1   **Homogenization of Rhizosphere Bacterial Communities by Pea (*Pisum sativum* L.)**

2   **Cultivated under Different Conservation Agricultural Practices in the Eastern Himalayas**

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19 **Abstract:**

20 Conservation agriculture offers a suitable system to harmonize agriculture with the environment,

21 especially in fragile ecosystems of North-East India. Soil microbes play pivotal roles in

22 ecosystem functioning and act as indispensable indicators of overall fitness of crop plant and soil

23 health. Here we demonstrated that altercations in residue management and tillage practices lead

24 to the development of differential bacterial communities forcing the pea plants to recruit special

25 groups of bacteria leading to highly homogenous rhizosphere communities. Pea rhizosphere and

26 bulk soil samples were collected, and bacterial community structure was estimated by 16S rRNA

27 gene amplicon sequencing and predictive functional analysis was performed using Tax4Fun. The

28 effect on pea plants was evident in the bacterial communities as the overall diversity of

29 rhizosphere samples was significantly higher to that of bulk soil samples. *Bacillus*,

30 *Staphylococcus*, *Planomicrobium*, *Enterobacter*, *Arthrobacter*, *Nitrobacter*, *Geobacter,* and

31 *Sphingomonas* were noticed as the most abundant genera in the rhizosphere and bulk soil

32 samples. The abundance of Firmicutes and Proteobacteria altered significantly in the rhizosphere

33 and bulk samples, which was further validated by qPCR. Selection of specific taxa by pea plant

34 was indicated by the higher values of mean proportion of *Rhizobium, Pseudomonas, Pantoea,*

35 *Nitrobacter, Enterobacter* and *Sphingomonas* in rhizosphere samples, and *Massilia,*

36 *Paenibacillus* and *Planomicrobium* in bulk soil samples. Tillage and residue management

37 treatments did not significantly alter the bacterial diversity, while their influence was observed

38 on the abundance of few genera. Recorded results revealed that pea plant selects specific taxa

39 into its rhizosphere plausibly to meet its requirements for nutrient uptake and stress amelioration

40 under the different tillage and residue management practices.

41   **Key words:** Pea, Conservation Agriculture, Residue management, rRNA amplicon sequencing,

42   Rhizosphere, Tillage

1. Introduction:

44   Intensive agriculture practiced to meet the food production needs of the ever-increasing

45   population in the changing climate is posing a severe strain to soil and environmental health.

46   Adopting climate-friendly practices appear to be the best strategy to achieve sustainability in

47   agriculture (Rahi, 2017). Conservation agriculture (CA) being an evolved agro-ecosystem

48   management approach for preserving and enriching the environmental resources while

49   improving and sustaining the crop productivity amidst possible environmental stresses (Godfray

50   and Garnett, 2014; Nelson et al., 2017; Muzangwa et al., 2017). Adoption of reduced-tillage or

51   no-till practices preserve soil structure and minimize erosion and loss of organic matter

52   (Hogenhout and Loria, 2008; Mus et al., 2016). Comprehensively, CA requires the simultaneous

53   application of viable crop rotations, minimal soil disturbances, and crop residue retention

54   (Nichols et al., 2015).

55   North-East India is known for its diverse, risk-prone, and fragile hills and degraded plateau

56   ecosystems. The region is characterized by fragility, marginality, inaccessibility, and ecosystem

57   diversity. Around 77% of the region's area is hilly and eroded plateaus, with only 12% area

58   under net cultivation. No-till or minimal tillage systems of CA are the alternatives to reconcile

59   agriculture with its environment in these fragile and degraded ecosystems (Ghosh et al., 2010).

60   Incorporation of crop rotation and diversifying cropping systems with pulse crops, including pea,

61   lentil, and chickpea is also an essential step towards CA, which increase the crop yield and

62   decrease the impact of intensive cultivation on the environment (Laik et al., 2014; Gan et al.,

63   2015). Legume crop like peas can enhance soil organic nitrogen contributed by the symbiotic

64   association of legume and rhizobia (Herridge et al., 2008; Rahi et al., 2012; Rahman et al.,

65   2014).

66   Soil microbial diversity plays a significant role in sustaining ecosystem functioning, as microbial

67   communities influence several ecosystem processes and services. Microbial communities are

68   highly sensitive to soil management practices, and a detailed microbial community analysis can

69   reveal various important indicators of soil quality (Fierer et al., 2007; Sánchez-Cañizares et al.,

70   2017). Previous studies have suggested CA practices like no-till alter microbial diversity and

71   activity significantly when compared to conventional tillage (Degrune et al., 2017; Babin et al.,

72   2019). Similarly, nutrient and residue management practices like the application of chemical

73   fertilizers often result in a change in soil pH and organic carbon content influencing the

74   endogenous microbial communities (Fierer et al., 2007). The rainfed agricultural lands in North-

75   East India is highly susceptible to soil erosion leading poor soil quality. Several strategies

76   including, no-till, minimal tillage and conventional tillage, in-situ residue retention, weed

77   biomass, green leaf manure, and farmyard manure, and rotation of peas and paddy are being

78   tested on experimental farms of the ICAR Research Complex for North Eastern Hill Region,

79   Umiam, Meghalaya, India (Das et al., 2014). Significant increase in soil organic carbon, soil

80   microbial biomass carbon, and dehydrogenase activity has been observed in no-till treatments in

81   comparison to conventional tillage in hills of North Eastern India (Das et al., 2014). Previous

82   studies on CA in North Eastern regions of India suggested that reduction in tillage intensity can

83   reverse soil degradation and improve soil quality, crop productivity and economic returns, but

84   little is known about its impact on the soil microbial community structure.

85   Crop plants are the other most crucial factor influencing the microbial communities in a CA

86   system (Maarastawi et al., 2018). Plant root exudates stimulate the microbial activity in the

87   rhizosphere, resulting in increased microbial active biomass and abundance in the rhizosphere by

88   several folds in comparison to surrounding bulk soil (Lugtenberg & Kamilova, 2009). During

89   this process of increased microbial activity in plant rhizosphere, some specific microorganisms

90   were also selected, leading to the build-up of plant-specific community in the rhizosphere (Hu et

91   al., 2018). These rhizosphere microorganisms perform various functions to support plant growth

92   and productivity by enhancing nutrient uptake and tolerance against stress, regulating the

93   immune system, and protecting against pathogens (Lundberg et al., 2012). It is expected that

94   crop plants select a specific community of bacteria in their rhizosphere to meet its nutrition and

95   stress requirements, which lead to homogenize the diverse communities developed in response to

96   the selection imposed by various agricultural practices. Based on this we hypothesize that crop

97   plants (i.e., pea) could be the most dominating selection factor in a long-term experiment with

98   multiple tillage and residue management practices in the acidic and iron-rich soils of Meghalaya

99   in the Eastern Himalayas.

100 **2. Materials and Methods**

101 **2.1. Sample collection:**

102 Different tillage and residue management treatments are maintained for the last eight years by

103 alternatively growing rice and pea in rotations in the experimental fields of the ICAR Research

104 Complex for NEH Region, Umiam, Meghalaya. Three different tillage treatments, i.e. zero

105 tillage; minimum tillage and conventional tillage were practiced during the cultivation of rice

106 and only zero tillage during pea cultivation. Six treatments of residue management including

107 100% NPK, 50% NPK, 50% NPK with in-situ residue retention (ISRR), 50% NPK with weed

108 biomass (WB), 50% NPK with green leaf manure (GLM) and 100% organic treatment i.e.

109 farmyard manure (@5t/ha), weed Biomass and rock phosphate (@150 kg/ha) were maintained

110   during rice cultivation. After harvesting rice, seeds of pea was sown at the seed rate of ~80 kg

111   per hectare by opening narrow troughs of optimum depth with the help of manually operated

112   furrow opener in between two rice lines thus giving a row to row spacing of 20 cm for pea. The

113   recommended dose of nutrients and seeds were placed in furrow and covered with soil: FYM

114   mixture (2:1 ratio) for better seed and soil contact. As a whole, only two treatments were

115   maintained for the pea crop, 100 % organic treatment was maintained in the same plots while

116   remaining treatments were replaced with a single inorganic treatment (20:60:40 N:P2O5: K2O

117   kg/ha). Bulk soil and Pea rhizosphere samples were collected from each treatment plot. Bulk soil

118   samples were collected in triplicates and pooled together from all the treatment plots. Three pea

119   plants growing in the mid-rows of each plot were uprooted using a shovel. The whole root

120   systems of the plants were separated from the loosely adhered soil by gentle shaking. The

121   samples from each plot were pooled together in a sterilized polythene bag and transported to the

122   laboratory under 4 °C.

