MICROBIAL SIGNATURES IN THE RHIZOSPHERE AND SURROUNDING BULK SOILS AND DIFFERENTIAL ABUNDANCE DUE TO WATERING FOR SWEET INDIAN MALLOW (*ABUTILON FRUTICOSUM*)

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Abstract. The present study aims to decipher microbiota signatures of rhizosphere soil of the medicinal plant *A. fruticosum* and surrounding bulk soil. The study also investigates differential response of microbes potentially serving to promote drought tolerance in the plant. Microbiomes of rhizosphere and bulk soils were collected after 0, 24 and 48 h of watering, deep sequenced and annotated to the different taxonomic ranks. The results strongly indicated higher relative abundance in rhizosphere soil microbiomes compared to those in the bulk soil of phyla Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes and Acidobacteria. Of these, growth of Acidobacteria and Firmicutes, in addition to Gemmatimonadetes, Melainabacteria, Elusimicrobia and Armatimonadetes, responded positively to watering across microbiome source. While Cyanobacteria was the only abundant phylum in bulk soil and showed lower abundance in rhizosphere soil due to watering. At the genus level, *Bacillus*, *Microvirga*, *Adhaeribacter*, *Sphingomonas*, *Arthrobacter* and *Pontibacter* are the most abundant in rhizosphere soil, while growth of genera *Ramlibacter*, *Haliangium*, *Gemmatimonas* and unidentified genera of taxon Acidobacteria significantly increased 24 h after watering. Results of the present study warrant comprehensive research to dissect factors influencing differential stress responses and plant-bacterial relationships in order to provide feasible soil management programs in the future.
Keywords: *drought, PGPB, OTU, alpha and beta indices, phylogenetic tree*

Introduction

Sweet Indian mallow (*Abutilon fruticosum*) is a drought tolerant perennial herb that belongs to the family Malvaceae (also known as Mallow family). This family is among the largest families of angiosperm that have high medicinal and economic potentials as many parts of this plant generate biological activities useful in the treatment of several human diseases, like piles, inflammation of the bladder, ulcers and rheumatism (Husain and Baquar, 1974; Patel and Rajput, 2013). Therefore, it is of crucial importance to monitor conservation processes of this important family and investigate ways to promote tolerance to drought stress in their native habitat. As plant species of genus *Abutilon* are not toxic to biological systems, they are eaten by wild and domesticated ungulates and birds. This medicinal plant is native to USA, Mexico, Africa and southwestern Asia including Saudi Arabia (Fryxell, 2002).

Desert plants harbor several metabolic strategies to tolerate drought stress, but very little is known about the possible contribution of the surrounding microflora in promoting abiotic stress tolerance in plants. Recent studies demonstrated that the microbiome in the surrounding root-soil interface, e.g., rhizosphere, plays a role in augmenting the plant's ability to withstand drought by changing microbiome structure (Xu et al., 2018). Rhizosphere is described as the hotspot of plant-microbe interaction

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within their soil environment. This spot is occupied by a diversity of microbial communities that are mutually influenced by intact plant and soil types (Philippot et al., 2013). For example, plant root exudes a variety of chemicals and nutritional compounds into the rhizosphere to attract a variety of microorganisms including bacteria and archaea (Mendes et al., 2013; Geng et al., 2018). Such attracted microbes harbor diverse metabolic potentialities that, in turn, promote plant growth and overall performance especially under abiotic stresses (Dennis et al., 2010; Dai et al., 2019). The latter interaction refers to the symbiotic relationship between plant and its intact microbes.

Some of the recent studies indicated that shifts in structure of microbiomes within the root endosphere and in the rhizosphere are in favor of the Actinobacteria and other Gram-positive taxa (Naylor et al., 2017; Timm et al., 2018; Xu et al., 2018, 2021; Xu and Coleman-Derr, 2019). Interestingly, these shifts are proportional to the strength and duration of drought and can rapidly diminish upon alleviation of the stress (Xu et al., 2018). Recent reports implicate the root external environment (rhizosphere) is involved in a wide range of stress responses in plant (Dai et al., 2019).

The present study aims at the detection of microbiota signature of rhizosphere soil of the medicinal plant *A. fruticosum* and its surrounding bulk soil, and differential response of microbes that can potentially serve in promoting plant growth under drought stress.

Materials and methods

Watering regime and collection of bulk and rhizosphere soils

The experiment was conducted 10 km away from Jeddah, Saudi Arabia in June (2021) in a spot in Southern Jeddah province that received no rainfall for at least three months prior experiment. We have assigned plots (1 m^2 each) of three single-grown plants of *Abutilon fruticosum* perennial herb with similar size, in addition to three neighboring bulk soil plots for the experiment. Distance between selected plots was ~10 m. Microbiomes of the two types of soils were harvested (between 10-30 cm depth) as previously described (Geng et al., 2018; Dai et al., 2019). Rhizosphere and bulk soil samples were collected at dawn after 0 (prior watering), 24 and 48 h of watering (25 l dH_2O/plot). The amount of water is based on the average amount received by rainfall in summer season. Harvested samples were immediately put in liquid nitrogen and transferred to the laboratory for storage until further DNA extraction.

Genomic DNA extraction and 16S rRNA sequencing

Total genomic DNAs of soil microbiome samples were extracted using CTAB/SDS method. DNA concentration and purity was monitored by electrophoresis on 1% agarose gels and concentration was adjusted to 1 ng/μL using sterile double-distilled water. Then, metagenomic DNA samples were shipped to Novogene (HK) Co., Ltd. (Singapore) for deep sequencing and bioinformatics analyses. The 16S rRNA gene at region V3-V4 was amplified using primers 515F/806R and PCR was carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). Amplicons were run on 2% agarose gel to detect quality and quantity and those with 400-450 bp length were chosen for further analysis. Then, amplicons were purified with Qiagen Gel Extraction kit (Qiagen, Germany) and libraries were generated with NEBNext® Ultra™ DNA Library Prep kit for Illumina platform. Deep sequencing generated ~250 bp paired-end (PE) raw reads that were quantified via Qubit and Q-PCR.

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Data processing, OTU analysis and taxonomic annotation

Nomenclature and grouping of raw read datasets were made for microbiome samples of surrounding bulk (S) and rhizosphere (R) soils of *Abutilon fruticosum* that were collected in three replicates at dawn after 0 (S11-S13 [group A] and R11-R13 [group D], respectively), 24 (S21-S23 [group B] and R21-R23 [group E], respectively) and 48 h (S31-S33 [group C] and R31-R33 [group F], respectively) of watering. The grouping type ABCDEF represent interaction between source of microbiome, e.g., rhizosphere and bulk soils, and time after watering, e.g., 0, 24 and 48 h. Raw data was also grouped based on source of microbiome regardless of time after watering, e.g., bulk (group J) and rhizosphere (group K) soils comprising grouping type JK, and on time after watering regardless of source of microbiome, e.g., 0 (group G), 24 (group H) and 48 h (group I) comprising grouping type GHI.

Raw data was then merged using FLASH (V1.2.7 (Magoč and Salzberg, 2011)) and raw tags were subjected to quality filtering to obtain high-quality clean tags (Bokulich et al., 2013) consulting QIIME software V1.7.0 (Caporaso et al., 2010). The tags were compared with the reference database (SILVA database, http://www.arb-silva.de/) in order to detect chimeric sequences for removal using UCHIME algorithm (Edgar et al., 2011). Then, effective tags were analyzed using Uparse software V7.0.1090 (Edgar, 2013), where sequences with $\geq 97\%$ similarity were assigned to the same OTU. Representative sequences of different OTUs were screened for annotation. Then, QIIME software V1.7.0 (Altschul et al., 1990) in Mothur method was performed against SSUrRNA dataset of SILVA database to annotate taxa with threshold of 0.8-1 at taxonomic levels including kingdom, phylum, genus and species and to obtain taxa-based abundance distribution (Quast et al., 2012).

Alpha diversity analysis

Statistical indices of alpha diversity (e.g., Shannon and Simpson) (at p value < 0.05) were generated with QIIME software V1.7.0 and displayed with R software V2.15.3 to detect richness and evenness of microbial communities of individual samples, respectively (Core Team, 2013; Li et al., 2013). Boxplots were formed to analyze significant differences in the two alpha diversity indices between (e.g., grouping type JK) and among (e.g., grouping types ABCDEF and GHI) groups using t-test and Tukey, respectively. Biodiversity rarefaction curves were also created by randomly selecting certain amount of sequencing data from each sample, then counting the number of the represented species (i.e., the number of OTUs). The sequencing data volume was detected for rationality of the overall analysis as steep curve indicates that many species remain to be discovered, while flat curve indicates that data volume is saturated and all species, except scarce species, were detected (Lundberg et al., 2013). Then, species accumulation boxplot or curve (Specaccum) was generated where gradual increase of the curve occurs with the increase of number of sequenced samples until the curve is flattened. This test determines the possibility of collected microbiomes to satisfy all microbes in the soil metagenomes.

Beta diversity analysis

Analysis of similarity (anosim) was performed by R software (Vegan package: anosim function) as a comparative tool of microbial communities at the groups level. Anosim boxplots were generated, where Y-axis represents the distance rank between

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samples, and X-axis represents the results between groups. R value generally stands between 0 and 1, where value close to 0 with $P \leq 0.05$ indicates no significant inter- or intra-group differences, while value close to 1 indicates that inter-group differences are greater than intra-group differences.

Beta diversity indices to assess the differences or distances between or among microbial communities were estimated using weighted and unweighted unifrac matrices of QIIME software V1.7.0. The data matrices have generated heatmaps of weighted and unweighted unifrac beta diversity and also used in measuring unweighted pair-group method with arithmetic means (UPGMA) as a hierarchical clustering method and in measuring principal coordinate analysis (PCoA) that ensures incorporation of ecological distance. UPGMA method is used to construct phylogenetic trees with relative abundance of each sample by phylum (Lozupone and Knight, 2005; Lozupone et al., 2007, 2011). PCoA is displayed by WGCNA package, stat packages and ggplot2 package in R software V2.15.3.

Evolutionary phylogenetic trees of representative sequences for individual samples and their grouping types at genus level (the top 100 genera in abundance) were also constructed using MUSCLE V3.8.31 (Edgar, 2004) and relative abundance of each genus in each tree was displayed. Finally, taxa of each sample or group at the taxonomic ranks phylum, genus and species were selected based on statistical significance at levels of sample (S11-S33 and R11-R33) and different grouping type (e.g., ABCDEF, JK and GHI) in order to detect the taxa with differential abundance and their proportions.

Results

In this study, deep sequencing of 16S rRNA partial-length gene was performed for microflora of rhizosphere soil of the medicinal plant *Abutilon fruticosum* and its surrounding bulk soil in order to explore microbe signatures in the two soil types and microbe's differential abundance due to watering. We have ensured that water is maintained and its amount (25 liters dH_2O/m^2) is enough to keep the soil moist for at least 48 h. This amount was based on the fact that maximum rainfall amount in the selected spot is ~25 cm, therefore, the amount of water to be received by individual plant in 1 m^2 plot equals 25000 cm^3. We speculated that microflora will immediately respond to watering by changing diversity, an approach to adapt to the new environmental condition. Therefore, we thought we will have the chance to detect differential growth patterns and responses of microbes after watering and elevating the stress. We also compared the influence of the plants interacting with their intact microbes with the microbes of surrounding bulk soil in terms of alpha and beta diversities.

Statistics of 16S rRNA sequencing data

Illumina MiSeq was used in analyzing microbiomes of rhizosphere soil of *Abutilon fruticosum* and surrounding bulk soil in three replicates after 0, 24 and 48 h of watering and raw statistical data is shown in *Table 1*. Nomenclature of microbiome grouping types was decided based on the interaction between source of microbiome and time after watering (e.g., grouping type ABCDEF and derivatives), regardless of time after watering (e.g., grouping type JK), and regardless of microbiome source (e.g., grouping type GHI and derivatives). The sequence length per read ranges

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between 409-416 bp with average raw, clean and effective tag numbers of 191474, 187706 and 142646, respectively. Average percentages of Q20 and Q30 with sequencing error rates of < 1 and 0.1% are 97.64 and 92.85%, respectively, while average percentage of effective tags in raw data is 68.58% (*Table 1*). The data in *Figure 1* indicates that average number of OTUs per sample is as high as 2563, while numbers of total, taxon and unique tags are 142646, 133598 and 9046, respectively. As expected, average percentage of archaeal sequences was far lower (0.43%) than that of bacterial sequences (99.57%) (*Fig. A1* in the *Appendix*). The data in *Figure A2* indicates that number of taxa taged per sample at the genus level was, expectedly, much higher than that at the species level. Sequences taged with unidentified species of a given genus refer to new species.

Table 1. *Statistics of raw sequencing data of microbiomes collected from surrounding bulk (S) and rhizosphere (R) soils of Abutilon fruticosum in three replicates after 0 (S11-S13 [group A] and R11-R13 [group D], respectively), 24 (S21-S23 [group B] and R21-R23 [group E], respectively) and 48 h (S31-S33 [group C] and R31-R33 [group F], respectively) of watering. Samples data were also grouped based on source of microbiome regardless of time after watering, e.g., bulk (group J) and rhizosphere (group K) soils and on time after watering regardless of source of microbiome, e.g., 0 (group G), 24 (group H) and 48 h (group I)*

Group			Sample Name	Raw PE (no.)	Raw tags (no.)	Clean tags (no.)	Effective tags (no.)	Base (nt no.)	Avg. len. (nt)	Q20 (%)	Q30 (%)	GC %	Effectivity %
A	J	G	S11	202,329	186,930	183,790	154,094	63,095,793	409	97.74	93.11	55.32	76.16
			S12	206,537	190,785	187,527	148,590	60,878,051	410	97.69	92.92	55.36	71.94
			S13	218,208	201,086	197,323	155,552	63,785,697	410	97.72	93.12	55.65	71.29
B		H	S21	197,737	183,095	179,734	139,042	57,092,281	411	97.69	92.96	55.76	70.32
			S22	209,771	194,101	190,670	154,235	63,478,777	412	97.66	92.86	55.36	73.53
			S23	208,498	192,432	188,865	146,495	60,307,978	412	97.74	93.10	55.4	70.26
C		I	S31	204,937	187,333	183,688	150,196	61,669,902	411	97.62	92.81	55.28	73.29
			S32	185,214	171,658	168,277	125,520	51,521,913	410	97.72	92.91	55.23	67.77
			S33	209,161	192,144	188,426	145,921	59,991,150	411	97.68	92.98	55.41	69.76
D	K	G	R11	208,662	191,660	187,621	144,935	60,221,366	416	97.62	92.83	55.85	69.46
			R12	206,340	189,234	185,407	137,133	56,884,636	415	97.63	92.87	56.20	66.46
			R13	217,927	200,569	196,680	149,840	62,190,891	415	97.63	92.84	56.17	68.76
E		H	R21	216,328	197,344	193,135	145,040	60,218,141	415	97.50	92.51	55.91	67.05
			R22	204,765	187,535	183,319	127,405	52,939,215	416	97.53	92.60	56.21	62.22
			R23	207,956	191,328	187,303	133,975	55,594,046	415	97.58	92.71	56.27	64.42
F		I	R31	204,624	189,285	185,563	131,411	54,479,082	415	97.72	92.92	55.92	64.22
			R32	219,603	199,398	194,673	139,841	57,776,395	413	97.48	92.50	56.67	63.68
			R33	216,950	200,607	196,714	138,410	57,370,972	415	97.65	92.77	56.31	63.80

Raw PE no. represents number of original paired-end (PE) reads after sequencing. Raw tags no. represents number of tags merged from PE reads. Clean tags no. represents number of tags after filtering. Effective tags no. represents number of tags after filtering chimera and can be finally used for subsequent analysis. Base nt no. is the number of bases of the Effective Tags. Avg. len. (nt) represents average length of Effective Tags. Q20 and Q30 are the percentages of bases whose quality value in Effective tags is greater than 20 (sequencing error rate is less than 1%) and 30 (sequencing error rate is less than 0.1%). GC (%) represents GC content in Effective Tags. Effectivity (%) represents the percentage of Effective Tags in Raw PE

Description of the raw sequencing data along with the recovered OTUs at different bacterial taxonomic levels (kingdom, class, order, family, genus and species) is shown in *Table S1*. The total number of OTUs across samples is 4562 of which number of archaeal OTUs is 29. Number of OTUs with average number of sequencing reads over

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1000 is 12. Of which, the largest average number of reads was assigned to OUT1 (~26343), followed by OTU4 (~4825), OTU7 (~3671) and OTU2 (2514). These OTUs refer to unidentified genus of taxon Cyanobacteria, unidentified species of genus *Microvirga*, *Bacillus niacini* and unidentified species of genus *Adhaeribacter*, respectively (*Table S1*).

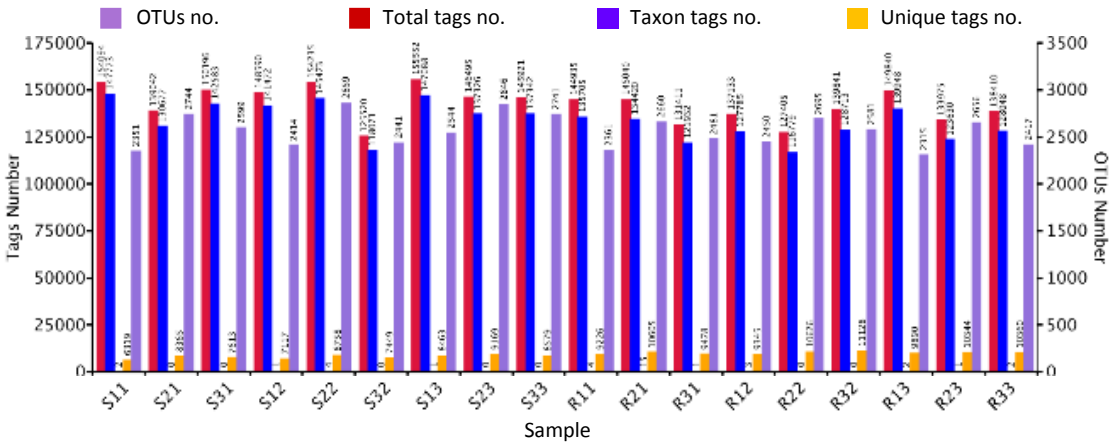

Figure 1. *Description of Tag and OTU information of microbiomes collected from surrounding bulk (S) and rhizosphere (R) soils of Abutilon fruticosum three replicates after 0 (S11-S13 and R11-R13, respectively), 24 (S21-S23 and R21-R23, respectively) and 48 h (S31-S33 and R31-R33, respectively) of watering. OTUs no. (Purple bars) refers to the number of OTUs to identify the numbers of OTUs in different samples. Total tags no. (Red bars) refer to the number of effective tags; Taxon Tags no. (Blue bars) refer to the number of annotated tags; Unique tags no. (Orange bars) refers to the number of tags with a frequency of 1 and only occurs in one sample*

Alpha diversity analysis

The results for the number of observed species (S_{obs}) at the individual level were not influenced by the source of the microbiome or time after watering, while those for Shannon and Simpson indices significantly indicated higher index values for individual bulk soil microbiomes compared with those of rhizosphere soil (*Fig. A3*). Similar results were reached at the grouping type level, where Shannon and Simpson values were significantly higher in grouping type DEF (or group K) than in grouping type ABC (or group J) (*Fig. 2*). This indicates that taxa richness and evenness are significantly higher in the rhizosphere soil than in the bulk soil.

In terms of microbiomes collected at different times after watering across microbiome source (e.g., grouping type GHI), there were no distinctive differences in both Shannon and Simpson indices (*Fig. 2*).

