Variation of soil bacterial communities along a chronosequence of *Eucalyptus* plantation

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Eucalyptus is harvested for wood and fiber production in many tropical and sub-tropical habitats globally. Plantation has been controversial because of its influence on the surrounding environment however the influence of massive *Eucalyptus* planting on soil microbial communities is unclear. Here we applied high-throughput sequencing of the 16S rRNA gene to assess the microbial community composition and diversity of planting chronosequences, involving two, five and ten years of *Eucalyptus* plantation, comparing to that of secondary-forest in South China. We found that significant changes in the composition of soil bacteria occurred when the forests were converted from secondary-forest to *Eucalyptus*. The bacterial community structure was clearly distinct from control and five year samples after *Eucalyptus* was grown for two and ten years, highlighting the influence of this plantation on local soil microbial communities. These groupings indicated a cycle of impact (two- and ten- year plantations) and low impact (five-year plantations) in this chronosequence of *Eucalyptus* plantation. Community patterns were underpinned by shifts in soil properties such as pH and phosphorus concentration. Concurrently, key soil taxonomic groups such as *Actinobacteria* showed abundance shifts, increasing in impacted plantations and decreasing in low impacted samples. Shifts in taxonomy were reflected in a shift in metabolic potential, including pathways for nutrient cycles such as carbon fixation, which changed in abundance over time following *Eucalyptus* plantation. Combined these results confirm that *Eucalyptus* plantation can change the community structure and diversity of soil microorganisms with strong implications for land-management and maintaining the health of these ecosystems.

1   **Variation of soil bacterial communities along a chronosequence of *Eucalyptus* plantation**

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13   **ABSTRACT:** *Eucalyptus* is harvested for wood and fiber production in many tropical and sub-tropical habitats

14   globally. Plantation has been controversial because of its influence on the surrounding environment however the

15   influence of massive *Eucalyptus* planting on soil microbial communities is unclear. Here we applied high-

16   throughput sequencing of the 16S rRNA gene to assess the microbial community composition and diversity of

17   planting chronosequences, involving two, five and ten years of *Eucalyptus* plantation, comparing to that of

18   secondary-forest in South China. We found that significant changes in the composition of soil bacteria occurred

19   when the forests were converted from secondary-forest to *Eucalyptus*. The bacterial community structure was

20   clearly distinct from control and five year samples after *Eucalyptus* was grown for two and ten years, highlighting

21   the influence of this plantation on local soil microbial communities. These groupings indicated a cycle of impact

22   (two- and ten- year plantations) and low impact (five-year plantations) in this chronosequence of *Eucalyptus*

23   plantation. Community patterns were underpinned by shifts in soil properties such as pH and phosphorus

24   concentration. Concurrently, key soil taxonomic groups such as *Actinobacteria* showed abundance shifts,

25   increasing in impacted plantations and decreasing in low impacted samples. Shifts in taxonomy were reflected

26   in a shift in metabolic potential, including pathways for nutrient cycles such as carbon fixation, which changed

27   in abundance over time following *Eucalyptus* plantation. Combined these results confirm that *Eucalyptus*

28   plantation can change the community structure and diversity of soil microorganisms with strong implications for

29   land-management and maintaining the health of these ecosystems.

30   **Introduction**

31   *Eucalyptus* (*Eucalyptus* spp.), a Myrtaceae species that is native to Australia, is now extensively

32   planted at the global scale because of its fast-growth and strong adaptability to the local

33   environment. It occupies approximately 20 million hectares within the tropical artificial forests

34 (Cook et al., 2016), while in China there are nearly 3.7 million ha of plantation, making the country

35 the second largest *Eucalyptus* plantation area (Versini et al., 2014; Zhang et al., 2016).

36 Accompanying notable economic benefits(Ahmed, 1989), long-term *Eucalyptus* plantation,

37 however, could induce some severe ecological impacts involving soil degradation and understory

38 diversity loss due to allelopathy (Zhang & Fu, 2009; Yang et al., 2017; Fang et al., 2009). Soil

39 degradation via allelopathy likely impacts soil microbial diversity at the community level due to

40 inhibition of key microbial taxa (Bertin et al., 2003).

41   In terrestrial ecosystems, soil microorganisms play crucial roles in ecological processes

42 including pedogenesis, organic matter decomposition and nutrient cycling (Hu et al., 2015; Zeng

43 et al., 2017; Hu et al., 2014). Soil bacteria are the most abundant and functionally diverse microbial

44 taxa that drive processes which mediate soil quality and decompose organic substances (He et al.,

45 2009), and the composition and diversity of soil bacterial communities are sensitive to

46 environmental factors like soil characteristics and vegetation type (Huang et al., 2008; Leecruz et

47 al., 2013). Plant productivity, diversity and community composition are driven directly or

48 indirectly by the strong interdependence between plants and soil microbes, whereby a series of

49 microbial metabolic activities that liberate available nutrients or other compounds can then be

50 acquired by plants (Heijden et al., 2008). Conversely, soil bacterial and fungal communities have

51 been shown to be affected by *Eucalyptus* plantations using PLFA (phospholipid fatty acid) analysis

52 since the community structure was impacted significantly with the variation of planting age (Chen

53 et al., 2013; Cao et al., 2010; Wu et al., 2013). This is contradictory to other studies reported which

54 demonstrate that *Eucalyptus* plantations reduced microbial biomass, soil organic carbon and

55 nitrogen concentrations during continuous planting (Behera & Sahani, 2003; Cortez et al.,

56 2014).Another relevant study of secondary tropical forest (SF) converted into *Eucalyptus*

57 plantations (EP) using high-throughput sequencing techniques revealed a significant difference

58 between SF and EP samples in bacterial composition and diversity (Lan et al., 2017). Given that

59 these tools can elucidate the impact of artificial planting, especially on the surrounding soil

60 ecosystem affected by the functioning of microbial communities if impacted (Zhang et al., 2017;

61 Zheng et al., 2017; Zhou et al., 2017; Lin et al., 2017), their application will better define the

62 response of soil microbial community diversity and function to land use shift and long-term

63 *Eucalyptus* plantation, which remains obscure.

64   In this study, we used high-throughput sequencing of the 16S rRNA gene to assess soil

65 bacterial diversity and community composition and shifts in functional potential along a

66 chronosequence of *Eucalyptus* plantations (2 years, 5 years and 10 years; a nearby secondary-

67 forest was simultaneously investigated as control). We highlighted the following questions: (a)

68 how soil bacterial diversity and community composition varied between different *Eucalyptus*

69 plantation stages and the secondary-forest. (b) what are the main factors driving the structure and

70 composition of bacterial communities along the plantation chronosequences and (c) how

71 functional profiles of bacterial communities involving nutrient cycling, metabolism and

72 degradation shifted by *Eucalyptus* plantation at different growing stages.

73 **Materials & Methods**

74 **Site information and soil sampling**

75 Soil samples were collected from *Eucalyptus* plantations in the Zhenlong Town of Huizhou City,

76 Guangdong Province, South China (22°96' N, 114°36' E) (Fig. 1) on June 8th, 2017. The climate of

77 this region belongs to the subtropical monsoon climate with an average annual precipitation of

78 2,000 mm, mainly from April to September, and the mean annual temperature was 22℃. The

79 current *Eucalyptus* forests have been planted for many years as a substitution after cutting down

80 all trees in previous secondary-forest.An adjacent secondary-forest which was defined as forest

81 lands formed naturally under the impact of human activities (Corlett, 1994), was investigated and

82 sampled as the control.

