Differential Responses of the Acidobacterial Community in the Topsoil and Subsoil to Fire Disturbance in *Pinus tabulaeformis* Stands

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Acidobacteria is found to be dominant and abundant in forest soil, and performs specific ecological functions (such as cellulose decomposition and photosynthetic capacity, etc.). However, relative limited is known about its changing patterns after a fire interruption. In this study, the response of soil *Acidobacteria* to a wildfire disturbance was investigated using the Illumina MiSeq sequencing system. The research area was classified by different severities of fire damage (high, moderate, and low severity, and an unburnt area), and samples were collected from various soil layers (0-10 cm as topsoil; 10-20 cm as subsoil). We obtained a total of 986,036 sequence reads; 31.77% of them belonged to *Acidobacteria*. Overall, 18 different *Acidobacteria* subgroups were detected, with subgroups 4, 6, 1, 3, and 2 the most abundant, accounting for 31.55%, 30.84%, 17.42%, 6.02%, and 5.81% of acidobacterial sequences across all samples, respectively. Although no significant differences in acidobacterial diversity were found in the same soil layer across different fire severities, we observed significantly lower numbers of reads, but higher Shannon and Simpson indices, in the topsoil of the high-severity fire area than in the subsoil. Non-metric multidimensional scaling (NMDS) analysis and permutational multivariate analysis of variance (PERMANOVA) also revealed significant differences in the acidobacterial community structure between the two soil layers. Soil pH, total nitrogen, NH_4^+-N, the Shannon index of understory vegetation and canopy density were the major drivers for acidobacterial community structure in the topsoil, while soil pH and organic matter were significant factors in the subsoil. A variance partitioning analysis (VPA) showed that edaphic factors explained the highest variation both in the topsoil (15.6%) and subsoil (56.3%). However, there are large gaps in the understanding of this field of research that still need to be explored in future studies.

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1   **ABSTRACT**

2   *Acidobacteria* is found to be dominant and abundant in forest soil, and performs specific

3   ecological functions (such as cellulose decomposition and photosynthetic capacity, etc.).

4   However, relative limited is known about its changing patterns after a fire interruption. In this

5   study, the response of soil *Acidobacteria* to a wildfire disturbance was investigated using the

6   Illumina MiSeq sequencing system. The research area was classified by different severities of

7   fire damage (high, moderate, and low severity, and an unburnt area), and samples were collected

8   from various soil layers (0-10 cm as topsoil; 10-20 cm as subsoil). We obtained a total of

9   986,036 sequence reads; 31.77% of them belonged to *Acidobacteria*. Overall, 18 different

10   *Acidobacteria* subgroups were detected, with subgroups 4, 6, 1, 3, and 2 the most abundant,

11   accounting for 31.55%, 30.84%, 17.42%, 6.02%, and 5.81% of acidobacterial sequences across

12   all samples, respectively. Although no significant differences in acidobacterial diversity were

13   found in the same soil layer across different fire severities, we observed significantly lower

14   numbers of reads, but higher Shannon and Simpson indices, in the topsoil of the high-severity

15   fire area than in the subsoil. Non-metric multidimensional scaling (NMDS) analysis and

16   permutational multivariate analysis of variance (PERMANOVA) also revealed significant

17   differences in the acidobacterial community structure between the two soil layers. Soil pH, total

18   nitrogen, NH_4^+-N, the Shannon index of understory vegetation and canopy density were the

19   major drivers for acidobacterial community structure in the topsoil, while soil pH and organic

20   matter were significant factors in the subsoil. A variance partitioning analysis (VPA) showed that

21   edaphic factors explained the highest variation both in the topsoil (15.6%) and subsoil (56.3%).

22   However, there are large gaps in the understanding of this field of research that still need to be

23   explored in future studies.

24   **INTRODUCTION**

25   Fire is a significant interfering factor in forest ecosystems. Heat from fire has strong direct and

26   indirect effects on vegetation (Knelman et al., 2015), animals (Wang & Yan, 2017), soil (Mikita-

27   Barbato et al., 2015), regional climate (Lehmann et al., 2014), and the water cycle (Chen et al.,

28   2013). As an important part of the forest ecosystem, soil microorganisms maintain ecosystem

29   stability through the role in driving organic matter decomposition and soil nutrient cycling

30   (Kristal & Kathleenk, 2010). Although the response of soil microorganisms to fire disturbance

31   has been studied extensively, the impacts of fire severity on bacterial communities is far from a

32   settled question. Pressler et al. (2018) concluded that there are insufficient studies to draw strong

33   conclusions about the role of fire severity on the bacterial community. Weber et al. (2014) found

34   inconsistent response of bacterial communities to fire severity. However, the above studies

35   focused on the overall changes in bacterial communities. Little attention has been paid to the

36   effects of a wildfire on specific bacterial phyla.