Rarefaction curves indicated that the maximum depth permitted to retain all samples in the dataset is ~120,000 sequence reads (*Fig. A4*). The curves were about to be flattened indicating that microbial data volume is almost saturated. This conclusion was confirmed via the species accumulation curve or Specaccum (*Fig. A5*), where the increase of number of individual sequenced samples resulted in the gradual increase of the curve until it is flattened indicating that number of samples (18) in this experiment is comprehensive.

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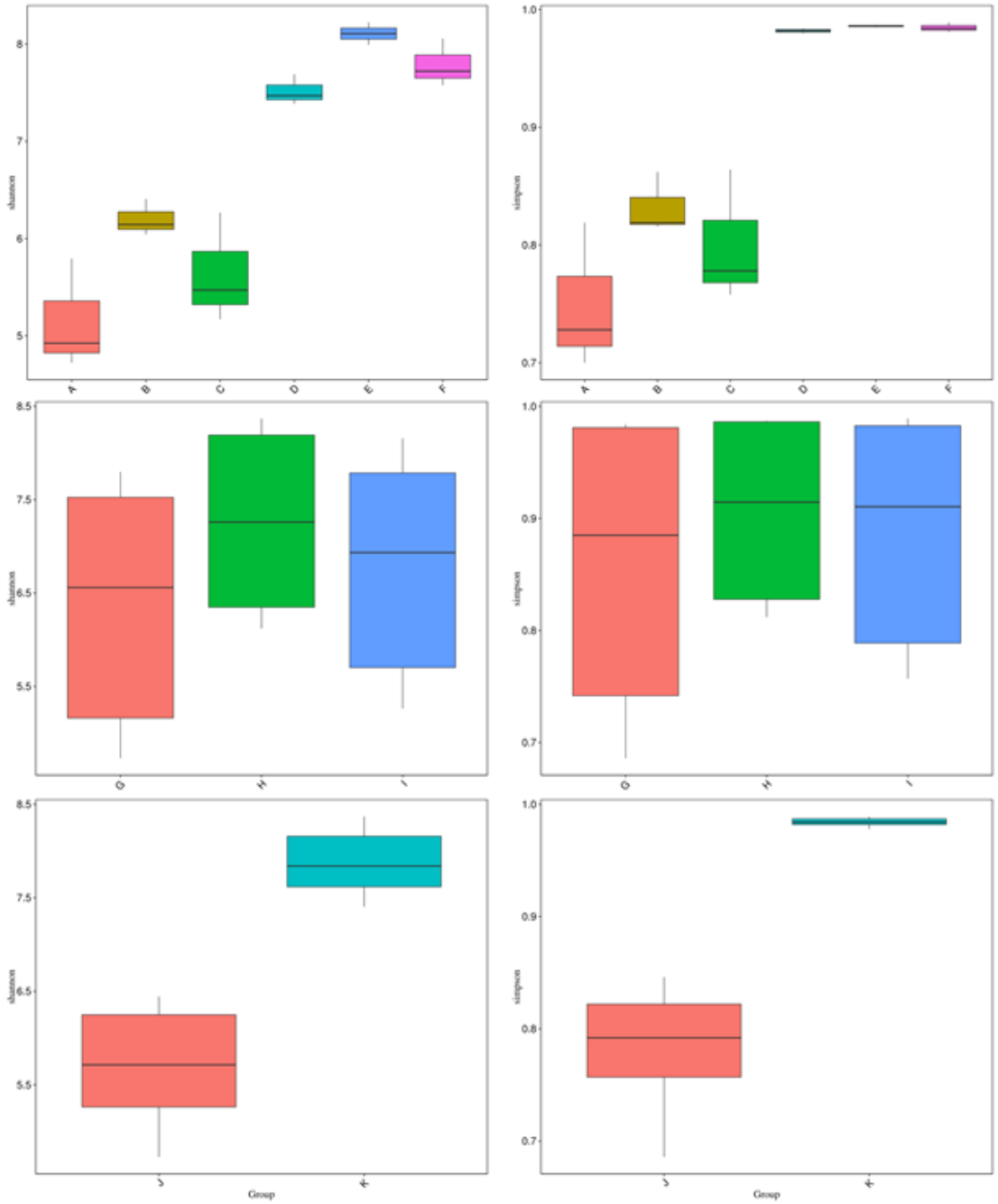

Figure 2. *Boxplots of Shannon and Simpson alpha diversity measures referring to taxa richness and evenness, respectively, of microbiomes collected from surrounding bulk (grouping type ABC) and rhizosphere (grouping type DEF) soils of Abutilon fruticosum after 0 (grouping type AD), 24 (grouping type BE) and 48 h (grouping type CF) of watering. Samples data were also grouped based on source of microbiome regardless of time after watering, e.g., bulk (group J) and rhizosphere (group K) soils and on time after watering regardless of source of microbiome, e.g., 0 (G), 24 (group H) and 48 h (group I)*

Beta diversity analysis

Anosim results at the level of grouping type and derivatives of JK, and grouping type GHI (e.g., GH, GI and HI) are shown in *Figure A6*. Anosim is a nonparametric test

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displayed as boxplots to be used in justifying the reason of dividing groups and evaluating whether variation among groups is significantly higher or lower than variation within groups. The results of anosim boxplots indicate that R values between groups G and H (R = 0.15, P = 0.081), between G and I (R = 0.046, P = 0.519) and between H and I (R = 0.46, P = 0.245) refer to insignificant inter and intra-group differences in global microbiome structure. On the other hand, anosim boxplot between groups J and K showed an R value of 1 (P = 0.001) indicating that inter-group differences are significantly greater than intra-group differences. This indicate that grouping of bulk soil (grouping type ABC or group J) and rhizosphere soil (grouping type CDE or group K) has high rationality (*Fig. A6*), while grouping of microbiomes regardless of microbiome source at the three time points 0 (grouping type AD or group G), 24 (grouping type BE or group H) and 48 h (grouping type CF or group I) after watering has low rationality. Therefore, we expected to detect very few taxa with significant abundance due to watering regardless of microbial soil source.

Heat maps of weighted and unweighted unifrac beta diversity measures at the individual (*Fig. A7*) and group (*Fig. 3*) levels as well as dendrogram trees or hierarchical clustering by phylum (*Fig. A8*) were generated.

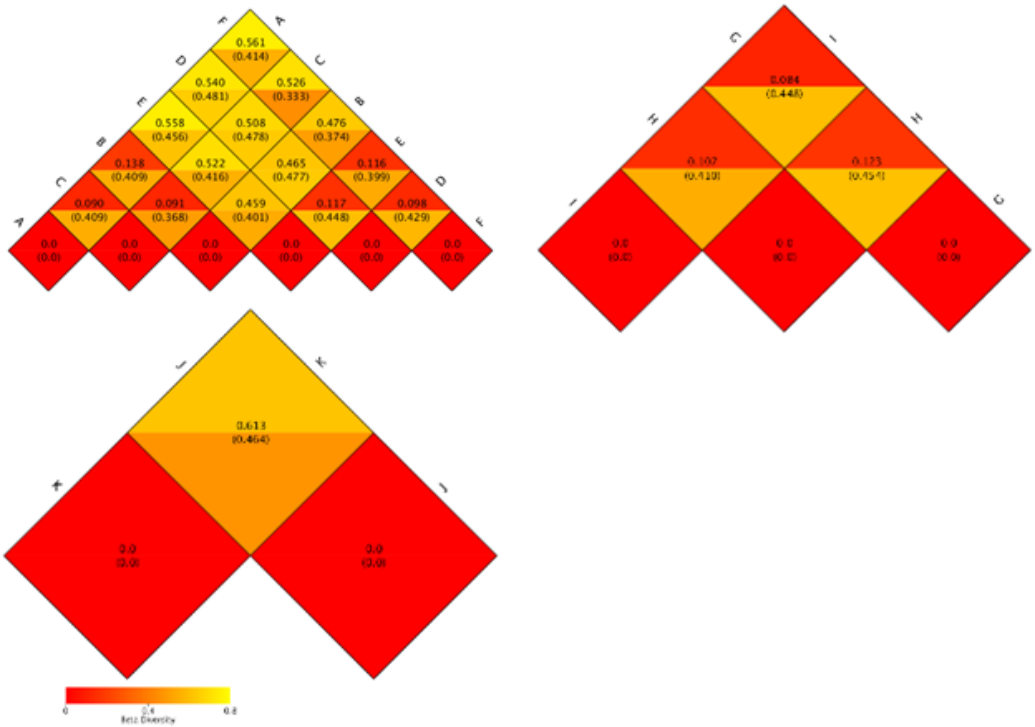

Figure 3. *Heatmaps of weighted (top record) and unweighted unifrac beta diversity measures of microbiomes collected from surrounding bulk (grouping type ABC) and rhizosphere (grouping type DEF) soils of Abutilon fruticosum after 0 (grouping type AD), 24 (grouping type BE) and 48 h (grouping type CF) of watering. Samples data were also grouped based on source of microbiome regardless of time after watering, e.g., bulk (group J) and rhizosphere (group K) soils and on time after watering regardless of source of microbiome, e.g., 0 (G), 24 (group H) and 48 h (group I)*

Weighted uniFrac refers to the relative abundance of sequences, while unweighted uniFrac refers to the unique species in the environmental samples (Lozupone et al.,

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2007, 2011). The results of weighted unifrac heat map and hierarchical cluster at the individual level indicated distinctive separation between microbiomes of bulk soil (S11-S33) and rhizosphere soil (R11-R33) samples. While, unweighted unifrac results indicated no distinctive distances based on the unique microbes in either source of soil microbiome samples (*Fig. A8*). The weighted and unweighted unifrac heatmaps in *Figure 3* for grouping types ABCDEF and JK align with those at the individual level, while those for grouping type GHI indicated no distinctive differences either with weighted or unweighted unifrac matrices. The latter results support our claim of focusing more on the interactive influence of time after watering and microbiome source in addition to the influence of microbiome source regardless of time after watering.

When uniFrac is measured solely, it refers to the phylogenetic information of environmental samples, while when coupled with standard multivariate statistical techniques, it refers to principal coordinates analysis (PCoA) to map distances among microbial communities. When similarity among individual samples or groups is high, then they are closely located. The results at the grouping types ABCDEF and JK in *Figure 4* indicate complete separation between microbiomes of bulk and rhizosphere soils as the latter (grouping type DEF or group K) are located in the positive direction of PCoA 1 (PC1), while bulk soil microbiomes (grouping type ABC or group J) are located in the negative direction of PCoA 1 (PC1). Interestingly, at the PCoA 2 (PC2) level, grouping type BE (or group H) is located in the positive direction, while grouping types AD (or group G) and CF (or group I) are located in the negative direction (*Fig. 4*). The latter results indicated the high tendency of gathering microbiomes collected after 24 h, e.g., grouping type BE (or group H), compared with microbiomes collected after 0 or 48 h of watering, e.g., grouping type AD (or group G) or CF (or group I), respectively. The latter conclusion is further supported by the existence of microbiome samples S11-S13 (or group A) and S31-S33 (or group C), on one hand, and existence of microbiome samples R11-R13 (or group D) and R31-R33 (or group F), on the other hand, in close vicinity. While, microbiome samples S21-S23 (or group B), on one hand, and R21-R23 (or group E), on the other hand, tend to be located far from their respective source (S or R) groups (*Fig. 4*).

Evolutionary phylogenetic trees of representative sequences by genus (the top 100 genera in abundance) for individual samples (*Fig. A9*) and their grouping types, e.g., ABCDEF (*Fig. A10*), JK (*Fig. A11*) and GHI (*Fig. A12*) were constructed. They indicated high abundance of the unidentified genera of *Cyanobacteria* and *Rickettsiales* families in bulk soil microbiomes, while genera *Bacillus*, *Ammoniphilus*, *Adhaeribacter*, *Pontibacter*, *Arthrobacter* and *Microvirga* in rhizosphere soil microbiomes (*Fig. A9-A11*). For grouping type GHI, only genus *Massilia* showed lower abundance in H group of microbiomes collected 24 h after watering compared with the other two groups G and I of microbiomes collected 0 and 48 h after watering, respectively (*Fig. A12*).

Results of relative abundance of individual microbiome samples as well as microbiome groups and grouping types in the bulk and rhizosphere soils of *A. fruticosum* at phylum level is shown in *Figures 5, A13, A18, A21* and *A24*. Abundance (of the phylogenetic trees) and relative abundance at the genus level is shown in *Figures 6, A9-A12, A14, A15, A19, A22* and *A25*, while relative abundance at the species level is shown in *Figures 7, A16, A17, A20, A23 and A26.*

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Relative abundance of individual microbiome samples was detected for selected taxa at the taxonomic ranks phylum, genus and species (*Figs. A13, A14* and *A16*, respectively). Taxa selection was based on statistical significance of relative abundance at the three taxonomic ranks for grouping types ABCDEF (*Figs. A18-A20*, respectively), JK (*Figs. A21-A23*, respectively) and GHI (*Figs. A24-A26*, respectively). Genera with differential abundance across grouping types belong to seven phyla as shown in the heat map of *Figure A15*. It is clear that the largest number of taxa with significantly different relative abundance at the three taxonomic levels was detected for grouping type JK. It is worth noting that species *Bacillus niacini* showed differences within replicates of rhizosphere soil microbiomes after 0 (e.g., samples S11-S13 or group D) and 48 h (e.g., samples S31-S33 or group F) of watering (*Fig. A17*), although this species showed significant high abundance in group K (*Figs. 7, A16* and *A23*).

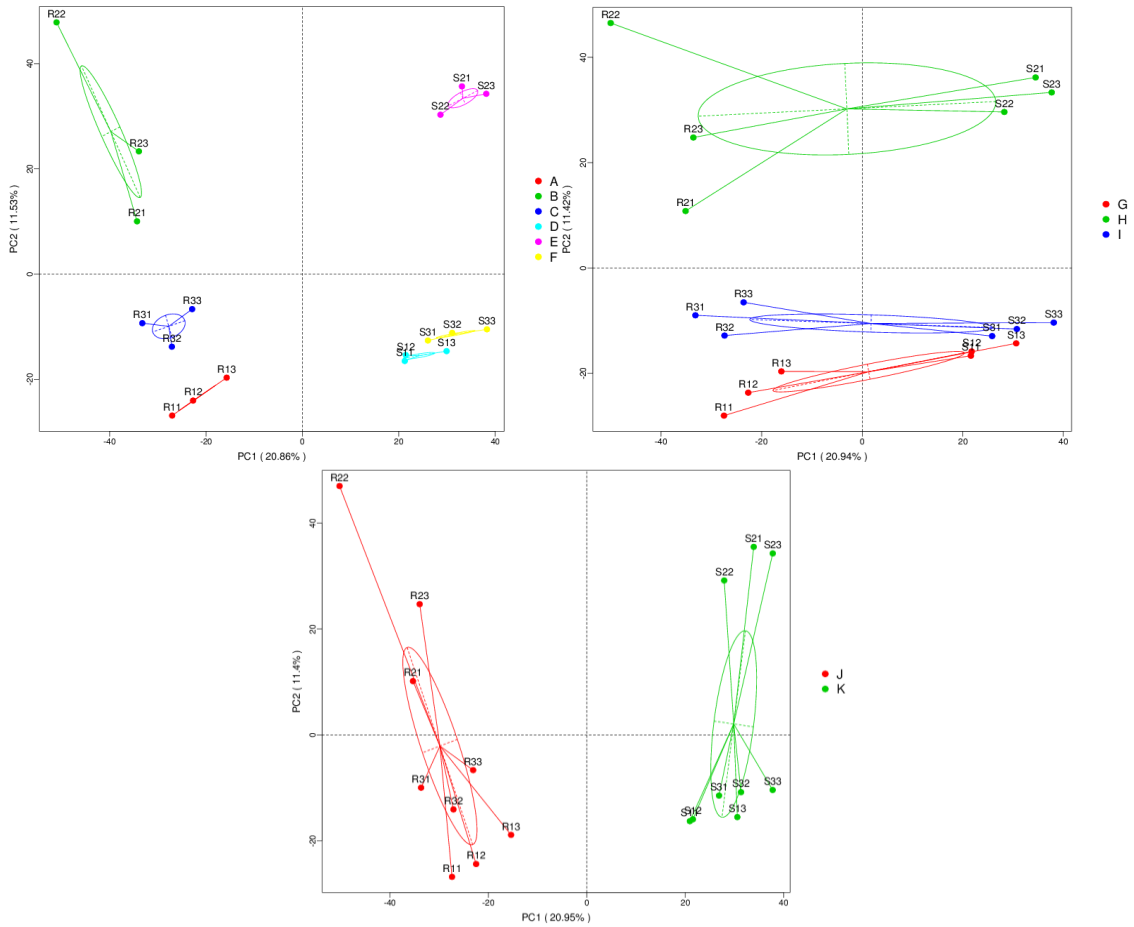

Figure 4. *Principle coordinate analysis (PCoA) based on OTU abundance of microbiomes collected from surrounding bulk (grouping type ABC) and rhizosphere (grouping type DEF) soils of Abutilon fruticosum after 0 (grouping type AD), 24 (grouping type BE) and 48 h (grouping type CF) of watering. Samples data were also grouped based on source of microbiome regardless of time after watering, e.g., bulk (group J) and rhizosphere (group K) soils and on time after watering regardless of source of microbiome, e.g., 0 (group G), 24 (group H) and 48 h (group I). A colored dot represents a given sample in one group, and similar colored dots refer to samples of the same group. X-axis is the first principal coordinate and Y-axis is the second. Number in brackets represents contributions of PCoA to differences among samples*

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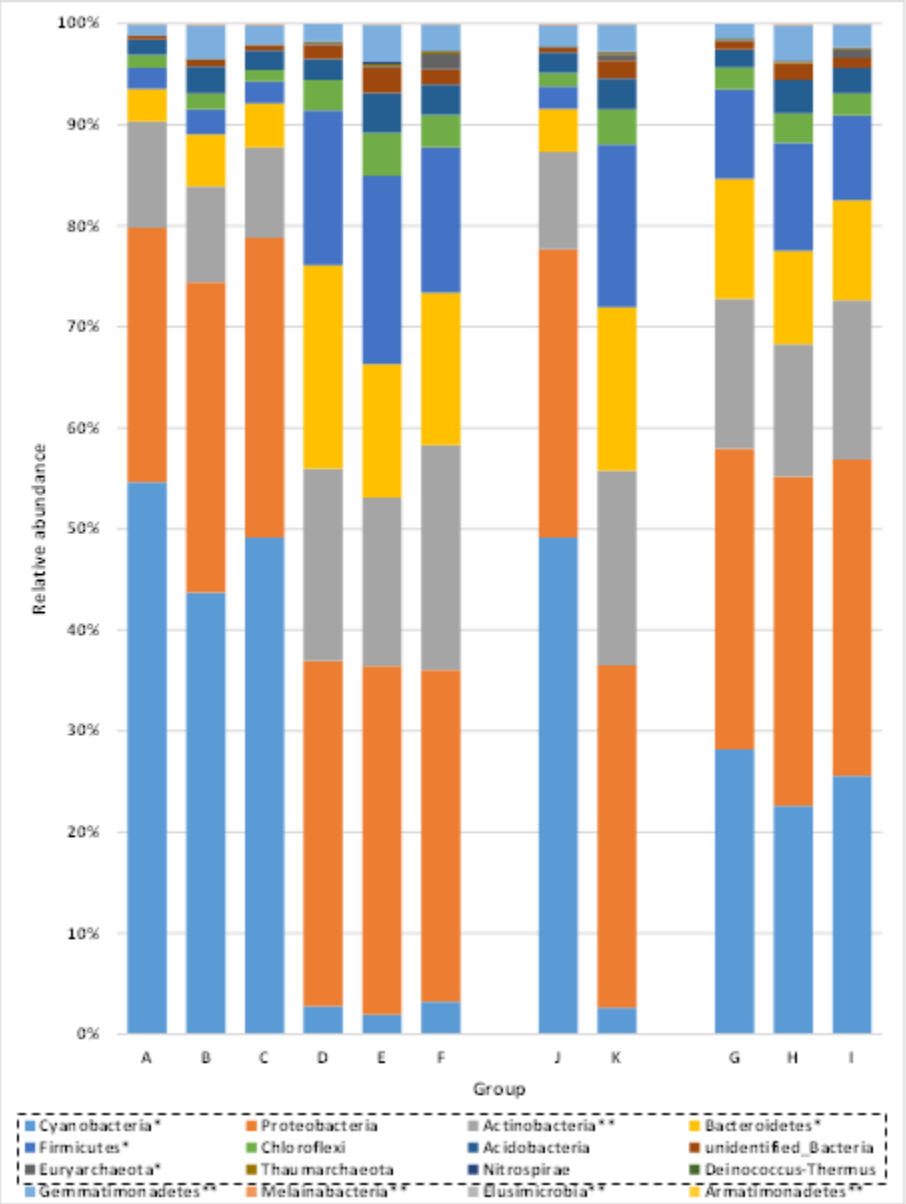