83   According to the space for time substitution procedure (Zheng et al., 2017), we selected four

84 forests including 2 years (2YR), 5 years (5YR) and 10 years (10YR) *Eucalyptus* plantations and

85 the secondary-forest. In each forest, three 20 x 20 m² plots were established in random locations

86 and soil samples were collected. Within each plot, the detritus and litter were swept away, and five

87 soil cores of 0~15 cm depth was collected, pooled and mixed into a composite sample. A total of

88 12 composite soil samples were collected from every different *Eucalyptus* plantation stages and

89 secondary-forest on a similar gradient and altitude approximately . Composite soil samples were

90 stored with ice bags and transported to the laboratory as soon as possible for downstream

91 treatments. All the samples were sieved through 2 mm mesh and divided into two groups, with

92 one group stored in 4℃ for soil properties measurement, and the other group frozen in -80℃ for

93 soil community DNA extraction.

94 **Measurement on soil physicochemical properties**

95 The soil pH was determined by a pH monitor with a soil to water ratio of 1:2.5. The soil organic

96 carbon (SOC) and total nitrogen (TN) were measured in $K_2Cr_2O_7$ oxidation and the Kjeldahl

97 method. Total phosphorus (TP) and total potassium (TK) were measured with the alum molybdate

98 yellow colorimetric method and the atomic absorption spectrophotometry. Available nitrogen

99 (AN) and available potassium (AK) were quantified using the alkali-hydrolyzed diffusing method

100 and flame photometer, respectively. Available phosphorus (AP) was immersed with 0.5mol/L

101 NaHCO$_3$, and then measured by Mo-Sb colorimetric method. Soil NH$_4^+$ and NO$_3^-$ were assayed

102 with a flow analyzer (AA3, SEAL analytical, Germany).

103 **Soil community DNA extraction and high-throughput sequencing**

104 The total genomic DNA of soil was extracted from 0.2 g of each sample using the Power Soil

105 DNA Isolation kit (MoBio Industries, Carlsbad, CA, USA) following the manufacturer's

106 instructions. Quality of the extracted DNA was determined with a NanoVue Plus

107 spectrophotometer (GE, New Jersey, USA) and 1% agarose gel electrophoresis. The V3-V4 hyper-

108 variable region of the 16S rRNA gene was amplified using primers 338F (5'-

109 ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The

110 following PCR reaction program consists of an initial denaturation at 95℃ for 3min, 28 cycles of

111 95℃ for 30s, 55℃ for 30s, 72℃ for 45s, then 72℃ for 10min and 10℃ until halted manually.

112 PCR products were pooled with an equimolar concentration and sequenced with length of 300bp

113 using a MiSeq sequencer and TruSeq chemistry (Illumina, San Diego, CA, USA) at Shanghai

114 Majorbio Bio-pharm Technology Co., Ltd.

115 **Bioinformatics analysis**

116 After sequencing, the primary analysis of raw FASTQ data was processed with the QIIME2

117 pipeline (version 2017.10; http://qiime2.org/)(Caporaso et al., 2010). Briefly, DADA2 (Callahan

118 et al., 2016) was used for error-correction, quality filtering, chimera removal and sequence variant

119 calling of the Illumina amplicon sequences, with reads truncated at 270bp, corresponding to a

120 quality score >20 . Resultant feature sequences (sOTUs) were summarized and then annotated

121 using an RDP classifier (Cole et al., 2009) pre-trained to the full-length Greengenes database

122 (version Aug,2013)(DeSantis et al., 2006). sOTU can be used interchangeably with amplicon

123 sequence variants that it refers to sequences that differ by >1 nucleotide. The predictive functional

124 categories of bacterial community were annotated through Phylogenetic Investigation of

125 Communities by Reconstruction of Unobserved States (PICRUSt) and generated metabolic

126 pathways depending on KEGG Orthologs at level 3 after importing the normalized sOTU table

127 (Langille et al., 2013). This sOTU table was generated in QIIME2 by performing closed reference

128 clustering of sOTU sequence variants against the Greengenes database (version Aug,2013,

129 99%)(DeSantis et al., 2006) using VSEARCH(Rognes et al., 2016). The raw data have been

130 deposited with the European Nucleotide Archive database (http://www.ebi.ac.uk/ena) under

131 accession code ERP109013.

132 **Statistical analysis**

133 The estimated alpha diversity including number of sOTUs, Shannon and Chao1 indices were

134 calculated within QIIME2 with a resampling depth of 27,838 sequences *per* sample to ensure even

135 sampling depth with statistical significance determined using Kruskal-Wallis tests. Using rarefied

136 sOTU table, the relative correlation between biotic and environmental similarity matrices was

137 calculated using Mantel-tests within QIIME2 using the vegan package of R(Oksanen J et al., 2007)

138 to elucidate which variables most influenced microbial community structure. Principal coordinates

139 analysis (PCoA) was carried out based on the weighted Unifrac distance (Lozupone et al.,

140 2011)between samples in QIIME2 with the significance of sample groupings determined using

141 Analysis of Similarities (ANOSIM)(Clarke, 1993). Environmental variables found to have the

142 most significant influence using the Mantel-tests were mapped as colour gradients to PCoA

143 ordinations. According to the results of mantel test, we choose pH(P=0.025, r=0.328),

144 TN(P=0.048, r=0.297), TP(P=0.041, r=0.303) and AP(P=0.104, r=0.22) to assess the association

145 between bacterial community structure and soil environmental factors by canonical

146 correspondence analysis (CCA) using the vegan package in R.

147   Differentially abundant features were determined by Linear discriminant analysis Effect Size

148 (LEfSe) in biobakery(Segata et al., 2011). Additionally, one-way analysis of variance (ANOVA)

149 was applied to evaluate significant differences based on Least Significant Difference (LSD)

150 method in species composition and soil chemistry among samples, with a least significant

151 difference ($P < 0.05$) using SPSS 19.0 (IBM Corporation, Armonk, NY, USA).

152 **Results**

153 **Soil physicochemical characteristics**

154 Soil physicochemical characteristics potentially affected by *Eucalyptus* plantations are shown in

155 Table 1. The soil pH and SOC in the secondary forest and 5YR *Eucalyptus* soil were higher

156 (p<0.01) than 2YR and 10YR *Eucalyptus* soils. By contrast, available phosphorus (AP) of 2YR,

157 10YR *Eucalyptus* soil were higher than 5YR and secondary-forest samples (p<0.05), and TP

158 increased significantly (p<0.01) with the time of *Eucalyptus* planting, while reached a stable value

159 between 5YR and 10YR soils. The content of TK decreased with plantation age of *Eucalyptus*, but

160 recovered higher than origin in the later plantation stage. The NH_4^+ content was changed

161 significantly (p<0.01) comparing the different age of *Eucalyptus* soil and secondary forest, which

162 showed the lowest value in 10YR soils. But there were no significant changes in soil TN, AN and

163 AK contents among the secondary forest and *Eucalyptus* plantations.

164 **Diversity of soil bacterial community**

165   In total, 427687 high-quality sequences were obtained from 12 soil samples in three different age

166   stages of *Eucalyptus* plantations and secondary-forest with reads quality trimmed to 270bp.

167   subsequent reads were normalized to 27838 sequences with rarefaction curves (Fig. S1)

168   determining that this depth was sufficient to describe the sample diversity.

169   The numbers of observed sOTUs were 648,642,684,643 in 2YR, 5YR, 10YR *Eucalyptus*

170   plantation and secondary-forest respectively (Fig. S1). The estimated Shannon indexes did not

171   significantly differ among different ages of *Eucalyptus* plantation and secondary forest (Fig. S2).