123   **2.2. Isolation of rhizosphere soil, DNA extraction, and next-generation sequencing:**

124   Roots of pea plants were transferred to the 15 ml sterilized centrifuge tube and submerged in the

125   10 ml phosphate buffer saline (PBS) to harvest rhizosphere soils. The tubes were subjected to

126   sonication for 60 seconds, and roots were transferred to a fresh centrifuge tube filled with 10 ml

127   PBS. Sonication step was repeated once again to remove the soil adhered to the roots. The

128   process of washing in PBS and sonication was repeated for a total of three times to get the total

129   rhizosphere soil into the PBS. The rhizosphere soil containing PBS was centrifuged at 5,000 x g

130   for 10 min. The soil pellet was used for the extraction of rhizosphere metagenomic DNA. Total

131   community DNA was extracted from bulk soil and rhizosphere soil samples using DNeasy

132   Power Soil kit (Qiagen-Hilden, Germany) following the manufacturer's instruction. The

133   concentration of resulting DNA was measured using Nanodrop-1000, (Thermo Scientific, USA)

134   and DNA concentration was normalized to 10☐ng/µl. The freshly extracted DNA was used as

135   the template for the amplification of the V4 region of bacterial 16S rRNA gene using universal

136   bacterial primers (Fadrosh et al., 2014). The amplicon sequencing (library preparation and

137   sequencing) was performed on the Illumina Miseq platform according to the manufacturer's

138   instructions (Illumina, USA).

139   **2.3. Bioinformatics and statistical analysis:**

140   Assembly of forward and reverse reads generated for each sample was carried out using FLASH

141   (Fast Length Adjustment of SHort reads) (Magoc and Salzberg, 2011). Bacterial diversity

142   analysis was done using standard QIIME (v1.9.0) pipeline (Zhernakova et al., 2016) on the

143   assembled sequences. These sequence reads were clustered into operational taxonomic units

144   (OTUs) using UCLUST algorithm (Edgar, 2010) and SILVA database (Quast et al., 2013) by

145   closed reference based OTU picking method keeping 97% sequence similarity threshold.

146   Representative sequences (repset) from each OTU were selected for taxonomic assignment.

147   Statistical analysis of the alpha diversity across different groups and was done using STAMP

148   (Parks et al., 2010). The differential abundance analysis of bacterial genera across the different

149   study groups was also done using STAMP. The abundance of bacterial phylum and genus was

150   represented using GraphPad Prism (GraphPad Software, La Jolla California USA). Beta diversity

151   analysis of the bacterial diversity present in the bulk and rhizosphere soil samples was done

152   using the online tool microbiome analyst (Dhariwal et al., 2017). R language based package

153   ggtern an extension of package ggplot2 was used to plot ternary diagrams of the differential

154   abundant OTUs in the bulk soil and rhizosphere soil samples. Presence of the shared and unique

155   bacterial genera across the bulk soil and rhizosphere samples was investigated using online tool

156 InteractiVenn (http://www.interactivenn.net). OTUs based predictive functional analysis of the

157 bacterial community was done using Tax4Fun package (Aßhauer et al., 2015) in R and KEGG

158 database. Also, Principal component analysis (PCA) based on the abundant predicted functions

159 was performed in the PAST3 (PAleontological STatistics) software (Øyvind et al., 2001).

160 **2.4. Absolute quantification of bulk soil and Pea rhizosphere bacteria:**

161 Quantitation of total bacteria and phylum Firmicutes was done using respective qPCR primers

162 (**Table 1**). Briefly, for each gene 10 μl reactions (in triplicate) were set containing suitable pair

163 of primers (0.5μM), 10 ng of metagenomic DNA and SYBR green master mix (Applied

164 Biosystems Inc. USA). The reactions were run on 7300 Real-time PCR system (Applied

165 Biosystems Inc. USA) using the following PCR conditions: initial denaturation at 95 °C for

166 10 min, followed by 40 cycles at 95 °C for 10 s, 60 °C for 1 min. Group-specific standard

167 curves were generated from serial dilutions of a known concentration of individual PCR

168 products. The amplification specificity of primers was checked by melt curve analysis performed

169 at the end of qPCR cycles. Mean values of the three replicate were used for enumerations of

170 tested gene copy numbers for each sample using standard curves.

171 **2.5. Data availability:**

172 The sequence data is made available at NCBI SRA submission with accession number

173 SUB5624752 (Bioproject ID: PRJNA544901).

174 **3. Results:**

175 A total of 2,686,925 raw sequence reads were generated for the bulk soil (n=18) and rhizosphere

176 soil (n=18) samples using the Illumina Miseq sequencing platform. Amongst these sequences,

177 2,650,426 (98.6%) sequences were assembled using FLASH and clustered into 15,048 OTUs.

178   Among these, 10,078 OTUs were represented by two or more than two sequences, while

179   remaining OTUs were singletons.

180   **3.1. Diversity analyses of Bacterial Communities**

181   Alpha diversity assessed by Observed OTUs, Shannon, Simpson, and Chao1 indices differed

182   significantly between rhizosphere and bulk soils (p>0.05) (**Fig. 1**). The rhizosphere exhibited a

183   strong influence on bacterial diversity across various treatments, as the values of diversity

184   indices were always significantly higher in rhizosphere samples in comparison to bulk soils (**Fig.**

185   **1**). The factors tillage and residue management practices do not influence alpha-diversity indices

186   (**Fig. 1**). Both bulk soil and rhizosphere soil samples from the organic treatment exhibited least

187   inter-sample variation in Shannon and Simpson alpha diversity indices, in comparison to other

188   residue management treatments (**Fig. 1h and1k**).

189   The beta diversity analysis based unweighted unifrac PCoA showed clustering of the bulk soil

190   and rhizosphere soil sample into two distinct clusters (**Fig. 2**). The total variation explained by

191   first was PCoA 34.8% (22% for PCoA1 and 12.8% for PCoA2), wherein all the bulk soil

192   samples were on the positive side of the PC2 except B15 and B03 while all the rhizosphere soil

193   samples were on the negative side of the axis (**Fig. 1e**).

194   **3.2. Taxonomic composition of bacterial communities:**

195   Total 71 bacterial phyla were detected in the bulk soil and rhizosphere samples in which,

196   Proteobacteria (32.5%), Firmicutes (29.4%), Acidobacteria (9.03%), Actinobacteria (7.2%),

197   Chloroflexi (4.7%), Nitrospirae (3.1%), Verrucomicrobia (2.7%), Thaumarchaeota (2.6%),

198   Bacteroidetes (2.1%) and Planctomycetes (1.03%) were highly abundant and constituted 95% of

199   the overall bacterial community (**Fig. 3a**). Group's specific abundance pattern was seen for

200   Proteobacteria and Firmicutes wherein, Firmicutes were highly abundant in bulk soil (~41.7%)

201   than rhizosphere (~17.8%) while, Proteobacteria were highly abundant in rhizosphere (~43.9%)

202   in comparison to bulk soil (~18.6%) samples (**Fig. 3a**).

203   Bacterial genera *Bacillus, Staphylococcus, Planomicrobium, Enterobacter, Arthrobacter,*

204   *Nitrosotalea, Massilia, Nitrobacter, Geobacter, Pseudomonas, Anaeromyxobacter,*

205   *Sphingomonas,* candidates genus *H16, Variibacter, Pantoea, Paenibacillus, Nitrospira,*

206   *Rhizobium, Sulfurovum, Haliangium, Acidibacter, Phenylobacterium, Hyphomicrobium,*

207   *Acinetobacter* and *Novosphingobium* were found dominant with a collective abundance more

208   than 0.5% (**Fig. 3b**). A high abundance of *Planomicrobium* and *Arthrobacter* was found in the

209   bulk soil samples and *Enterobacter* in the rhizosphere soil samples. *Arthrobacter* was highly

210   abundant (in a range of 7% to 18%) in the samples B01, B04, B12, B13 and R09 (6.9%), while

211   remaining samples showed a lesser abundance (0.1 to 4%). Notably, for bulk soil sample B06,

212   B07, B15, B16, B18, and rhizosphere samples R03, R06 and R07 have a high abundance of

213   *Bacillus* (**Fig. 3b**).

