

COMPARISON OF C AND N MINERALIZATION AND METAGENOME ANALYSIS OF RHIZOSPHERE SOILS BELONGING TO DIFFERENT *Colchicum* L. SPECIES

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Abstract: This study is aimed at determining the characteristics and microbiota of soil upon which some Turkish *Colchicum* (Colchicaceae) species naturally grows. For this aim the rhizosphere soil samples of *Colchicum balansae* Planch., *Colchicum triphyllum* Kunze and *Colchicum variegatum* L. were analysed in this research. The carbon mineralization rate of *C. balansae* soil at $p < 0.05$ is significantly different from that of the other two soils. In terms of nitrogen mineralization, significant difference exists between all the three soils ($p < 0.001$). *Colchicum variegatum* rhizosphere was found to have the highest bacterial diversity. The results revealed that 254 bacterial species were common to the three rhizosphere soils, 35.60% of the bacterial species were unique to *C. variegatum* soil while 21.89% and 22.67% of the bacterial species were unique to *C. balansae* and *C. triphyllum* soil respectively. It was found that *C. variegatum* and *C. balansae* collected from areas close to each other had the highest number of common bacterial species, while *C. triphyllum* from the distant region shared 75 with *C. variegatum* and 19 with *C. balansae*. Metagenomics analysis reveals that in the rhizosphere of *C. variegatum*, *C. balansae* and *C. triphyllum*, Actinobacteria is dominant at phylum level. Likewise, in *C. variegatum* soil, *Nitrosocosmicus* and halophilic *Halobacter* were found to be the dominant archaea. In the soils of *C. triphyllum* and *C. balansae* Saccharomycetales were detected, while *Cryptococcus neoformans* var. *grubii* H99 was exclusively detected in *C. balansae* soil. Significant difference ($p < 0.05$) was observed in *C. variegatum* rhizosphere soil in terms of organic carbon (C%) and carbon mineralization from the other two soil samples. Significant differences were observed in all three soils in terms of nitrogen content, and the *C. triphyllum* rhizosphere soil was significantly different from the others in terms of available phosphorus content ($p < 0.05$). This study showed that biological as well as the physico-chemical properties of the rhizosphere soil regulate soil microbial diversity and density and by extension influences their activity which evidently manifests itself in carbon and nitrogen mineralisation.

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Özet: Bu çalışma, bazı *Colchicum* L. (Colchicaceae) türlerinin doğal olarak yetiştiği toprağın özelliklerini ve mikrobiyotasını belirlemeyi amaçlamaktadır. Bu amaç ile *Colchicum balansae* Planch., *Colchicum triphyllum* Kunze ve *Colchicum variegatum* L.'nin rizosfer toprak örnekleri analiz edilmiştir. *Colchicum balansae* toprağının karbon mineralizasyon oranı diğer iki toprağından önemli ölçüde farklıdır ($p < 0,05$). Azot mineralizasyonu açısından, her üç toprak arasında önemli bir fark vardır ($p < 0,001$). *Colchicum variegatum* rizosferinin en yüksek bakteri çeşitliliğine sahip olduğu belirlendi. 254 bakteri türü üç rizosfer toprağında ortak, bakteri türlerinin %35,60'ı *C. variegatum* toprağına özgüydü, bakteri türlerinin sırasıyla %21,89'u ve %22,67'si *C. balansae* ve *C. triphyllum* toprağına özgüydü. Birbirine yakın bölgelerden toplanan *C. variegatum* ve *C. balansae*'nin en fazla ortak bakteri türüne sahip olduğu (116), uzak bölgeden gelen *C. triphyllum*'un ise *C. variegatum* ile 75 ve *C. balansae* ile 19'unu paylaştığı tespit edildi. Metagenomik analiz, *C. variegatum*, *C. balansae* ve *C. triphyllum*'un rizosferinde Actinobacteria'nın baskın bakteri filumu olduğunu ortaya koymaktadır. Bunun yanında *C. variegatum* toprağında *Nitrosocosmicus* ve halofilik *Halobacter*'in baskın arke olduğu bulunmuştur. *Colchicum triphyllum* ve *C. balansae* topraklarında Saccharomycetales tespit edilirken, *Cryptococcus neoformans* var. *grubii* H99 sadece *C. balansae* toprağında tespit edildi. *Colchicum variegatum* rizosferinde diğer iki toprak örneğinden organik karbon (%C) ve karbon mineralizasyonu bakımından önemli derecede farklılık ($p < 0,05$) gözlenmiştir. Toprakta azot içeriği bakımından her üç toprakta anlamlı farklılık gözlenmiş olup yarıyıllı fosfor içeriklerinde ise *C. triphyllum* diğerlerinden anlamlı derecede farklıdır ($p < 0,05$). Bu çalışma, rizosfer toprağının biyolojik ve fiziko-kimyasal özelliklerinin, toprak mikrobiyal çeşitliliği ve yoğunluğunun karbon ve azot mineralizasyonuna olan etkilerini göstermektedir.



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Introduction

Organisms are colonised by microbes, which were found to have significant impacts on the development and health of their hosts (Berg *et al.* 2016, Mendes & Raaijmakers 2015). The microbiome determining the health, development and productivity of a plant is generally regarded as the plant's second genome (Lakshmanan *et al.* 2014). Endosphere, rhizosphere and phyllosphere of plants provide microbial communities with specific functions. Substantial implication on the ecosystem arises due to fluctuations in plant - soil microbe interactions, because soil microbes influence plant growth and survivability through different mechanisms (Compant *et al.* 2019). To predict future ecosystem function, series of comprehensive study on microbial communities and plants as well as their reaction towards climate change became crucial (Classen *et al.* 2015). The properties of ecosystem are shaped and maintained via the interaction of soil microbes with one another and with the plant. Landscape patterns of animal and plant composition, abundance and diversity can be shaped by the aforesaid microbial interactions (Berg *et al.* 2010, van der Putten *et al.* 2013). Metagenomics analysis reveals that through conventional methods, only 5% of bacteria has been successfully cultivated (Mendes & Raaijmakers 2015). Berg *et al.* (2016) on studying the colonization of the rhizosphere of *Solanum distichum* Schumach. & Thonn. (Nightshade) and *Matricaria chamomilla* L. (Chamomile) by microbes note that specific microbial communities are selected by different plants. As a result of exudate variation relative to plant developmental stages, specific microbial communities are selected at a time (Chaparro *et al.* 2013). As microbes proliferate in close proximity with a variety of host plants, fluctuations in environmental conditions influence their interactions amongst themselves and their plant hosts (Braga *et al.* 2016).

When the movement of soil organic matter in nature is examined, it is understood that each stage is interconnected and even dependent on each other. Organic wastes are first broken into small pieces by the soil fauna and mixed into the soil, so it is possible for other soil creatures to break down and decompose more easily. While all factors are at the optimum level, the change of even one factor affects this cycle, which is reflected in carbon mineralization (Ayilara *et al.* 2020).

Interactions between microbial communities in soil and plants are not unidirectional, the plant influences the type of microbial community that will become established around it by exuding nutrients and these microbial communities will establish a positive, neutral or negative relationship with the host plant (Thrall *et al.* 2007). In their interactions with plants, prokaryotic and eukaryotic microbes have evolved a myriad of cooperative and competitive mechanisms shaping microbial assemblages on plants (Hassani *et al.* 2018).

