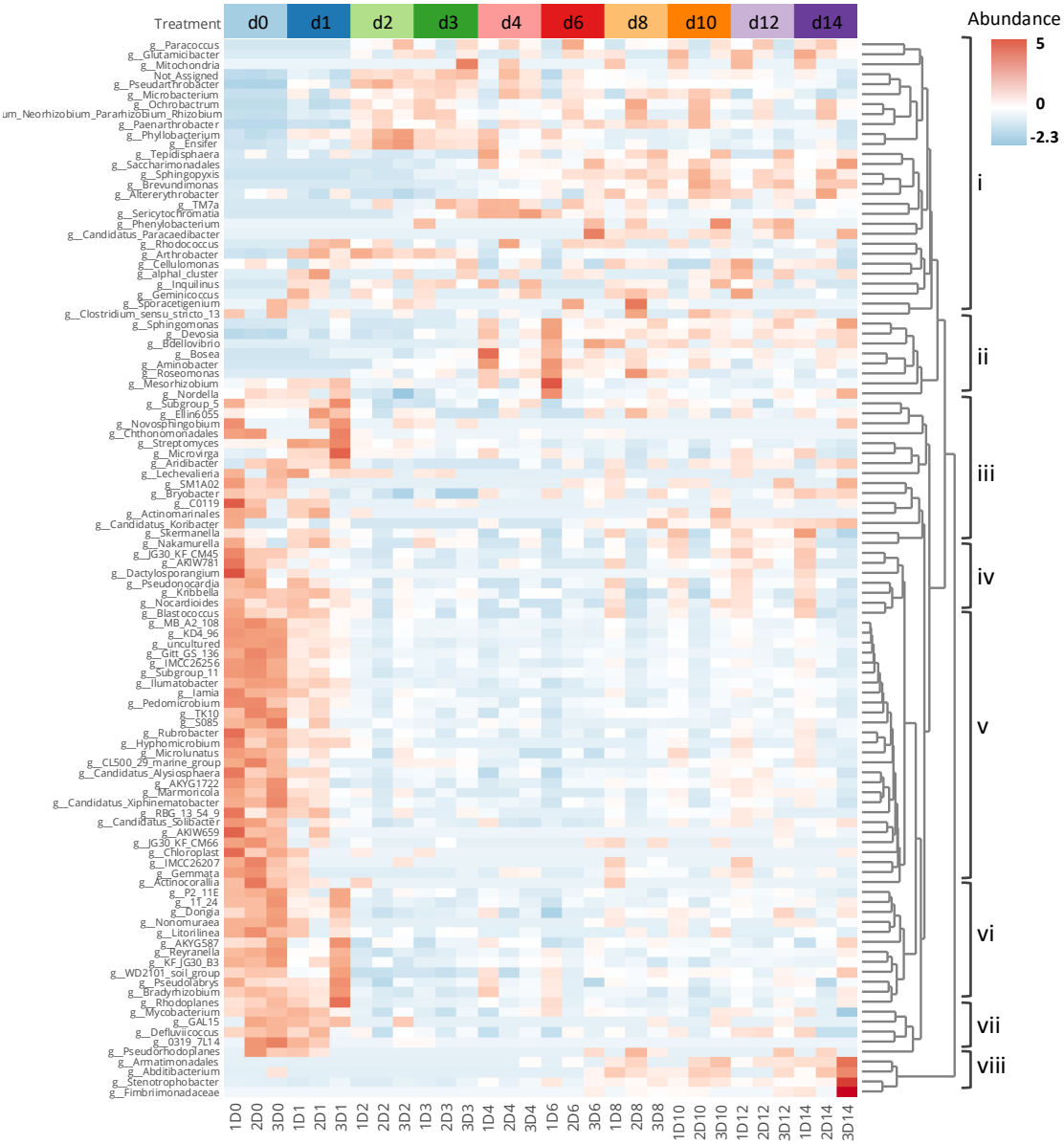

Fig. 4: Heatmap showing abundance of the fifty most prevalent genera following addition of REC to bulk soil.