172   The Chao1 index increased along the *Eucalyptus* plantations however this trend was not

173   statistically significant (Fig. S2).

174   **Structure and composition of bacterial communities**

175   When using weighted Unifrac Samples formed distinct clusters in the PCoA plot (Fig.1) based

176   on planting year, one cluster consisting of samples from 2 year and 10 year plantations and one

177   with samples from 5 year and SF plantations (Fig. 2). This however was primarily explained by

178   axis 2 (18.69%) with grouping being less clear along the primary axis, which explained 47.38%

179   of variation. Interestingly, when we used unweighted Unifrac samples grouped more clearly by

180   plantation year indicating that diversity is to some degree partitioned by age, however this is

181   primarily being realized in less abundant taxa (Fig. S3). Further statistical analysis revealed that

182   overall, plantation year did not significantly explain beta-diversity patterns (anosim result,

183   *P*=0.073), however the difference between the 2YR/10YR cluster and the 5YR/SF cluster was

184   significant (*P*=0.014). As the 2YR and 10YR samples strongly differ from the control non-

185   plantation SF samples, we have termed this cluster "impacted". However, as the community in

186   the 5YR samples groups with the control SF plantation we have termed this group "low

187   impacted" (refer to Discussion). Mantel tests showed that pH and AP were primary

188   environmental drivers of beta-diversity patterns (Table 2) and clearly differ between the 5YR/SF

189   low impacted cluster and 2YR/10YR impacted cluster (Fig. 3).

190   Sequences variants were classified into 29 phyla, 85 classes, 150 orders, 236 families and

191   344 genera, including some unclassified or no rank species. The dominant phyla included

192   *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Planctomycetes*, *WPS-2* and

193   *Verrucomicrobia* (Fig. 4), whose relative abundance were above 2%. The *Proteobacteria* had the

194   highest abundance, especially in secondary forest, but decreased with the increased years of

195   *Eucalyptus* plantation, accounting for 44.22%, 41.3%, 39.75% and 37.34% respectively (Fig. 4)

196   however this difference was not statistically significant (ANOVA P > 0.05, Table 3). The

197   relative abundance of *Actinobacteria* increased significantly (ANOVA P < 0.05) in 2YR

198   *Eucalyptus* plantations, but recovered to the SF abundance in later plantation years (Fig. 4, Table

199   3). *WPS-2* climbed significantly in 2YR and 10YR but was detected no significant variation in

200   5YR and secondary-forest (Fig. 4, Table 3). In comparison with the secondary-forest soil, the

201   relative abundance of *Cyanobacteria*, *TM6* and *Tenericutes* in *Eucalyptus* forest showed a

202   significant decrease (ANOVA P < 0.05), but not with the increasing year of *Eucalyptus*

203   plantation relative to SF. However, the *Gemmatimonadetes* and *AD3* were significantly higher in

204   *Eucalyptus* plantation than that in secondary forest (ANOVA P < 0.05, Fig. 4, Table 3).

205   At the genus level (Table S1, approximately 50% were listed), the dominant genera in both

206   *Eucalyptus* plantation soil and secondary forest were *Rhodoplanes*, and lineages within the order

207   *Actinomycetales* and family *Rhodospirillaceae*, however these showed no significant variation

208   between samples. Abundant genera (>2%) displaying significant differences in abundance

209   between samples based on one-way ANOVA (*P*<0.05) were listed in Table S1. *Candidatus*

210   *Koribacter* increased in abundance in the 10YR(1.94%) sample relative to SF, however did not

211   significantly change in the three treatment groups. The relative abundance of *Bradyrhizobium*

212   showed a significant drop in the 10YR(1.86%) sample, but was consistent across early stages of

213   *Eucalyptus* plantation. Additionally, the *Conexibacter* in 5YR(0.14%) decreased significantly

214   compared with the 2YR(0.48%) sample, although was not significantly different between

215   *Eucalyptus* samples and SF. By contrast, *Skermanella* in the 5YR(0.13%) soil decreased relative

216   to SF(1.21%), but recovered in 10YR(0.53%) samples. *Azospirillum* increased significantly in

217   2YR *Eucalyptus* samples (0.98%), while relative abundance decreased back to the original level

218   in 5 and 10 year plantations.

219   Overall 16 taxonomic groups were overrepresented in samples forming the impacted cluster

220   and 19 taxonomic groups were overrepresented in the low impacted cluster samples (Fig. 5) as

221   determined by LEfSe LDA scores (Fig. S4). In particular, taxa at different phylogenetic levels

222   within the *Actinobacteria* phylum were significantly different in abundance between the impacted

223   and low impacted clusters (Fig. 5). These included the *Thermoleophilia* order, *Acidimicrobiia*

224   order, *Solirubrobacterales* class, *Acidimicrobiales* class, *Pseudonocardiaceae* family and

225   *Nocardiaceae* family. Moreover, At the genus level, *Bradyrhizobium* were found to predominate

226   in soils of low impacted cluster, as well as *Myxococcales* at the order level. The plots of features

227   above with statistically significant differences between clusters were put into supplementary

228   materials (Fig. S5;Fig. S6;Fig. S7).

229   **Relationships between bacterial community and soil characteristics**

230   CCA (Canonical Correlation Analysis) was carried out to identify the main soil characteristics

231   driving community patterns in *Eucalyptus* plantation and secondary forest soil samples. According

232   to the Mantel test results (Table 2), four soil properties demonstrated a strong relationship to beta

233   diversity patterns and are included as vectors in the CCA analysis (Fig. 6). The first two axes of

234   the CCA explain 31.14% and 26.58%, of the variability respectively (Fig. 6). The result showed

235   that pH was positively associated with 5YR and SF soil samples (the low impacted cluster), but

236   negatively associated with 2YR and 10YR and explained 89.66% of CCA1. In addition, likewise

237   on CCA1, TP and AP were positively correlated with the 2YR and 10YR soils (impacted cluster),

238   while contrary to the 5YR and SF samples.

239   **Function prediction of bacteria**

240   The potential metabolic functions of soil taxa were predicted using PICRUSt, a software package

241   that uses the genomic composition of organisms closely related to the 16S rDNA composition of

242   the sampled community and the predicted metagenome correlated to an actual metagenome with

243   a Spearman's R of 0.81 for soil microbial communities (Langille et al., 2013). Related genes

244   collapsed into KEGG pathways at level 3 of the KEGG hierarchy with a mean NSTI score of 0.12

245   and varied significantly between clusters of *Eucalyptus* plantation with 20% of pathways having a

246   corrected P-value < 0.05 (Table S2).

247   In particular nitrogen metabolism, amino acid metabolism and energy metabolism,

248   degradation in bisphenol, chlorocyclohexane and chlorobenzene, chloroalkane and chloroalkene

249   decreased significantly in the impacted cluster (Fig. 7, Table S2). Additionally, glycoysis and TCA

250   cycle in impacted increased significantly than that in low impacted samples.

251   **Discussion**

252   Overall what makes this study site, a model system for the influence of *Eucalyptus* plantation,

253   interesting is that during a chronosequence of *Eucalyptus* plantation, composition and function of

254   soil bacteria community were shifted in impacted years but lower impact was observed in the five-

255   year plantation. Furthermore, soil properties were altered significantly, which were treated as a

256   main factor driving community variation.