Recently, Turkey has been shown to be the richest country in terms of *Colchicum* L. diversity. Persson (2007) reported that the genus is represented with a total of 99 species in the world and approximately half of the 50 endemic species are distributed in Turkey (Uysal &

Kaya 2019). From time immemorial, *Colchicum* species have been employed in treating numerous disorders due to their anti-inflammatory, therapeutic and anti-tumoral activities (Alali *et al.* 2007). According to their flowering time, *Colchicum* species have been categorized into two categories, viz. Hysanthous in which flowering occurs in autumn while leaves and fruits appear in spring and Synanthous in which both the flowers and the leaves emerge together in spring. *Colchicum variegatum* and *C. balansae* fall under the autumn flowering type while *C. triphyllum* falls under the spring flowering type (Toplan *et al.* 2016). *Colchicum* species have been found to be rich in phenolic acids, alkaloids, flavonoids, fatty acids and tannins (Evans 2002). The study is of great importance in terms of determining the microbial activities that reflect environmental changes in the most accurate and precise way and understanding the ecology of these plants under the threat of global warming.

Materials and Methods

Sample collection

The present study aimed to reveal and compare the carbon mineralization and microbial diversity of the rhizosphere soils of three *Colchicum* species, one of which is endemic, in different regions of Turkey.

The rhizosphere soils of *C. variegatum*, *C. balansae* and *C. triphyllum* were separately collected from Muğla-Kale Road (37°15'34.3" N, 28°40'11.9" E) (Mediterranean climate), Datça-Knidos Road (36°43'31" N, 27°35'49" E) (Mediterranean climate) and Niğde-Çamardı (37°50'16" N, 35°1'26" E) (terrestrial climate) Turkey, respectively. For each plant species, the rhizosphere soils were collected from each of the localities and soils of each locality were mixed homogeneously and analysed in triplicate.

Physico-chemical analysis

The soil samples were air dried. The air-dried soil samples were sieved and their pH values were measured (Jackson 1958). The organic carbon content of the samples were determined by the Anne method (Duchauffour 1970), and total nitrogen contents were evaluated by the Kjeldahl method (Bremner 1965). Available phosphorus content (ppm) of the samples were determined following the description of Olsen (1954). Soil texture was identified according to Bouyoucos (1951), while CaCO₃ content (Allison & Moodie, 1965) and field capacity (Demiralay, 1993) were determined with Scheibler calcimeter and 1/3 atm pressurized pump, respectively.

Bacterial and fungal count determination

Total bacterial count of each soil sample was determined using the Petroff-hausser counting chamber. 10 g of soil sample was added into 95 mL sodium pyrophosphate solution (0.1% w/v) contained in a beaker. The mixture was homogenized at room temperature on an orbital shaker at 100 rpm for 30 minutes. The homogenized mixture was serially diluted (10⁻¹ to 10⁻⁶) in sterile distilled water, 100 µl aliquots from the dilution were plated on

appropriate culture media [Tryptone Soya Agar (TSA) (Oxoid PO0163A, Basingstoke, Hampshire, England) for enumeration of total aerobic mesophilic and spore forming bacteria and modified Czapek Dox Agar (Oxoid CM0097) for fungal counts] and incubated at 30°C. Aerobic mesophilic bacteria and spore forming bacteria were evaluated after 2 days of incubation, while fungal colony count was determined after 3 days incubation.

Carbon and nitrogen mineralization determination

In a 750 mL glass jar, 80 g of the air-dried soil was moistened up to 80% of its field capacity. A 50 mL beaker containing 10 mL of 1M NaOH was placed at the middle of the jar containing the moistened soil. The jar was tightly sealed and incubated at 28°C. Empty vessels served as blank control. After every 3 days of incubation, 2 mL of BaCl₂ was added to the jar containing the NaOH, a few drops of 0.1% phenolphthalein solution – an indicator – was added upon the emergent solution and 1M HCl was titrated upon the solution (Alef & Nannipieri 1995). In place of the beaker that has been removed from the jar, another new beaker containing 10 mL 1M NaOH was placed, the jar was tightly closed and taken back to the incubator. The carbon mineralized through microbial activity was expressed in mg per 100 g of dry soil [mg C/100 g of dry soil]. The ratio of the carbon that undergoes mineralization to the total soil carbon is specified as the "Mineralization rate".

For nitrogen mineralization, soils moistened at 80% of the field capacity in jars closed with two layers of cloth in order to ensure correct and sufficient aeration were placed in an incubator at 28°C. Soil samples were moistened every 2-3 days and incubated for 42 days. Measurements were made on the first and 42nd days of incubation. To determine mineral nitrogen (NH₄⁺ NO₃⁻) using the Parnas-Wagner procedure, 200 mL of 1 N calcium chloride was dispensed onto an appropriate amount of the soil sample, the mixture was agitated for 1 hour and distilled. Through dividing the amount of mineral nitrogen after 42 days of incubation by the amount of soil organic nitrogen, the nitrogen mineralization ratio of the soil was determined (Gökçeoğlu 1979, Lemee 1967).

Metagenomic Studies

Total DNA from the soil samples were isolated by using ZymoBIOMICSTM DNA Miniprep kit (Catalog number: D4300), (Zymo Research, USA) as described by the manufacturer. The forward and reverse primers: 27F (5'-AGAGTTTGATCCTGGCTCAG-3'), 1492R (5'-TACGGCTACCTTGTTACGACTT-3'), 21F (5'-TTCCGGTTGATCCYGCCGGA-3'), 958R (5'-YCCGGCGTTGAMTCCAATT-3'), EukF (5'-AACCTGGTTGATCCTGCCAGT-3'), EukR (5'-TGATCCTTCTGCAGGTTCCACTAC-3') were used for the amplification of the 16S rRNA gene of bacteria, fungi and archaea, respectively. DNA purity and concentration were analysed through 260/280 nm absorbance measure using NanoDrop spectrophotometer 2000 (Thermo Fischer scientific, USA).

Sequence Analysis and Evaluation of the Results

A sequencing protocol of 48 hours (R9.4) was performed with the aid of MinION™ [version 0.46.1.9 (R9.4)] control software. The read data was obtained based on the 1.2.2 rev 1.5 workflow and software Metrichor™ agent (version 0.16.37960). After the sequencing was completed, the results obtained in fast5 format were converted to fastq format using the guppy v3.1.5 software (base-calling and de-multiplexing). Barcode and adapter sequences were cleared using Porechop v. 0.2.3 software, and universal primers and labels were deleted by deleting 45 bases from both ends of the sequences. After the sequences were cleaned, the reads at 1300-1500 bp length were filtered and the remaining readings were excluded from analysis.

The cleaned readings were analysed with a customized workflow using the mothur v. 1.39.5 platform. Sequences were purified from chimeric structures, by measuring the distances between them with the similarity matrix to be aligned relative to each other, the readings showing more than 99% similarity were clustered and operational taxonomic units (OTUs) were formed. The generated OTUs were compared according to the RDP 16S rRNA database and their taxonomic annotations were performed and the OTUs that were determined as the same genus were associated and statistical results were obtained. Primer sequences used in sequencing were extracted from the raw data obtained from the Miniseq device. Primer-free reads were matched to each other. The matched reads were searched in the bacteria taxon database. The biome format was obtained and converted to grass format. QIIME II software (Bolyen 2019) was used for Alpha-beta diversity analysis from the grass table. Basic coordinate analyses were also executed relative to the organisms with whose OTUs are matched and their quantitative values were obtained.

Statistical analysis

All data obtained from the experiment were statistically analysed using SPSS windows (version 15.0, 2006). To determine significance differences existing between the different soil samples, One-Way ANOVA and Tukey HSD multiple comparisons tests were conducted. Differences between treatments were considered significant at p≤0.05.