3.3. Population dynamics of the most dominant genera

To determine the effect of REC addition on bacterial community further we tracked relative proportions of the fifty most dominant genera. Population density trajectories fell into 8 clusters (Fig. 4). The clusters contain OTUs that either increased rapidly, increased slowly, remained constant or decreased in dominance over time. Selected examples of fast growing, slower growing and dominant bacteria were plotted (Fig. 5). *Pseudarthrobacter, Allorhizobium, Neorhizobium, Pararhizobium Rhizobium* group and *Paenarthrobacter,* showed very low relative abundance in the bulk soil community but grew quickly in response to REC, the trait of cluster i (Fig. 5 i, $_{a\text{-}c}$). This shows that these were rare microbiota in the bulk soil but responded to rhizosphere carbon, dominating the community till day 14. Cluster ii contained taxa of intermediate prominence that increased in the presence of REC, including *Mesorhizobium* and *Sphingomonas* (Fig. 5 ii, $_{a\text{-}b}$). Taxa like *Skermanella* were predominant in bulk soil and maintained their proportion of the community over time, while *Streptomyces* responded to the REC initially but receded (Cluster iii). *Kribella* and *Rubrobacter* initially decreased but rebounded after termination of REC addition (Clusters iv & v). Taxa such as *Mycobacterium* in Cluster-vii maintained population density (Fig. 5 vii). Clusters vi and viii presented interesting cases. *Bradyrhizobium* (Cluster-vi) decreased in predominance and rebounded, but not to the original level. Members of this genus are known to grow slowly and may have simply been overrun by the fast-growing taxa. *Stenotrophobacter* (Cluster viii), while not responding numerically to REC, did grow after termination of REC addition (Fig. 5 viii). Some rare taxa increased upon introduction of REC. This indicates that REC had a positive effect on the activity or growth of bacteria that were less abundant in bulk soil. These shifts in population density of individual taxa could not be due to nutrient availability alone, but also to interactions among taxa, for example growth suppression. Diverse bacteria in soil are

known to display possitive or negative interactions with other taxa, either promoting or suppressing their growth (Gupta et al. 2024) .