Figure 5. *Relative abundance at the phylum level within different grouping types of taxa that showed significant differences in microbiomes collected from surrounding bulk (grouping type ABC) and rhizosphere (grouping type DEF) soils of Abutilon fruticosum after 0 (grouping type AD), 24 (grouping type BE) and 48 h (grouping type CF) of watering. Samples data were also grouped based on based on source of microbiome regardless of time after watering, e.g., bulk soil (group J) and rhizosphere soil (group K), and time after watering regardless of source of microbiome, e.g., 0 (group G), 24 (group H) and 48 h (group I). Taxa with (*) refer to occurrence of significant differences within grouping type ABCDEF, taxa with (**) refer to occurrence of significant differences within grouping type GHI, while first 12 taxa (inside the dotted black box) refer to occurrence of significant differences between grouping type JK. Statistical analysis is shown in Metastat boxplots of Figures A18-A26*

For grouping type analysis, relative abundance of microbiomes was studied for taxa at the three taxonomic levels, e.g., phylum, genus and species (*Figs. 5-7*, respectively). The results of grouping type ABCDEF significantly indicated higher relative abundance

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in rhizosphere soil microbiomes compared with those in bulk soil of two bacterial phyla, e.g., Bacteroidetes (e.g., groups D vs. A) and Firmicutes (e.g., groups E vs. B) and an archaeal phylum, e.g., Euryarchaeota (e.g., group F vs. grouping type ABCDE) (*Figs. 5* and *A18*). Opposite results were reached for phylum Cyanobacteria that significantly showed higher relative abundance in bulk soil microbiomes compared with their respective ones in rhizosphere soil (e.g., groups B vs. E and groups C vs. F). The results of the above-mentioned four phyla of grouping type ABCDEF almost align with theirs at the grouping type JK (*Figs. 5* and *A21*). Grouping type JK for the other eight phyla (seven bacterial and one archaeal) significantly showed higher relative abundance in rhizosphere soil microbiomes (group K) compared with those in bulk soil (group J). These phyla include Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria and Deinococcus-Thermus and a new unidentified bacteria as well as the archaeal phylum Thaumarchaeota (*Fig. A21*).

At the genus level, *Ramlibacter* of grouping type ABCDEF showed significantly higher relative abundance in microbiomes of group C than that in group A (*Figs. 6* and *A19*). Other genera include *Planococcus*, *Procabacter* and *Raineyella.* Relative abundance of genus *Planococcus* in rhizosphere soil microbiomes of group E was significantly higher than that in bulk soil microbiomes of group B. Relative abundance of genus *Procabacter* in rhizosphere soil microbiomes of group D was significantly higher than that of group F, while that of genus *Raineyella* showed unexplainable significant differences (*Fig. A19*). The results of grouping type JK indicated that relative abundance in 10, out of 12, phyla was significantly higher in rhizosphere soil microbiomes (group K) than those in bulk soil (group J). These genera are *Bacillus*, *Microvirga*, *Adhaeribacter*, *Pontibacter*, *Nocardioides*, *Arthrobacter*, *Sphingomonas*, *Ammoniphilus*, *Rubellimicrobium* and unidentified bacteria. While, opposite results were reached for unidentified genera of taxa Cyanobacteria and Rickettsiales (*Figs. 6* and *A22*).

At the species level, relative abundance of *Acidobacteria* bacterium WWH111 and *Bdellovibrio* sp. oral clone CA006 was significantly higher in microbiomes of group A than that of group D, while in group D than that group F of bacterium YC-ZSS-LKJ66 (*Figs. 7* and *A20*). In terms of the significant differences in relative abundance in grouping type JK at the species level, eight taxa showed higher significance values in rhizosphere soil microbiomes (group K) compared with those of bulk soil (group J). These taxa are *Bacillus niacini*, *Arthrobacter subterraneus*, *Blastococcus aggregatus*, soil bacterium WF55, *Bacillus selenatarsenatis*, *Sphingomonas wittichii*, *Bacillus funiculus* and *Rhodocista* sp. SCSIO 13435 (*Figs. 7* and *A23*). Opposite results were reached for *Amycolatopsis ruanii*, *Massilia albidiflava*, *Streptomyces mutabilis* and *Aquabacterium citratiphilum*, where relative abundance was significantly higher in bulk soil microbiomes (group J) compared with those in rhizosphere soil (group K). Interestingly, genus *Massilia* showed lower abundance regardless of soil microbiome sources 24 h after watering compared with microbiome sources collected 0 and 48 h after watering (*Fig. A11*).

The results of grouping type GHI indicated significant differences of relative abundance for five phyla, e.g., Acidobacteria, Gemmatimonadetes, Melainabacteria, Elusimicrobia and Armatimonadetes (*Figs. 5* and *A24*). Interestingly, relative abundance of these phyla significantly increased after 24 h of watering regardless of microbiome source (group H), while original abundance was significantly restored 48 h after watering (group I) for the two phyla Elusimicrobia and Gemmatimonadetes. Phylum Elusimicrobia is known as a rare bacterial phylum in the matagenomic data (Wei et al., 2017). Similar results were reached at the genus and species levels for other

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taxa, where relative abundance of the four genera *Ramlibacter*, *Haliangium*, *Gemmatimonas* and unidentified genera of taxon Acidobacteria (*Figs. 6* and *A25*) as well as the two species of *Acidobacteria* bacterium LP6 and SCN69-37 (*Figs. 7* and *A26*) significantly increased after 24 h of watering (group H).

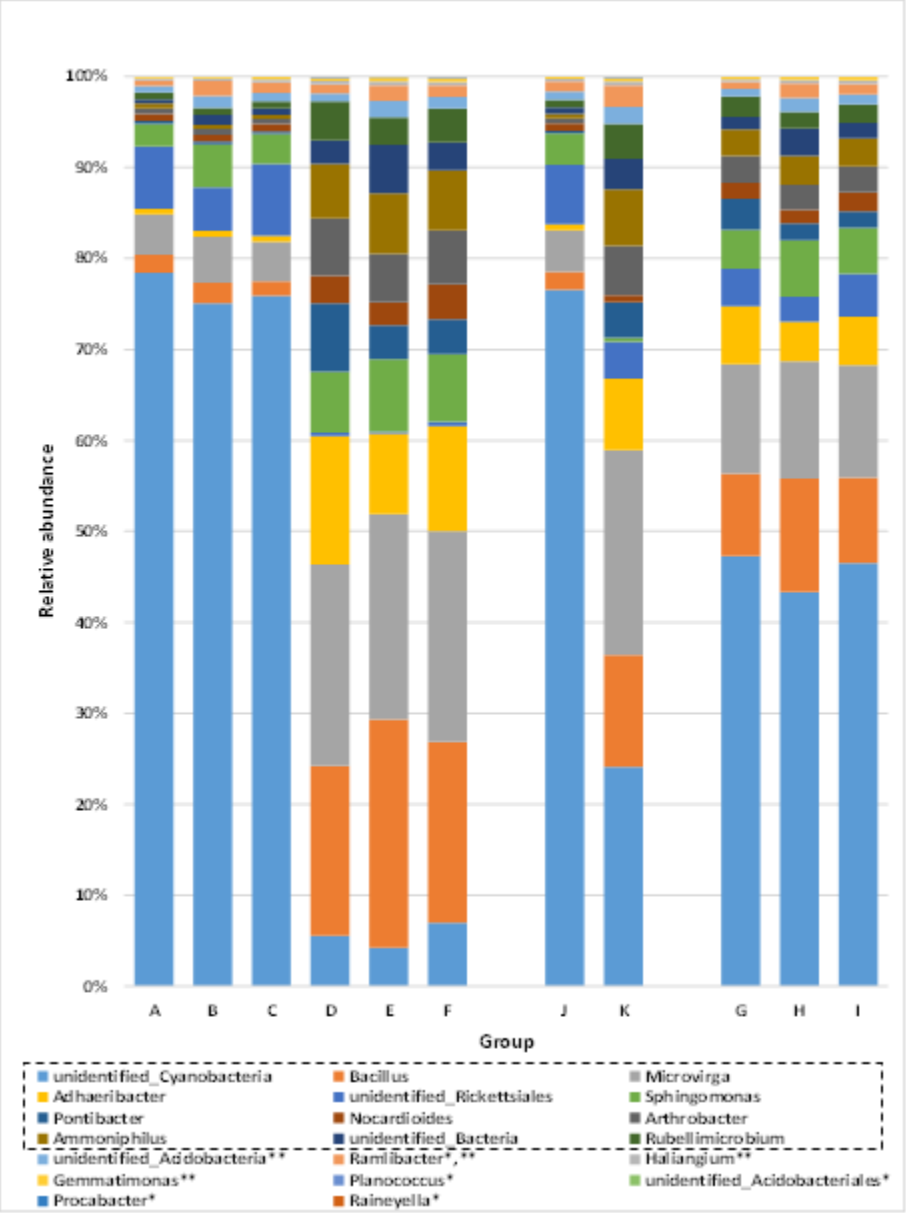

Figure 6. *Relative abundance at the genus level within different grouping types of taxa that showed significant differences in microbiomes collected from surrounding bulk (grouping type ABC) and rhizosphere (grouping type DEF) soils of Abutilon fruticosum after 0 (grouping type AD), 24 (grouping type BE) and 48 h (grouping type CF) of watering. Samples data were also grouped based on based on source of microbiome regardless of time after watering, e.g., bulk soil (group J) and rhizosphere soil (group K), and time after watering regardless of source of microbiome, e.g., 0 (group G), 24 (group H) and 48 h (group I). Taxa with (*) refer to occurrence of significant differences within grouping type ABCDEF, taxa with (**) refer to occurrence of significant differences within grouping type GHI, while first 12 taxa (inside the dotted black box) refer to occurrence of significant differences between grouping type JK. Statistical analysis is shown in Metastat boxplots of Figures A18-A26*

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Discussion

Differential abundance due to microbiome source

(a) Phylum level

Rhizosphere soil is a composite of root surface soil and soil around roots, while bulk soil refers to the area near the selected plants where the closest growing flora is at least 10 m^2 away. In the present study, relative abundance of phylum Cyanobacteria (*Figs 5* and *A21*) or its descendent unidentified genus of family Cyanobacteria (*Fig. 6* and *A22*) is significantly higher in bulk soil compared with that in rhizosphere soil. Cyanobacteria is an above-ground phylum and genera of Gram-negative bacteria representing a major component of the surface soil biota that has internal membranes of flattened sacs, named thylakoids, to obtain energy via photosynthesis (Liesack et al., 2000; Sinha and Häder, 2008; Liberton et al., 2013; Ranjan et al., 2016). Then, this microbe can exist independently in bulk soil as its favorite option, but it can also initiate symbiotic associations in rhizosphere soil with a diverse range of plant kingdom members as the alternative option. The symbiotic association represents a phenomenon similar to legume-rhizobium symbiosis (Nilsson et al., 2005) as microbes of this phylum can act as a cyanobiont receiving its main carbon source from the host, while providing, in return, nitrogen-rich products (citrulline and glutamate) to the host (Lindblad et al., 1991; Bergman et al., 2007; Cuddy et al., 2012). The microbe also has a positive role in plant growth by promoting substances such as IAA and provides organic matter, amino acids, vitamins and oxygen to plant rhizosphere. The microbe can also ameliorate salinity and solubilize phosphates to be available to the plant roots (Prasanna et al., 2009). We speculate that this association will not be mandatory after watering and plant will be able to survive independently until water diminishes again or, otherwise, the plant might associate with other microflora that have alternative mechanisms to promote its growth under normal or drought condition.

Prior phylogenetic studies of Cyanobacteria revealed the natural occurrence of 30 OTUs in the soil. In the present study, a number of 32 OTUs were generated of which OTU1 comprises the most abundant taxon across bulk and rhizosphere soils (*Table S1*). Interestingly, none of the OTUs of this phylum was identified in the present study down the phylum level indicating that new versions of this microbe exist in the rhizosphere soil of *A. fruticosum* and surrounding bulk soil. A scientific evidence indicates that each individual plant root might host a unique taxon of cyanobionts (Costa et al., 1999; Gehringer et al., 2010). This observation might, at least, explain the high variation in the cyanobiont diversity in rhizosphere soil of *A. fruticosum*.

Phyla Bacteroidetes, Firmicutes, Actinobacteria, Acidobacteria, Chloroflexi and Proteobacteria were proven to mutually dominate rhizospheres of several plants possibly as a result of plant's specific root exudates (Lundberg et al., 2012; Dai et al., 2019; Zhang et al., 2019). In the present study, these bacterial phyla were proven to favor rhizosphere soil for growth (*Fig. 5* and *A21*) indicating that root exudates of *A. fruticosum* represents a main factor influencing proper growth of these bacteria. Many reports indicated that microbial abundance in rhizosphere soil can be as high as 10-1000 times that in bulk soil (Miransari, 2011; Zuo et al., 2021). In the present study, microbial abundance in the bulk soil is far less than that of rhizosphere soil of *A. fruticosum*, especially for the most abundant taxa like Cyanobacteria (*Table 1; Figs. 5-7*).

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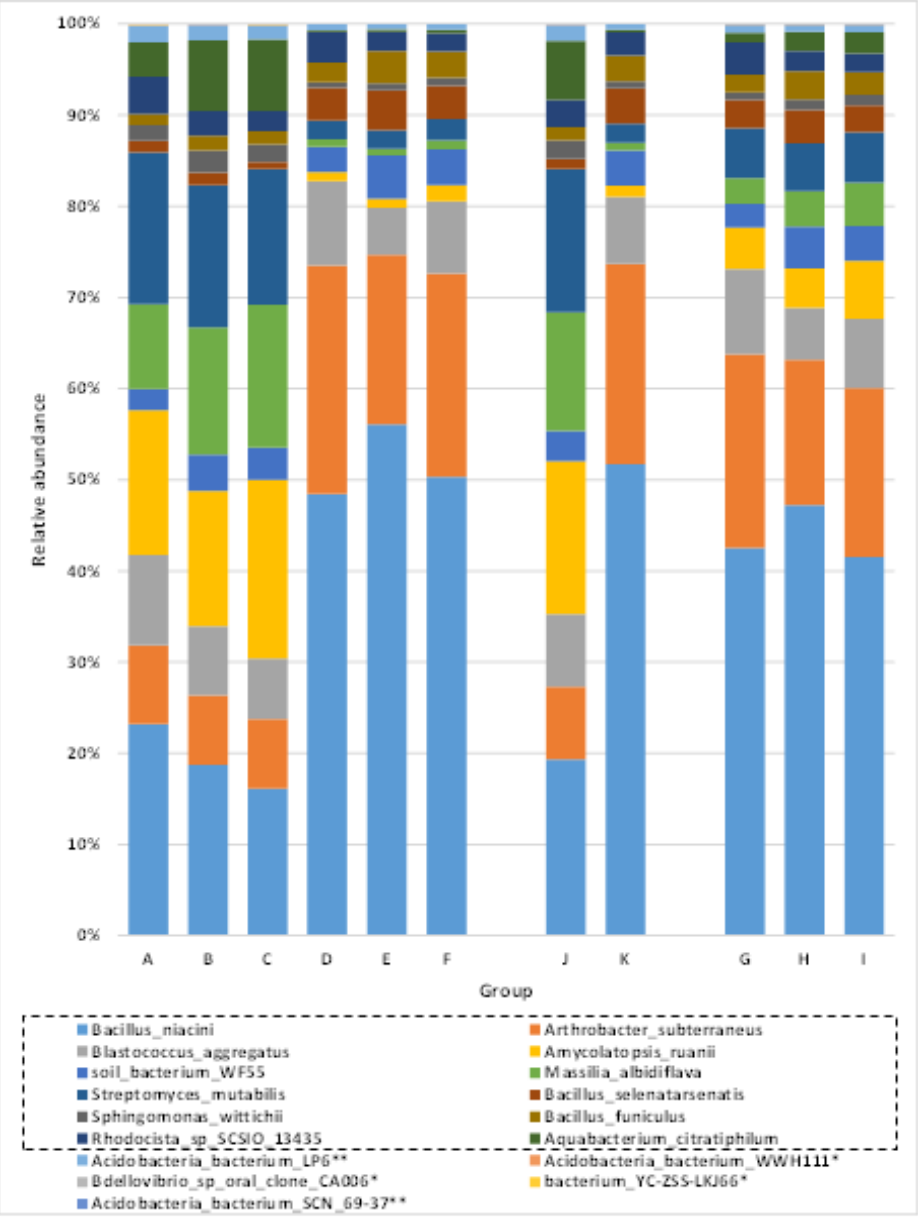

Figure 7. *Relative abundance at the species level within different grouping types of taxa that showed significant differences in microbiomes collected from surrounding bulk (grouping type ABC) and rhizosphere (grouping type DEF) soils of Abutilon fruticosum after 0 (grouping type AD), 24 (grouping type BE) and 48 h (grouping type CF) of watering. Samples data were also grouped based on based on source of microbiome regardless of time after watering, e.g., bulk soil (group J) and rhizosphere soil (group K), and time after watering regardless of source of microbiome, e.g., 0 (group G), 24 (group H) and 48 h (group I). Taxa with (*) refer to occurrence of significant differences within grouping type ABCDEF, taxa with (**) refer to occurrence of significant differences within grouping type GHI, while first 12 taxa (inside the dotted black box) refer to occurrence of significant differences between grouping type JK. Statistical analysis is shown in Metastat boxplots of Figures A18-A26*

Among other factors influencing high abundance of phyla in rhizosphere soil of *A. fruticosum*, Lopes et al. (2021) indicated that range of soil pH implies shaping of soil microbiomes. In terms of the differential abundance of bacteria in rhizosphere and bulk

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soils, it was proven that microbes that favor high soil pH grow better in rhizosphere soil, while those favoring low pH (≤5) grow better in bulk soil (Chodak et al., 2015). As soil pH in the western region of Saudi Arabia was estimated to be > 5, this might explain the high abundance of members of these phyla in rhizosphere soil. In addition, symbiotic association of microbes of these phyla might be a favorite option to the plant under watering condition. Other factors may also refer to the consequence of natural co-existence of the two highly abundant phyla Acidobacteria and Proteobacteria that are responsible for sustainability of the other phyla in the rhizosphere whose abundance after watering (Wei et al., 2017). Thus, these phyla will perform parallel, in terms of growth rate, to those of the phyla Acidobacteria and Proteobacteria. Highly abundant Phyla Firmicutes and Bacteroidetes also co-exist in the soil in order to imply minimal competition for resources through cooperation and/or specialization (Wei et al., 2017), where Firmicutes, for example, favors lipid nutrients in biogas reaction, whereas Bacteroidetes favors starch nutrients (Kampmann et al., 2012).