Results

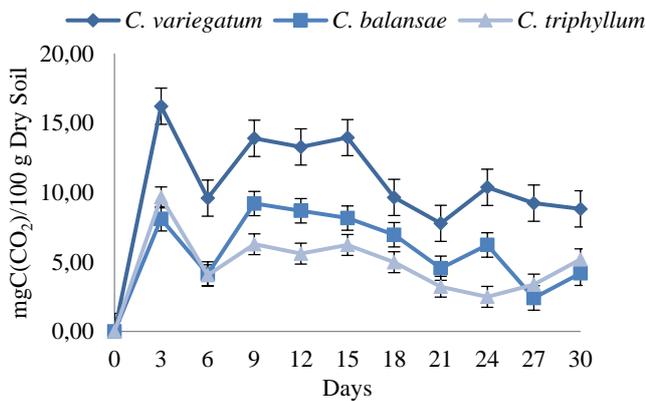
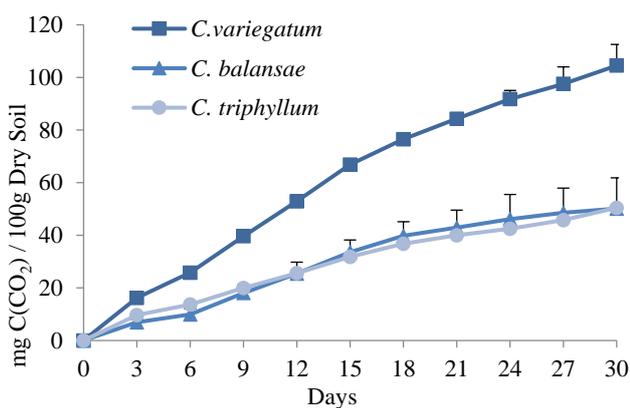
The physico-chemical characteristics of the analysed soil samples are presented in Table 1.

Daily carbon mineralization curves clearly show the changes that occurred during organic matter decomposition in the soils (Fig. 1). According to the curves of cumulative carbon mineralization, the carbon mineralization in the rhizosphere soil of *C. variegatum* was higher than that of others (Fig. 2). The rhizosphere of *C. balansae* was found to have the highest carbon mineralization rate (3.66%) and a carbon content of 1.37% which is lower than that of its counterparts (Fig. 3). This signifies that the organic matter present in this rhizosphere soil is easily mineralised.

Table 1. Physico-chemical characteristics of the soil samples analysed.

	<i>C. variegatum</i>	<i>C. balansae</i>	<i>C. triphyllum</i>
Sandy (%)	63.52 ± 0.01* c**	68.43 ± 0.01 b	79.05 ± 0.01 a
Silt (%)	32.32 ± 0.01 a	20.24 ± 0.01 c	13.61 ± 0.01 b
Clay (%)	4.16 ± 0.01 c	11.33 ± 0.01 a	7.34 ± 0.01 b
Texture	Sandy loam	Sandy loam	Loamy sand
Field capacity (%)	23.57 ± 0.01 a	18.57 ± 0.01 b	18.21 ± 0.01 c
CaCO ₃	0.53 ± 0.37 b	0.19 ± 0.10 b	9.31 ± 0.49 a
pH	7.64 ± 0.14 a	7.63 ± 0.04 a	7.86 ± 0.12 a
C (%)	4.00 ± 0.06 a	1.37 ± 0.17 b	1.87 ± 0.12 b
N (%)	0.24 ± 0.06 a	0.04 ± 0.01 c	0.17 ± 0.04 b
C/N	16.68 ± 0.34 c	34.67 ± 2.91 b	10.99 ± 0.70 a
P ₂ O ₅ (kg/da)	7.17 ± 0.14 b	6.04 ± 0.48 b	14.76 ± 0.91 a

*Values represent the means ± standard error of triplicate analysis. **Different letter in each row denote significant differences ($p \leq 0.05$) by Tukey's post-hoc test in ANOVA.

**Fig. 1.** Daily carbon mineralization, [mg C (CO₂)/100 g Dry Soil]**Fig. 2.** Cumulative carbon mineralization [mg C (CO₂) / 100 g Dry Soil]

After 42 days of incubation, *C. variegatum* rhizosphere soil was found to have the lowest nitrogen mineralization rate followed by that of *C. triphyllum* while *C. balansae* rhizosphere soil was found to exhibit the highest nitrogen mineralization rate (Table 2).

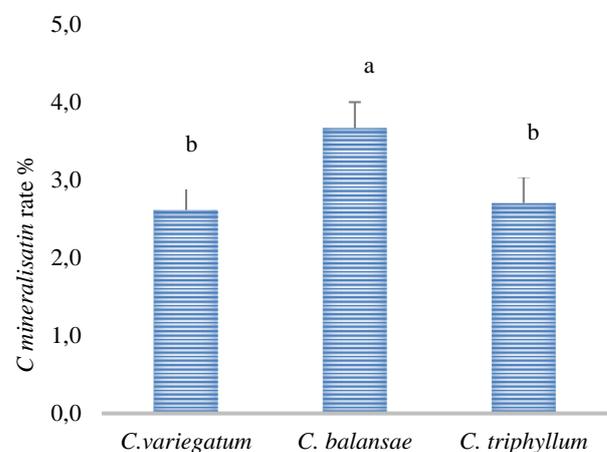
The *C. variegatum* rhizosphere soil sample was found to exhibit total aerobic mesophilic bacterial count of 1.3×10^4 cfu/mL, spore forming bacterial count of 2×10^4 cfu/mL and fungal count of 4.3×10^4 cfu/mL.

Colchicum balansae rhizosphere soil sample was found to exhibit total aerobic mesophilic bacterial count of 0.7×10^4 cfu/mL, spore forming bacterial count of greater than 0.5×10^4 cfu/mL and fungal count of 1.3×10^4 cfu/mL. While the rhizosphere soil sample of *C. triphyllum* was found to have aerobic mesophilic bacterial count of 1.4×10^4 cfu/mL, spore forming bacterial count of greater than 1×10^4 cfu/mL and fungal count of 6.7×10^4 cfu/mL.

The read counts of *C. variegatum*, *C. balansae* and *C. triphyllum* rhizosphere soil samples were determined to be 32,400, 27,800 and 32,200 respectively. The total OTUs of *C. variegatum*, *C. balansae* and *C. triphyllum* were determined to be 21,483, 18,747 and 22,961 respectively.

The rhizosphere soil of *C. balansae* was found to have the highest proportion of eukaryotic microorganisms with *Cryptococcus neoformans* var. *grubii* H99 been dominant with an abundance of 45%, the 4-7% human DNA found in this rhizosphere can be attributed to contamination of the sample with human remains due to handling or anthropogenic activities in the area (Fig. 4).

Since eukaryotic microorganisms have the ability to decompose polysaccharides that the Bacteria domain cannot biologically break down into smaller constituents with their exoenzymes, the diversity and proportional excess of eukaryotic microorganisms seen in *C. balansae* soil support the high carbon and nitrogen mineralization rates in this soil. In the rhizosphere soil of *C. triphyllum* Saccharomycetales – which play a substantial role in carbon cycle – were found to be the dominant eukaryotes with an abundance of 41%, so also *Beta vulgaris* subsp. *vulgaris* L. was found to be 31% abundant in the rhizosphere soil sample of *C. triphyllum* (Fig. 5), this emanate from *B. vulgaris* subsp. *vulgaris* plants growing at the location from which the samples were collected.