214   Comparative analysis at higher (phylum) and lower (genus) taxonomic ranks revealed the

215   presence of differentially abundant bacterial taxa in bulk soil and rhizosphere soil samples. Phyla

216   Proteobacteria and Bacteroidetes were abundant in rhizosphere soil while Firmicutes,

217   Chloroflexi, Nitrospirae, and Planctomycetes were highly abundant in bulk soil (t-test, $p<0.05$,

218   Benjamini-Hochberg FDR corrections) (**Fig. 4a**). The abundance of genera *Rhizobium,*

219   *Pseudomonas, Pantoea, Paenibacillus, Nitrobacter, Enterobacter,* and *Sphingomonas* were

220   significantly higher in rhizosphere soils, while *Massilia, Paenibacillus* and *Planomicrobium*

221   were high in bulk soils (t-test, $p<0.05$) (**Fig. 4b**). Overall, the presence of 917 bacterial genera

222   was observed in both rhizosphere and bulk soil samples. Among 917 genera 60% (551 nos.)

223   were shared between the bulk soil and rhizosphere soil samples, while ~29% (267 nos.) were

224   unique to the rhizosphere, and ~11% (99 nos.) genera were exclusive to bulk soil sample (**Fig.**

225   **4c**).

226   **3.3. Quantitative analysis of bacterial community:**

227   The average total bacterial abundance in the rhizosphere (8.9 x 10^9 copies/gm) and bulk soil

228   (9.42 x 10^9 copies/gm) samples was statistically not different from each other (**Fig. 5a**).

229   Quantitative enumeration of the genus Firmicutes depicted the significantly higher copy number

230   (3.76 x 10^9 copies/gm) in the bulk soil samples in comparison to the rhizosphere samples (1.78 x

231   10^9 copies/gm) (**Fig. 5b**). The mean relative abundance of Firmicutes detected in 16S rRNA gene

232   amplicon sequencing data was ~18% in the rhizosphere samples and ~42% in the bulk soil

233   samples (**Fig. 5c**).

234   **3.4. Effect of soil management practices on the bulk soil and pea rhizosphere microbiome:**

235   Bulk soil microbial community showed the differential abundance of *Pseudolabrys, Roseiarcus,*

236   and *Tumebacillus* across the residue management practices (ANOVA, p<0.05). The abundance

237   of *Pseudolabrys* and *Tumebacillus* was high in samples from 100% NPK and organic treatments,

238   while *Roseiarcus* was abundant in 50% NPK and organic treatment field samples (**Fig. 6a**).

239   Similarly, in the bacterial community analysis of the rhizosphere soil bacterial genera

240   *Anaerolinea, Hydrogenispora,* and *Syntrophorhabdus* were differentially abundant across the

241   groups and having higher abundance in the organic treatment farm **(Fig. 6b)**.

242   Three different soil management practices revealed enrichment of specific OTUs in the bulk soil

243   and rhizospheric soil (ANOVA, p<0.05). Among the bulk soil samples 12 OTUs belonging to

244   four taxa were differentially abundant in conventional tillage, 66 OTUs from 32 taxa in

245   minimum tillage and only six OTUs from four taxa in zero tillage (**Fig. 7a**). Similarly, in the

246   rhizosphere soil samples 295 OTUs of 96 taxa, 17 OTUs of the 13 taxa and 82 OTUs of the 37

247   taxa were significantly enriched in the conventional tillage, minimum tillage and zero tillage,

248   respectively (**Fig. 7b**). At the genera-level bacterial community analysis across three tillage

249   treatment fields, 11 bacterial genera showed differential abundance (**Table 2**). All these genera

250   showed higher abundance in the conventional tillage fields.

251   **3.5. Predictive functional analysis of the microbial community:**

252   The functional contributions of the bacteria were predicted based on OTUs, and the results

253   revealed the presence of 398 different functional classes. Among these classes, transporters, two-

254   component system, secretion system, ABC transporters, transcription factors, peptidases,

255   ribosome, methane metabolism, quorum sensing, and bacterial motility proteins were the top 10

256   highly represented classes. The presence of 6596 KEGG orthologues (KO) was predicted across

257   all samples, belonging to metabolism, environmental information processing, cellular processes,

258   human diseases, genetic information processing, and organismal systems. Principle component

259   analysis based on the highly abundant functions clustered the bulk soil and rhizosphere samples

260   into separate groups (**Fig 8**). Majority of the rhizosphere samples are placed on the negative side

261   of PC1 and positive side of PC2, while most of the bulk samples were on the positive side of

262   PC1 and negative side of PC2 (**Fig 8**). Bacterial genes associated with iron complex outer

263   membrane receptor protein, cobalt-zinc-cadmium resistance protein (CzcA), RNA polymerase

264   sigma-70 factor, ribonuclease E, translation initiation factor IF-2, serine/threonine protein kinase,

265   hydrophobic/amphiphilic exporter-1, beta-glucosidase and multiple sugar transport system

266   permease protein showed high negative values for PC1 and positive for PC2 (Table S3).

267   Rhizosphere samples revealed high abundance of genes involved in nitrogen fixation (*NifQ*) by

268   64%, enterobactin (siderophore) production by 39%, plant hormone IAA production (tryptophan

269   2-monooxygenase) by 10%, phosphate metabolism (inorganic phosphate transporter) by 40% in

270   comparison to bulk soil samples (**Supp file S1**).

271   **4. Discussion:**

272   Agriculture is a continually improving system to make it resilient and sustainable for the long

273   term and meet the ever-increasing human requirements. Conservation agriculture offers a

274   framework to improve soil structure, save water, enhance soil nutrient supply and cycling,

275   increase yield, and maintain soil biodiversity. This study was designed to investigate the effect of

276   long-term exposure to various tillage and residue management practices on the bacterial

277   community structures of the bulk soils, and how pea plant shapes the rhizosphere communities.

278   Soil bacterial diversity is known to be influenced by the numerous environmental factors

279   including soil pH (Lauber et al., 2009; Cho et al., 2016; Sánchez-Cañizares et al., 2017),

280   chemistry of soil organic matter (Lupatini et al., 2017), plant species grown in the soil

281   (Yamamoto et al., 2018), secretion of different root exudates by the plant species (Sasse et al.,

282   2017; Huang et al., 2019) and processing of the soil before cultivation of the plants (Moreno-

283   Espíndola et al., 2018). Majority of the soils in North-East regions of India are acidic (pH = 5.0

284   to 6.0) and rich in organic carbon, which makes them deficient in available phosphorous,

285   medium to low in available potassium, and highly rich in iron and aluminum (Thakuria et al.,

286   2016). Our results showed the dominance of Proteobacteria, Firmicutes, Acidobacteria and

287   Actinobacteria in both bulk and rhizosphere soils (Fig 3), and are in agreement to the previous

288   report on the dominance of copiotrophic microorganisms like Proteobacteria, Firmicutes, and

289   Actinobacteria in rich organic environments, and soils with low pH harbour more Acidobacteria

290   (Fierer et al., 2007).

291   Significant differences were observed in the overall alpha diversity in the rhizosphere and bulk

292   soil samples (**Fig. 1**), demonstrating higher species abundance and evenness (based on Shannon

293   and Simpson indices) in rhizosphere samples. There was no significant difference in bacterial

294   richness and evenness among the different tillage and residue management treatments

295   ($P > 0.05$) in both rhizosphere and bulk soils (**Fig. 1**), indicating that plant rhizosphere effect

296   is the key driver for alpha diversity. Plants can alter the microbial communities by secreting a

297   variety of nutrients and bioactive molecules into the rhizosphere (Hu et al., 2018; Huang et al.,

298   2019). The enrichment of specific OTUs in the pea rhizosphere leading to the increased diversity

299   was further confirmed, as all the rhizosphere samples were grouped in a small cluster, in

300   comparison to a loose clustering of bulk soil samples in the PCoA biplot (**Fig. 2**). The built-up of

301   homogeneous bacterial communities in most of the rhizosphere samples can be attributed to the

302   selection pressure of the pea roots, which continually release a large number of border cells and

303   mucilage (Ropitaux et al., 2019), and pose a strong rhizosphere effect on the bacteria. Impact of

304   crop plant on the diversity of rhizosphere microbes has also been observed for different crops

305   including barley, cotton, maize and wheat (Yurgel et al., 2018; Babin et al., 2019; Kerdraon et

306   al., 2019).