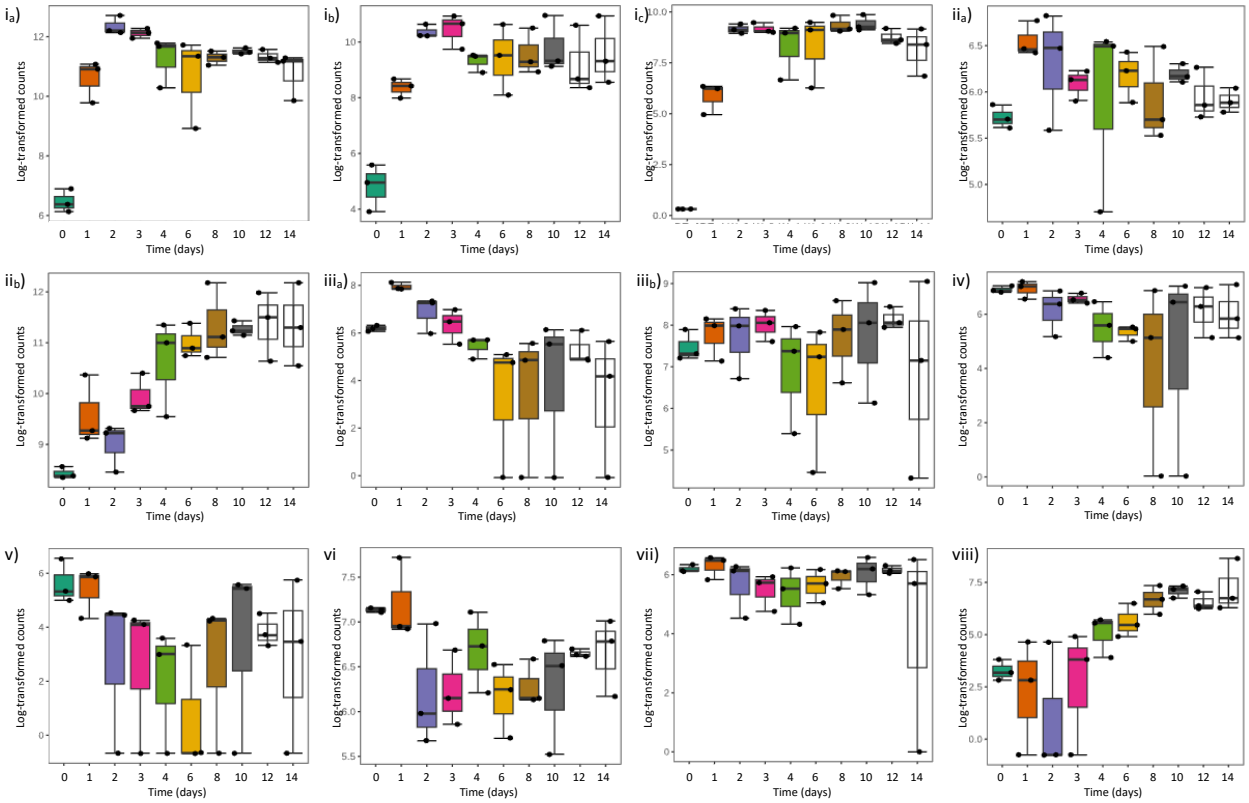

Fig. 5: Relative proportions of selected genera representing clusters i – viii of the heat map (Fig. 4). *Pseudarthrobacter* (i_a), *Allorhizobium, Neorhizobium, Pararhizobium Rhizobium* group (i_b), *Paenarthrobacter* (i_c), *Mesorhizobium* (ii_a), *Sphingomonas* (ii_b), *Streptomyces* (iii_a), *Skermanella* (iii_b), *Kribbella* (iv), *Rubrobacter* (v), *Bradyrhizobium* (vi), *Mycobacterium* (vii), and *Stenotrophobacter* (viii).

3.4. Identification of potential keystone genera

To reveal genera with potential as keystone members of the community, we sought possible interactions among dominant taxa. For this we made a correlation network at $p<0.01$ using

SECOM-2 and 100 permutations at genus level (Fig. 6). Several genera in Clusters i and ii appeared to correlate with a large proportion of the community over time, including *Sphingomonas, Pseudarthrobacter, Allorhizobium-Rhizobium* group, and *Stenotrophomonas.* These four genera had the highest number of node-specific interactions. Other rapid growers such as *Phylobacterium* and *Mycobacterium* did not make the cutoff criteria for interactions. Interestingly, *Streptomyces* are known for production of various antibiotics, and correlated with many genera, such as *Paenarthrobacter, Microvirga* and *Sphingomonas* that are not known as antibiotic producers. *Bdellovibrio* is known for the predation of other bacteria, but correlated with few other genera. Together with a small number of other bacteriovorous genera, *Bdellovibrio* is unique in ecological impact among bacteria (Pląskowska and Zakrzewska-Czerwińska 2023).