**Fig. 3.** Carbon mineralization rate (%)

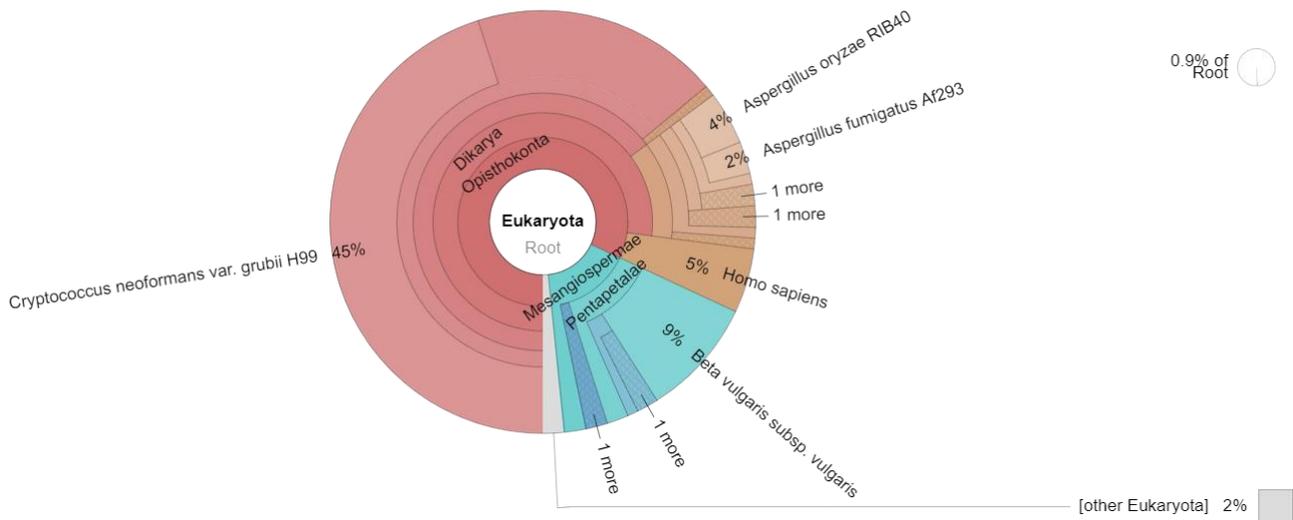


Fig. 4. Composition and abundance of domain Eukaryota in rhizosphere soil of *Colchicum balansae*.

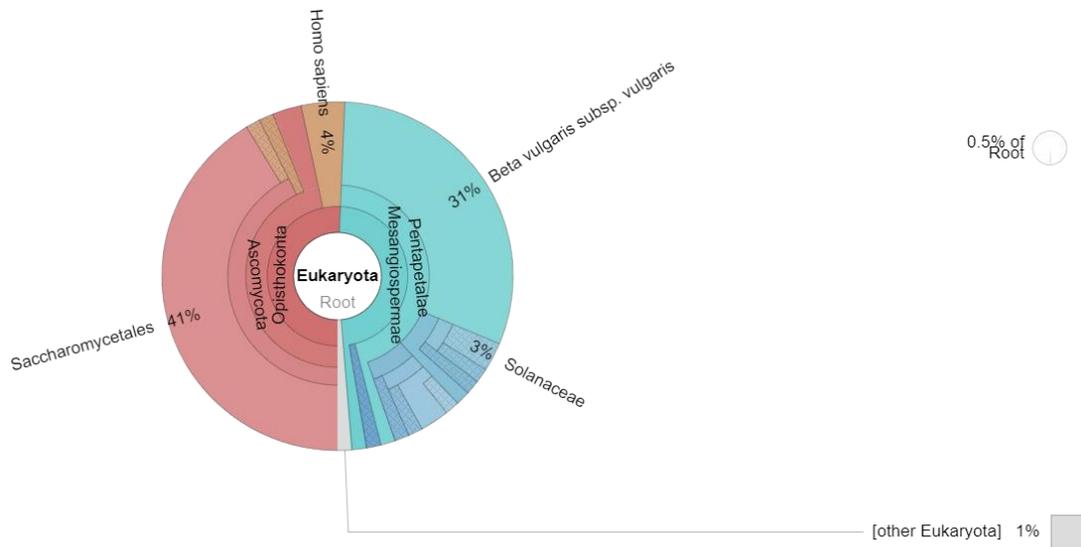


Fig. 5. Composition and abundance of domain Eukaryota in rhizosphere soil of *C. triphyllum*.

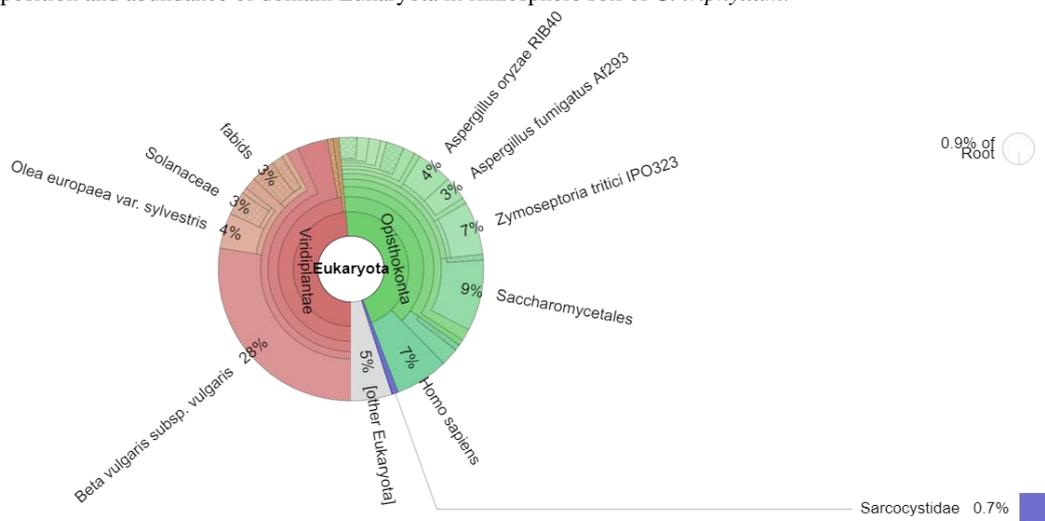


Fig. 6. Composition and abundance of domain Eukaryota in rhizosphere soil of *C. variegatum*

While the rhizosphere soil of *C. variegatum* was found to be the least in eukaryotic microbes abundance with Saccharomycetales been prominent (Fig. 6).

Figs 7-9 presents the percentage abundance of Archaea present in the analysed soil samples. *Haloprofundus* sp. MHR1 was found to be common to both *C. variegatum* and *C. balansae* rhizosphere soil samples occurring in 20% and 7% abundance respectively. Likewise, *Thermococcus* was found to be common to the rhizosphere soil samples of *C. balansae* and *C. triphyllum* at percentage abundance of 7% and 8% respectively. So also, the archea *Candidatus Nitrosocosmicus franklandus* were found to be common to the rhizosphere soil samples of *C. variegatum* (20% abundance) and *C. triphyllum* (33% abundance). *Candidatus nitrosونيا*, was found to be unique *C. variegatum* rhizosphere soil sample.

The abundance of bacterial phyla of the analysed rhizosphere soil was presented in Figs 10-12. It can be deduced that environmental factors as well as plant type

influences the quantity, quality and diversity of soil microbial content.

Metagenomic analysis reveals the diversity in bacterial phyla amongst the rhizosphere of the 3 different plants with dominance of Actinobacteria, Proteobacteria, Chloroflexi and Acidobacteria (Fig. 13).

Actinobacteria were found to be the dominant class in the rhizosphere of *C. balansae* and *C. triphyllum* while in the rhizosphere of *C. variegatum*, the dominant class was found to be Thermoleophilia (Fig. 14).