307   Majority of members of microbial communities in the host plant are horizontally acquired from

308   the surrounding environment, and the soil is the main reservoir of a plant rhizosphere

309   microbiome (Wagner et al., 2016; Sánchez-Cañizares et al., 2017). Our results on pea

310   rhizosphere and bulk soils are consistent with this, as 551 (60%) genera of 917 were common in

311   bulk and rhizosphere soil samples (**Fig. 4c**). Dominance of Proteobacteria recorded in pea

312   rhizosphere samples, with significant increase in the abundance of genera including

313   *Pseudomonas*, *Rhizobium*, *Pantoae*, *Enterobacter* and *Sphingomonas* known for plant growth

314   promoting attributes, was in agreement with the previous studies on the rhizosphere microbiome

315   analysis (Mendes et al., 2013; Weinert et al., 2011; Yurgelet al., 2018; Goss-Souza et al., 2019).

316   Pea-*Rhizobium* symbiosis is well documented in Indian soils (Rahi et al., 2012), hence increase

317   in the abundance of *Rhizobium* in pea rhizosphere was expected. Higher abundance of

318   *Pseudomonas* and *Sphingomonas* was also reported in the rhizospheres of crop plants like

319   lettuce, wheat and maize (Schreiter et al., 2014; Kerdraon et al., 2019). In addition to

320   Proteobacteria, a significantly higher abundance of Bacteroidetes and Planctomycetes was also

321   recorded in the rhizosphere soil in comparison to bulk soil samples (**Figs. 3-4**), positive

322   correlations with increased abundance of Bacteroidetes and Planctomycetes have been observed

323   to increase in organic carbon and phosphorus concentrations, respectively in rhizosphere of

324   soybean (Goss-Souza et al., 2019). The genus *Nitrobacter* was at higher abundance in pea

325   rhizosphere samples than bulk soils (Fig 4b), suggesting its enrichment by host plant as

326   *Nitrobacter* converts nitrite to nitrate making nitrogen more readily available to the host plant

327   (Richardson et al., 2009; Hubbard et al., 2018).

328   The abundance of Firmicutes represented by members of genera like *Bacillus*, *Staphylococcus*

329   and, *Planomicrobium*, witness a significant decrease in pea rhizosphere in comparison to bulk

330   soil samples (**Figs. 3-5**), previous studies have also indicated negative correlation of plant growth

331   and high abundance of Firmicutes in the soil (Zhang et al., 2014; Kumar et al., 2018). The

332   consistently higher relative proportion of genus *Planomicrobium* in the majority of bulk soil

333   samples (**Fig. 3**) also confirms its dominance in soils (Hue et al., 2019). The abundance of

334   bacterial phyla Chloroflexi and Nitrospirae was significantly higher in bulk soil to rhizosphere

335   samples (**Fig. 4a**), which is in agreement with the previous study on the impact of land use

336   intensity and plant functional identity on microbial communities (Schöps et al., 2018). The

337 members of Chloroflexi are known for the decomposition of organic matter (Wu et al., 2016),

338 which is expected to be high in the bulk soils because of different organic inputs and paddy crop

339 residue in most of the treatments. Nitrospirae are slow-growing microbial K-strategists adapted

340 to low substrate concentrations (Daims et al., 2015), and could not cope up other fast-growing

341 bacterial communities in the nutrient-rich pea rhizosphere environment. Nitrospirae are involved

342 in nitrification process (oxidation of nitrite to nitrate) and have been reported to be dominant in

343 N-fertilized treatment in paddy soil (Kumar et al., 2018). Significantly high abundance of

344 *Massilia* and *Paenibacillus* was observed in bulk soil than rhizosphere samples (**Fig. 4b**), which

345 can be attributed to presence of high cellulosic biomass in most of the treatment leading to the

346 selection of the members of genera with potential to degrade cellulose (Ofek et al., 2012; Grady

347 et al., 2016).

348 Minor differences were observed in the bacterial community composition in response to residue

349 management treatments in both bulk and rhizosphere soils (**Fig. 6**), exhibiting the complex

350 responses of microbial communities to fertilizer applications (Hartmann et al., 2015).

351 Differential abundance of members of genera *Pseudolabrys*, *Roseiarcus* and *Tumebacillus* were

352 recorded in bulk soils samples, and *Anaerolineae*, *Hydrogenispora* and *Syntrophorhabdus* in

353 rhizosphere samples across the residue management treatments (**Fig. 6**). The higher proportion of

354 *Pseudolabrys* in 100% NPK and organic treatments, indicate its specific enrichment in these

355 treatments, *Pseudolabrys* is a member of Rhizobiales, reported from soil and lettuce rhizosphere

356 (Cipriano et al., 2016). *Roseiarcus* is a methanotroph isolated for acidic peat soil (Kulichevskaya

357 et al., 2014), the function of this genera is not well understood and was abundant in the organic

358 treatment (**Fig. 6**). Increased abundance of *Tumebacillus* in organic treatment in the acidic soils

359 of Meghalaya, suggesting that in acidic soils aluminum stress activates *Tumebacillus* to improve

360   aluminum stress tolerance in host plants (Lian et al., 2019). Among the rhizosphere sample

361   significant increase has been observed in the relative abundance of *Anaerolineae*,

362   *Hydrogenispora* and *Syntrophorhabdus* in the organic treatment (**Fig. 6**), the organic treatment

363   increase soil carbon content and nutrients, which are the key drivers for soil microbial

364   community (Fierer et al., 2007; Bei et al., 2018). All of these genera are anaerobic bacteria

365   capable of decomposing diverse carbon sources under anoxic environments and have identified

366   as the unique core taxa for rice soils (Jiao et al., 2019).

367   Though pea was cultivated under zero-tillage, influence of previous tillage treatments during rice

368   cultivation was observed on the prevalence of few specific OTUs (**Fig. 7**), suggesting the

369   plausible effect of tillage treatments on the bacterial community structure. This can be attributed

370   to the fact that tillage practices alter soil bulk density, pore structure, water availability, soil

371   organic carbon, etc (Zhang et al., 2018). However, the impact to tillage treatments was not

372   pronounced, and only 0.56% OTUs in the bulk soil, and 2.60% OTUs in the rhizosphere soil

373   were enriched across various tillage treatments (**Fig. 7**). Increased abundance of OTUs assigned

374   to *Acidothermus, Anaeromyxobacter, Bryobacter, Flavobacterium, Lysobacter, Nitrobacter,*

375   *Pedomicrobium, Rhizomicrobium, Pseudolabrys, Rhodomicrobium,* and *Sideroxydans* was

376   recorded in the rhizosphere soil samples in the conventional tillage in comparison to minimal and

377   zero tillage (**Table 2**). Severe soil disturbance in conventional tillage accelerates soil organic

378   matter oxidation hence expected to enrich a few members of bacteria (Ding et al., 2011).

379   Higher abundance of genes related to nitrogen fixation, phytohormone and siderophore

380   production, phosphate metabolism, etc. in the rhizosphere soil (**Supp file S2**) substantiate our

381   earlier conclusions on the selection of bacterial communities with plant growth promoting

382   potential in the rhizosphere (**Fig. 3**)**.** Principle component analysis based on the highly abundant

383 functions clustered the bulk soil and rhizosphere samples into separate groups, indicating

384 selection of specific bacterial communities in rhizosphere based on their putative functions (**Fig.**

385 **8**). In addition to high abundance of plant growth promotion genes, bacterial genes associated

386 with iron complex outer membrane receptor protein, cobalt-zinc-cadmium resistance protein

387 (CzcA), RNA polymerase sigma-70 factor and ribonuclease E were also abundant in rhizosphere

388 soil as compared to the bulk soil (**Supp file S2**), revealing the possible role of bacterial

389 communities in abiotic stress (low pH and high aluminum and iron toxicity) amelioration.

390 **6. Conclusions:**

391 Our work showed that pea plant is the most dominating selection factor shaping the microbial

392 communities under diverse residue management and tillage treatments. Soil management and

393 residue management practices also affect the bacterial community structure in both bulk soil and

394 pea rhizosphere. However, their influence was not significantly different. Enrichment of bacterial

395 taxa known for plant growth promotion attributes and removal of toxic elements from soil was

396 recorded in the rhizosphere indicating selection of rhizosphere communities by the plant to meet

397 its requirements of nutrient uptake and combating stress. Predictive functional analysis also

398 revealed the plausible enrichment of plant growth promoting and stress tolerance genes in the

399 pea rhizosphere.