37   *Acidobacteria* have been recognized as being ubiquitous and abundant in forest soil (Jones

38   et al., 2009; Mbuthia et al., 2015). Based on 16S rRNA sequencing, 10% to 50% of the obtained

39   soil microbial sequences have been assigned to this phylum (Navarrete et al., 2013; Liu et al.,

40   2016). Currently, *Acidobacteria* are classified into 26 subgroups (Barns et al., 2007); subgroups

41   1 to 4 and subgroup 6 are found to be predominant in soils (Naether 2012; Zhang et al., 2014).

42   Although bacteria of this phylum are difficult to cultivate (Liu et al., 2016), molecular techniques

43   provide an opportunity to gain an insight into their ecological and physiological characteristics

44   (Kielak et al., 2016). In previous studies, *Acidobacteria* have been observed to exhibit

45   oligotrophy that is found to be related to some ecosystem processes, such as the decomposition

46   of organic matter and carbon cycling (Jones et al., 2009; Griffiths et al., 2011; Eichorst et al.,

47   2011; Sul et al., 2013) and their phylogenetic diversity is considered to be comparable to that of

48   the phylum *Proteobacteria* (Liu et al., 2016). However, not all members of the subgroups show

49   consistent biological characteristics. For example, the gene sequences of *Acidobacteria* are also

50   detected in neutral or even alkaline environments (Xiong et al., 2012). In addition, de Castro et al.

51   (2013) reported that several isolates of this phylum are capable of growth in a eutrophic

52   environment. Furthermore, Belova et al (2018) reported two acidobacterial strains (SBC82^T and

53   CCO287) were assigned to a novel genus and species and members of this genus colonize acidic

54 soils and peatlands and specialize in degrading complex polysaccharides. Hausmann et al (2018)

55 showed by genome-centric metagenomics and metatranscriptomics that members of the

56 *Acidobacteria* phylum have a putative role in peatland sulfur cycling. Eichorst et al (2018)

57 performed a large-scale comparative genome analysis of acidobacterial subdivisions and

58 observed that low- and high-affinity respiratory oxygen reductases in multiple genomes,

59 suggesting the capacity for growing across different oxygen gradients. They also detected the

60 capacity of many acidobacterial genomes to use multiple carbohydrates as well as inorganic and

61 organic nitrogen sources. Diamond et al (2019) also reported that acidobacteria play important

62 roles in C cycling in soils.

63 Studies of the influence of fire on the abundance of *Acidobacteria* have shown inconsistent

64 results, with reports of a great decrease (Xiang et al., 2014), a significant increase in lightly burnt

65 unvegetated soils (Knelman et al., 2015), and minor changes at the phylum level, but with a

66 significantly lower abundance of subgroup 1 after burning (Shen et al., 2016). The differences in

67 these results may be attributable to the sampling time, location, fire severity, vegetation type, etc.,

68 as well as the variable biological characteristics of the *Acidobacteria* subgroups (Liu et al., 2016).

69 For example, Zhang et al (2014) reported that the relative abundances of subgroup 1, 2, 3

70 increased with the altitude, but the relative abundances of subgroup 4, 5, 6, 16, 17 decreased

71 with the altitude. Several researchers also found that the effects of soil pH on *Acidobacteria*

72 subgroups are not exactly the same (Jones et al., 2009; Turlapati et al., 2013; Kielak et al., 2016).