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Table 2. Nitrogen mineralization (mg/kg) rates (%).

	Day 1 (mg/kg) NH ₄ -N	NO ₃ -N3	Day 42 (mg/kg) NH ₄ -N	NO ₃ -N	N min rate (%)
C. variegatum	26.99 ± 0.50* b**	5.12 ± 0.21 b	38.80 ± 2.11 a	30.06 ± 1.64 b	2.87 ± 0.14 c
C. balansae	28.82 ± 0.40 a	4.33 ± 0.53 b	39.25 ± 0.44 a	13.75 ± 0.31 c	13.25 ± 0.17 a
C. triphyllum	24.72 ± 0.11 c	74.21 ± 7.10 a	37.37 ± 1.57 a	84.81 ± 3.09 a	7.19 ± 0.10 b

*Values represent the means ± standard error of triplicate analysis. **Different letter in each column denotes significant differences as $p \leq 0.05$ by Tukey's post-hoc test in ANOVA.

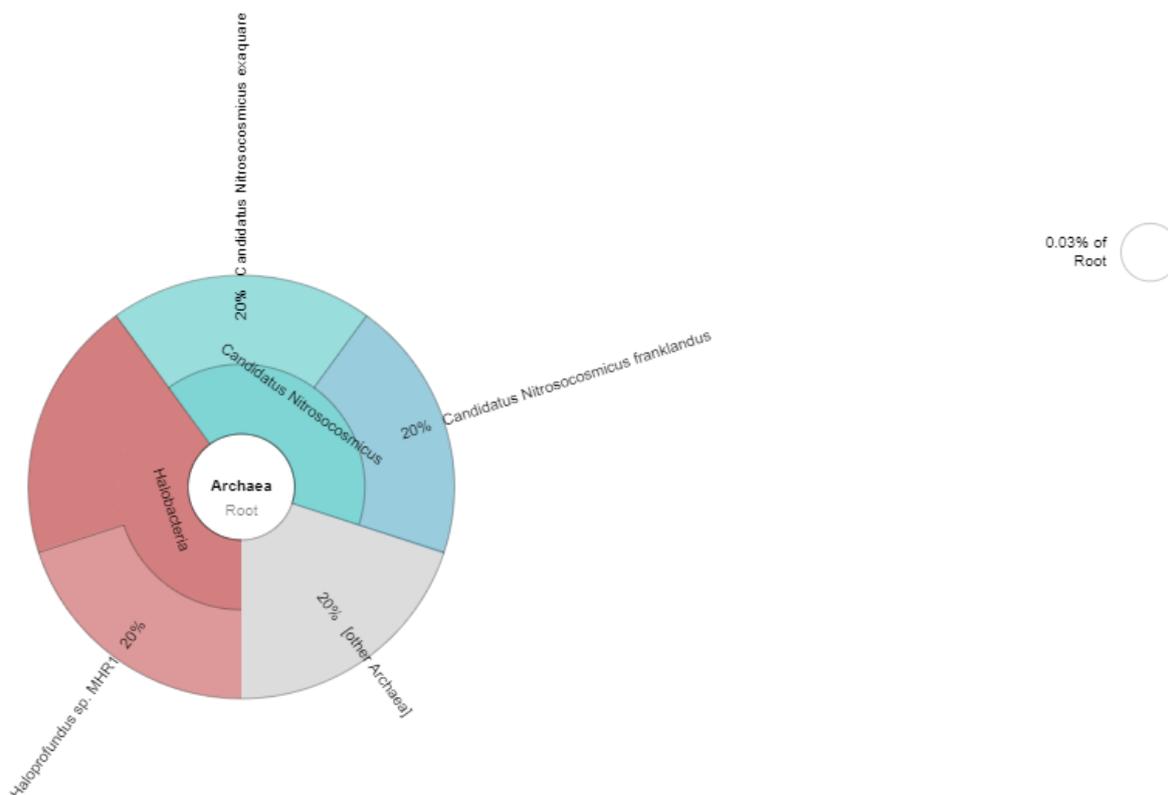


Fig. 7. Composition and abundance of domain Archaea in *C. variegatum* rhizosphere.

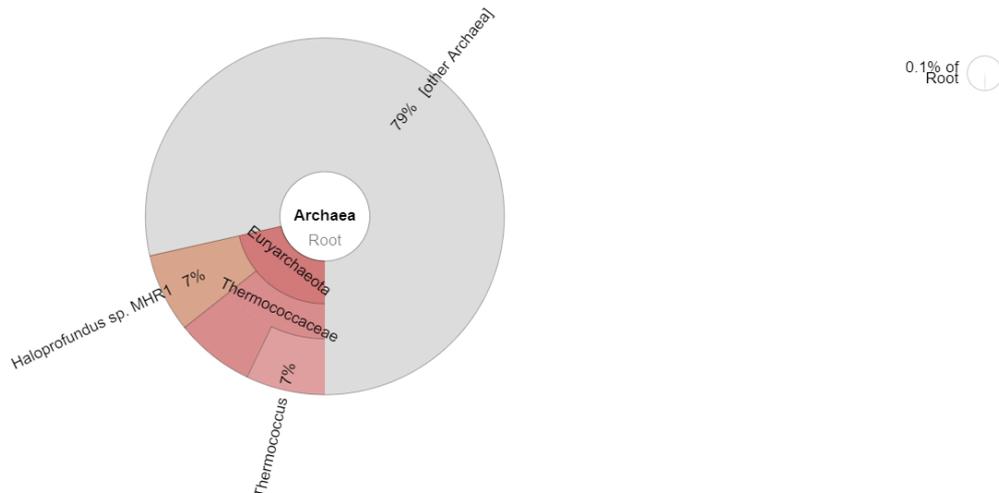


Fig. 8. Composition and abundance of domain Archaea in *C. balansae* rhizosphere.

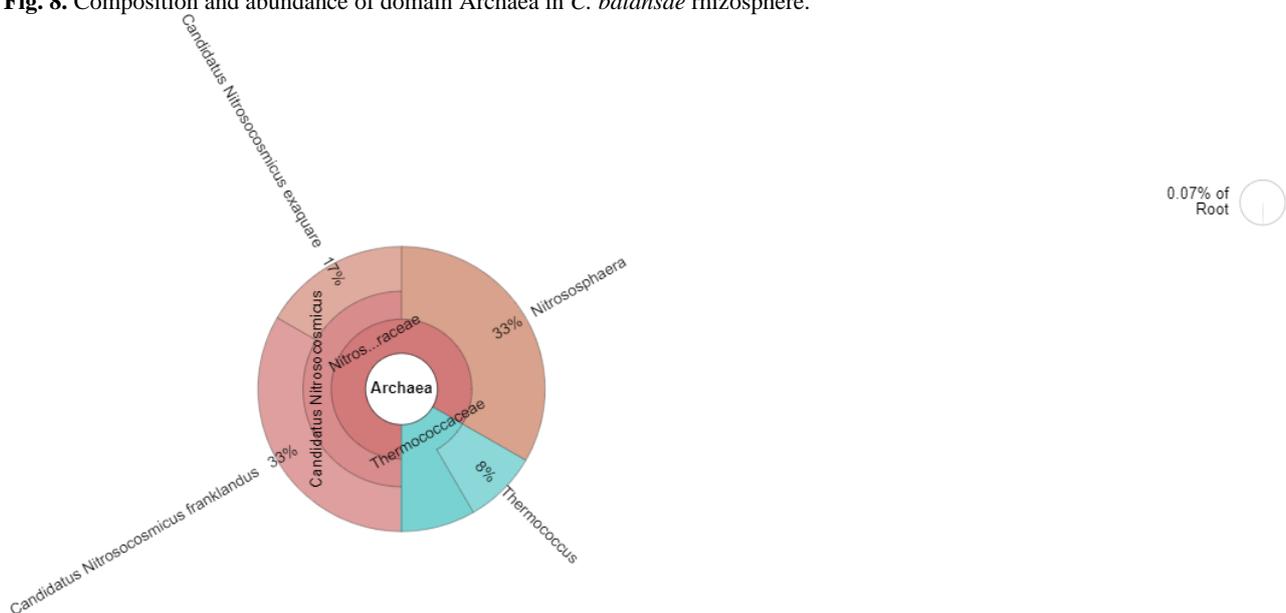


Fig. 9. Composition and abundance of domain Archaea in *C. triphyllum* rhizosphere.