403 **Competing interests**

404 The authors declare that they have no competing interests.

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610   **Figure legends:**

611   **Fig. 1** Bar charts with standard error bars illustrating the values of alpha diversity measures,

612   Observed OTUs (a-c) Chao1 (d-f), Shannon (g-i) and Simpson (j-l) in the bulk soil and pea

613   rhizosphere samples from different tillage and residue management practices.

614   **Fig. 2** PCoA bi-plot based on relative abundance of bacterial OTUs exhibiting the beta diversity

615   among the bulk soil and pea rhizosphere samples.

616   **Fig. 3** Relative abundance of bacteria in the bulk soil and pea rhizosphere samples at the phylum

617   (a) and genus (b) levels.

618   **Fig. 4** Differentially abundant bacterial taxa across the bulk soil and pea rhizosphere samples. A)

619   At the phylum level. B) At the genera level C) Venn diagram representing the number of shared

620   and unique OTUs in the bulk soil and rhizosphere soil samples.

621   **Fig. 5** Abundance of total bacteria and phylum Firmicutes in the bulk soil and rhizosphere soil

622   samples. A) Total bacterial abundance in the bulk soil and rhizosphere soil samples, B) qPCR

623   based and C) NGS based relative abundance of phylum Firmicutes in the bulk soil and

624   rhizosphere soil samples.

625   **Fig. 6** Differentially abundant bacterial taxa detected across the different residue management

626   practice groups in the a) bulk soil and b) pea rhizosphere samples.

627   **Fig. 7** Ternary plot depicting the number of OTUs enriched in the zero tillage, minimum tillage

628   and conventional tillage plots in the a) bulk soil and b) pea rhizosphere samples. Each circle

629   depicts one individual OTU. The size of the circle reflects its relative abundance. Family or

630   genus level taxonomy of the OTUs specifically enriched in the bulk soil (c) and rhizosphere soil

631   (d) in respective tillage treatments. For the rhizosphere soil samples taxonomy of OTUs having

632   abundance more than 0.05% is represented.

633   **Fig. 8** PCA bi-plot based on relative abundance predictive metabolic potentials of bacterial

634   communities in the bulk soil and pea rhizosphere samples.

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639   **Table 1:** qPCR primers used in this study

Bacteria taxa	Primer	Primer sequence (5'→3')	Amplicon size	Annealing temperature
Bacteria	Bact341F[#]	CCTACGGGAGGCAGCAG	177	60
	Bact 518R[#]	ATTACCGCGGCTGCTGG		
Firmicutes	Firm934F[*]	GGAGYATGTGGTTTAATTCGAAGCA		
	Firm1060R[*]	AGCTGACGACAACCATGCAC	126	60

640   ([#]Suryavanshi et al, 2016; [*]Gua et al., 2008)

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663 **Table 2**: Differentially abundant bacterial genera across the three different tillage treatments practices

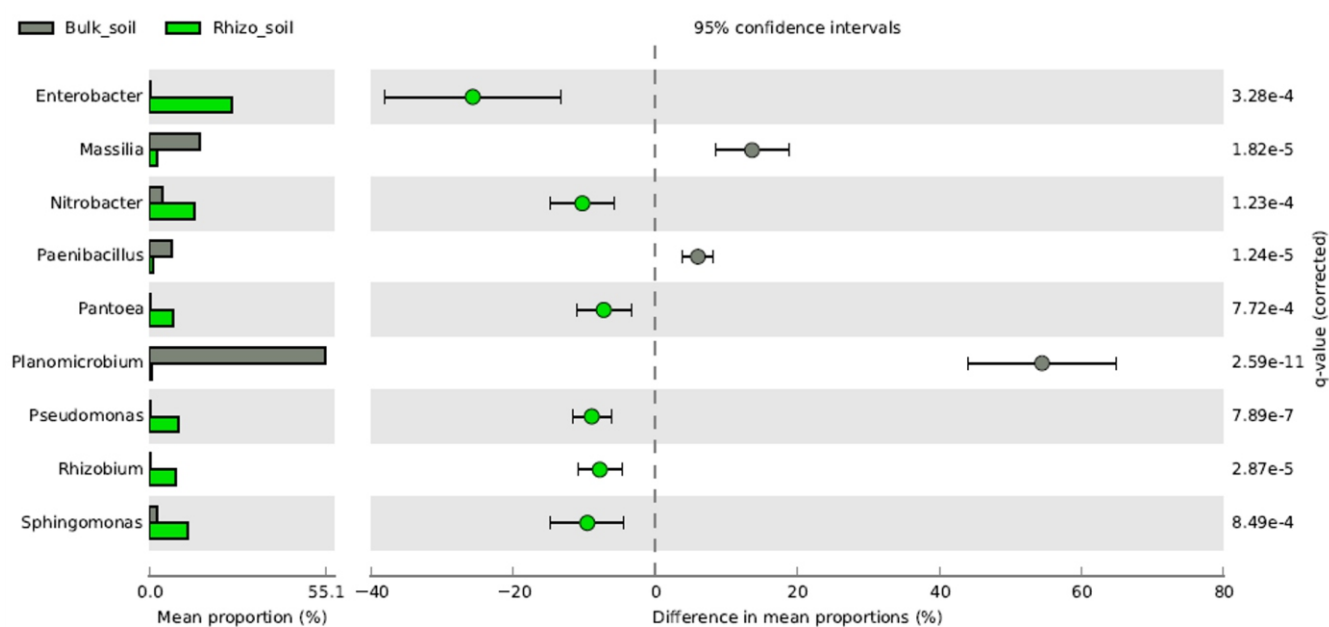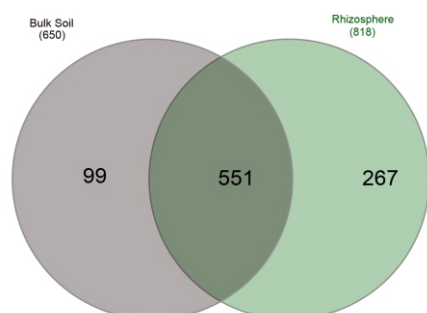| Genera	Zero tillage (Mean ± STD)	Conventional tillage (Mean ± STD)	Minimum tillage (Mean ± STD)	p-values (ANOVA)
Flavobacterium	0.989 ± 0.684	2.197 ± 0.428	0.523 ± 0.54	0.001
Lysobacter	0.639 ± 0.426	1.194 ± 0.354	0.269 ± 0.174	0.002
Pseudolabrys	1.071 ± 0.533	1.298 ± 0.158	0.345 ± 0.36	0.003
Rhodomicrobium	0.437 ± 0.253	0.888 ± 0.18	0.299 ± 0.264	0.003
Pedomicrobium	0.297 ± 0.164	0.578 ± 0.197	0.194 ± 0.15	0.008
Acidothermus	0.421 ± 0.233	0.836 ± 0.418	0.2 ± 0.127	0.01
Bryobacter	1.285 ± 0.663	1.54 ± 0.32	0.473 ± 0.463	0.011
Anaeromyxobacter	1.763 ± 1.184	3.445 ± 1.398	1.107 ± 0.885	0.018
Rhizomicrobium	0.72 ± 0.645	1.179 ± 0.423	0.274 ± 0.224	0.025
Nitrobacter	4.096 ± 2.36	5.912 ± 1.874	2.104 ± 1.812	0.031
Sideroxydans	0.401 ± 0.366	0.629 ± 0.121	0.195 ± 0.196	0.047

664

665

666

667

668

669

(a)

(b)

Rhizosphere and bulk soil samples

(a)

(b)

(c)

(a)

Bulk CT

Bulk ZT

Bulk CT

Bulk ZT

Bulk MT

Bulk ZT

Bulk MT

- ● Bulk CT
- ● Bulk MT
- ● Bulk ZT

(b)