73 Xiang et al (2014) reported that the relative abundance of *Acidobacteria* is negatively correlated

74 with soil pH; however, Liu et al. (2016) found a significantly positive relationship between the

75 abundance of *Acidobacteria* and soil pH. Although significant findings have been made

76 concerning the distribution of *Acidobacteria* after fire disturbance (Xiang et al., 2014; Shen et

77 al., 2016; Sun et al., 2016), knowledge of the response pattern at the subgroup level is still

78 limited. In this study, we try to control other variables to be unified and mainly to explore the

79 influence of different fire severities on *Acidobacteria.*

80 In the context of the high relative abundance and special ecological characteristics of

81 *Acidobacteria*, we aimed to gain an insight into the response of this phylum (especially at the

82 subgroup level) to wildfire. We assessed the response of *Acidobacteria* to different fire severities

83 (high, moderate and low severity, and unburnt) at two soil depths (0-10 cm as the topsoil and 10-

84 20 cm as the subsoil). We sought to address three questions: (1) How does the acidobacterial

85 community composition vary in the topsoil and subsoil after burning at different severities? (2)

86 What are the main factors that affect changes in soil acidobacterial communities after a wildfire?

87 (3) Are the driving factors in the topsoil and subsoil the same?

88

89 **MATERIALS & METHODS**

90

91 **Site Description and Soil Sampling**

92 This study was conducted in Pingquan County, Hebei province, northern China

93 (118°22′~118°37′E, 41°01′~41°21′N) (Fig. S1). As a typical coniferous tree species in North

94 China, *Pinus tabulaeformis* is widely distributed in this area (Zhu et al., 2015). The oil content of

95 the branches and leaves of *Pinus tabulaeformis* is high, and the trees belong to the flammable

96 forest type (Niu, 2011). The soil types were classified as brown soil and cinnamon soil (Li et al.,

97 2015). The climate is semi-humid and semi-humid continental monsoon, with a mean annual

98 temperature of 7.3°C. The mean annual precipitation in this region is 550 mm, of which 70% is

99 summer rainfall (July-September) (Li et al., 2015).

100 In April 2015, a 56.33 ha wildfire occurred in this region. The burned area was divided into

101 different regions according to the fire severity (high, moderate and low severity, and a nearby

102 unburnt area) (Zheng et al., 2012). The percentages of different severity levels are about 55.56%

103 (high), 33.33% (moderate), 11.11% (low). The classification standard of fire severity is shown in

104 Table 1 and we also provide a set of photographs to show the visual perception of different fire

105 severity areas (Fig. 1). Soil samples were collected 6 months after the fire.

106   Three plots (20 m x 20 m) were established in each of four distinct patches, representing the

107   different fire intensity levels (high, moderate, low and unburned). There were 12 plots

108   in total, with similar slopes (20~23°), aspects (37~42°) and elevations (1119~1143 m). In each

109   plot, five random soil cores at a depth of 0-10 cm and 10-20 cm were taken. Before sample

110   collection, the litter, charred debris, and ash layer were removed. Then, the five soil cores from

111   the same layer were mixed into one soil sample. In total, 24 composite samples (12 topsoil; 12

112   subsoil) were collected. All samples were transported to the laboratory in an ice box for further

113   analysis. Samples were divided into two parts: one part was stored at 4°C for biogeochemical

       analysis, and the other was stored at $-20℃$ for DNA analysis.

114   **Plant Diversity and Structure and Soil Physicochemical Analyses**

115   The plant species, plant height, canopy density and tree diameter at breast height (DBH>5cm)

116   were recorded in each plot (Table S1). Then, ten quadrats were randomly established in each

117   plot (five 1×1 m quadrants for herbs; five 5×5 m quadrats for shrubs). The diversity of the

118   understory vegetation (i.e. herbs and shrubs) was evaluated using the Shannon-Wiener index

119

120

121   (H'$_{Vegetation}$), Simpson index (D'$_{Vegetation}$) and Pielou's evenness index (J'$_{Vegetation}$) (Yu et al.,
       Soil moisture (SM) was determined by oven-drying the samples at 105°C until they were
       2012).
122   of constant weight. pH was determined using a pH meter for a 1:2.5 ratio of fresh soil to

123   deionized water at 20°C. Soil organic matter (OM) content was measured by dichromate

124   oxidation (Xue et al., 2014), and total nitrogen (TN) was measured with the Kjeldahl method

125   (Walkley & Black, 1934). The ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N)

126   contents were also measured (Crooke & Simpson, 1971; Best, 1976).

127

128   **DNA Extraction and PCR Amplification**

129   Genomic DNA was extracted from 0.5 g of fresh soil using an E.Z.N.A.® soil DNA kit (Omega

130   Bio-tek, Norcross, GA, USA), following the manufacturer's instructions. The quality of the

131   DNA extracted was checked by 1% agarose gel electrophoresis and spectrophotometry (the ratio

132   of optical density at 260 nm/280 nm). All extracted DNA samples were stored at −20°C for

133   further analysis.