Fig. 15 presents the percentage composition of bacteria genera in the three rhizosphere soils analysed. In *C. balansae* and *C. triphyllum* rhizosphere, *Microvirga* – belonging to the methylobacteriaceae – abundance was found to be higher in comparison to that found in the rhizosphere of *C. variegatum*. The percentage abundance of *Azospirillum* and *Nitrospira* genera were found to be higher in the rhizosphere of *C. balansae*.

Fig. 16 shows the bacterial species recorded in the three rhizospheres that were analysed metagenomically. The most bacterial species were found in the *C. variegatum* rhizosphere. Among all three rhizosphere soils, it was determined that 35.60% of the bacterial species in *C. variegatum* rhizosphere soil, and 21.89% and 22.67% of *C. balansae* and *C. triphyllum*, respectively, and they were unique bacterial species. It has been also observed that 254 bacterial species were common to the three rhizosphere soils. It was determined that 116 bacterial species in *C. variegatum* and *C. balansae* soils collected from regions close to each other were common only in the rhizosphere soil among the

analysed soils. It was also found that 75 bacterial species were common to *C. variegatum* and *C. triphyllum* while 19 bacteria were found to be common to the *C. triphyllum* and *C. balansae* rhizosphere soils.

The dominant species in *C. variegatum* rhizosphere where *Conexibacter woesei* DSM 14684 (2%), *Plantactinospira* spp. BC1 (1%), *Burkholderia pseudomallei* (0,8%), *Frankia alni* (0,8%). In the rhizosphere of *C. balansae*; *C. woesei* DSM 14684 (3%), *Plantactinospira* sp. BC1 (3%), *Solibacter usitatus* (1%), *M. dispar* (1%), *Baekduia soli* (1%) were found to be the dominant species while in the rhizosphere of *C. triphyllum* the dominant species were *Plantactinospira* spp. BC1 (3%), *Mycoplasma dispar* (3%), *Kribbella flavida* (2%), *C. woesei* DSM 14684 (2%) and *Rubrobacter indicoceni* (2%).

Alpha-Beta diversity analysis of the three analysed soil samples shows that *C. balansae* with Shannon index of 8.325 has greater diversity than *C. variegatum* and *C. triphyllum* whose index is 8.305 and 8.210 respectively (Fig. 17).

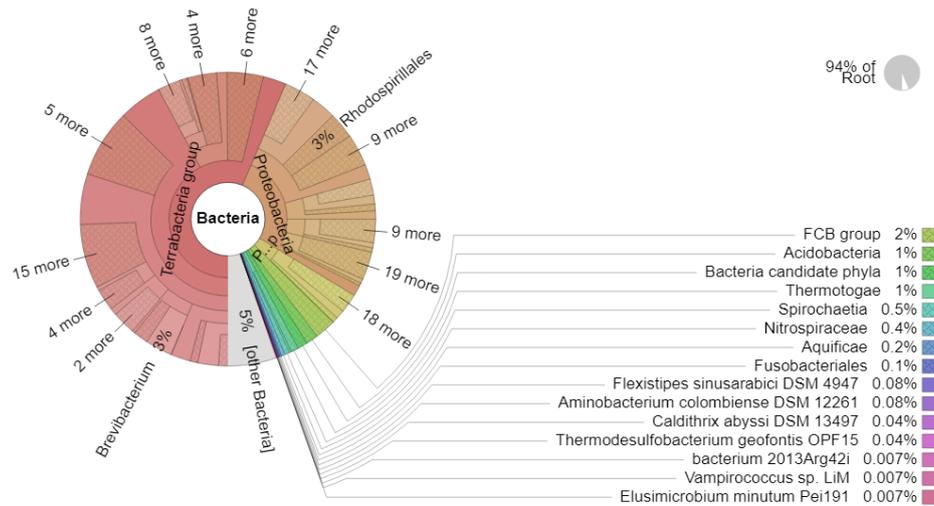


Fig. 10. Composition and abundance of domain Bacteria in *C. variegatum* rhizosphere

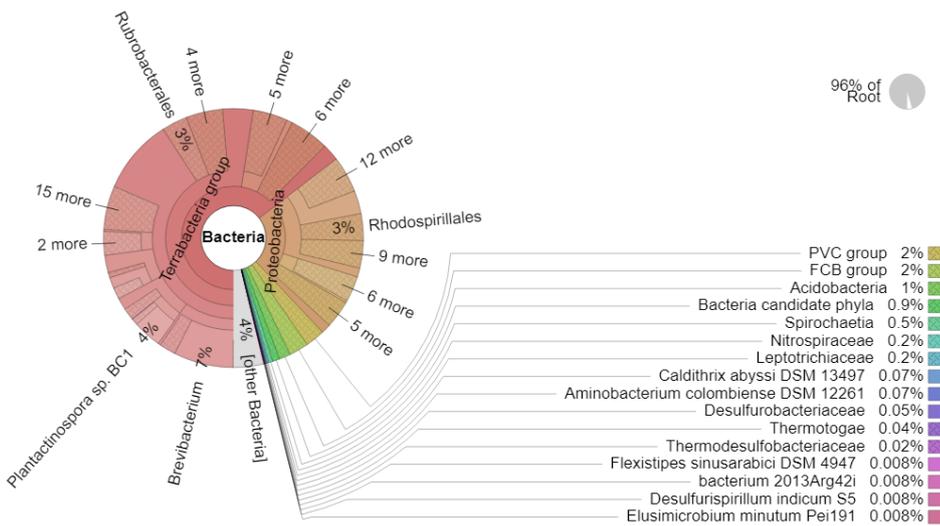


Fig. 11. Composition and abundance of domain Bacteria in *C. balansae* rhizosphere

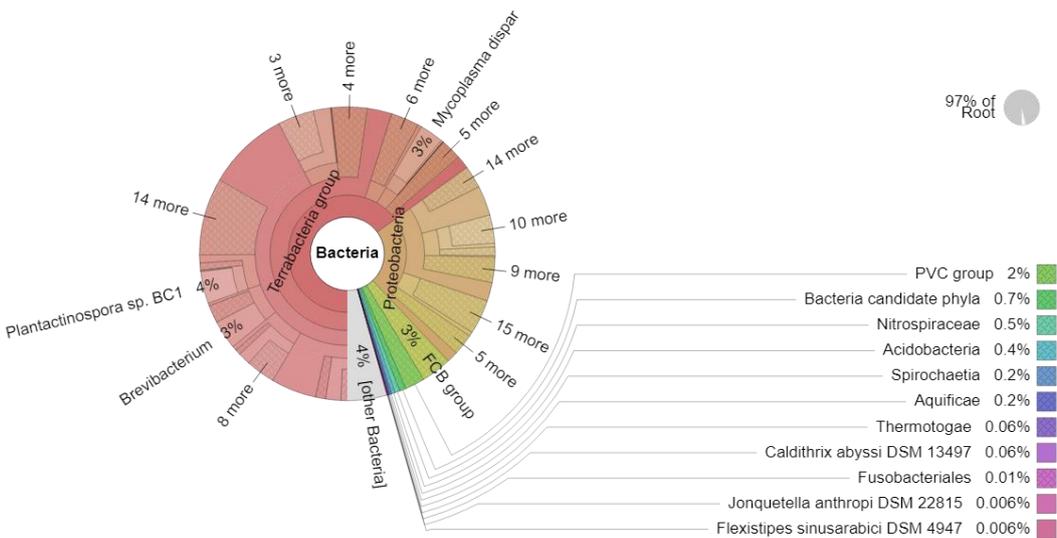


Fig. 12. Composition and abundance of domain Bacteria in *C. triphyllum* rhizosphere