Phylum Acidobacteria was also reported to have genes for colonization in the rhizosphere to establish symbiotic relationships with plants (Kalam et al., 2020). Other genes encode enzymes for carbon metabolism, like the degradation of complex carbohydrate polymers, metabolic intermediates in the soil (Belova et al., 2018), enzymes for inorganic and organic sources of nitrogen metabolism (Eichorst et al., 2018), sulfur metabolism (Tank and Bryant, 2015) as well as enzymes for acid tolerance to help it survive in highly acidic conditions (Sun et al., 2012). The above-mentioned characteristics of the microbe indicate why Acidobacteria favors growth in rhizosphere soil in the present study (*Fig. 5* and *A21*).

Nonetheless, Lazcano et al. (2021) claimed in strawberry that archaeal phylum Thaumarchaeota, and bacterial phyla Chloroflexi and Firmicutes and Acidobacteria exhibited higher abundance in bulk soil relative to rhizosphere soil, while Proteobacteria, Bacteroidetes and Actinobacteria showed opposite results. Previous reports on several other plants indicated that phylum Acidobacteria showed higher abundance in the bulk soil (Walker et al., 2010), while phyla Actinobacteria (Deiglmayr et al., 2006), Bacteroidetes (Schmidt et al., 2008) and Proteobacteria (Mukhtar et al., 2021) showed higher abundance in rhizosphere soil. Interestingly, Na et al. (2019) indicated that phyla Acidobacteria, Chloroflexi, and Cyanobacteria are enriched in rhizosphere of broomcorn millet at the jointing stage, while decreased as the plants continue growing. We refer these contradicting results to the natural growth dynamics of microbiota responding differently to the surrounding plants and environmental conditions.

(b) Genus and species levels

Descendent genera of phyla Bacteroidetes (e.g., *Adhaeribacter* and *Pontibacter*), Firmicutes (e.g., *Bacillus* and *Ammoniphilus*), Actinobacteria (e.g., *Nocardioides* and *Arthrobacter*), and Proteobacteria (e.g., *Microvirga*, *Sphingomonas* and *Rubellimicrobium*), as well as *Bacillus niacini*, *B. funiculus*, *B. selenatarsenatis*, *Arthrobacter subterraneus*, *Blastococcus aggregatus*, soil bacterium WF55, *Sphingomonas wittichii* and *Rhodocista* sp. SCSIO 13435 showed significant higher microbial abundance in rhizosphere soil compared with that of bulk soil (*Figs. 6, 7, A22* and *A23*). While, opposite results were reached for descendent of phylum Proteobacteria, e.g., unidentified genus of family Rickettsiales, *Acidobacteria* bacterium

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WWH111, *Bdellovibrio* sp. oral clone CA006, *Amycolatopsis ruanii*, *Massilia albidiflava*, *Streptomyces mutabilis* and *Aquabacterium citratiphilum*.

Genus *Adhaeribacter* (Bacteroidetes) was proposed to predominantly exist in rhizosphere of Broomcorn Millet (Na et al., 2019) aligning with the results of the present study (*Table 1; Figs. 6* and *A22*). Genus *Pontibacter* (Bacteroidetes) was reported to possess menaquinone-7 (MK-7) as predominant respiratory quinone that is essential for the production of human vitamin K-2 (Joshi et al., 2012), while this genus was recently reported to contribute to the hormetic responses of soil alkaline phosphatase (a potential endpoint) when low levels of cadmium (stressor) presented in soils (Fan et al., 2018).

Other reports indicated that abundance of genus *Bacillus* (Firmicutes) and members of Actinobacteria in non-cultivated bulk soil is higher than that of members of Proteobacteria, while these taxa showed similar abundance in rhizosphere soil of *Capsicum annum* plant (Marasco et al., 2013). Nonetheless, abundance of genera *Streptomyces* (Actinobacteria) and *Microvirga* (Proteobacteria) was high in *Calotropis procera* rhizosphere, while that of genera *Bacillus* and *Ammoniphilus* (Firmicutes) decreased. Interestingly, the latter three genera act as plant growth promoting bacteria (PGPB) (Wang et al., 2017). In phylum Actinobacteria, genus *Nocardioides* showed higher abundance in maize rhizosphere soil (Piutti et al., 2003), while genus *Arthrobacter* was proven to be an important fraction of the microflora that promotes plant growth especially under drought stress. Members of genus *Arthrobacter* are resistant to desiccation and can survive for a long time under starvation condition (Chukwuneme et al., 2020). In the present study, the genus *Arthrobacter* showed no differential response to watering.

Aligning with the results of the present study, genus *Sphingomonas* (Proteobacteria) is more abundant in rhizosphere and is known to promote growth of *Arabidopsis thaliana*. However, it can survive in bulk soil by taking energy from degrading organic pollutants (Luo et al., 2019). Genus *Sphingomonas* is also known for its ability to tolerate drought stress and promote tolerance in the neighboring roots (Luo et al., 2019). However, this genus showed no differential response to watering in the present study. Information regarding genus *Rubellimicrobium* (Proteobacteria) is rare except that it was shown to be highly enriched in oil-contaminated and non-contaminated rhizosphere soil of barley and alfalfa compared with its level in sandy clean soil (Kumar et al., 2018). Family Rickettsiales was proven to be highly abundant in bulk soil (*Figs. 6* and *A22*), however, there is no information in the literature to support its role in the soil.

At species level, no solid information regarding the roles of most highly abundant bacteria in rhizosphere soil (*Figs. 7* and *A23*), except that *Sphingomonas wittichii* is among bacteria that make bioremediation and has the ability to degrade xenobiotic compounds dibenzofuran and dibenzo-p-dioxin (Coronado et al., 2014), while *Bacillus niacini* and *Streptomyces mutabilis* were reported to be a potential plant growth promoting (PGP) rhizobacteria (Suralta et al., 2018; Bhattacharyya et al., 2020) with the ability, as a potential bio-fertilizers, to fix nitrogen and solubilize phosphorous (Latif et al., 2020).

Contradiction of the results at the genus level can be due to the fact that microflora react differentially with the root system of the different interacting plant or otherwise abundance of members of the same genus in different soil types differs at species level. Wang et al. (2017) indicated that plant genotype and growth stage, plant disease

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incidence, soil nutrient contents, soil enzyme activities and pH are main factors affecting abundance of microbes in different soil types.

Differential abundance due to watering

Generally speaking, Gram-positive (diderm) bacteria are known to be more drought tolerant than Gram-negative (monoderm) bacteria as the first has stronger cell wall and drought avoidance strategies (ex., spore formation) (Potts, 1994). Although drought-induced microbial enrichment refers to the boundaries between monoderm and diderm lineages, Xu et al. (2018) indicated that the discriminating factors can to the structure and properties of cell wall, rather than the presence or absence of outer membrane. Accordingly, the latter authors confirmed that root microbiomes of different sources under normal watering conditions prefer to be colonized by diderm bacteria, rather than by monoderm bacteria.

Watering of drought-stressed plants was speculated to rapidly disturb natural progression of microbiome development, which can be eventually restored after water is diminished. Xu et al. (2018) speculated that a maximum of one week following watering is a maximum time to restore microbiome pattern of drought-treated plant roots. In the present study, two days after watering were enough to recover the natural growth pattern of some microbes at phylum and genus levels and to restore their previous or natural growth pattern under drought condition (*Figs. A24* and *A25*), while it seems that this short period is not enough for the majority of microflora to do both actions, e.g., induction of disturbed growth pattern, and restoration of natural growth pattern of drought-stressed microbes. This supports our claim that growth dynamics of microbiota respond differently to the genotypes of interacting plant and environmental conditions.

The results of the present study for phylum Cyanobacteria showed significant increase of relative abundance in bulk soil groups B and C due to watering compared with those of rhizosphere soil groups E and F, respectively (*Figs. 5* and *A18*). This indicates that members of this phylum responded positively to watering in the present study possibly due to the fact that roots of *A. fruticosum* reduced carbon exudation rate to complement drought stress pressure (Reid and Mexal, 1977). Besides, the microbe itself might have strong alterative mechanisms for independent survival when water becomes available.

Relative abundance of phylum Bacteroidetes increased before watering in rhizosphere soil microbiome group D compared with that of bulk soil microbiome group A (*Figs. 5* and *A18*). This might indicate that watering abolished differential microbial abundance between the two types of soil, although the overall growth across time after watering was in favor to the rhizosphere soil (*Figs. 5* and *A21*). While, relative abundance of phylum Firmicutes and its descendent genus *Planococcus* showed significant increase in microbiome abundance of rhizosphere soil group E after 24 h of watering compared with that of the respective bulk soil group B (*Figs. 5, 6, A18* and *A19*). In addition, relative abundance of descendent unidentified taxon of taxon Acidobacteria (*Figs. 6* and *A25*) and descendent genus of phylum Proteobacteria, e.g., *Haliangium* (*Figs. 6* and *A25*), as well as the two species of genus Acidobacteria, e.g., LP6 and SCN69-37 (*Figs. 7* and *A26*), significantly increased 24 h after watering regardless of microbiome source. This might refer to the instant response of some microbes to watering regardless of soil type. Although not statistically proven, the results for the other descendent genus of phylum Proteobacteria, e.g., *Massilia* indicated

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lower abundance level (*Fig. A11*). Interestingly, the other descendent genera of phylum Proteobacteria, e.g., *Ramlibacter* and *Procabacter*, indicated significant increase in microbiome abundance of groups C and D compared with that in groups A and F, respectively (*Figs. 6* and *A19*). This indicates that *Ramlibacter* benefitted from watering in the rhizosphere soil, while abundance of *Procabacter* was declined due to watering. Regardless of microbiome source, abundance of *Ramlibacter* increased significantly 24 h after watering supporting the latter results in *Figure A19* that this genus benefits from watering. Results of archaeal phylum Euryarchaeota, which has no distinctive differentially abundant descendent genera at any grouping type, indicated that relative abundance was significantly increased in rhizosphere soil 48 h after watering (*Figs. 5* and *A18*). This indicates that this phylum also benefited from watering but responded later than bacterial phylum Firmicutes (*Figs. 5* and *A18*).

Previous studies indicated that drought significantly affects microbial composition in the rhizosphere and mitigates the adverse effect of drought stress on plant growth, which can be used as an emerging strategy for improving drought stress tolerance in plants (Xu et al., 2018). Genus *Planococcus* was reported to exist in rhizosphere and root endosphere of halophytes indicating that this genus is salt tolerant (Mukhtar et al., 2021) and can promote growth of plants when exists in their rhizosphere soil (Rajput et al., 2013). In addition, *Bacillus*-generated biofertilizers are highly tolerant to diverse environmental stresses due to their ability to form spores, which are resistant to desiccation (Barnard et al., 2013). Bacterial responses to drought and rewetting stress are based on genetic makeup of the bacteria, the surrounding environment, e.g., contents of soil N and organic C and heavy metal, and host-microbe interaction (Chodak et al., 2015). Stress tolerant bacteria, in turn, induce physiological response in the plant root vicinity in order to help them alleviate adverse effects of drought stress (Bokhari et al., 2019). In maize seedlings, responses include stimulation of ribosomal synthesis and cell proliferation (Vardharajula et al., 2011). Relative abundance of Actinobacteria under drought was declared to be higher within the rhizosphere than that in the surrounding bulk soil (Naylor et al., 2017) in alignment with the results of the present study. It was reported that Actinomycetes has the ability to survive in unfavorable environments (Passari et al., 2015), thus, it is likely that relative abundance in bulk soil is quite high as well. The phylum is also known to produce bioactive secondary metabolites acting as plant growth promoting (PGP) and nitrogenous compounds in non-legumes and to make P solubilization via production of various organic acid including citric acid, gluconic acid, lactic acid, malic acid, oxalic acid, propionic acid, and succinic acid which aid in promoting plant growth (Sathya et al., 2017). Several other phyla, Bacteroidetes, Cyanobacteria, Firmicutes, and Proteobacteria were also reported to act as plant growth-promoting bacteria (Yadav et al., 2018). Jang et al. (2020) indicated that phyla affected by drought stress and the presence of plants include Armatimonadetes, Firmicutes and Proteobacteria. The authors indicated that relative abundance of phylum Armatimonadetes and Proteobacteria in bulk soil was lower under drought condition than that under watered conditions and vice versa in the rhizosphere soil of rice. Thus, the three phyla Armatimonadetes, Firmicutes and Proteobacteria are considered in several reports to have potential significance in assisting plants to withstand drought stress (Ma et al., 2020). These results are consistent with those of many other reports on various plants (Chodak et al., 2015; Bu et al., 2018; Gumiere et al., 2019). More recently, attention was given to the promising phylum Armatimonadetes as it is proposed to have insurance mechanisms against fungal invasions and drought stress in

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avocado rhizosphere (Bejarano-Bolívar et al., 2021). However, bacterial members of Armatimonadetes are difficult to isolate or purely (Hu et al., 2014). Then, it is highly recommended to consult culturomics approaches in order to explore proper culture conditions and recover pure cultures of members of this phylum for further beneficial use in improving plant tolerance against drought stress.

Drought and rewetting stress significantly alter structure of soil metagenomes (Chodak et al., 2015). The authors indicated that abundance of Gram-positive bacterial phyla, like Firmicutes and Actinobacteria, increases after the stress, whereas Gram-negative bacteria, like Proteobacteria, Bacteroidetes, Chloroflexi, Gammaproteobacteria, decreases. It seems that plant growth stage affects relative abundance of rhizosphere microbes under drought stress where abundance of Actinobacteria and Acidobacteria significantly increased at early stages in peanut, while that of Cyanobacteria and Gemmatimonadetes increased at flowering stage (Dai et al., 2019).

Finally, isolation of selected plant growth-promoting bacteria (PGPB) in this study using culturomics approach is highly recommended for soil management program to improve the tolerance of cultivated plant crops to drought stress.

Conclusion

In conclusion, the present study was able to detect the differential microbiota signatures in the rhizosphere of the wild plant *Abutilon fruticosum* and surrounding bulk soil. After watering plots of drought-stressed plants, some soil microbes responded positively and two days were enough for these microbes to restore their natural growth pattern. Such dynamics of microbial growth patterns act in restoring disturbed abundances due to the changes in the environmental condition. We also tried to raise the possible roles of Gram staining properties under drought stress and after watering, but it seems that this factor cannot be considered universally in justifying differential enrichment where phylum Proteobacteria, for example, is Gram-negative, yet members of this phylum (e.g., genera *Ramlibacter* and *Procabacter*) responded differently to watering. Therefore, the present study warrants comprehensive research to dissect other factors that can influence differential stress responses as well as tolerance of soil microbiota, and to shape accurate plant-bacterial relationships in order to help making more proper soil management decisions.

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APPENDIX

This manuscript has an electronic appendix with taxonomic data (OTUs).

Figure A1. *Abundance at the kingdom level (bacteria and archaea) of microbiomes collected from surrounding bulk (S) and rhizosphere (R) soils of Abutilon fruticosum in three replicates after 0 (S11-S13 and R11-R13, respectively), 24 (S21-S23 and R21-R23, respectively) and 48 h (S31-S33 and R31-R33, respectively) of watering. Samples data were also grouped based on source of microbiome regardless of time after watering, e.g., bulk soil (group J) and rhizosphere soil (group K)*

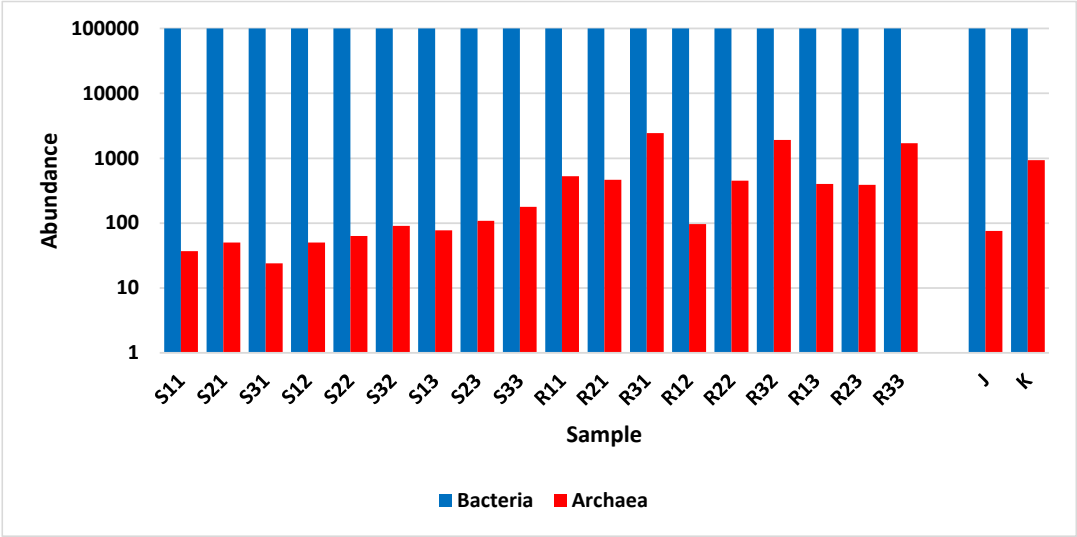

Figure A2. *Number of sequences of taxa taged at different taxonomic levels (kingdom, phylum, class, order, family, genus and species) of microbiomes collected from surrounding bulk (S) and rhizosphere (R) soils of Abutilon fruticosum in three replicates after 0 (S11-S13 and R11-R13, respectively), 24 (S21-S23 and R21-R23, respectively) and 48 h (S31-S33 and R31-R33, respectively) of watering*

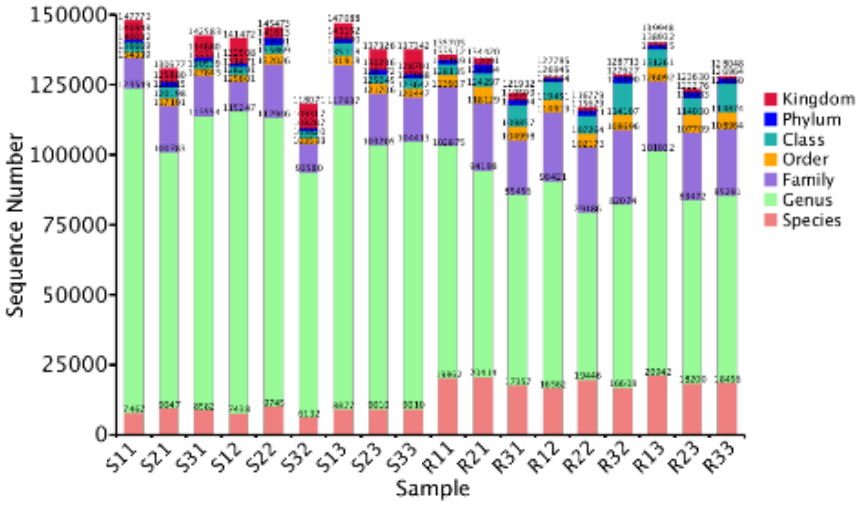

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Figure A3. *Records of observed species (S_{obs}), Shannon and Simpson alpha diversity indices of microbiomes collected from surrounding bulk (S) and rhizosphere (R) soils of Abutilon fruticosum in three replicates after (S11-S13 and R11-R13, respectively), 24 (S21-S23 and R21-R23, respectively) and 48 h (S31-S33 and R31-R33, respectively) of watering*