257   **Soil properties**

258   Key soil properties such as pH, SOC and phosphorus concentration (TP and AP) were altered

259   between secondary forest and *Eucalyptus* plantations or shifted with plantation time indicating the

260   influence of *Eucalyptus* on resident soil microbial communities. Soil pH as an indicator of soil

261   condition is affected easily by understory vegetation and plantation. *Eucalyptus* plantations

262   generally may lead to soil acidification when converted from original vegetation, as observed after

263   ten years(Rhoades & Binkley, 1996; Sicardi et al., 2004). In our study, the lower pH values were

264   shown in 2YR and 10YR *Eucalyptus* soil compared with secondary-forest, which may be a

265   consequence of the accelerated extraction of cations and of compounds released from decaying

266   leaf litter (Soumare et al., 2016). Consistent with our results, the amount of soil SOC has been

267   shown to decrease after planting *Eucalyptus* (Behera & Sahani, 2003; Cook et al., 2016; Zhang et

268   al., 2015) but also to increase in some conditions (Zhang et al., 2012). Infertile soil organic matter

269   may decrease soil structural stability leading to erosion (Behera & Sahani, 2003). The investigation

270   of soil properties indicated that soil SOC content in 2YR and 10YR was less than that in secondary-

271   forest. Nevertheless, the highest soil SOC value in 5YR *Eucalyptus* plantation may be caused by

272   low decomposition rates of microbial communities (Zheng et al., 2017). Soil SOC and other soil

273   nutrients differed between *Eucalyptus* soil and original forest, such as TP and AP, or stabilized

274   under certain conditions (TP), probably partly arising from processes such as litter fall

275   decomposition, leaching and mineralization (Falkiner & Smith, 1997; Jaiyeoba, 1998; Laclaua et

276   al., 2003). Phosphorus concentrations may exhibit an increase during the process of decomposition

277   of leaf litter (Ribeiro et al., 2002; Han et al., 2011).

278   **Community diversity patterns**

279   Several of the above variables were identified as key drivers in determining microbial community

280   structure and underpinning the clustering of samples into groups determined by plantation age. pH

281   has been identified as an important variable in determining microbial diversity patterns on a

282   number of scales (Fierer & Jackson, 2006) and is a factor in determining plantation soil community

283   composition (Zhou et al., 2017). Nutrient concentration (P, N) is also generally a major driver of

284   microbial diversity patterns (Leff et al., 2015) including in forest habitats (Liu et al., 2012) and

285   *Eucalyptus* plantations (Lan et al., 2017) and our results are consistent with this.

286   The low explanatory power of CCA1 and CCA2 may suggest that some relative factors were

287   not measured and that these also play a role in determining community diversity patterns.

288   Overall, our beta-diversity patterns showed a cycle of impact (2YR/10YR), which was

289   defined as a strong dissimilarity to the native soil conditions in the control SF, and low impacted

290   (5YR), where soil microbial diversity patterns resolved to a high-degree of similarity to control SF

291   samples. This was not stable over time as samples were low impacted after 5 years then displayed

292   community profiles indicative of higher impact after 10 years. The lower impact of soil

293   communities has been observed following *Eucalyptus* plantations recently (Chen et al., 2013)

294   however short-term fluctuations in microbial diversity are not well described in plantation systems.

295   The mechanism for this is potentially driven by patterns mediated by a lowering of soil pH and

296   increases in nutrient concentration as a consequence of leaf litter and tree-root inputs, such as

297 allelochemicals, as described above (Khan et al., 1999; Mitchell et al., 2012) in the impacted
298 samples. Indeed, following experimental addition of *Eucalyptus* leaves to control SF soil nutrient
299 concentrations increased however this experiment was only conducted on a short time-scale (<6
300 months,Table S3). The 5 years samples, being defined by a higher pH and lower nutrient
301 concentrations, were potentially less influenced by inputs derived from plant material. Collectively
302 this highlights the importance of managing leaf litter, tree harvesting and nutrient input in
303 influencing soil chemical properties and microbial community composition and that microbial
304 responses to plantation are not stable over time.

305   Alpha diversity of soil bacterial community in *Eucalyptus* plantations has been found to be
306 higher than native forest environment (Silveira et al., 2006; Lan et al., 2017). However, we found
307 no significant differences in bacterial alpha-diversity (species richness, diversity indices) between
308 *Eucalyptus* and other forest sites even though there were alterations in the structure of soil bacterial
309 community. Thus the above inputs drive shifts in relative abundance rather than presence absence
310 of community members. This homogeneity of alpha-diversity has been observed in similar
311 plantations (Kerfahi et al., 2016).

312 **Taxonomic composition shifts**

313 The most abundant phyla, *Proteobacteria*, *Actinobacteria*, *Acidobacteria* and *Verrucomicrobia*
314 are well-represented in most forest soils (Ederson Da C Jesus et al., 2009; Fierer et al., 2007; Lan
315 et al., 2017; Spain et al., 2009), and the distribution of dominant bacteria were stable after
316 *Eucalyptus* plantation in our study. The dominance of *Proteobacteria* and *Acidobacteria* as
317 generally observed in most of soils indicated low impact by land-use type(Rampelotto et al., 2013),
318 which may explain the small degree of variation among treatments of both of these phyla.
319 *Acidobacteria* have been reported in oligotrophic habitats with low nutrient concentrations, as well
320 as a wide range of metabolic organic matter, low C mineralization rate and ability to tolerate
321 fluctuations in adverse soil conditions (Aislabie & Deslippe, 2013; Fierer et al., 2007; Rampelotto
322 et al., 2013). Since *Proteobacteria* and *Acidobabcteira* could be considered as indicators of soil
323 trophic level organisms (Smit et al., 2001; Zhang et al., 2017), the change from secondary-forest
324 to *Eucalyptus* or stand age of *Eucalyptus* generated little effect on declining soil nutrition and
325 metabolizing organic resource within the ten-year *Eucalyptus* plantation. Bacteria belonging to the
326 phylum *Actinobacteria* were more dominant in impacted soils. *Actinobacteria* have been widely
327 reported as playing critical roles in exuding antibiotics and secondary metabolites (Quirinoa et al.,
328 2009; Lauber et al., 2013), and their increased abundance in impacted samples could have
329 consequences on these processes. Degradation of SOC in soils could lead to the high level of

330 *Actinobacteria* observed in impacted soils (2YR and 10YR), because of its higher ability to
331 consume organic carbon pools and its copiotrophs lifestyle (Rampelotto et al., 2013; Zhang et al.,
332 2017; He et al., 2012). Significant variation in the genus *Bradyrhizobium* between impacted and
333 low impacted clusters illustrates the low ability of nitrogen-fixing in the *Eucalyptus* plantation
334 (Silva et al., 2014). Likewise, *Cyanobacteria* was beneficial to nitrogen fixation and stabilized
335 structure of soil by potentially binding particles in the terrestrial ecosystems (Eldridge & Leys,
336 2003; Bowker et al., 2010). Hence, it was probable that soil in *Eucalyptus* plantations had less
337 tolerance from wind and water erosion than in the secondary-forest. Accordingly, we suggest that
338 land-use altered the soil structure and composition, contributing toward the productivity of soil.
339 Combined, the shifts in abundance of taxonomic groups with functional significance highlight the
340 impact of *Eucalyptus* plantation on local soils.

341 **Shifts in functional potential**

342 Taxonomic shifts between samples were reflected in the shifts in metabolic gene potential between
343 the impacted and low impacted group, the result of which suggested a decrease in overrepresented
344 functions in 2YR and 10YR impacted soils compared to low impacted potentially leading to an
345 accumulation of metabolic products and nutrients. According to the gene families identified by
346 PICRUSt analysis, we hypothesize that a decline in content of soil organic matter caused by
347 breaking up macro-aggregates (Hoosbeek et al., 2006) likely triggered the increase of soil bacterial
348 capacity to fix carbon when artificial plantation was sustained for a few years, which may partially
349 explain microbes impacted by *Eucalyptus* plantings (Chen et al., 2013; Cortez et al., 2014).
350 Overall, shifts in microbial function were evident between clusters indicating that pH and nutrient
351 shifts as a consequence of plantation will likely impact microbial function in these soils.