Rhizo CT

Rhizo ZT

Rhizo CT

Rhizo ZT

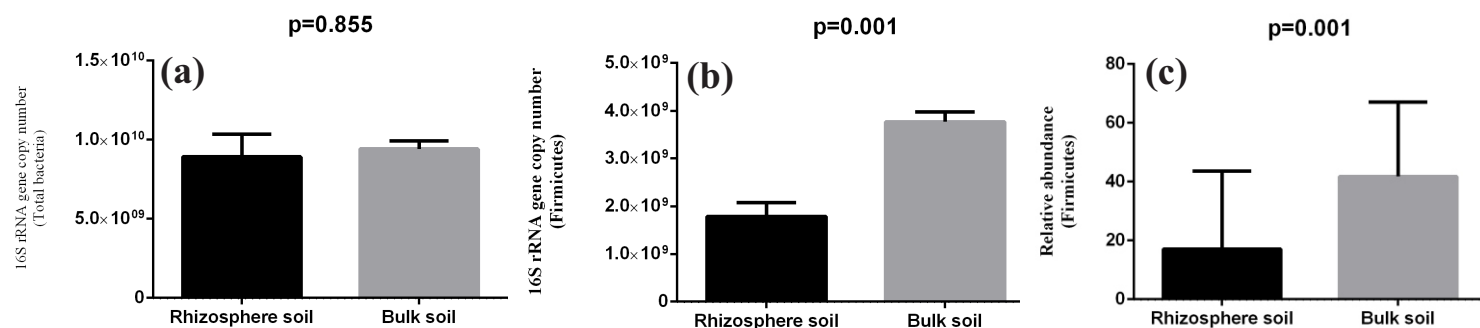Rhizo MT

Rhizo ZT

Rhizo MT

- ● Rhizo CT
- ● Rhizo MT
- ● Rhizo ZT
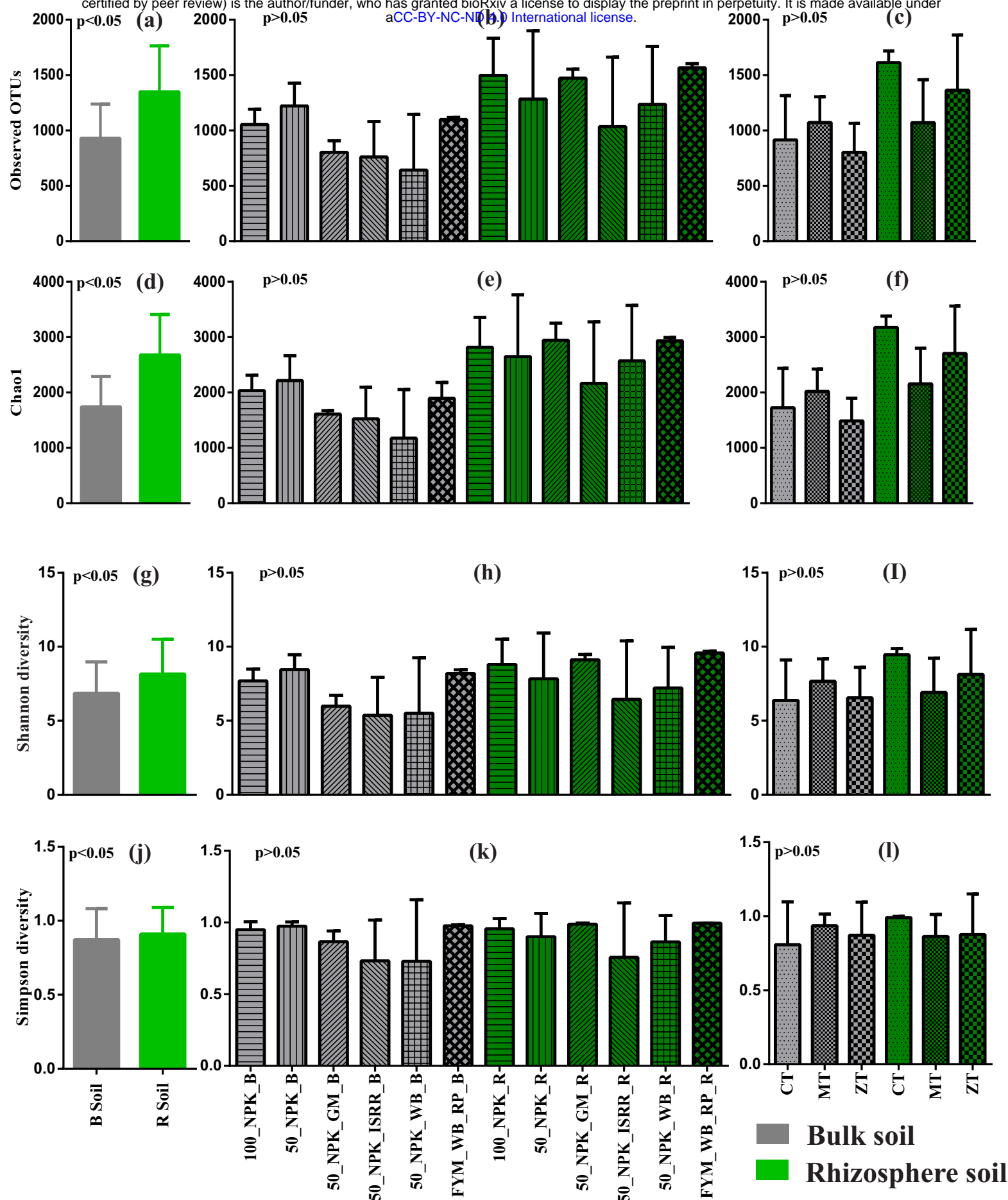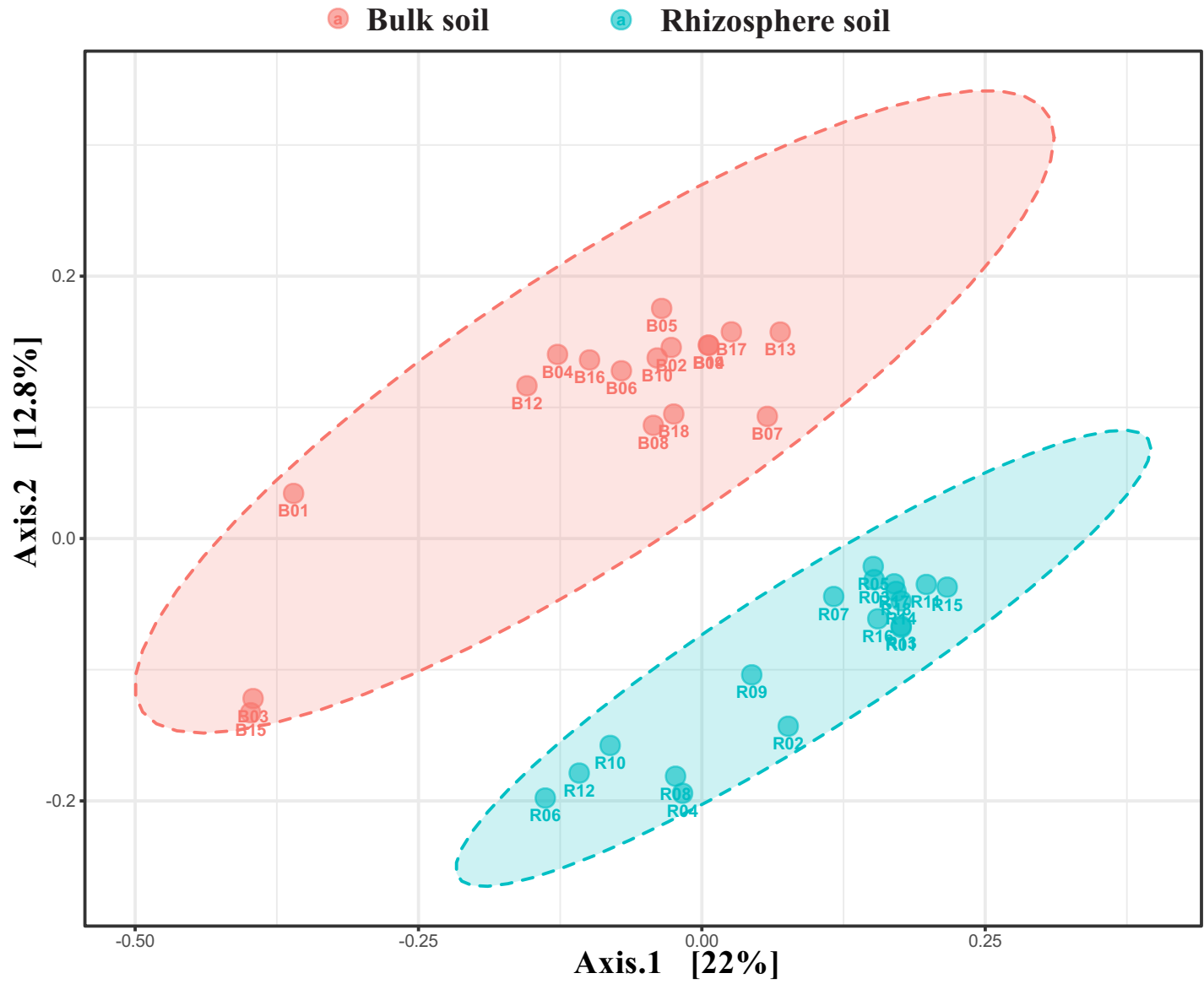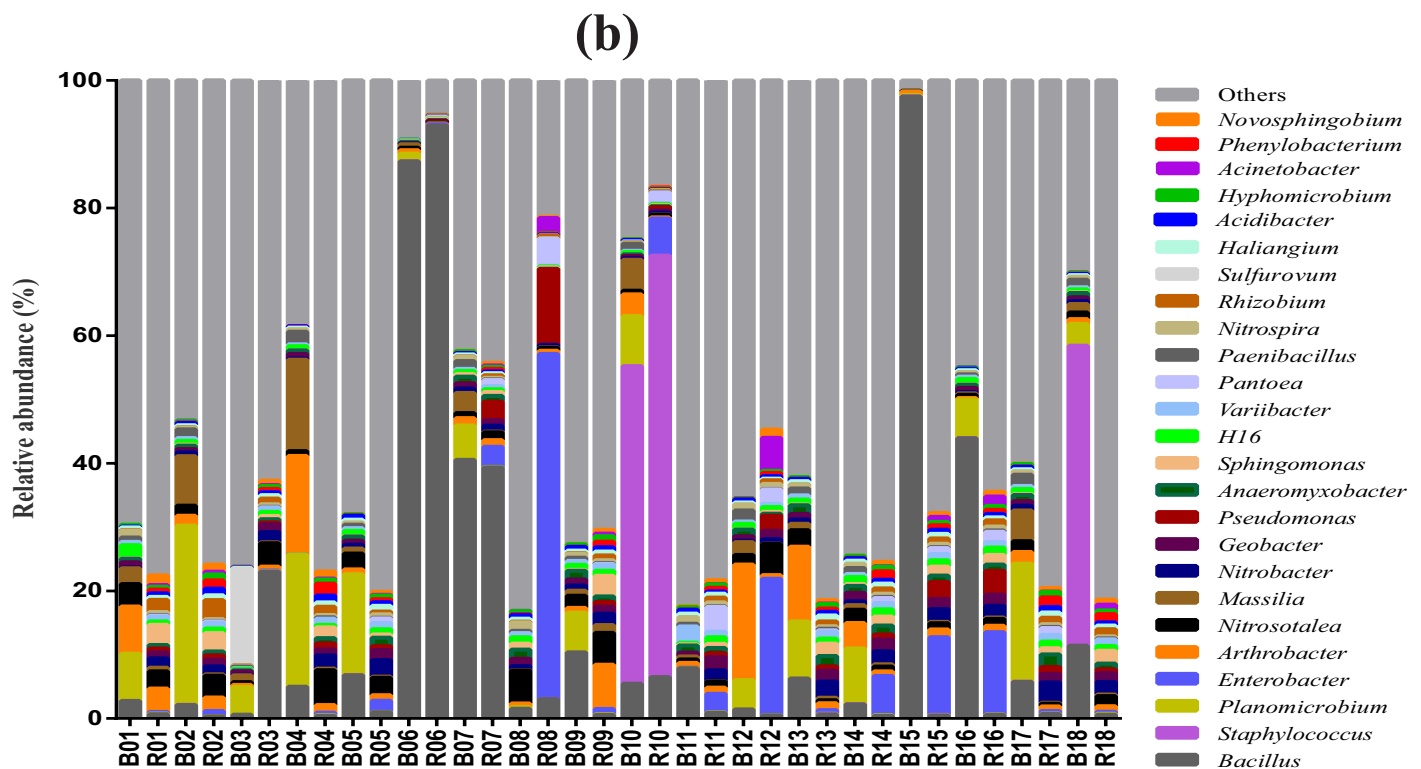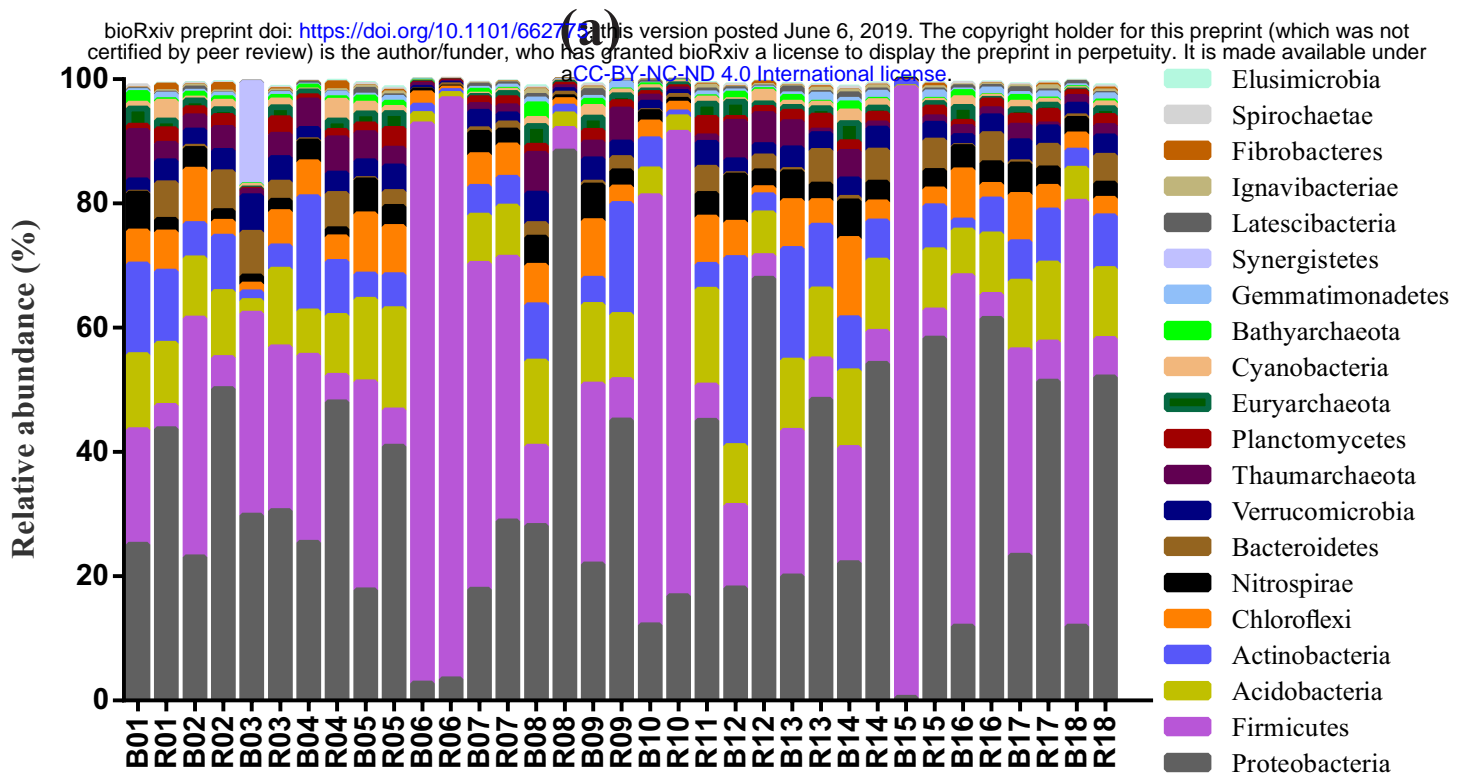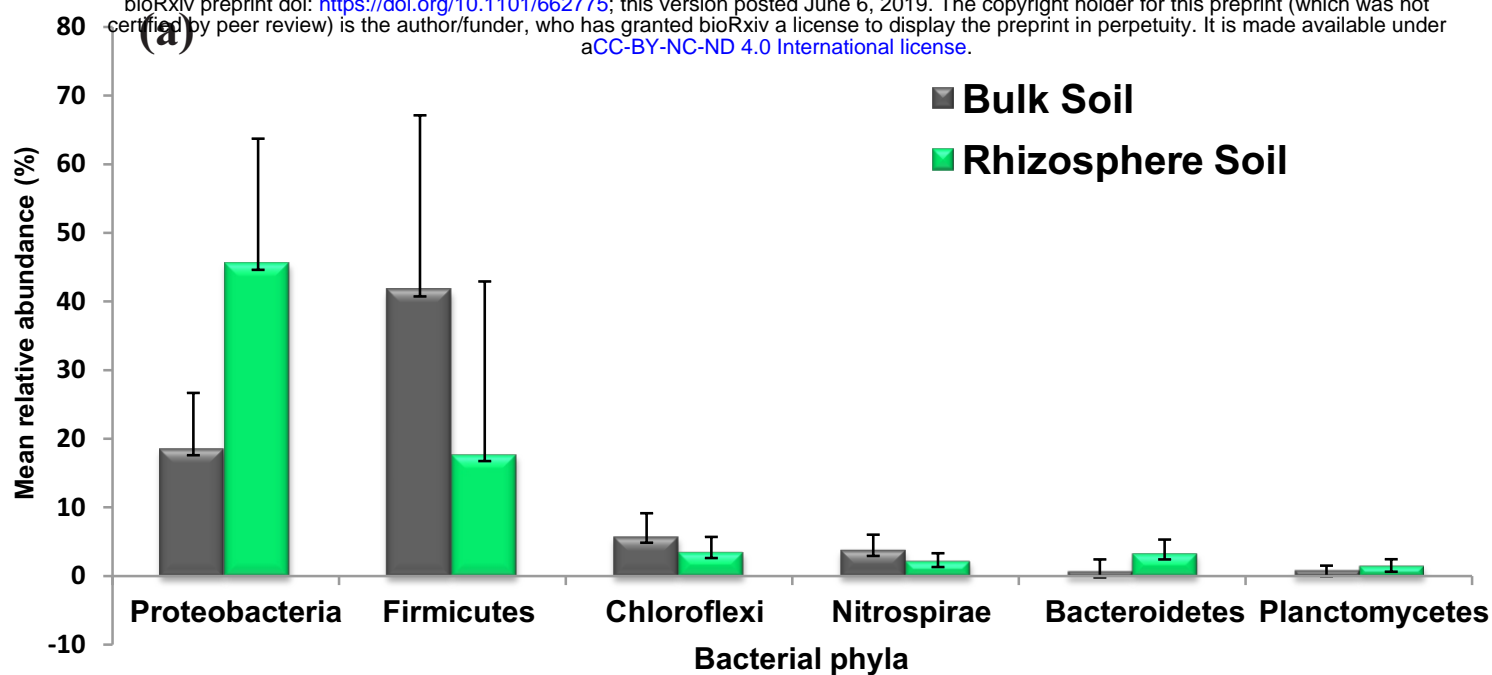
(c)
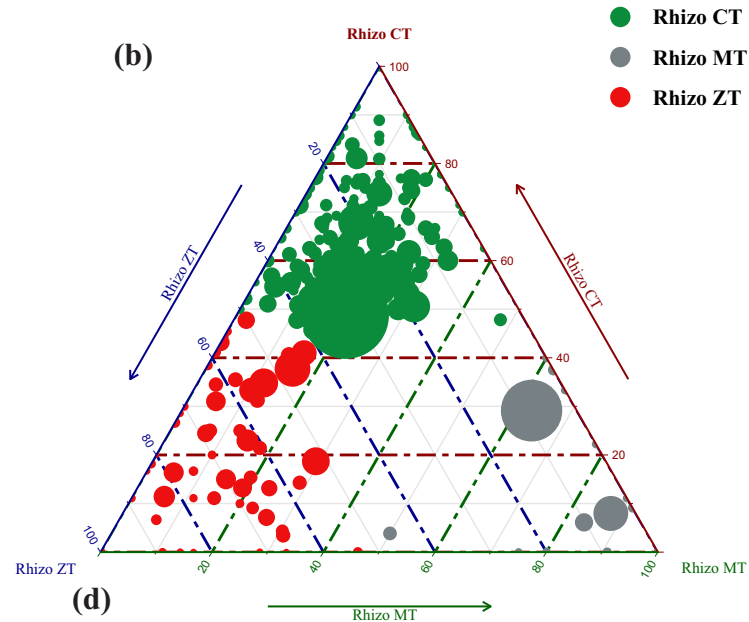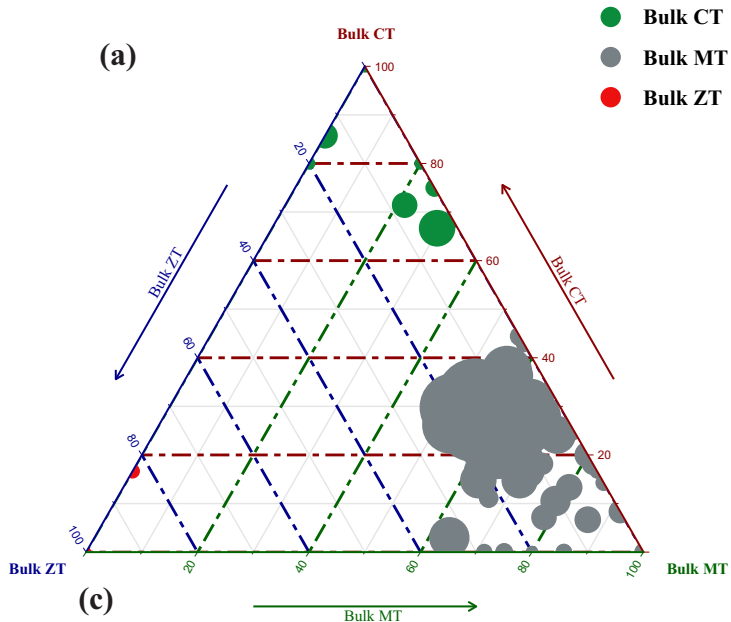
Bulk Soil CT	f_Christensenellaceae	g_Ferritrophicum	g_Thermoactinomyces
f_Anaerolineaceae	f_Lachnospiraceae	g_Fonticella	**Bulk Soil ZT**
f_Nitrospiraceae	f_Nitrosomonadaceae	g_Gaiella	f_Desulfurellaceae
g_Luedemannella	f_Sandaracinaceae	g_Geobacter	f_Planococcaceae
g_Oryzihumus	f_Syntrophaceae	g_GOUTB8	g_Planomicrobium
Bulk Soil MT	g_Anaeromyxobacter	g_Haliangium	g_Syntrophus
f_Acetobacteraceae	g_Bdellovibrio	g_Nitrospira	
f_Acidobacteriaceae	g_Blastochloris	g_Paludibaculum	
f_Anaerolineaceae	g_Bradyrhizobium	g_Ruminiclostridium	
f_Beijerinckiaceae	g_Bryobacter	g_Solibacter	
f_Blastocatellaceae	g_Burkholderia	g_Spirochaeta	
f_Chitinophagaceae	g_Desulfatiglans	g_Syntrophorhabdus	