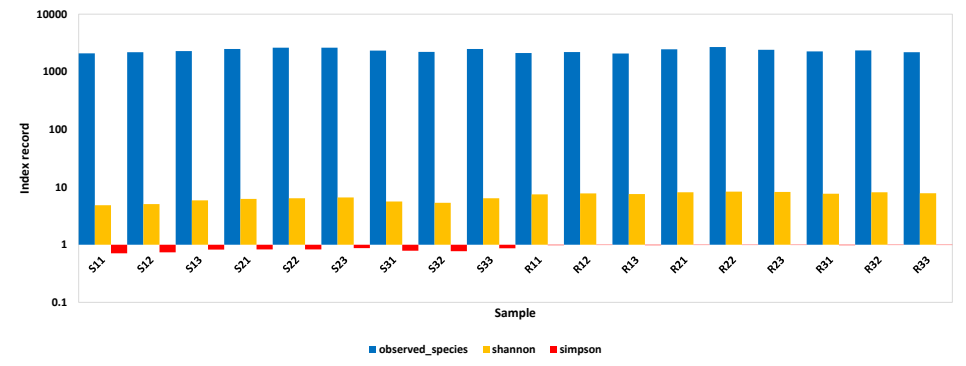

Figure A4. *Number of observed species as a rarefaction measure to describe the maximum depth permitted to retain all samples in the dataset (~120,000 sequence reads) for studying taxonomic relative abundance of microbial community as a beta diversity measure*

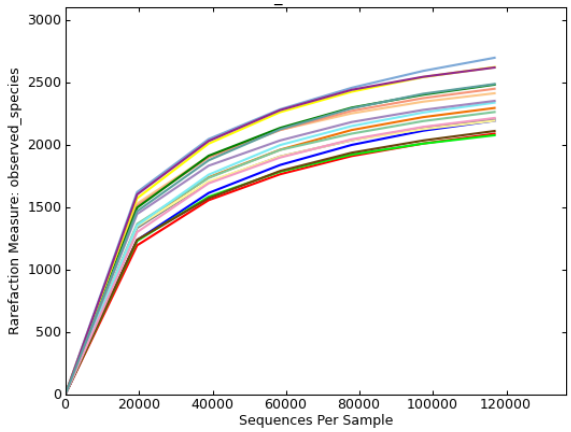

Figure A5. *Specaccum or species accumulation curve of 18 microbiome samples collected from Abutilon fruticosum rhizosphere soil and surrounding bulk soil*

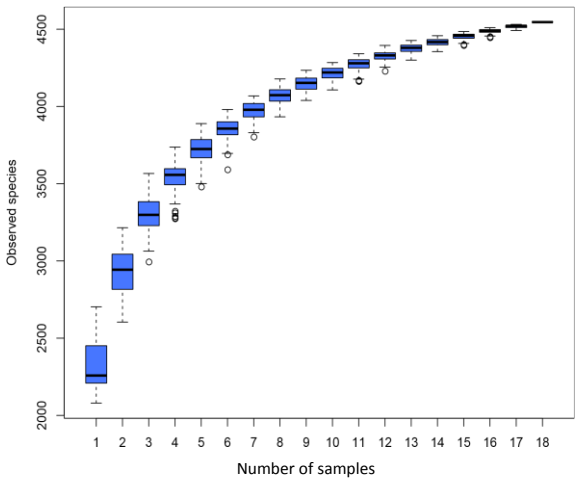

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Figure A6. *Anosim plots of microbiomes collected from surrounding bulk and rhizosphere soils of Abutilon fruticosum regardless of time after watering, e.g., bulk soil (group J) and rhizosphere soil (group K). Samples data were also grouped based on time after watering regardless of microbiome source, e.g., 0 (group G), 24 (group H) and 48 h (group I)*

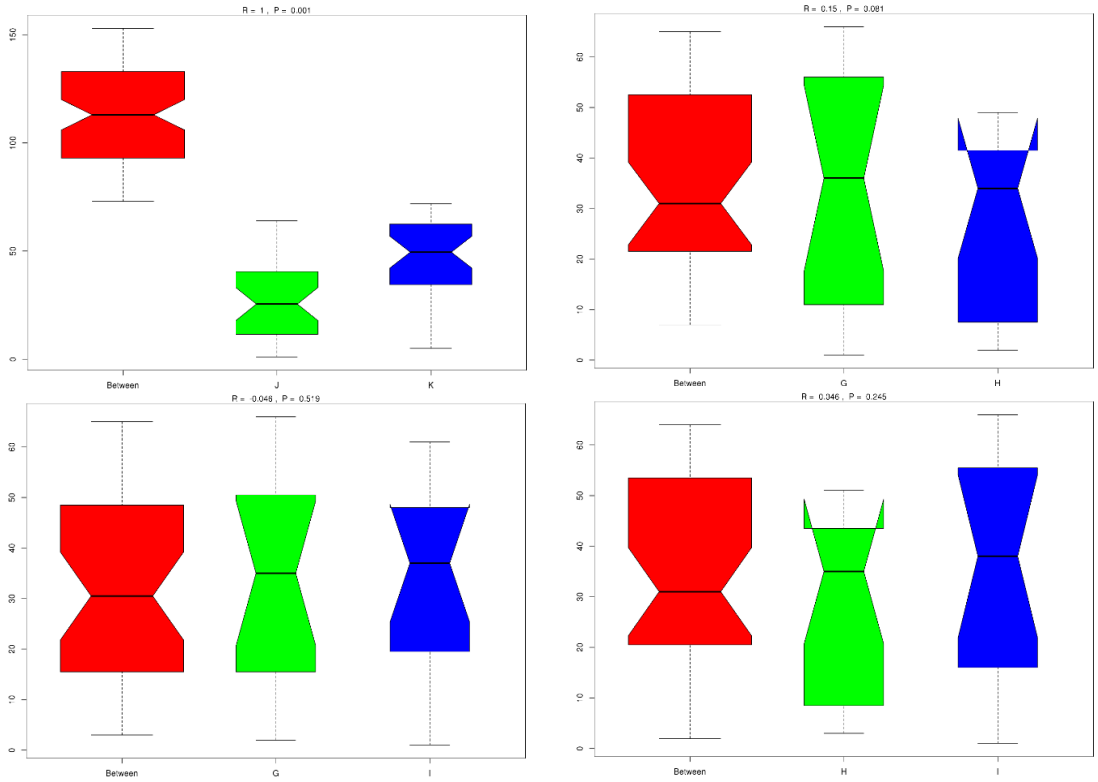

Figure A7. *Heatmap of weighted (top records) and unweighted (bottom records) unifrac beta diversity measures of microbiomes collected from surrounding bulk (S) and rhizosphere (R) soils of Abutilon fruticosum in three replicates after 0 (S11-S13 and R11-R13, respectively), 24 (S21-S23 and R21-R23, respectively) and 48 h (S31-S33 and R31-R33, respectively) of watering*

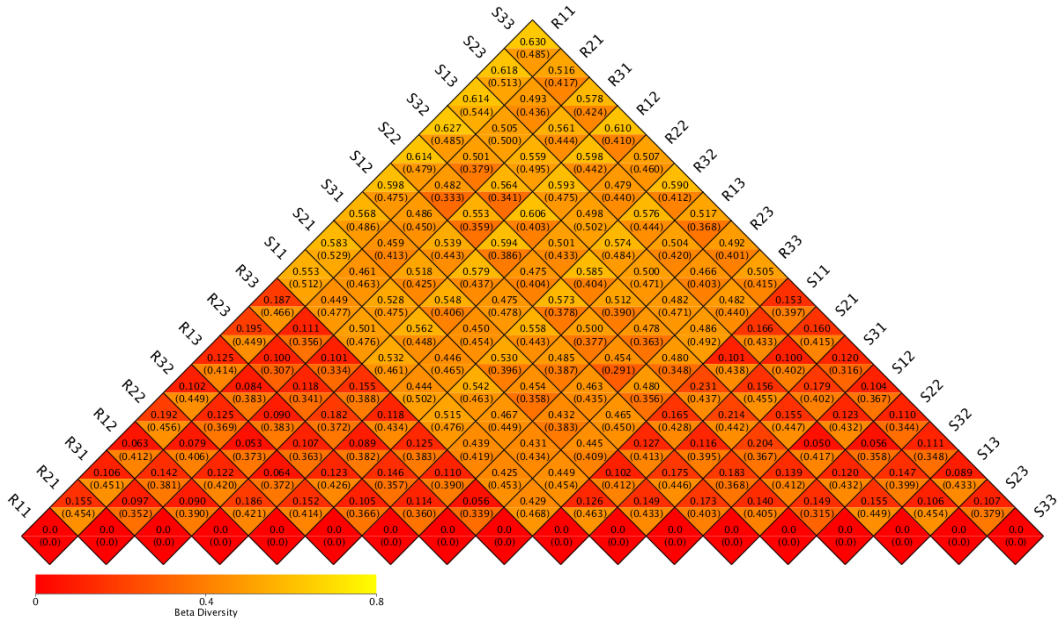

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Figure A8. *Dendrogram trees of unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis describing the calculated weighted (a) and unweighted (b) unifrac beta diversity distances of microbiomes collected from surrounding bulk (S) and rhizosphere (R) soils of Abutilon fruticosum in three replicates after 0 (S11-S13 and R11-R13, respectively), 24 (S21-S23 and R21-R23, respectively) and 48 h (S31-S33 and R31-R33, respectively) of watering*

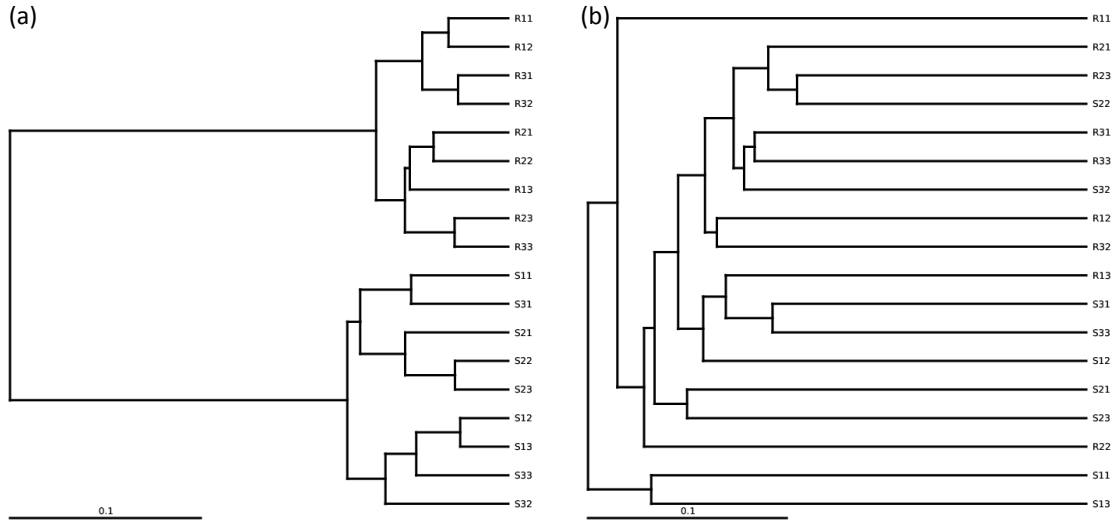

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Figure A9. *Genus evolutionary tree describing abundances of microbiome samples collected from surrounding bulk (S) and rhizosphere (R) soils of Abutilon fruticosum in three replicates after 0 (S11-S13 and R11-R13, respectively), 24 (S21-S23 and R21-R23, respectively) and 48 h (S31-S33 and R31-R33, respectively) of watering*

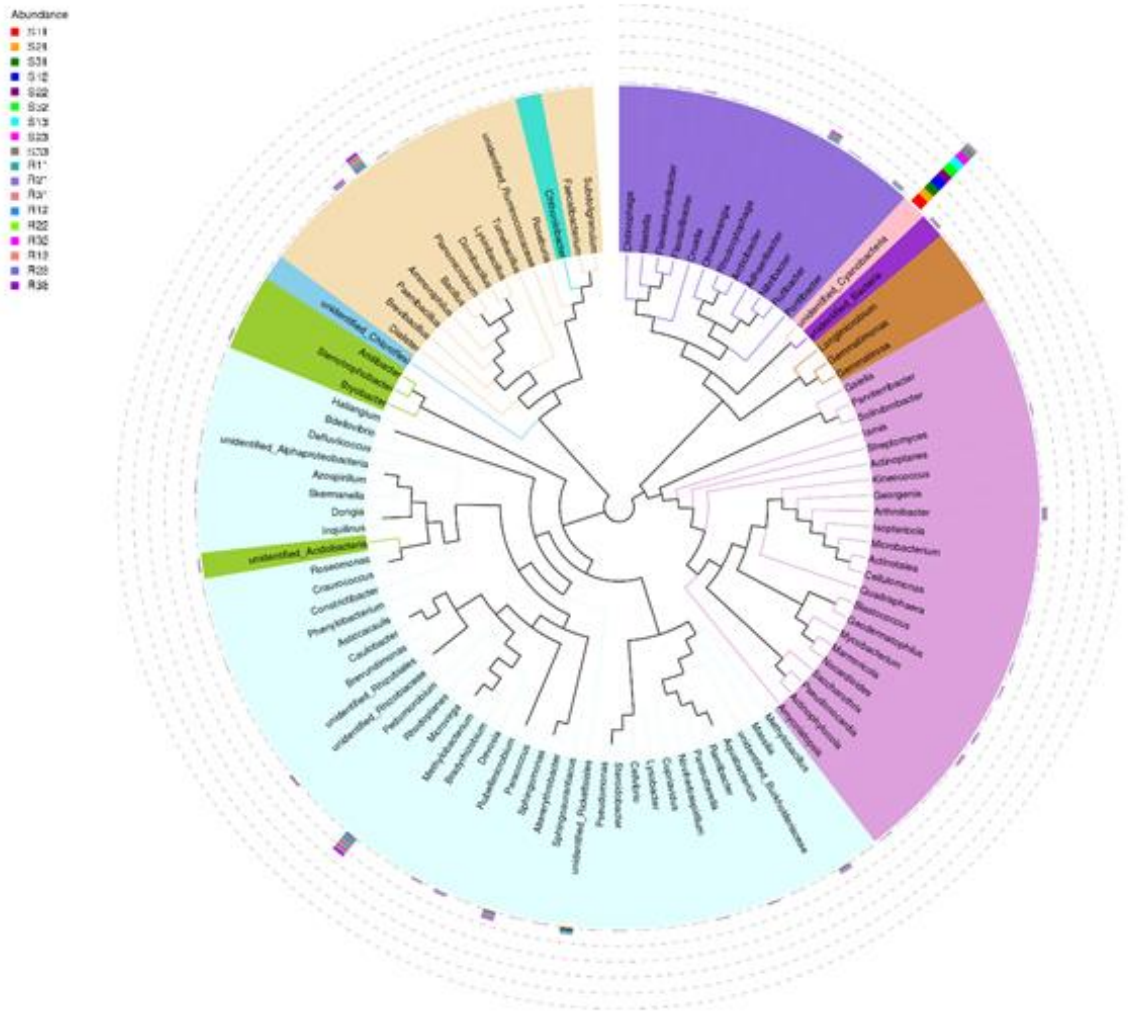

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Figure A10. *Genus evolutionary tree describing abundances of microbiomes collected from surrounding bulk and rhizosphere soils of Abutilon fruticosum after 0 (groups A and D, respectively), 24 (groups B and E, respectively) and 48 h (groups C and F, respectively) of watering*

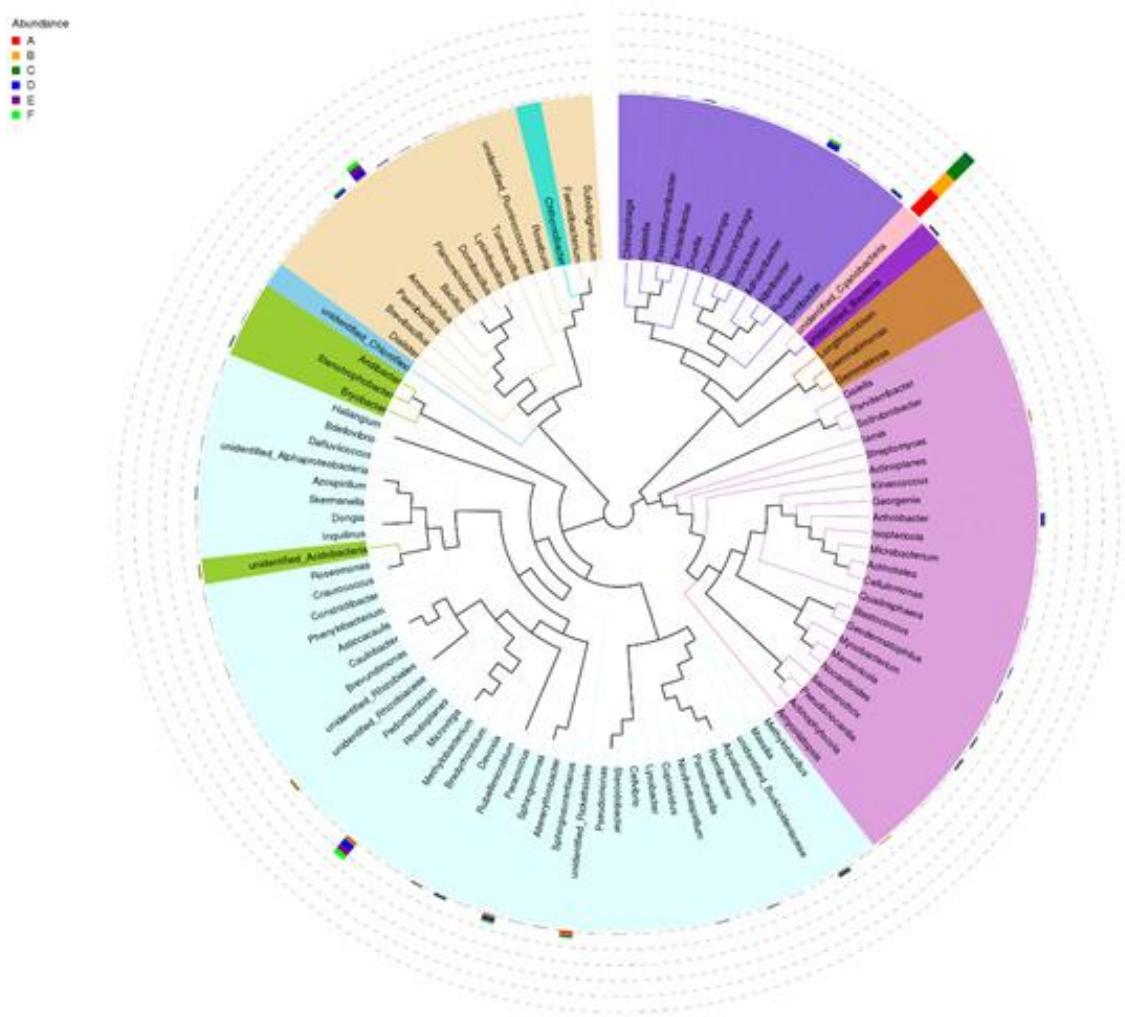

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Figure A11. *Genus evolutionary tree describing abundances of microbiomes collected from surrounding bulk (group J) and rhizosphere (K) soils of Abutilon fruticosum regardless of time after watering*