352 **Conclusions**

353 Within a chronosequence of *Eucalyptus* plantation, soil microbial community structure shifted
354 significantly in soils two and ten year's after plantation compared with secondary forest. Following
355 five years of plantation, low impact was observed with the community showing a high-degree of
356 similarity to control soils. The main factors which drove this partitioning were variation in pH and
357 nutrient concentrations, such as phosphorus potentially resultant from leaf litter and plant inputs.

358 Microbial communities were not stable over the time-scale measured highlighting the need
359 to understand microbial responses to plantation over varying time-scales to manage ecological
360 outcomes for plantations, such as stable, healthy soil microbial communities.

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363

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Figure 1

Sampling design and position in the Zhenlong Town of Huizhou City, Guangdong Province, South China (22°96' N, 114°36' E).

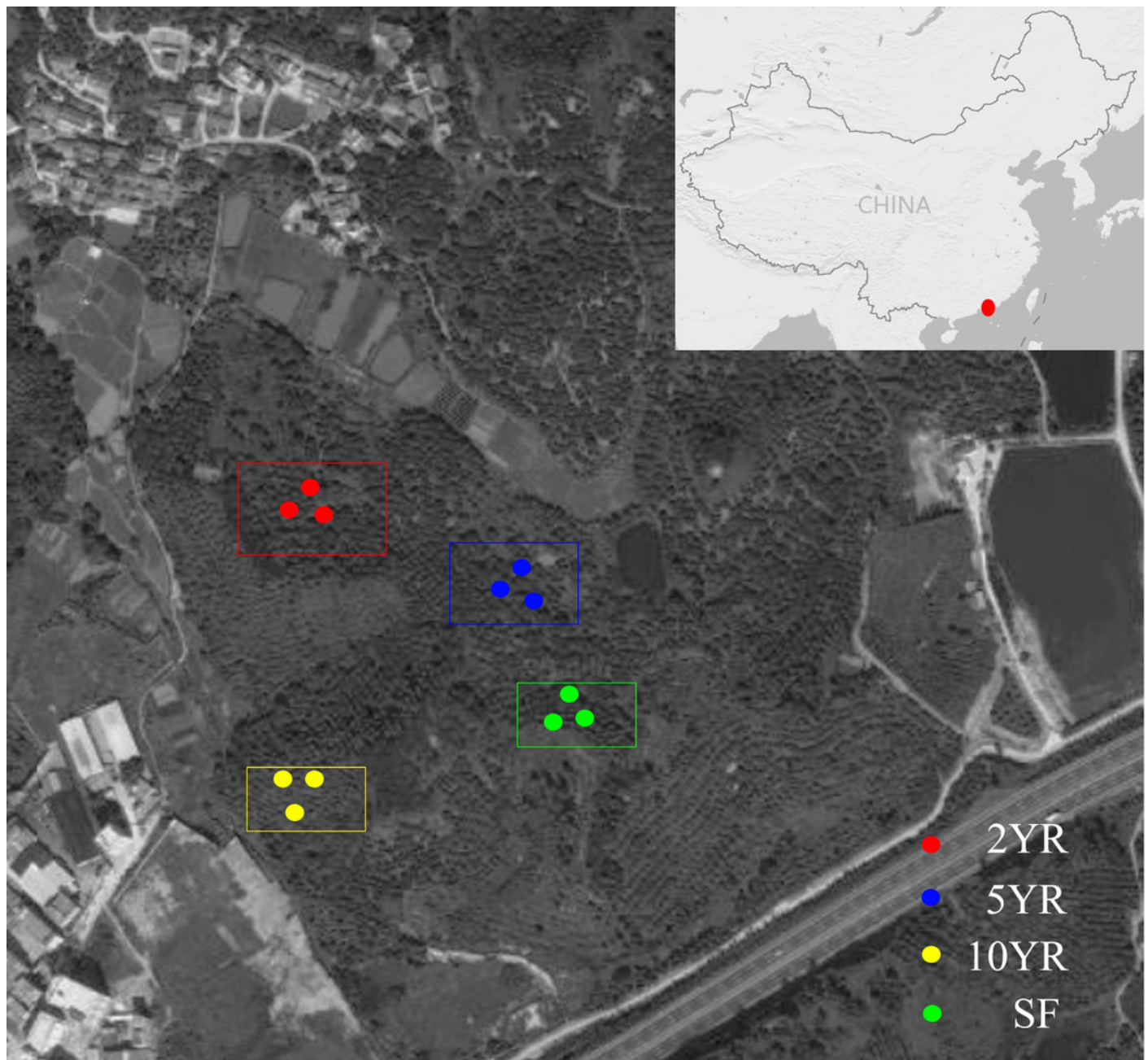



Figure 2

Principal coordinates analysis of bacterial community composition (weighted UniFrac dissimilarity) in *Eucalyptus* plantations and secondary forest soil