(d)

Rhizo Soil CT	g_Clostridium	g_Planctomyces	g_Variovorax
f_Acidobacteriaceae	g_Flavobacterium	g_Pseudolabrys	**Rhizo Soil MT**
f_Nitrospiraceae	g_Gaiella	g_Pseudomonas	g_Pantoea
f_Comamonadaceae	g_Gemmatimonas	g_Pseudorhodoferax	**Rhizo Soil ZT**
f_Nitrosomonadaceae	g_Geobacter	g_Reyranella	f_Acidobacteriaceae
f_Nitrospiraceae	g_Lysobacter	g_Rhizomicrobium	f_Chitinophagaceae
f_Xanthobacteraceae	g_Nitrobacter	g_Rhodomicrobium	g_Acidovorax
g_Acidibacter	g_Nitrospira	g_Sideroxydans	g_Anaeromyxobacter
g_Acidovorax	g_Novosphingobium	g_Sphingobacterium	g_Planctomyces
g_Anaeromyxobacter	g_Pantoea	g_Sphingobium	g_Sphingobium
g_Bradyrhizobium	g_Pedobacter	g_Sphingopyxis	g_Steroidobacter
g_Burkholderia	g_Phenylobacterium	g_Steroidobacter	

PC2 (14.9%)

PC1 (54.4%)

Bulk_soil   Rhizo_soil