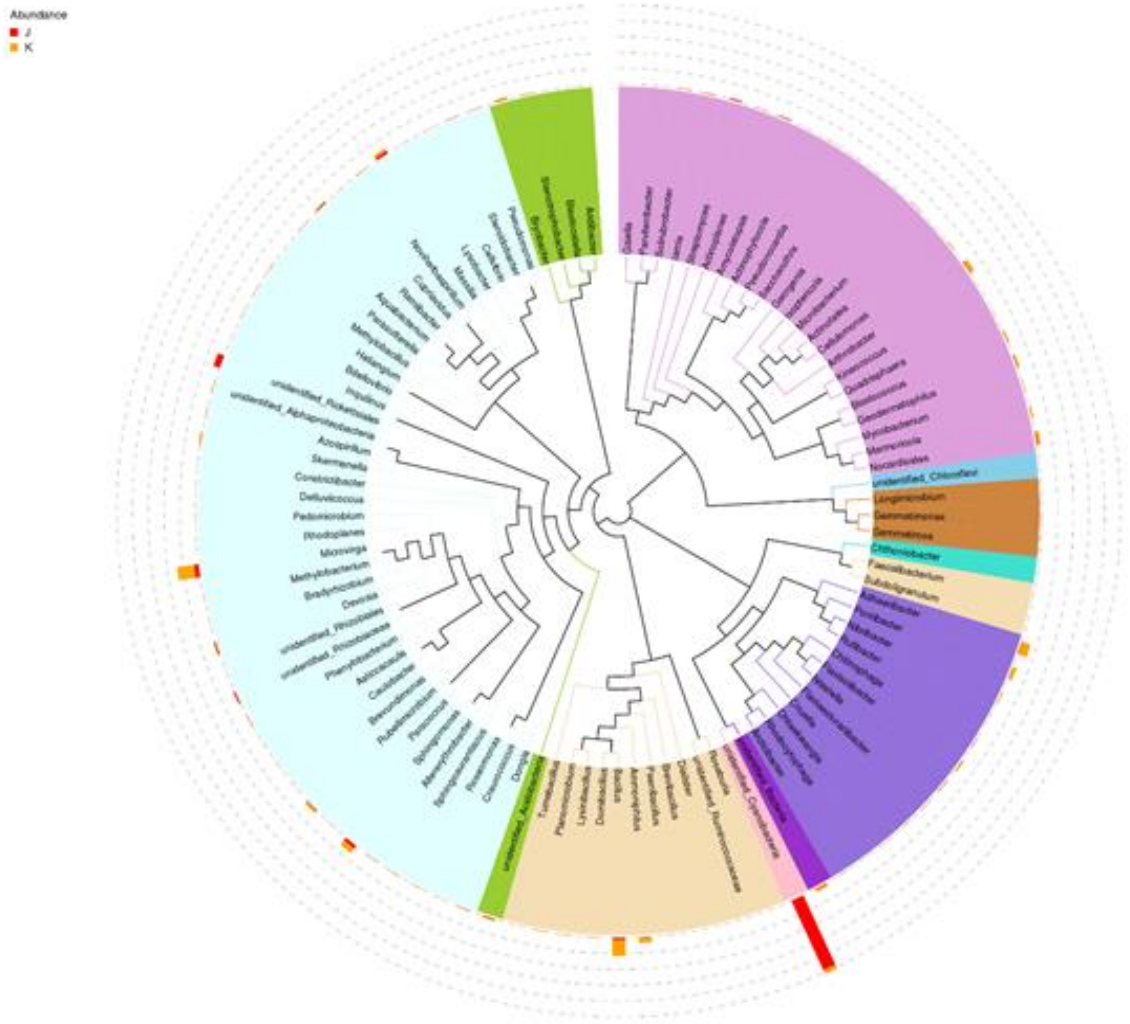

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Figure A12. *Genus evolutionary tree describing abundances of microbiomes collected from surrounding bulk and rhizosphere soils of Abutilon fruticosum after 0 (grouping type AD or group G), 24 (grouping type BE or group H) and 48 h (grouping type CF or group I) of watering regardless of source of microbiome*

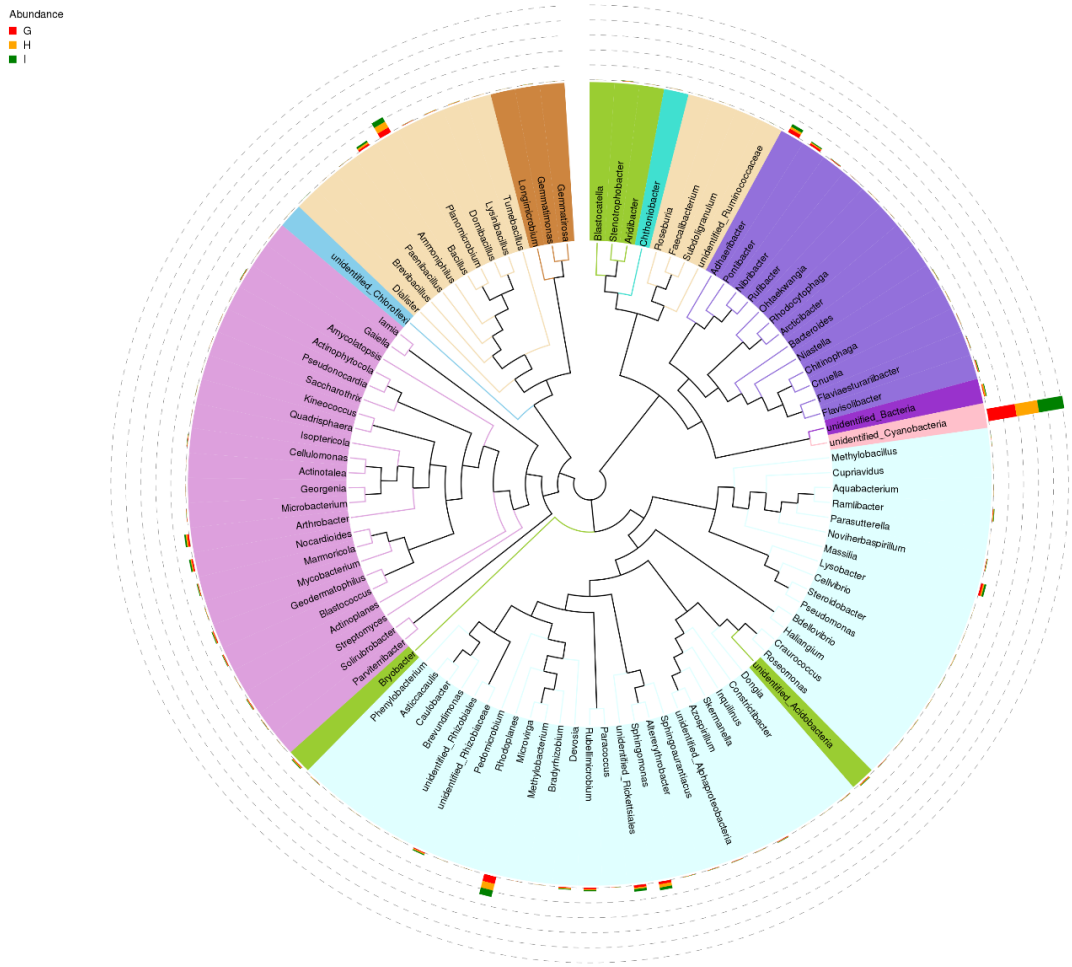

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Figure A13. *Relative abundance of individual samples at the phylum level emphasizing taxa that showed significant differences within different types of grouping of microbiomes collected from surrounding bulk (S) and rhizosphere (R) soils of Abutilon fruticosum in three replicates after 0 (S11-S13 [A] and R11-R13 [D], respectively), 24 (S21-S23 [B] and R21-R23 [E], respectively) and 48 h (S31-S33 [C] and R31-R33 [F], respectively) of watering. Samples data were also grouped based on based on source of microbiome regardless of time after watering, e.g., bulk soil (group J) and rhizosphere soil (group K), and time after watering regardless of source of microbiome, e.g., 0 (group G), 24 (group H) and 48 h (group I). Taxa with (*) refer to occurrence of significant differences within grouping type ABCDEF, taxa with (**) refer to occurrence of significant differences within grouping type GHI, while first 12 taxa (inside the dotted black box) refer to occurrence of significant differences within grouping type JK. Statistical analysis is shown in Metastat boxplots of Figures S18-S26*

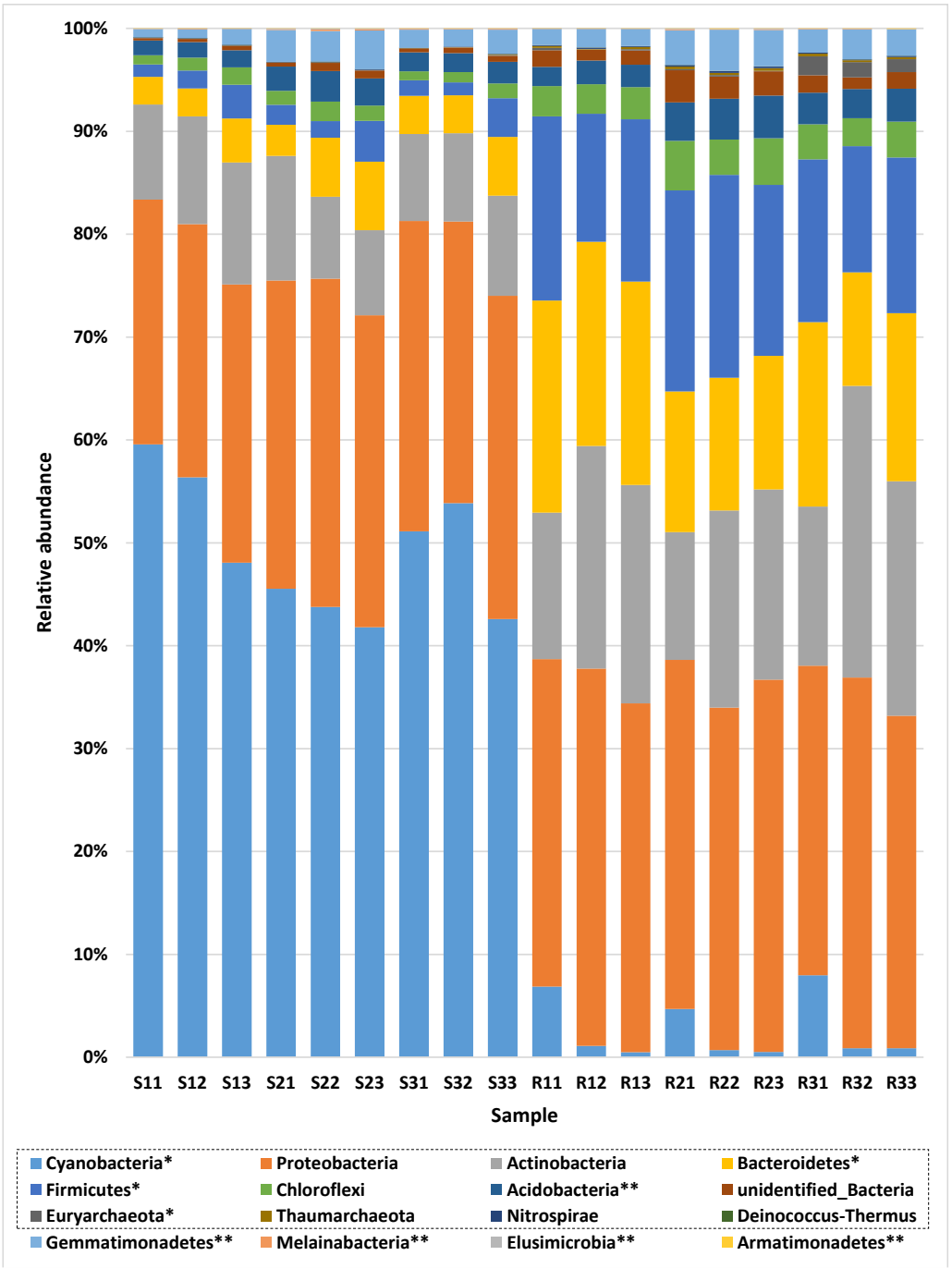

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Figure A14. *Relative abundance of individual samples at the genus level emphasizing taxa that showed significant differences within different types of grouping of microbiomes collected from surrounding bulk (S) and rhizosphere (R) soils of Abutilon fruticosum in three replicates after 0 (S11-S13 [A] and R11-R13 [D], respectively), 24 (S21-S23 [B] and R21-R23 [E], respectively) and 48 h (S31-S33 [C] and R31-R33 [F], respectively) of watering. Samples data were also grouped based on based on source of microbiome regardless of time after watering, e.g., bulk soil (group J) and rhizosphere soil (group K), and time after watering regardless of source of microbiome, e.g., 0 (group G), 24 (group H) and 48 h (group I). Taxa with (*) refer to occurrence of significant differences within grouping type ABCDEF, taxa with (**) refer to occurrence of significant differences within grouping type GHI, while first 12 taxa (inside the dotted black box) refer to occurrence of significant differences within grouping type JK. Statistical analysis is shown in Metastat boxplots of Figures S18-S26*

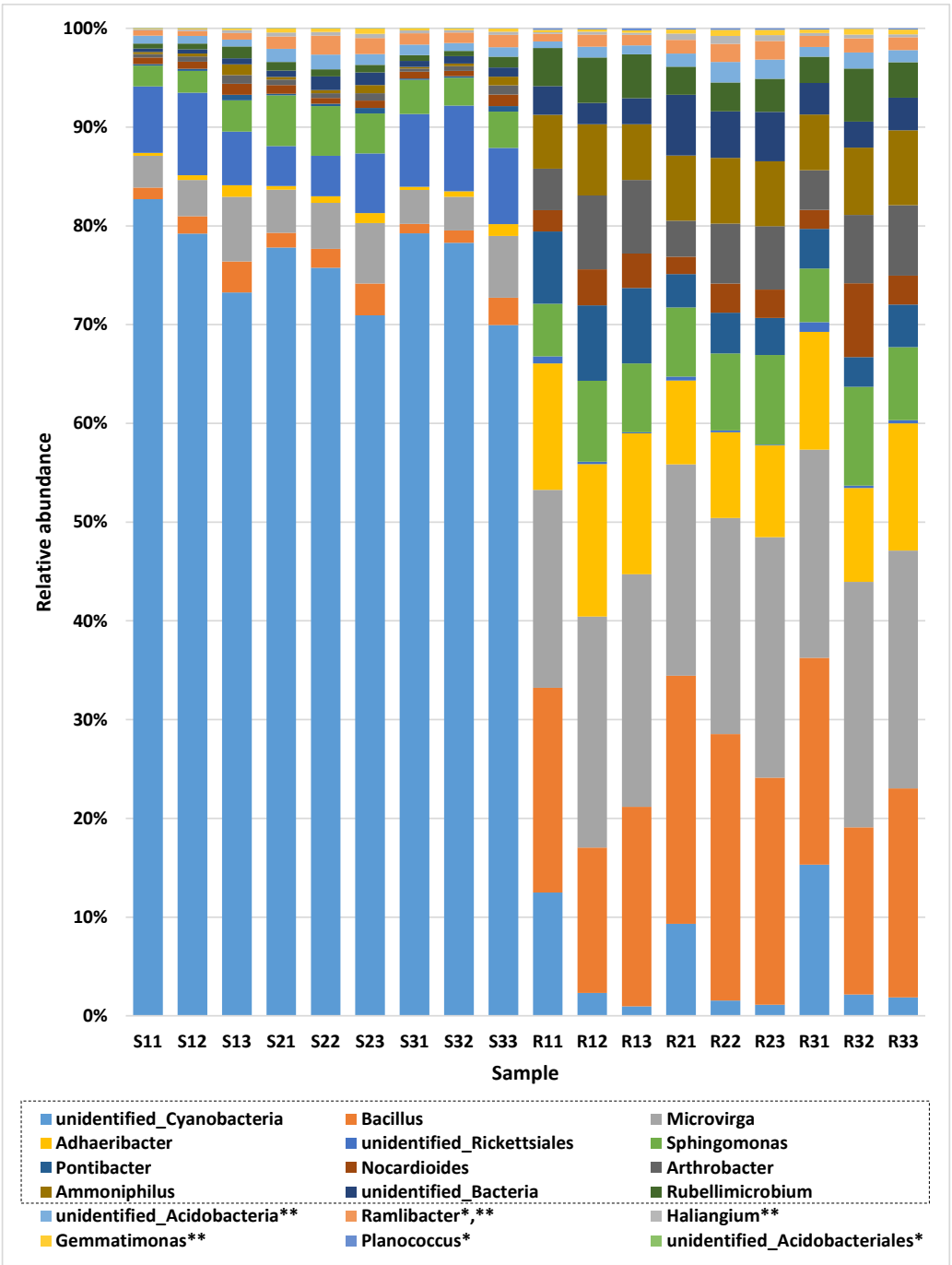

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Figure A15. *Heat map referring to the most abundant 35 genera and their phylum of individual samples microbiomes collected from collected from surrounding bulk (S) and rhizosphere (R) soils of Abutilon fruticosum in three replicates after 0 (S11-S13 and R11-R13, respectively), 24 (S21-S23 and R21-R23, respectively) and 48 h (S31-S33 and R31-R33, respectively) of watering*

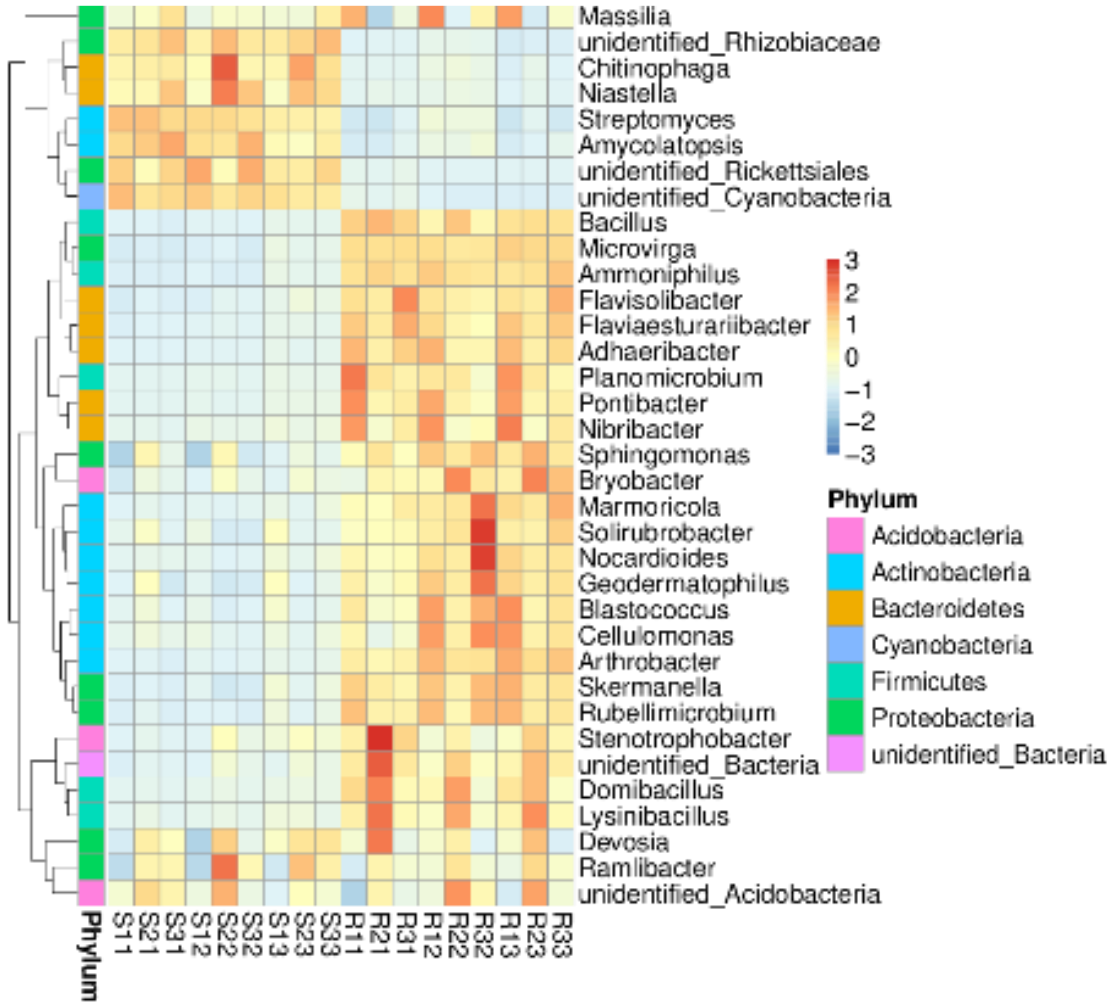

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Figure A16. *Relative abundance of individual samples at the species level emphasizing taxa that showed significant differences within different types of grouping of microbiomes collected from surrounding bulk (S) and rhizosphere (R) soils of Abutilon fruticosum in three replicates after 0 (S11-S13 [A] and R11-R13 [D], respectively), 24 (S21-S23 [B] and R21-R23 [E], respectively) and 48 h (S31-S33 [C] and R31-R33 [F], respectively) of watering. Samples data were also grouped based on based on source of microbiome regardless of time after watering, e.g., bulk soil (group J) and rhizosphere soil (group K), and time after watering regardless of source of microbiome, e.g., 0 (group G), 24 (group H) and 48 h (group I). Taxa with (*) refer to occurrence of significant differences within grouping type ABCDEF, taxa with (**) refer to occurrence of significant differences within grouping type GHI, while first 12 taxa (inside the dotted black box) refer to occurrence of significant differences within grouping type JK. Statistical analysis is shown in Metastat boxplots of Figures S18-S26*