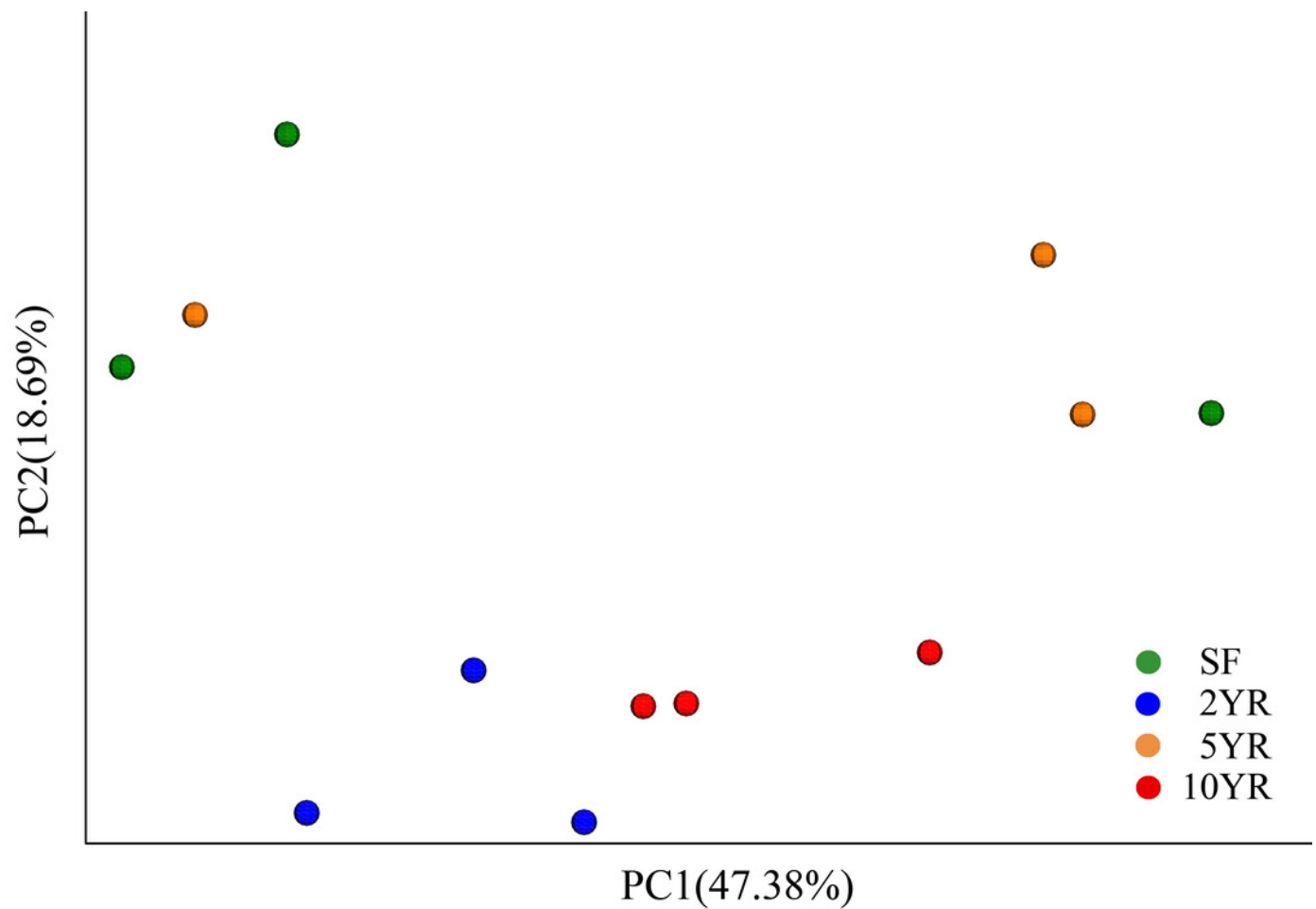



Figure 3

PCoA with color gradients mapped to a) pH and b) AP. Circles denote clusters defined as impacted and low impacted.

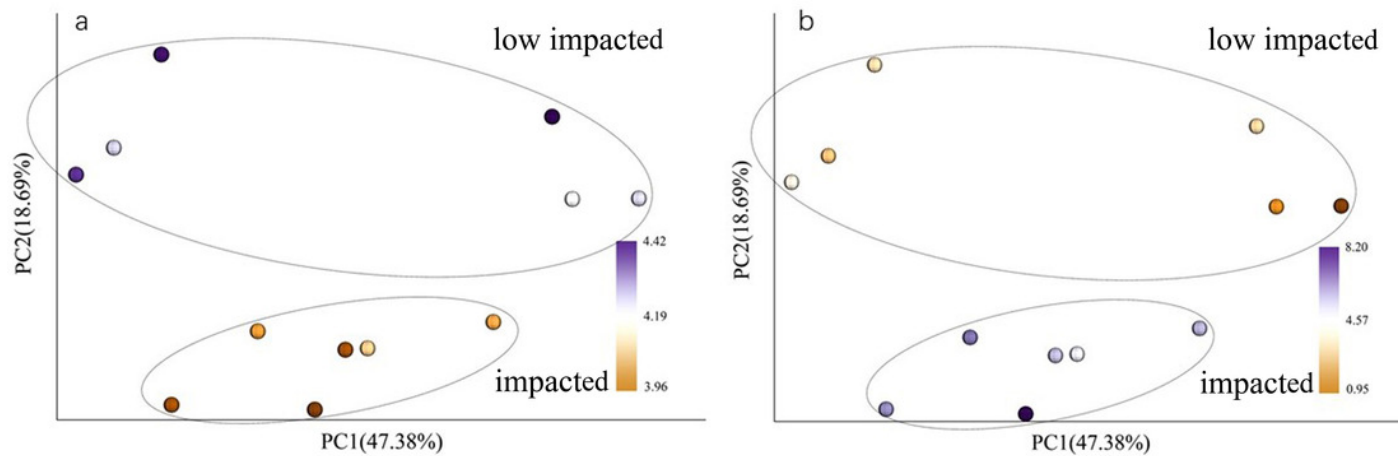



Figure 4

Relative abundance of bacterial phyla (three samples including in each treatments)

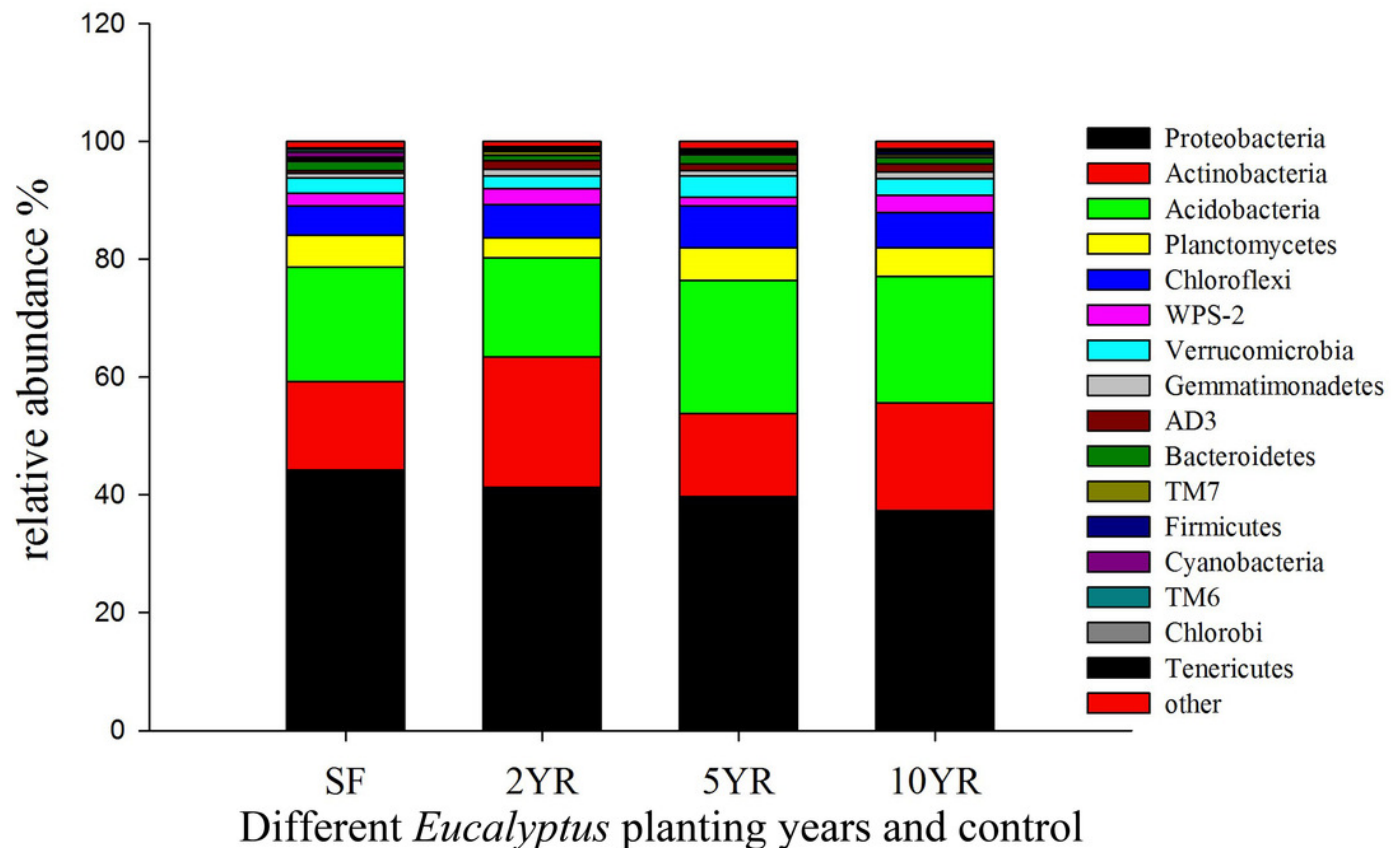



Figure 5

Differently abundant taxa abundance shown within phylogenetic lineages (LEfSe analysis) between impacted (comprised by 2YR and 10YR) and low impacted (comprised by 5YR and SF) clusters.