134   To assess the bacterial composition, the V3–V4 hypervariable regions of the bacterial 16S

135   rRNA genes were amplified by PCR (95°C for 5 min; 33 cycles at 95°C for 30 s, 56°C for 30 s

136   and 72 °C for 40 s; with a final extension of 72°C for 10 min), using the universal primers

137   forward 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and reverse 806R (5′-

138   GGACTACHVGGGTWTCTAAT-3′) (Liu et al., 2018). These primers contained an 8

139   nucleotide barcode sequence unique to each sample. PCR reactions were performed in triplicate

140   in a 50 μL mixture containing 5 μL of 10× Pyrobest Buffer, 4 μL of 2.5 mM dNTPs, 2 μL of

141   each primer (10 μM), 0.3 μL of Pyrobest DNA Polymerase (2.5 U/μL; TaKaRa code DR005A)

142   and 30 ng of template DNA. Then, the mixture was used to perform sequencing. The raw reads

143   were deposited into the National Center for Biotechnology Information Sequence Read Archive

144   database (accession number: SRP158101).

145   **Illumina MiSeq Sequencing**

146   Amplicons were extracted from 2% agarose gels and purified using an AxyPrep DNA Gel

147   Extraction kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's

148   instructions, and quantified using QuantiFluor-ST (Promega, Madison City, WI, USA). Purified

149   amplicons were pooled in equimolar amounts and paired-end sequenced (2×300) on an Illumina

150   MiSeq platform (Beijing Allwegene Technology Co., Ltd, China) according to standard

151   protocols.

152

153   **Processing of Sequencing Data**

154   The extraction of high-quality sequences was performed with the QIIME package (Quantitative

155   Insights Into Microbial Ecology) (version 1.2.1) (http://qiime.org/). Raw sequences were selected

156   on the basis of sequence length, quality, primer, and tag. The raw sequences were selected and

157   the low-quality sequences were removed as follows: (1) 300 bp reads were truncated at any site

158   receiving a mean quality score of <20 over a 10 bp sliding window, truncated reads that were

159   shorter than 50 bp were discarded; (2) exact barcode matching, 2 nucleotide mismatch in primer

160   matching, reads containing ambiguous characters were removed; (3) only sequences with an

161   overlap longer than 10 bp were assembled according to their overlap sequence. Reads that could

162   not be assembled were discarded. Chimeric sequences were identified and removed based on the

163   UCHIME algorithm (Edgar., 2011). The unique sequence set was classified into operational

164   taxonomic units (OTUs) under the threshold of 97% identity using UCLUST (Edgar., 2010) and

165   then the taxonomic annotations were conducted for each OTU based on the Silva119 16S rRNA

166   database using a confidence threshold of 90% (Quast et al., 2013). OTUs with less than five

167   reads were removed to reduce the risk of artificially inflating richness due to sequencing errors

168   (Chen et al., 2017).

169

Data Analysis

171   We performed separate one-way analysis of variance (ANOVA) with Tukey's test (equal

172   variances) or Dunnett's T3 test (unequal variances) to determine the effects of wildfire of

173   varying severity on soil physicochemical properties and the composition and diversity of the

174   acidobacterial community in different soil layers (0-10 and 10-20 cm). The differences in the

175   composition and diversity of the acidobacterial community between the topsoil and subsoil

176   within different fire severities were also compared by independent-sample t-test. Pearson

177   correlation analysis was used to determine relationships between the abundance of *Acidobacteria*

178   subgroups and environmental variables. Differences were considered significant with a P value

179   of 0.05 as the threshold. All tests were carried out using SPSS 19.0 for Windows.