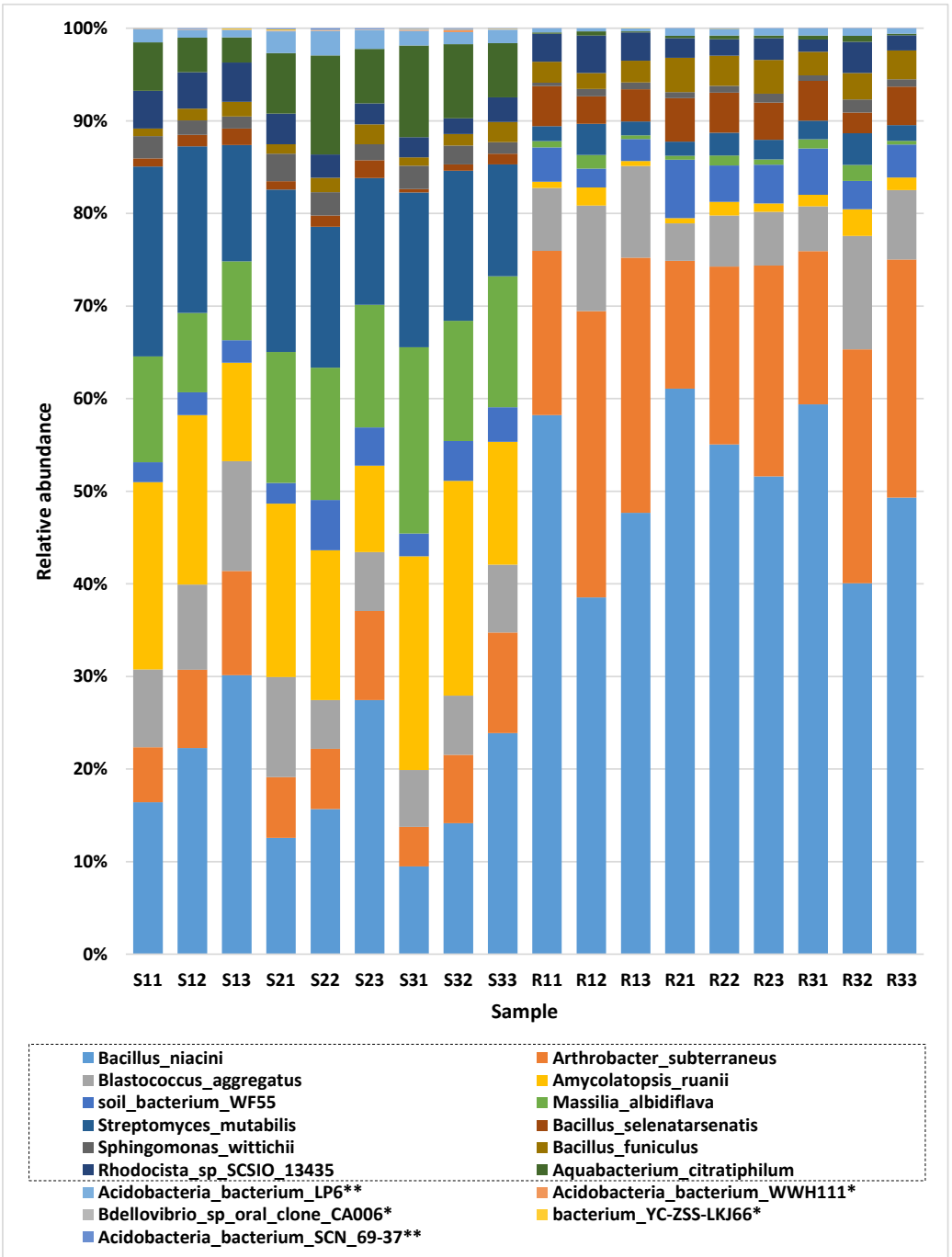

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Figure A17. *Differential abundance of Bacillus niacini of individual samples collected from surrounding bulk (S) and rhizosphere (R) soils of Abutilon fruticosum in three replicates after 0 (S11-S13 [A] and R11-R13 [D], respectively), 24 (S21-S23 [B] and R21-R23 [E], respectively) and 48 h (S31-R33 [C] and S31-S33 [F], respectively) of watering*

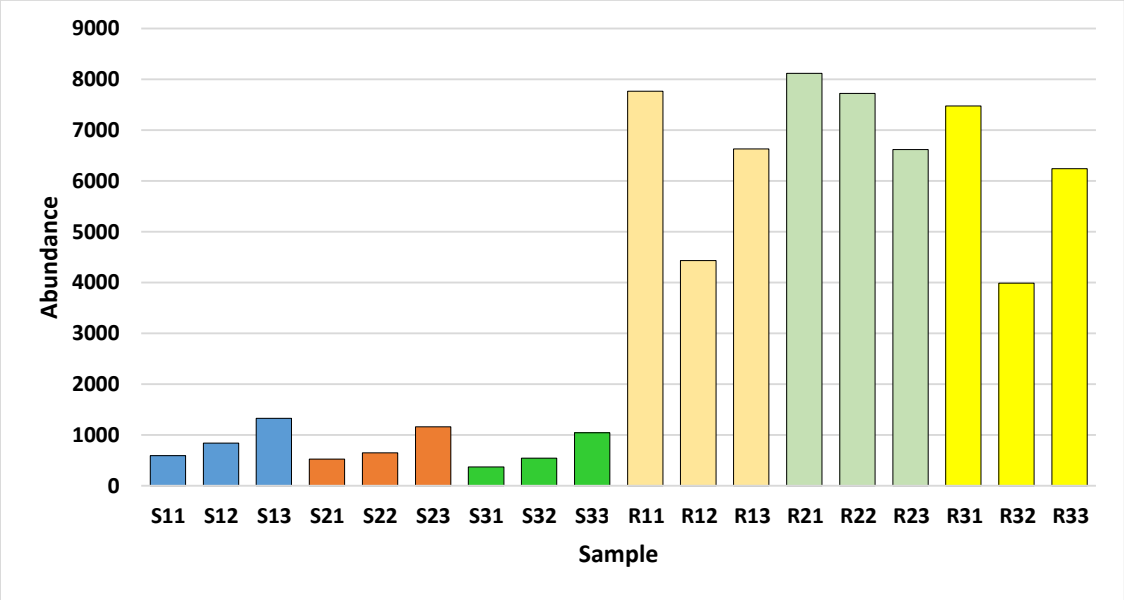

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Figure A18. *Metastat boxplots at the phylum level for the taxa that showed significant differences within grouping type ABCDEF of microbiomes collected from surrounding bulk (grouping type ABC) and rhizosphere (grouping type DEF) soils of Abutilon fruticosum after 0 (grouping type AD), 24 (grouping type BE) and 48 h (grouping type CF) of watering*

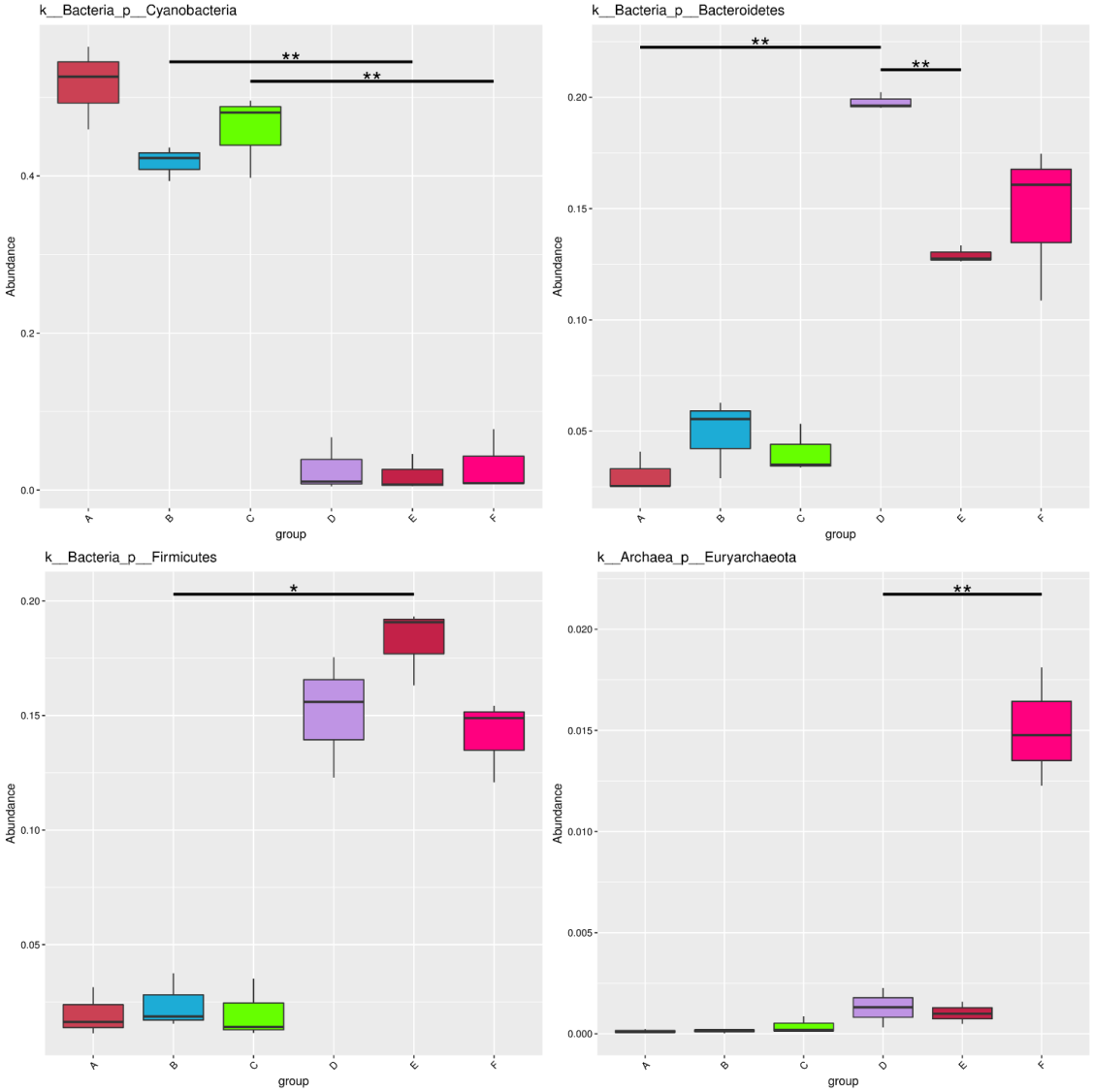

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Figure A19. *Metastat boxplots at the genus level for the taxa that showed significant differences within grouping type ABCDEF of microbiomes collected from surrounding bulk (grouping type ABC) and rhizosphere (grouping type DEF) soils of Abutilon fruticosum after 0 (grouping type AD), 24 (grouping type BE) and 48 h (grouping type CF) of watering*

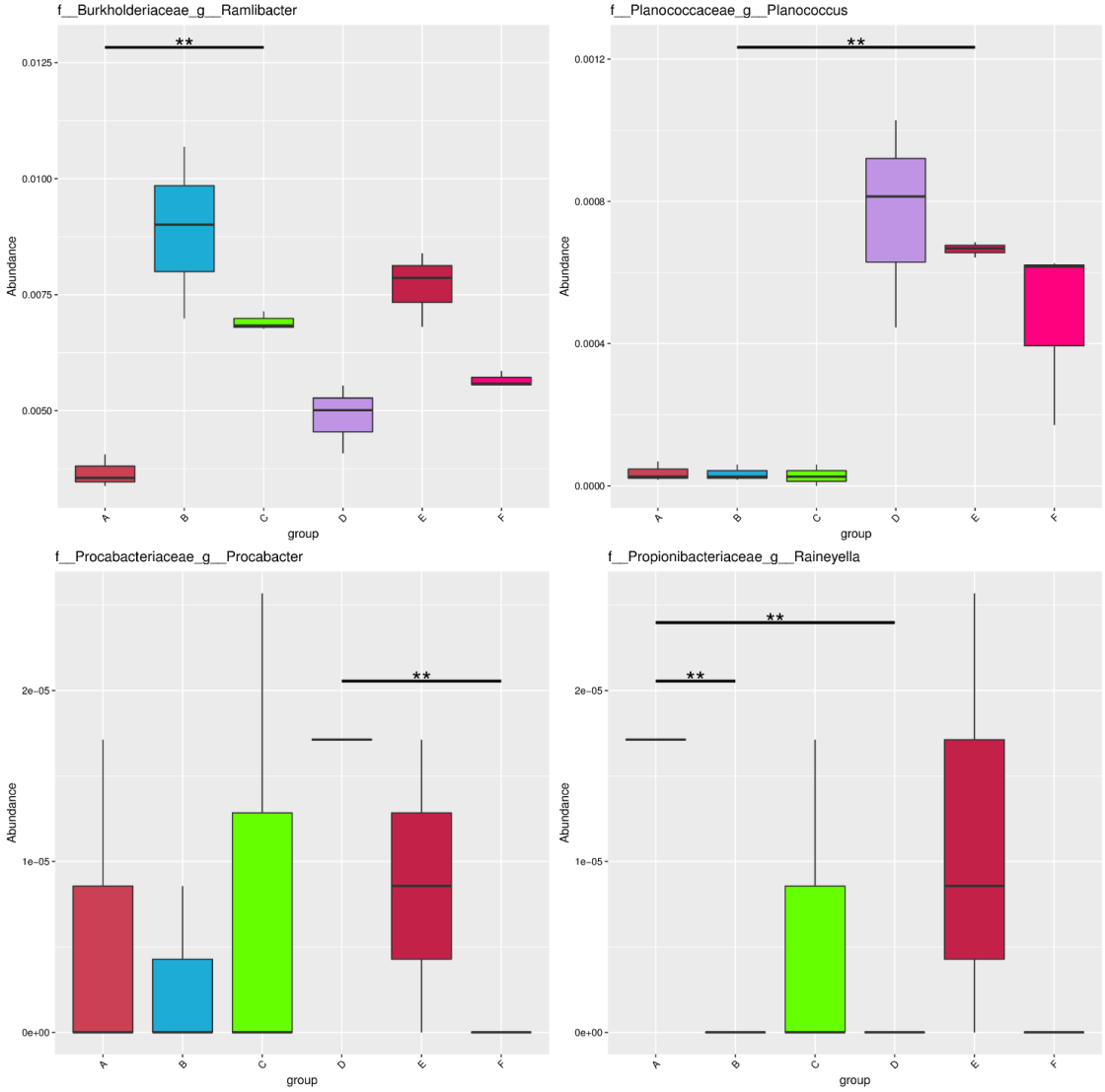

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Figure A20. *Metastat boxplots at the species level for the taxa that showed significant differences within grouping type ABCDEF of microbiomes collected from surrounding bulk (grouping type ABC) and rhizosphere (grouping type DEF) soils of Abutilon fruticosum after 0 (grouping type AD), 24 (grouping type BE) and 48 h (grouping type CF) of watering*

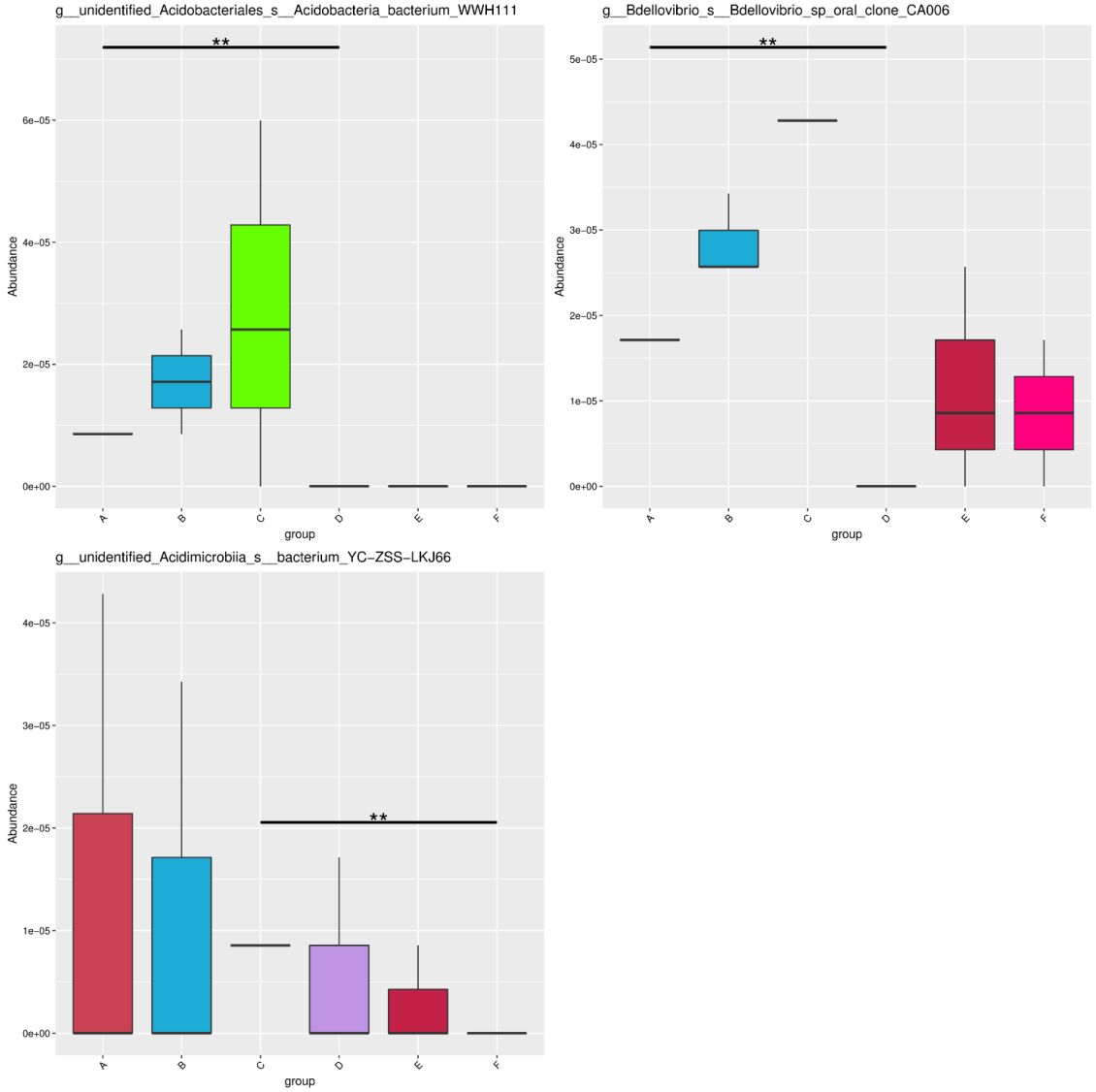

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Figure A21. *Metastat boxplots at the phylum level for the top 12 taxa that showed significant differences between groups of microbiomes collected from surrounding bulk (group J) and rhizosphere (group K) soils of Abutilon fruticosum regardless of time after watering*