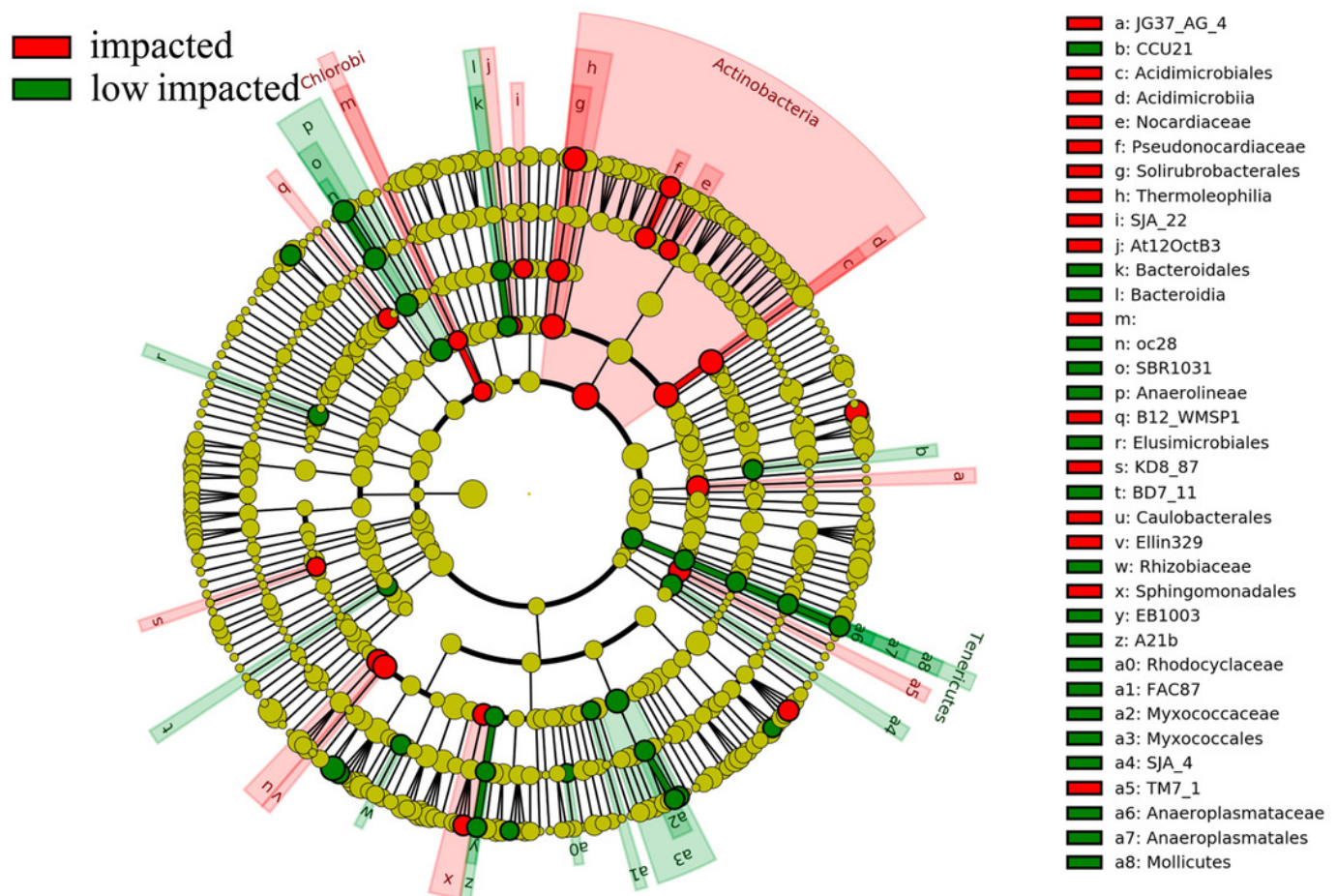



Figure 6

Canonical Correlation Analysis (CCA) of the abundance of sOTUs in bacterial community and soil environmental variables of the *Eucalyptus* and secondary forest.