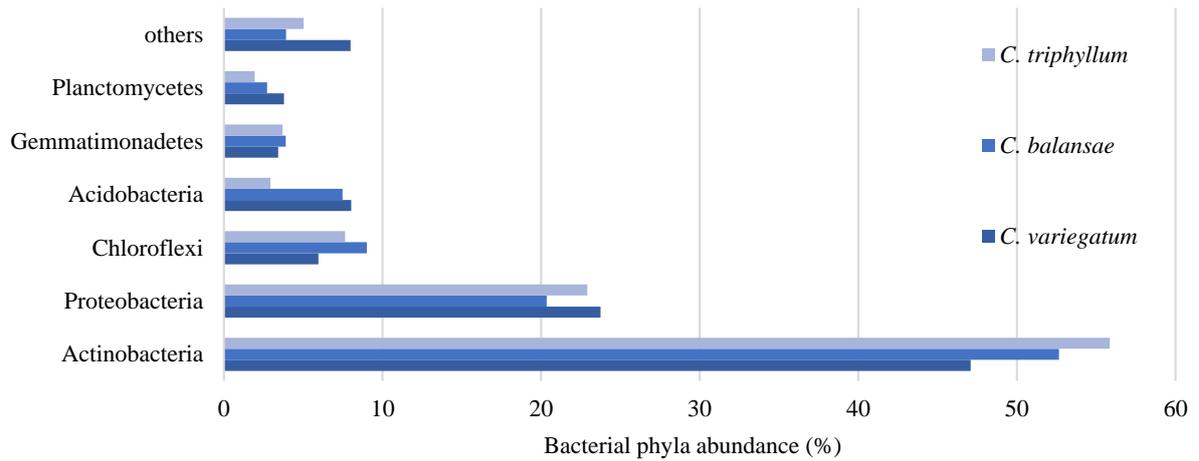


Fig. 13. Taxonomic distribution of identified bacteria in rhizosphere soil samples at phyla-level.

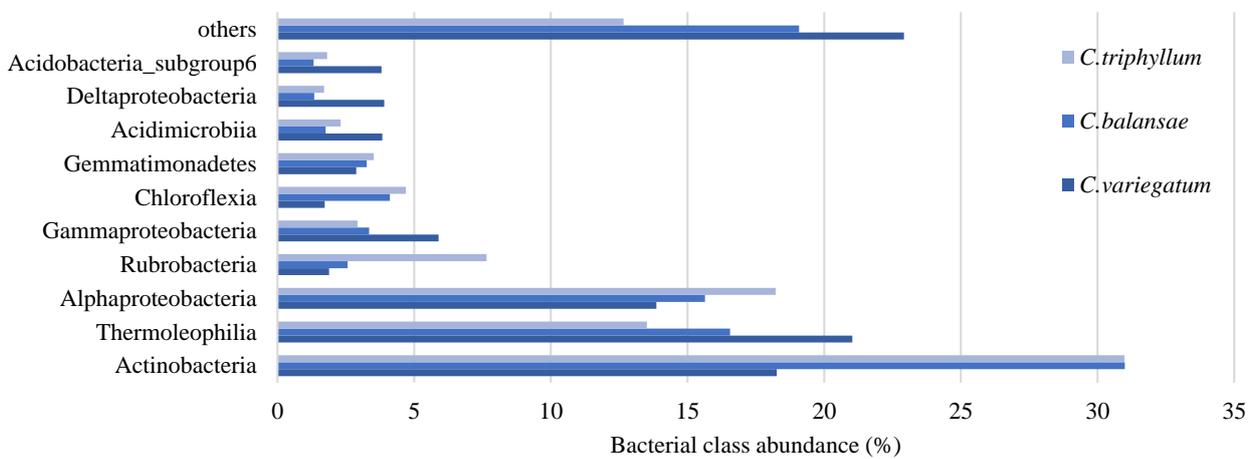


Fig. 14. Taxonomic distribution of identified bacteria in rhizosphere soil samples at class-level.

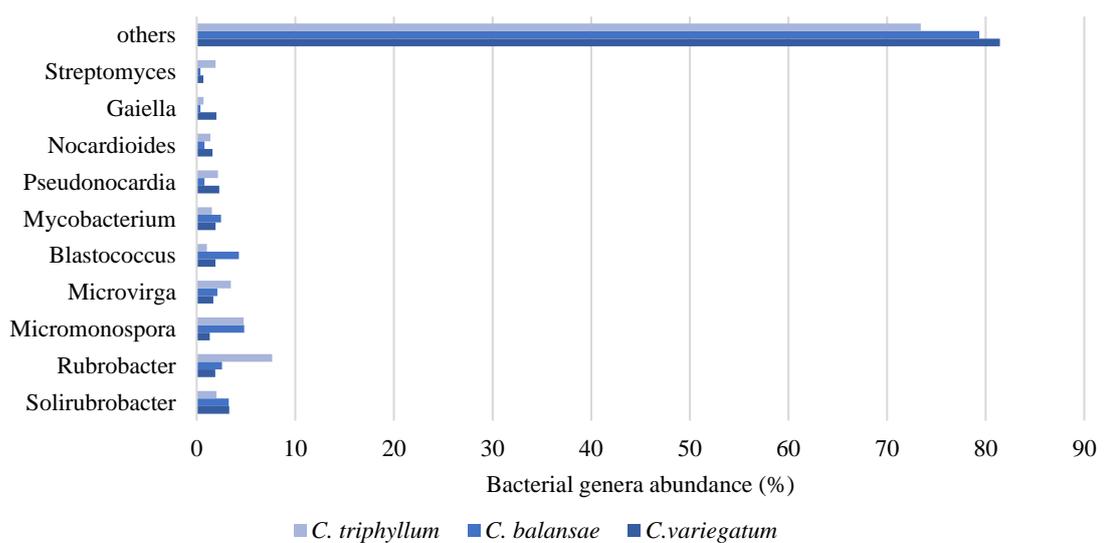


Fig. 15. Taxonomic distribution of identified bacteria in rhizosphere soil samples at genera-level.

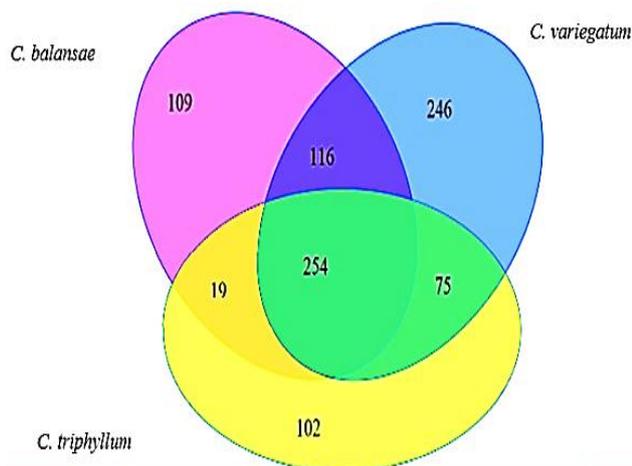


Fig. 16. Diversity of bacteria at species-level in the analysed rhizosphere soil.