180   To correct for sampling efficiency, we used a randomly selected subset of 6,199

181   acidobacterial sequences (the minimum sample size among 24 samples) per sample for

182   downstream analysis. Shannon-Wiener (H'$_{Acidobacteria}$) and Simpson (D'$_{Acidobacteria}$) indices of the

183   acidobacterial community were calculated using the OTUs with 97% identity. Non-metric

184   multidimensional scaling (NMDS) analysis based on Bray-Curtis and Weighted-Unifrac distance

185   and permutational multivariate analysis of variance (PERMANOVA) were used to compare the

186   composition of the acidobacterial community between different fire-damaged areas. Redundancy

187   analysis (RDA) was carried out to assess the relationships between soil properties and

188   acidobacterial community data (relative abundance of OTUs), and only environmental factors

189   with variance inflation factor values of less than 20 were selected for the model (Xiang et al.,

190   2014). A variance partitioning analysis (VPA) was further performed to quantify the effects of

191   environmental factors on acidobacterial community structure. The above analyses were carried

192   out with the Vegan package (Jari et al., 2018) in R (R Core Team, 2017).

193

194   **RESULTS**

195

196   **Soil Properties and Understory Vegetation**

197   Table 2 presents basic information on the physical and chemical properties of the soil after

198   burning. One-way ANOVA showed that soil OM, TN, ammonium nitrogen (NH$_4^+$-N), and

199   nitrate nitrogen (NO$_3^-$-N) in both soil layers significantly decreased with an increase in fire

200   severity. However, soil pH showed the opposite trend in that it increased after burning.

201   Interestingly, the patterns of change of SM in the topsoil and subsoil were inconsistent. The

202   highest value of SM was observed in the topsoil of the high-severity fire area.

203   As shown in Fig. S2 and Table S2, the diversity of the understory vegetation was

204   significantly changed after a high-severity wildfire. The highest value of Shannon-Wiener

205   (H'$_{Vegetation}$) and Simpson (D'$_{Vegetation}$) indices and the lowest value of Pielou's evenness index

206   (J'$_{Vegetation}$) were observed in the high-severity area compared with the other three types of plots

207 (*P*<0.05). However, no significant differences were found among the moderate-severity, low-

208 severity, and unburnt areas.

209

***Acidobacteria* Community Composition and Structure**

211 A total of 986,036 reads were detected using 24 samples through Illumina MiSeq sequencing.

212 Of these reads, *Acidobacteria* is the most abundant phylum with a abundance of 31.77% (Fig

213 S3). Each sample contained 6,199 to 27,585 acidobacterial reads (mean=13,053), with different

214 phylotypes (OTUs) ranging from 173 to 370 (mean=309; total=7425). The *Acidobacteria*

215 sequences obtained were further classified into 18 subgroups. Among them, subgroups 4, 6, 1, 3

216 and 2 were found to be dominant (abundance >5%) and common to the 24 libraries, which

217 accounted for 31.55%, 30.84%, 17.42%, 6.02% and 5.81% of the total acidobacterial sequences

218 across all soils, respectively (Fig. 2). The other 13 subgroups (subgroups 5, 7, 10-13, 15, 17, 18,

219 20, 22, 25, 26) were also found, but at a relatively low abundance (<5%).

220   The acidobacterial diversity patterns along a fire severity gradient were analyzed by the

221 number of reads, number of OTUs, Shannon index and Simpson index. We found no significant

222 differences in the same soil layer among different fire severities by one-way ANOVA (Fig. 3;

223 Table S3). However, independent-sample *t*-tests showed a significantly lower number of reads,

224 but higher Shannon and Simpson indices in the topsoil of the high-severity fire area compared

225 with the subsoil (*P*<0.05) (Fig. 3; Table S4).

226   NMDS analysis was used to distinguish differences in the acidobacterial community

227 structure between the two soil layers. Figure S4 shows that the acidobacterial community

228 structure of topsoil and subsoil is clearly divided into two parts. The PERMANOVA further

229 confirmed that the acidobacterial community structure in the topsoil was significantly different

230 from that in the subsoil (R^2=0.1317, *P*=0.048).

231

Relationship Between the Acidobacterial Community Structure and Environmental

Factors

RDA was used to examine the relationships between the soil acidobacterial community structure and environmental factors. Based on the test of the variance inflation factor, soil TN, NH_4^+-N, pH, the diversity of understory vegetation ($H'_{Vegetation}$, $D'_{Vegetation}$ and $J'_{Vegetation}$), plant DBH, plant height and canopy density were selected for the RDA model of topsoil; and soil OM, NO_3^--N, SM, pH, the diversity of understory vegetation ($H'_{Vegetation}$, $D'_{Vegetation}$ and $J'_{Vegetation}$), plant DBH and plant height were adopted for the RDA model of subsoil. Permutation test results indicated that the effects of soil pH, TN, NH_4^+-N, $H'_{Vegetation}$ and canopy density on the soil acidobacterial community structure reached a significant level in topsoil ($P<0.05$) (Fig. 4a). Meanwhile, soil pH and OM were found to be significant factors that influenced soil acidobacterial community structure in the subsoil (Fig. 4c).