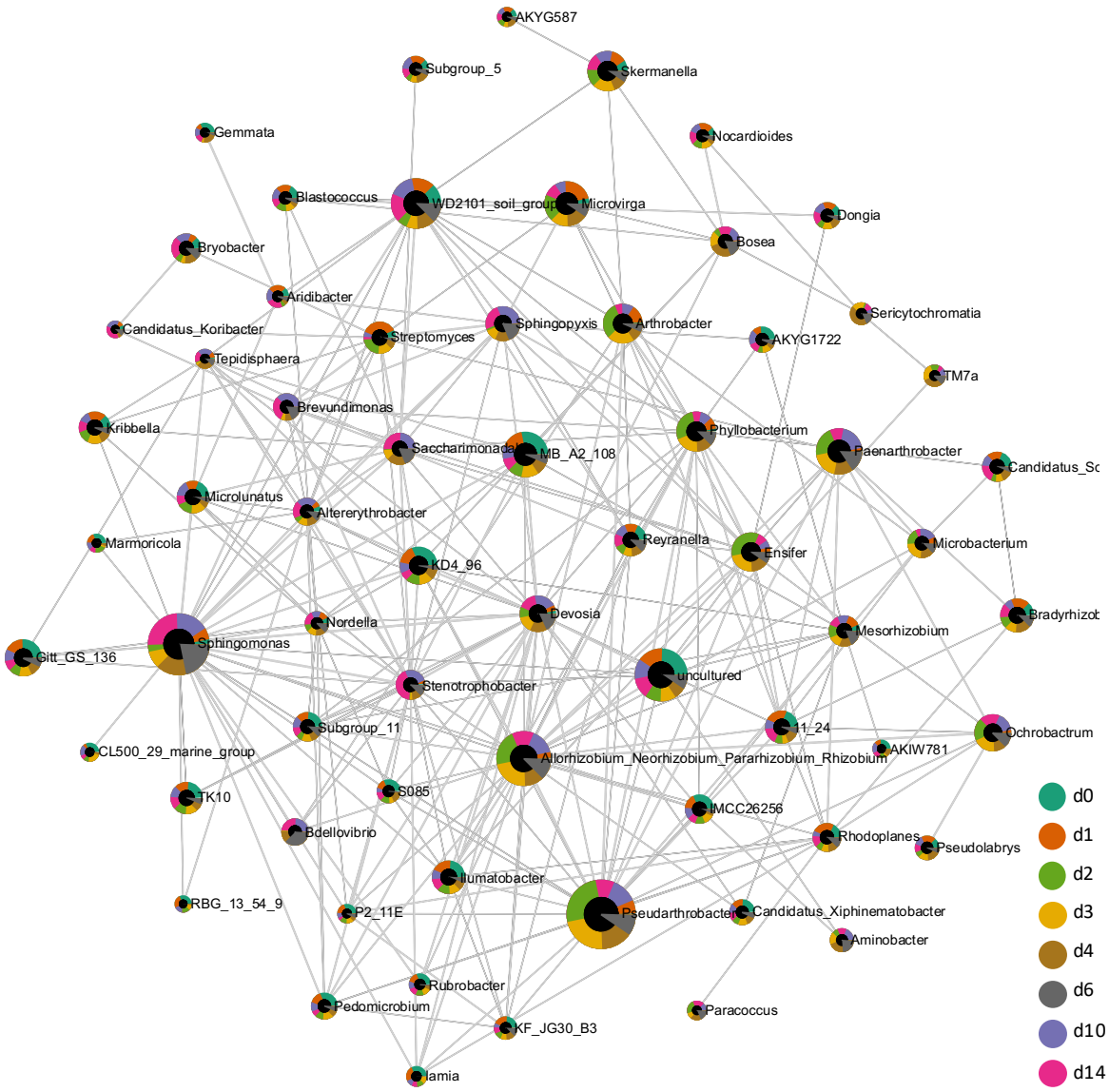

Fig. 6. Correlation Network ($p < 0.01$) of genera using Secom (Pearson 2), 100 permutations and a correlation threshold of 0.3. Lines connecting taxa indicate a high degree of correlation in proportions of the respective communities over time.

3.5. The dormant nature of the *Bacillus* population

The Bacillaceae occur widely in soil as supported by culture dependent (Mandic-Mulec et al. 2015) and culture-independent data (Delgado-Baquerizo et al. 2018). While endospore formers constituted a large part of the culturable count (Fig. 2), they were detected at 0.01-0.2% to below detection limit in DNA extracts. Bacillaceae are well known as fast-growing saprophytes, so we expected numbers to increase after REC introduction. The soil contained rapidly growing *B. cereus sensu lato* as seen by large colony size within 24h when plated on MYP agar. Surprisingly, their numbers in soil remained constant, indicating suppression by other microbiota, for example antibiotic-producing actinobacteria. As DNA data sets for some samples did not contain any Bacillaceae, we looked at the relationship of the entire Bacillota (Firmicutes) to other Phyla (Fig. 7). The most predominant phyla here were Pseudomonadota (Proteobacteria) and Actinobacteriota (Actinobacteria) (Fig. S1). Both correlated negatively with Bacillota, while several rare phyla correlated positively like Acidobacteriota.