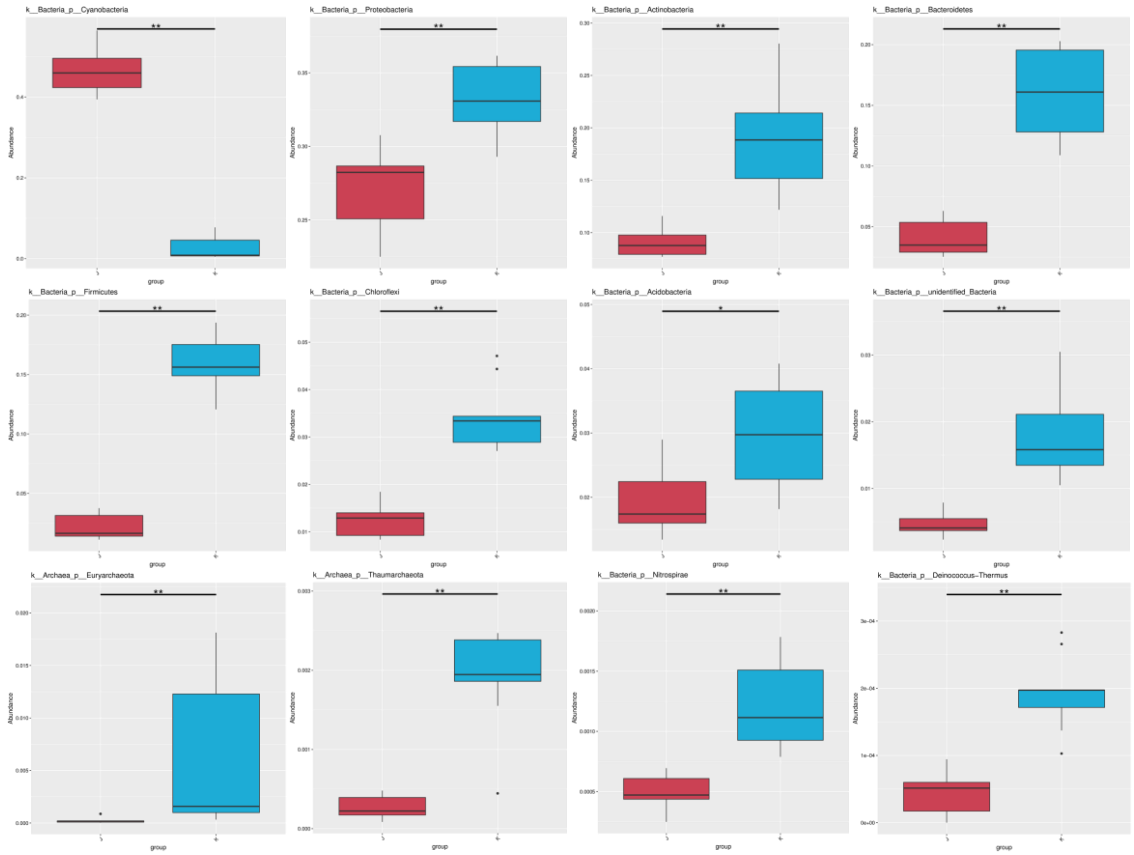

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Figure A22. *Metastat boxplots at the genus level for the top 12 taxa that showed significant differences between groups of microbiomes collected from surrounding bulk (group J) and rhizosphere (group K) soils of Abutilon fruticosum regardless of time after watering*

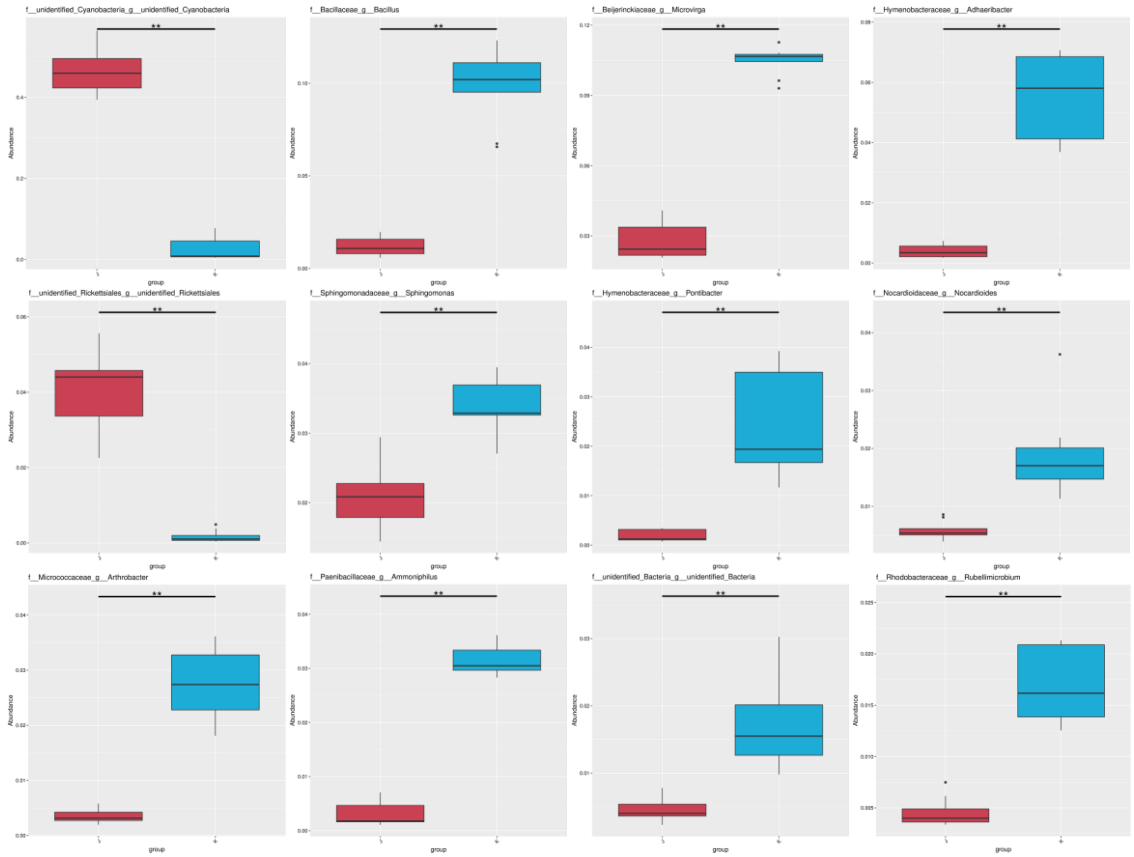

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Figure A23. *Metastat boxplots at the species level for the top 12 taxa that showed significant differences between groups of microbiomes collected from surrounding bulk (group J) and rhizosphere (group K) soils of Abutilon fruticosum regardless of time after watering*

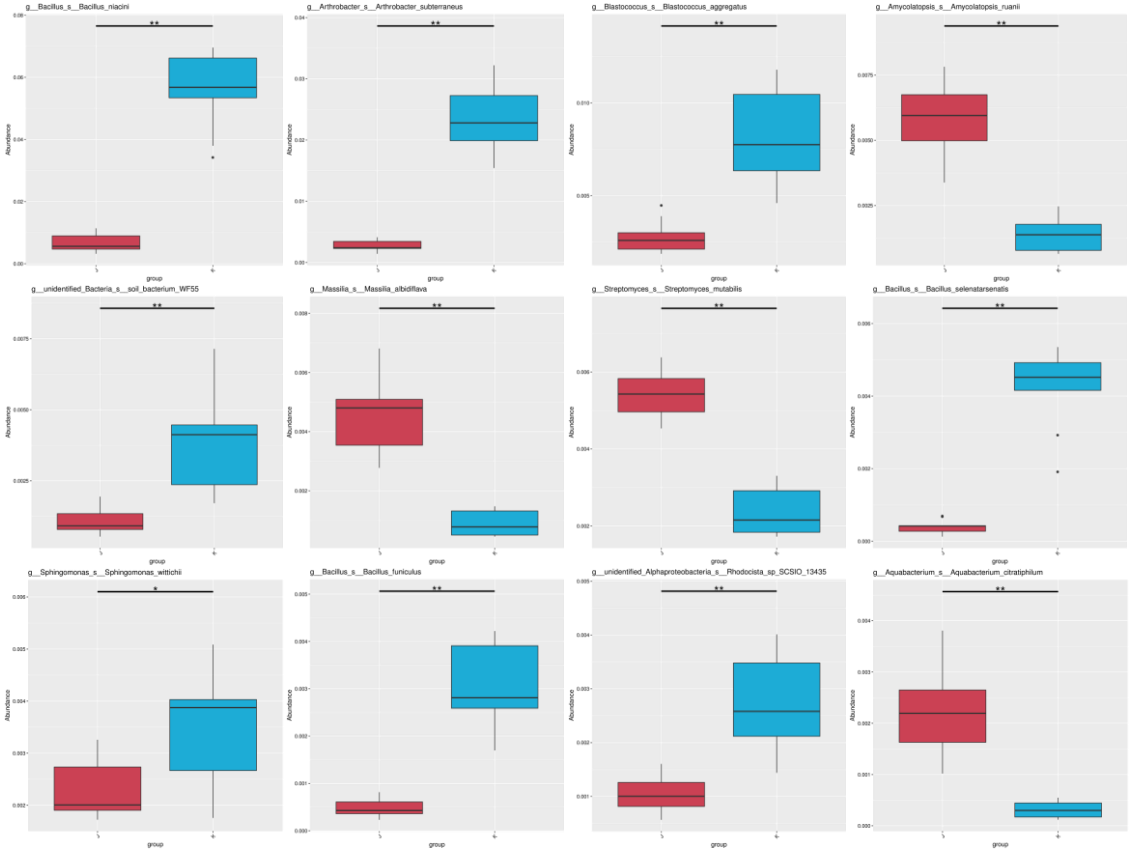

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Figure A24. *Metastat boxplots at the phylum level for the taxa that showed significant differences within grouping type GHI of microbiomes collected from surrounding bulk and rhizosphere soils of Abutilon fruticosum after 0 (group G), 24 (group H) and 48 h (group I) of watering regardless of source of microbiome*

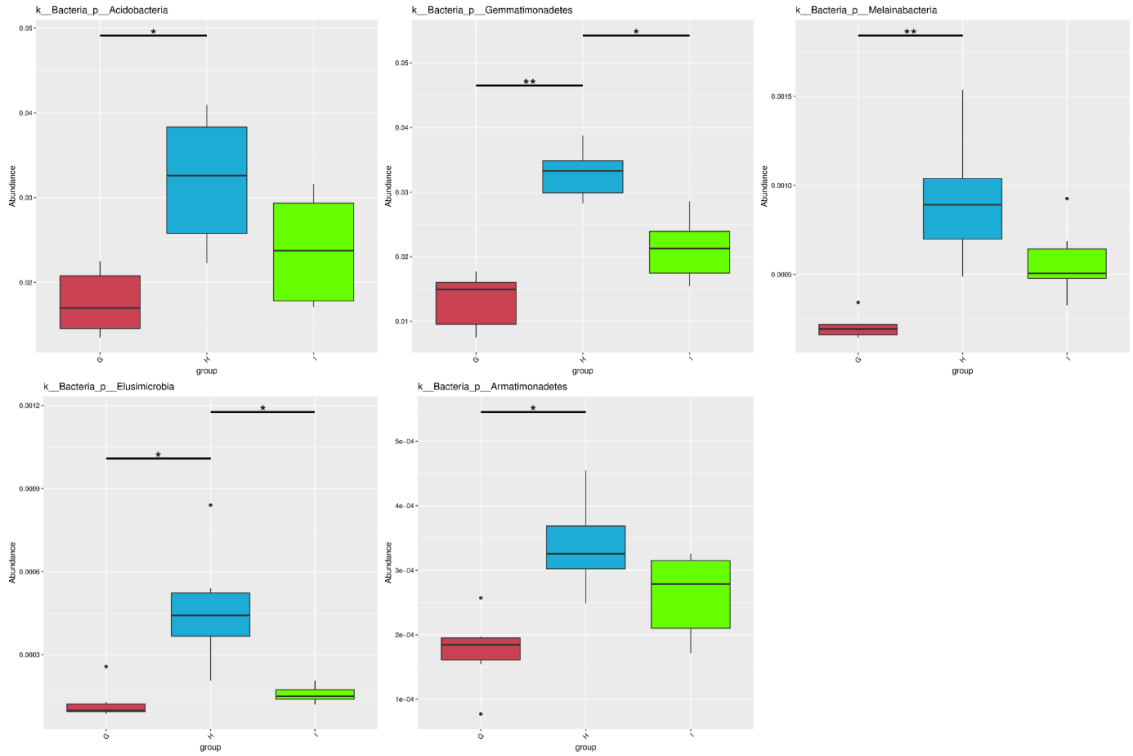

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Figure A25. *Metastat boxplots at the genus level for the taxa that showed significant differences within grouping type GHI of microbiomes collected from surrounding bulk and rhizosphere soils of Abutilon fruticosum after 0 (group G), 24 (group H) and 48 h (group I) of watering regardless of source of microbiome*

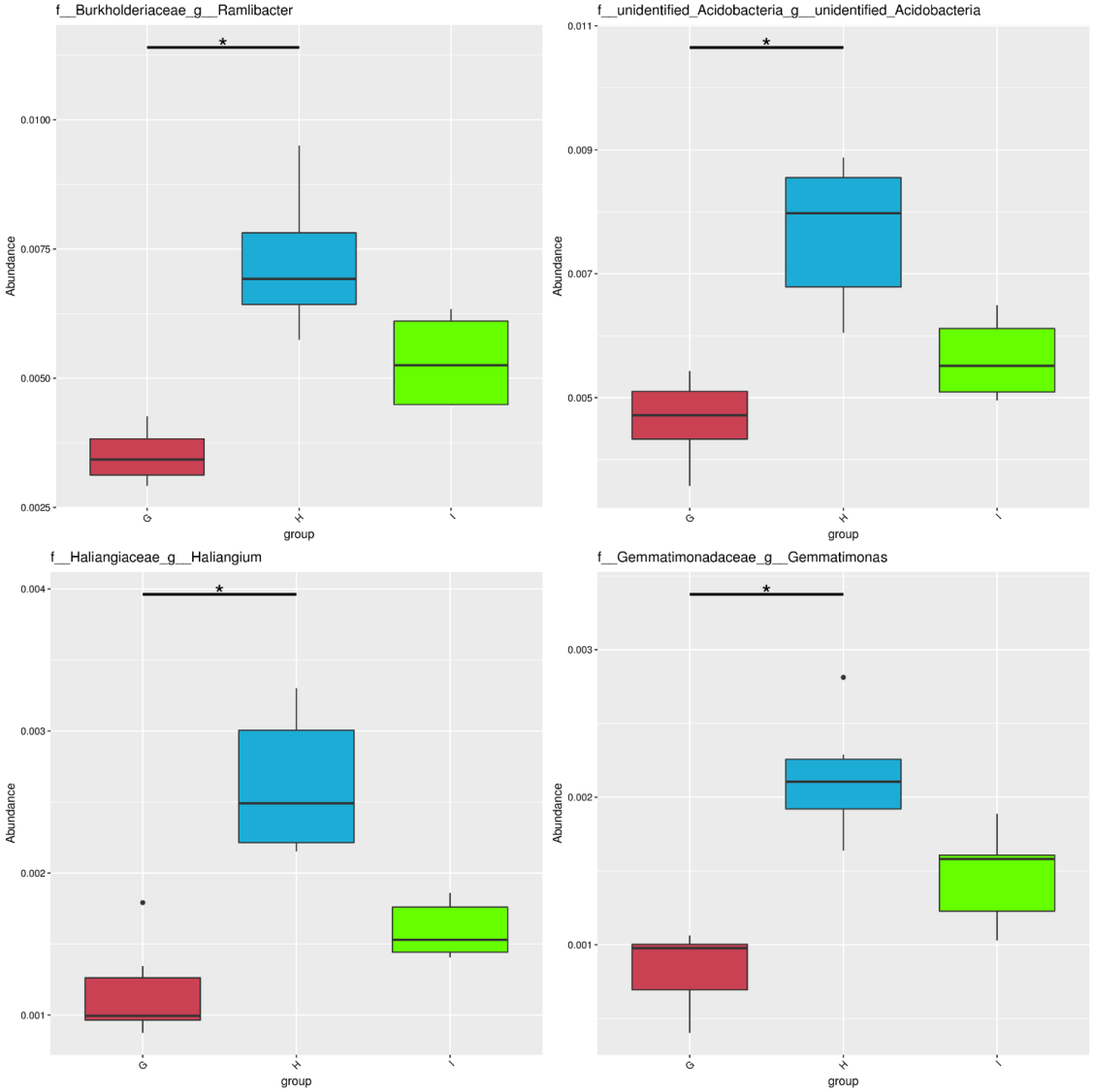

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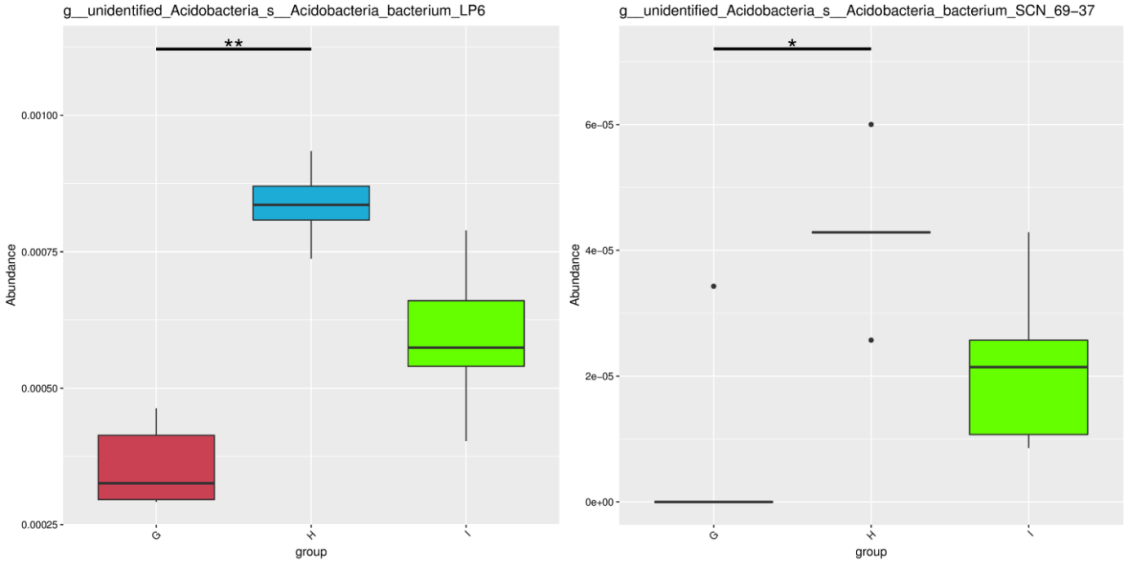
Figure A26. *Metastat boxplots at the species level for the taxa that showed significant differences within grouping type GHI of microbiomes collected from surrounding bulk and rhizosphere soils of Abutilon fruticosum after 0 (group G), 24 (group H) and 48 h (group I) of watering regardless of source of microbiome*



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