On the basis of RDA, we classified the environmental factors selected by the model into three parts. TN, NH_4^+-N, pH, OM, NO_3^--N and SM were classified as edaphic factors. $H'_{Vegetation}$, $D'_{Vegetation}$ and $J'_{Vegetation}$ were classified as understory vegetation diversity factors. Canopy density, DBH, and plant height were classified as plant community structure factors. VPA was conducted to assess the relative contributions of edaphic factors, understory vegetation diversity, and plant community structure to the acidobacterial community structure. In the topsoil, the combination of these variables explained 23.6% of the observed variation in soil acidobacterial community structure. Edaphic factors explained the largest fraction of the variation (15.6%), with an independent effect of 9.8%. Understory vegetation diversity and plant community structure independently explained 5.8% and 1.2% of the variation, respectively (Fig. 4b). In the subsoil, 69.4% of the variation was explained by the combination of the variables, with edaphic factors accounting for the largest fraction of the variation (56.3%) (Fig. 4d). The results showed that edaphic factors were the main factors affecting the acidobacterial community structure both in topsoil and subsoil. However, a large number of the factors involved especially in the topsoil, are still unknown.

259   Consistent with the results of the VPA, we also found different relationships between the

260   relative abundance of *Acidobacteria* subgroups and soil factors. For example, in the topsoil, the

261   relative abundance of *Acidobacteria* subgroup 1 was significantly positively correlated with

262   NH_4^+-N (r=0.698, p<0.05), while the relative abundance of subgroup 7 was significantly

263   negatively related to NH_4^+-N (r=-0.655, p<0.05). In the subsoil, the relative abundance of

264   *Acidobacteria* subgroups 1 (r=0.849, p<0.01) and 3 (r=0.607, p<0.05) were found to be

265   significantly positively correlated with OM. By contrast, the relative abundance of subgroups 4

266   (r=-0.630, p<0.05), 10 (r=-0.577, p<0.05), 18 (r=-0.651, p<0.05) and 20 (r=-0.582, p<0.05)

267   showed an opposite relationship with OM. We also found that not all *Acidobacteria* subgroups

268   showed a consistent relationship with soil pH in the two soil layers. Except for *Acidobacteria*

269   subgroups 3 and 4, the relationships between other subgroups and soil pH were found to be

270   consistent in the two soil layers. However, the dominant subgroups 3 and 4 showed a negative

271   relationship in the topsoil（subgroup3: r=-0.059; subgroup4: r=-0.092）, but a positive

272   relationship in the subsoil (subgroup3: r=0.025; subgroup4: r=0.803) (Table S5; Table S6).

273

274   **DISCUSSION**

275   Soil *Acidobacteria* are widespread and abundant in natural ecosystems (Mbuthia et al., 2015; Liu

276   et al., 2016), and have been analyzed in different environments, such as in wastewater treatment

277   processes (Crocetti et al., 2002), hot springs (Hobel et al., 2005), sand dunes (Smith et al., 2004),

278   an acidic sphagnum peat bog (Dedysh et al., 2006), Amazon forest soils (Navarrete et al., 2015)

279   and Chinese fir plantations (Liu et al., 2017). However, the responses of the acidobacterial

280   community to a fire disturbance are still limited. In this study, we analyzed the variation of

281   *Acidobacteria* in a *P. tabulaeformis* forest after a wildfire. The results of Illumina MiSeq

282   sequencing showed that 31.77% of the reads belonged to *Acidobacteria* across all the soil

283   samples. Overall, 18 different *Acidobacteria* subgroups were detected in this study, with

284   subgroups 4, 6, 1, 3 and 2 found to be dominant. This finding is similar to that of Zhang et al.

285 (2014), who also found 19 known subdivisions in Shennongjia Mountain, Central China, of

286 which subdivisions 6, 1, 2, 3, 4 showed relative high abundance.