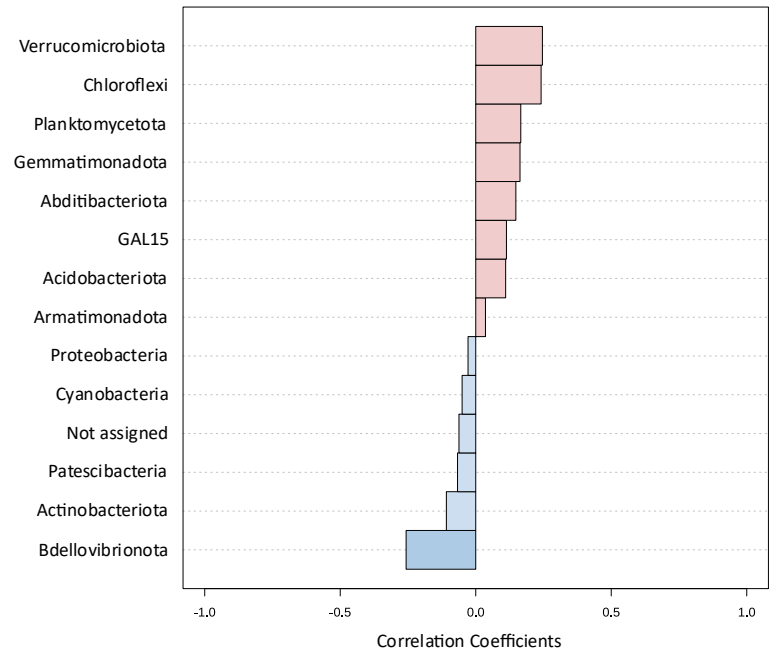

Fig. 7. Correlation of the entire Bacillota (Firmicutes) to the top 14 Phyla.

4. Discussion

The bulk soil bacterial community shifted upon exposure to REC, with a decrease in diversity indicated by Shannon index. The initial shifts were due to an increase in the abundance of multiple rapidly growing taxa, supporting our hypothesis. Community composition continued to change after termination of REC addition, with partial restoration of diversity. While multiple rapidly growing taxa increased in proportion, *Bacillus* did not respond to nutrient influx as expected, maintaining a fixed small population predominated by endospores.

4.1. **Bacterial community composition shifts upon REC addition.**

The introduction of REC was associated with shifts in community composition as indicated by changes in Beta diversity over time. This is in agreement with rhizosphere studies in wheat (Fan et al. 2018) and soybean (Mendes et al. 2014), where communities were less complex than in the surrounding bulk soil. The largest shifts occurred within 24h of the first and second REC addition, but community composition continued to shift after no REC was added, indicating continued community dynamics due to the initial shift, likely due to shifts in the balance of power among various taxa. As reported by others, the predominant phyla were Pseudomonadota, Actinomycetota and Acidobacteriota (Fan et al. 2017). The Acidobacteriota, Planctomycetota and Chloroflexi decreased, while the Pseudomonadota remained constant and the Actinomycetota increased overall, in agreement with other reports (Donn et al. 2015; Fan et al. 2017) (data not shown). Importantly, taxa within these phyla changed substantially. Within the α-Proteobacteria, Sphingomonadaceae and Rhizobiaceae increased, while Xanthobacteraceae decreased (Fig. S1). The increase in Actinomycetota included increases in Micrococcaceae such as *Paenarthrobacter* and *Pseudarthrobacter,* but decreases in Kitasatosporales (Streptomycetales) such as *Streptomyces*

(Fig. S1). These changes reflect substantial growth of specific taxa when exposed to REC. Therefore, bacteria in bulk soil exposed to a growing root would either find the newly provided niches beneficial or non-beneficial. The root zone (rhizosphere and endosphere) would select for taxa from the surrounding pool, called the "root filtration effect" by Luo et al (Luo et al. 2022).