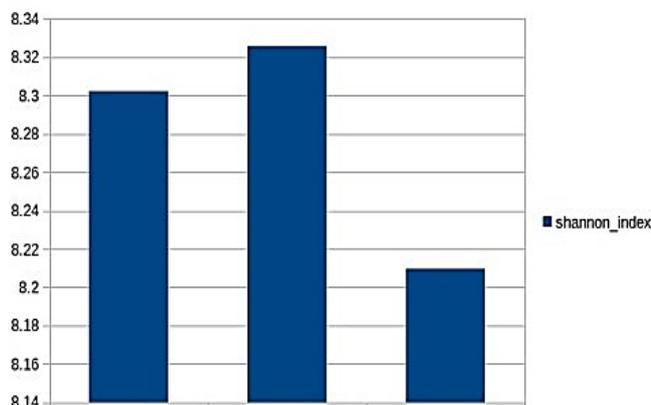


Fig. 17. Shannon index of the analysed rhizosphere soil samples.

Discussion

According to the daily mineralization curves, carbon mineralization was higher in the first three days and started declining on the 6th day. This shows that organic matter decomposing easily decreased in all three soils. The reversal of the graph between the days reflects that the organic matter in the soil is reduced to forms that decompose more easily thereby making them readily available for microbial utilization. From the 15th day, the curve started to move downwards rapidly in all the three analysed soils. This is due to insufficiency in organic matter utilizable by microorganisms.

The high carbon mineralization observed in *C. balansae* soil indicates that the microbial contents of it that play significant role in mineralization somehow differ from that of its counterparts in terms of quantity and quality. The rate of carbon mineralization recorded in the rhizosphere of *C. triphyllum* (1.87% C) and *C. variegatum* (4.0% C) is found to be 2.7% and 2.61% respectively. It can be deduced that high carbon content does not influence mineralization rate.

Plant and animal remains of the three rhizosphere soils were found at different proportions. The fact that the

highest number of eukaryotic microorganisms and the lowest plant residue are in *C. balansae* indicates that the abundance of plant and animal residues affect soil microbiota.

The abundance of the archaea *Halobacteria* was respectively found to be 40% and 7% in the rhizosphere of *C. variegatum* and *C. balansae* while in that of *C. triphyllum* none was found (Figs 7-9). This can be attributed to the fact that the bacteria, a halotolerant is usually found in coastal areas where salinity is high. In addition, *Nitrososphaera* whose percentage abundance was found to be 33% in *C. triphyllum* soil was not found in the other two soils. In *C. variegatum* and *C. triphyllum*, *Nitrosocosmicus* was found to be 40% and 50% abundant respectively. In *C. balansae* and *C. triphyllum*, Thermococcaeae was respectively determined to be 14% and 17% abundant. These results show that environmental factors such as plant type and location influence microbial diversity in the plant rhizosphere.

Actinobacteria is the most important because of the significant role it plays in agriculture. In soil, Actinobacteria act more like fungi, breaking down organic matter thereby making it readily available for plant utilization. The order Actinomycetales was thought for many years to be a group of fungi, thus the name “mycetales” which denotes mycelium. Some members of soil Actinobacteria establish symbiotic relationship with plants, where the plant is supplied with utilizable nitrogen by the bacteria. In addition to antibiotics synthesis, Streptomyces - a prominent member of the Actinobacteria group -and other members of the Actinobacteria group play a substantial role in soil buffering (Hogan 2010). Also, in the rhizosphere of *C. variegatum*, *C. balansae* and *C. triphyllum*, Acidobacteria was found at a percentage abundance of 8.02, 7.473 and 2.935% respectively. It was determined that *Acidobacteria* found in all soils belonging to different physiological characteristics were not sufficiently represented in culture (Barns *et al.* 2007). *Acidobacteria* are generally regarded as oligotrophic due to their growth in low organic environments (Kielak *et al.* 2016).

The high rate of nitrogen mineralization recorded in *C. balansae* rhizosphere – despite having low nitrogen content - is attributed to the proliferation of *Azospirillum* and *Nitrospira* within it, these bacterial genera were found to play significant role in nitrogen cycle. *Azospirillum* converts atmospheric nitrogen into utilizable nitrogen for plant assimilation thereby promoting plant growth (Katsy 2014, Madigan *et al.* 2003, Rosenberg 2013). *Azospirillum* can also alter the form of plant nutrients such as nitrogen and phosphorus to make them more available to plants. However, the amount of nitrogen fixed by *Azospirillum* via biological nitrogen fixation is still debated (Bashan & de-Bashan 2010). By synthesizing antioxidants, *Azospirillum* aid plants in alleviating stress due to drought or flood (Fukami *et al.* 2018). *Nitrospira* - a ubiquitous bacteria -in the second step of nitrogen fixation plays a prominent role in nitrogen fixation by oxidizing nitrite (Koch *et al.* 2015). Members of the genus

were assumed to be nitrite oxidizers because of their possession of nitrite oxidoreductase genes (Pester *et al.* 2014). Since the discovery of nitrifying bacteria, it was acknowledged that nitrification is a two-step process, though it is energetically unfavourable for one organism to accomplish the two steps (Costa *et al.* 2006). Recently, *Nitrospira* -Comammox bacteria- with the ability of performing the two steps of nitrification has been reported (Daims *et al.* 2015, Palomo *et al.* 2016, van Kessel *et al.* 2015). The manner through which bacteria contribute to nitrogen cycle was redefined with the discovery - within *Nitrospira* genus - of Comammox bacteria, thereby making future studies towards it crucial (van Kessel *et al.* 2015). With these new findings, there is the likelihood of adopting the complete nitrification process in engineered systems because of its low emission of greenhouse gases (Rodriguez-Caballero *et al.* 2013).

Solibacter usitatus -found in *C. balansae* rhizosphere- reduces nitrate because of its possession of norB gene. Phylogenetically candidatus *S. usitatus* and *Koribacter versatilis* are closely related. A substantial role is played by *Koribacter* in regulating CO₂ emission. Previous studies have shown that 20% of CO₂ emitted into the atmosphere is reduced by CO-oxidizing bacteria (Campbell 2014, Kishimoto *et al.* 1991). The high mineralization rate recorded in *C. balansae* rhizosphere despite its low nitrogen and carbon content can be attributed to the high abundance of the aforesaid bacteria.

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Conclusion

Carbon and nitrogen mineralization in *C. balansae*, *C. variegatum* and *C. triphyllum* rhizosphere were evaluated in this study. Soil microbial diversity and density were demonstrated to be influenced by biological as well as the soil physico-chemical properties. Carbon and nitrogen mineralization rates not only depend on the soil physico-chemical properties, but also vary depending on the type and density of microorganisms in the soil. *C. balansae* soil exhibited the highest carbon and nitrogen mineralization rate despite having lower carbon and nitrogen content than the other two soils.

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Author Contributions: Concept: İ.E., C.D., S.D., Desing: İ.E., C.D., S.D., Execution: İ.E., C.D., S.D., Material supplying: İ.E., C.D., S.D., Data acquisition: İ.E., C.D., S.D., Data analysis/interpretation: İ.E., C.D., S.D., Writing: İ.E., C.D., Z.K.S., S.D., Critical review: İ.E., C.D., S.D.

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