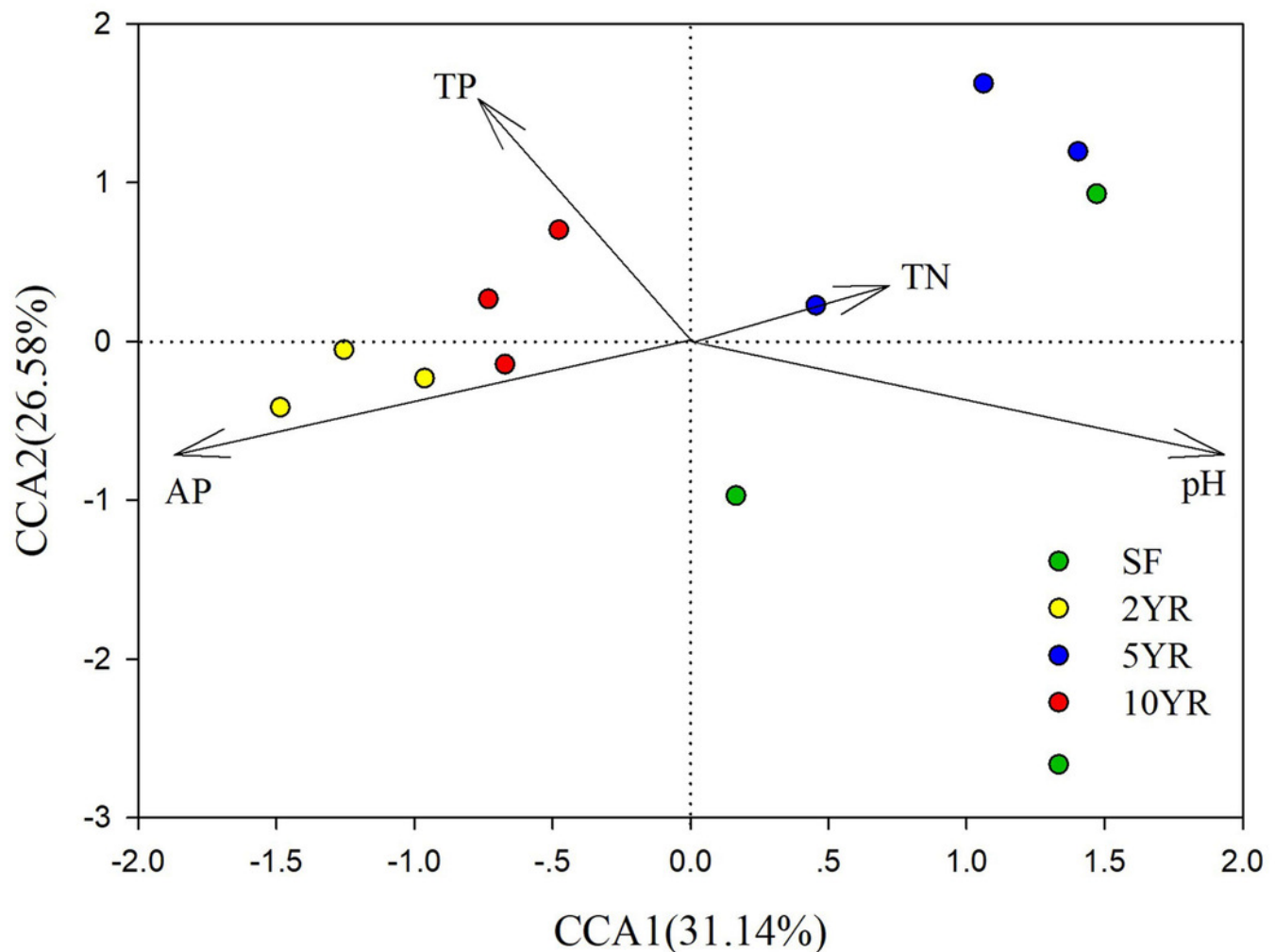
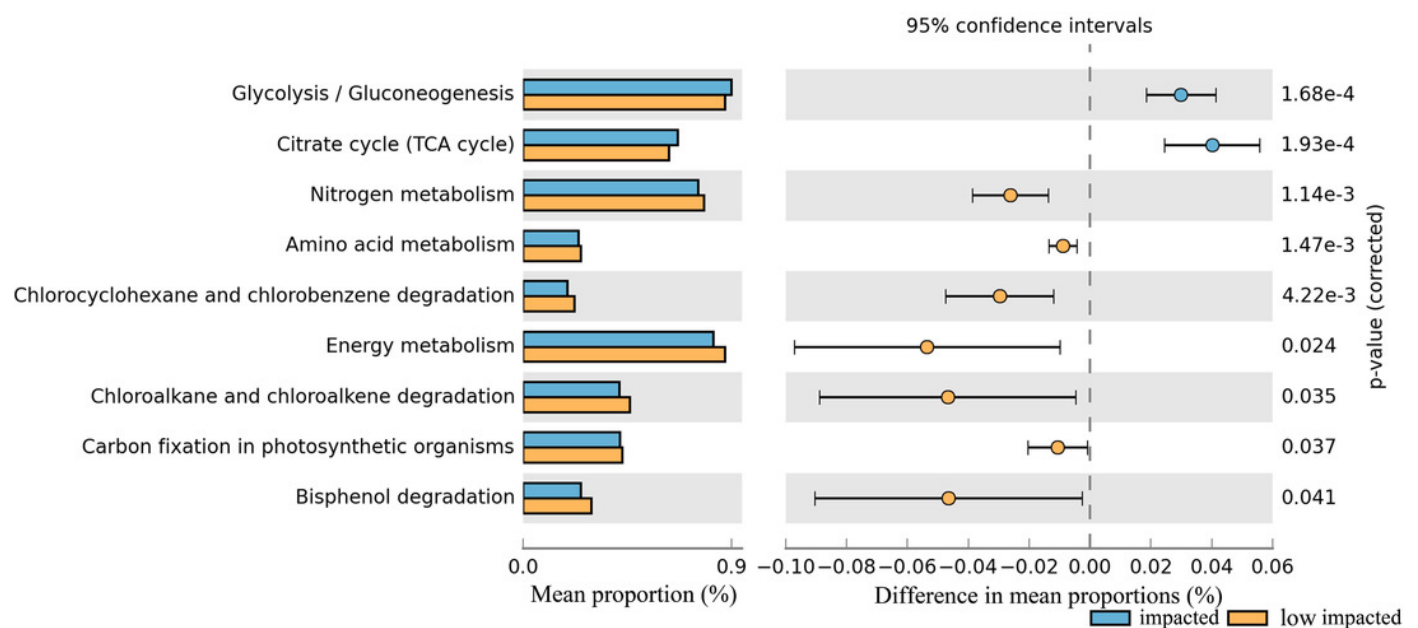



Figure 7

Relative abundance of predicted soil bacterial functions in impacted and low impacted clusters predicted by PICRUSt using KEGG Orthologs. Pathways presented here are relevant to soil ecosystem function and compound degradation, full data supplied in Table

Physicochemical properties in *Eucalyptus* plantations of different age and secondary forest

Note: different letters in rows indicate significant difference between the samples at *P* < 0.05, n=3.

Sample	pH	SOC (g/kg)	TN (g/kg)	TP (g/kg)	TK (g/kg)	AN (mg/kg)	NH4 (mg/kg)	NO3 (mg/kg)	AP (mg/kg)	AK (mg/kg)
SF	4.34±0.09a	60.32±12.05a	2.14±0.62a	0.22±0.03c	13.8±1.77ab	150.28±43a	21.82±2.64b	7.32±1.59b	2.98±1.78b	100.32±57.11a
2YR	4.01±0.06b	50.57±6.4b	1.74±0.04a	0.27±0.01b	11.67±2.04b	143.22±2.94a	15.87±3.81c	22.73±9.45a	7.17±0.91a	63.83±7.14a
5YR	4.29±0.12a	61.92±2.47a	2.15±0.13a	0.32±0.01a	8.2±0.42c	164.58±25.23a	32.8±2.98a	10.37±4.68ab	3.15±0.59b	55.51±11.63a
10YR	4.06±0.07b	42.41±6.16b	1.58±0.16a	0.32±0a	15.08±0.52a	133.12±19.52a	8.85±2.35d	21.21±8.51a	5.62±0.54a	91.63±6.39a

Results of mantel test between different soil properties and community composition.

Note: "r" is symbol for Spearman rho.

Soil properties	r	P-value
pH	0.328	0.025
TP	0.303	0.041
TN	0.297	0.048
AP	0.22	0.104
NH4	0.119	0.387
NO3	0.114	0.406
AN	0.094	0.541
SOC	0.085	0.556
AK	0.032	0.863
TK	-0.012	0.913

Differences in relative abundance of bacterial phyla in different stages of *Eucalyptus* plantation and secondary forest.

Note: different letters in rows indicate significant difference between the samples at *P* < 0.05, n=3.

Phylum/Treatment	SF	2YR	5YR	10YR
Proteobacteria	44.22±10.95a	41.3±2.21a	39.75±8.33a	37.34±1.48a
Actinobacteria	15.04±3.63b	22.18±2.38a	14.09±3.49b	18.37±4.15ab
Acidobacteria	19.47±6.67a	16.81±2.07a	22.56±4.54a	21.4±1.76a
Planctomycetes	5.38±2.7a	3.33±1.13a	5.51±2.16a	4.79±1.09a
Chloroflexi	5.03±2.47a	5.72±0.31a	7.22±2.78a	6.07±1.84a
WPS-2	2.1±0.09b	2.75±0.17a	1.41±0.56b	2.9±0.28a
Verrucomicrobia	2.64±1.69a	2.09±0.96a	3.6±1.86a	2.87±0.16a
Gemmatimonadetes	0.75±0.21b	1.11±0.04a	0.92±0.01a	1.13±0.18a
AD3	0.40±0.25b	1.49±0.46a	1.13±0.20a	1.38±0.68a
Bacteroidetes	1.57±0.85a	0.9±0.07a	1.58±0.75a	1.05±0.15a
TM7	0.39±0.1b	0.71±0.12a	0.31±0.08b	0.52±0.19ab
Firmicutes	0.33±0.17a	0.2±0.08a	0.21±0.1a	0.44±0.23a
Cyanobacteria	0.96±0.63a	0.29±0.10b	0.23±0.12b	0.23±0.04b
TM6	0.36±0.13a	0.16±0.04b	0.24±0.09b	0.16±0.05b
Chlorobi	0.01±0.02b	0.05±0.04ab	0.03±0.02ab	0.07±0.03a
Tenericutes	0.27±0.02a	0.00±0.01c	0.07±0.05b	0.02±0.02bc