The shifts in beta-diversity of the communities (Fig. 3c) included a change in predominance of a million-fold and more of genera such as *Pseudarthrobacter* (Fig. 5) within 48h following addition of REC. Several of these had high degree of node-specific interaction in the correlation network, specifically *Pseudarthrobacter, Sphingomonas, Allorhizobium-Rhizobium group*, and *Stenotrophobacter*. While these taxa were rare in bulk soil, species rarity is an integral part of community assembly, and when a new species enters a community it may remain rare at the beginning, forming part of the rare taxa. This is because the dominant species already occupy the space and are well adapted to the environment. Upon change in conditions, such as the introduction of REC from growing roots, some may benefit and increase in proportion (Gudelj et al. 2010). The taxa benefitting from exudate carbon, and displaying high degrees of node-specific interactions have potential as keystone genera (Zhou et al. 2011). Keystone taxa are critical in community structure and function (Power et al. 1996). They can also have low initial abundance, but change in predominance during change in conditions.

Arthrobacter chlorophenolicus plays a role in nitrogen fixation, phosphate solubilization and iron uptake, improving chlorophyll content (Kumar et al. 2014). *Pseudarthrobacter* sp. are reported to increase the height of wheat on their own as well as in combination with other soil bacteria like *Enterobacter* sp. and *B. megaterium*. The nitrogen fixing Rhizobium group co-inoculated with *Pseudomonas* increased wheat yield by up to 42% in wheat rhizosphere (Afzal et al. 2014).

Rhizobium also play a role in phosphate solubilization in wheat field soil (Abd-Alla 1994). *Paenarthrobacter* produce siderophores that helps in iron uptake, solubilize phosphate, produce IAA, and fix nitrogen (Özdoğan et al. 2022)... Li et al., 2022 reported that *Stenotrophobacter* are keystone taxa of rhizosphere of licorice and aid in high yield of apples (Li et al. 2022). The roles of these keystone taxa in the development of wheat rhizosphere communities should be evaluated in future studies.

As some taxa benefited from the introduction of REC, others decreased in prominence (Fig. 4). Decrease in species prominence can occur due to various processes and factors including stochastic processes, and biotic and abiotic interactions, without involving any specific physiological traits (Ai et al. 2013). Bacterial predominance is not only a function of growth, as some may be subject to suppressing effects of others. Some antibiotic-producing bacteria excrete more of the secondary metabolites in response to nutrient availability, so shifts in antibiotic excretion would lead to suppression of recently successful taxa. Niu *et al.* had grown a synthetic mixture of seven strains on axenic seedlings and found relative abundance of each species varied over the period of their experiments (Niu et al. 2017). Thus, species of low abundance in one set of conditions can initiate changes in a community when conditions change, in agreement with (Hajishengallis et al. 2011).

4.2. Some rare bacteria in bulk soil respond to root exudate compounds by increasing in number, becoming predominant.

Abundance of specific carbon sources should support growth of bacterial populations able to utilize them, while populations of other taxa will decrease (Demoling et al. 2007; Sánchez-Clemente et al. 2020). Here the addition of REC to bulk soil microbiota correlated with increase in populations of diverse rapidly growing taxa including *Pseudarthrobacter, Phylobacterium,*

Ensifer and the *Rhizobium* group (Fig. 4 cluster i). *Paenarthrobacter* increased 10^5-fold within 24h and another 100 -fold in the following 24h (Fig. 5 i_a), as did other taxa in cluster i. This shift to predominance of select taxa was supported by decrease in Shannon diversity, while Chao1 remained unchanged. These taxa occurred in very low proportions in the bulk soil, forming part of the so-called "rare microbial community members" (Pedrós-Alió 2012). Rare microbial community members include transient taxa or taxa on the way to extinction, while others may be dormant. Such dormant taxa may be awaiting a change in conditions in which to grow, acting as seed banks (Epstein 2009; Lennon and Jones 2011). Such rare taxa can greatly affect community dynamics upon change in conditions by growing from obscurity to abundance, termed "conditionally rare taxa" (CRT) by Shade et al (Shade et al. 2014). CRT are critical for ecosystem function over time (Chen et al. 2023), and respond sensitively to change in conditions (Hajishengallis et al. 2011). During growth of *Setaria viridis, Stipa bungeana,* and *Bothriochloa ischaemum* in soil on the Loess Plateau of China, rhizosphere microbial community composition shifted substantially from bulk soil, with increased relative abundance of *Proteobacteria* and *Bacteroidetes,* and decrease of *Actinobacteria* and *Acidobacteria* (Sun et al. 2018). Shifts in community composition impact the functional diversity such as nutrient transformation, and affect plant roots directly (Kandeler et al. 2002). Rare microbiota in coastal sands were also shown to respond to environmental changes, including nutrient availability (Gobet et al. 2012). The shift in nutrient avaibility through introduction of REC likely caused the CRT to act as a seed bank for the rapidly shifting community. Thus roots growing into bulk soil can be viewed as starters or initiators of environmental changes by providing nutrients to stimulate CRT to become active, akin to a germination factor to members of the seed bank.

4.3. **Some fast-growing taxa do not increase upon introduction of REC.**

Oligotrophs are highly adaptative in nutrient poor environment while copiotrophs grow in response to excessive nutrients (Koch 2001). Bulk soil does not receive substantial input of organic matter and would, therefore, be expected to benefit oligotrophs over copiotrophs. The infusion of organic material through root exudation should benefit copiotrophs, leading to a change in community composition, as seen in these results (Fig. 3c & 5). *Bacillus* and other Bacillaceae are saprophytes and copiotrophs, forming large colonies on agar surfaces in under 24h (Vilain et al. 2006). The infusion of sugars, amino- and organic acids into soil would be expected to support growth of diverse *Bacillus*. Surprisingly, the culturable count of *B. cereus sensu lato* did not increase upon infusion of REC. Bacillaceae constituted between 0.01-0.2% to below detection limit in DNA extracts, with no change during the 14d incubation period. These results show that the soil contained *Bacillus*, but that these did not respond to nutrient infusion by increasing population density. Selected *Bacillus* isolates were inoculated into water supplemented with REC at the standard application, as described in section 2.1, and growth to high optical density was observed within hours (Fig. 2d). This indicates that at least some of the *Bacillus* in the soil were able to grow using the REC cocktail as nutrient source. These results indicate that some factors other than nutrient restriction hindered growth of the *Bacillus* in this soil. Suggesting that the Bacillaceae in this soil were collectively susceptible to suppression by other bacteria in the soil. Several Bacillaceae produce antibiotics active against related taxa. *B. cereus* produces cereulide that suppresses other *Bacillus* (Tempelaars et al. 2011). Likewise, taxa like *Streptomyces* produce diverse antibiotics. *S. sannanensis* releases antibiotics effective against only Gram-positive bacteria (Singh et al. 2014). *Bacillus* are more susceptible to antibiotics than many other bacteria (Luna et al. 2007). In addition to antibiotics, vegetative *Bacillus* cells could have been targeted by bacteriovores such as *Bdellovibrio* (Stolp and Starr 1963; Pląskowska and Zakrzewska-

Czerwińska 2023). Bacteriophages abound in soil and could impact *Bacillus*. Diverse protozoa act as predators of bacteria, impacting their dominance and creating space for other bacterial species (Jousset et al. 2009; Winter et al. 2010).

5. Conclusion

Supplementation of root exudate cocktail (REC) into bulk soil was associated with profound shifts in community composition, with initial decrease in Shannon diversity, followed by further community shifts. These results show that the nutrients introduced to bulk soil by growing roots contribute to shifts in bulk soil bacterial community, which responds by developing into rhizosphere community. These shifts were dominated by many of the taxa able to grow rapidly, while the rapidly growing *Bacillus* did not change in prevalence. Factors associated with the dormant nature of the *Bacillus* endospore populations in wheat-field soils should be investigated.

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Figure S1 Twenty most prevalent taxa as a) Class, b) Order, and c) Family.