287   Fire has a strong effect on the diversity of the soil microbial community. A decrease of

288 soil bacterial diversity can be observed after burning, especially after a high-severity fire

289 (Ferrenberg et al., 2013; Xiang et al., 2014). Mabuhay et al. (2006) reported that the burnt areas

290 still showed very low microbial community diversity even 1 year after a fire. Xiang et al. (2014)

291 observed that the diversity of the bacterial community returned to match that of the unburnt

292 controls 11 years after the fire. Microbial diversity tends to recover gradually over a long time

293 (Shen et al., 2016). However, we found no significant differences of the acidobacterial α-

294 diversity in the topsoil or subsoil (Fig. 3). We speculated that the soil acidobacterial community

295 might recover in a much faster time. Interestingly, although the number of reads in the

296 topsoil was significantly lower than that in the subsoil, a significantly higher acidobacterial α-

297 diversity (i.e. Shannon and Simpson indices) was observed in the topsoil than in the subsoil of

298 high severity plots (Fig. 3). This phenomenon may be due to the edaphic and vegetable

299 heterogeneity caused by fire, enabling bacterial diversity to be maintained and even improved

300 with the change of microbial competition for resources (Chesson, 2000; Otsuka et al., 2008; Artz

301 et al., 2009).

302   Numerous studies have demonstrated that soil microbial communities are closely related to

303 aboveground vegetation and soil properties (Bardgett 2005; Chu et al., 2016). Among them,

304 Acidobacteria were found to play a role in promoting soil carbon cycling, as it can degrade

305 complex plant derived polysaccharides such as cellulose and lignin (Huang et al., 2015). Soil pH

306 has often been reported as a dominant factor in shifting acidobacterial communities (Xiong et al.,

307 2012; Xiang et al., 2014; Chu et al., 2016). This conclusion has also been confirmed in this study,

308 where we found that soil pH was a key factor both in the topsoil and subsoil (Fig. 4a; Fig. 4c).

309 Previous studies have shown that different subgroups presented inconsistent correlations with

310 respect to soil pH. For example, Turlapati et al. (2013) reported that subgroups 1, 2, 3, 12, and 13

311 were positively correlated with soil pH, but subgroups 4, 6, 7, 10, 11, 16, 17, 18, 22, and 25

312   showed a negative correlation. However, an opposite relationship was reported in the review

313   article by Kielak and colleagues (Kielak et al., 2016), who showed that pH had a negative

314   relationship with the abundance of subgroups 1, 2, 3, 12, 13, and 15, but a positive correlation

315   with subgroups 4, 6, 7, 10, 11, 16, 17, 18, 22, and 25. In this study, we found not only that the

316   relationships between *Acidobacteria* subgroups and soil pH were inconsistent in the same soil

317   layer, but also that certain subgroups had different relationships with soil pH in different soil

318   layers. For instance, the dominant subgroups 3 and 4 showed a negative relationship with soil pH

319   in the topsoil, but a positive relationship in the subsoil (Table S5; Table S6). We hypothesized

320   that the varied responses of the *Acidobacteria* subgroups to soil pH represented the key

321   determinant of the different relationships between *Acidobacteria* and soil pH. However, the

322   relationship between *Acidobacteria* subgroups and soil properties with the change of soil depth

323   still needs further study.

324   Soil nitrogen and OM were also found to be important parameters in driving the

325   acidobacterial community structures in the topsoil and subsoil, respectively (Fig. 4a; Fig. 4c).

326   Ward et al. (2009) reported that *Acidobacteria* played an important role in nitrogen cycling, and

327   subgroup 1 had related correlations with nitrate and nitrite. In this study, we also found a

328   significant relationship between subgroup 1 and NH_4^+-N in the topsoil (Table S5). *Acidobacteria*

329   are usually considered to be oligotrophic (Fierer et al., 2007; Jones et al., 2009). Navarrete et al.

330   (2015) found a significant positive relationship between the abundance of *Acidobacteria* and soil

331   OM. Liu et al. (2016) also observed a larger abundance of *Acidobacteria* in high organic carbon

332   content soils than in low carbon content soils in the black zone of northeast China. It has been

333   pointed out that not all subgroups or OTUs of this phylum would be oligotrophic (Fierer et al.,

334   2007; Chu et al., 2016). In this study, subgroups 4, 10, 18, and 20 were found to be significantly

335   negatively related to soil OM in the subsoil which suggesting oligotrophy(Table S6). However, a

336   significant positive relationship was also detected between the dominant subgroups 1 and 3 and

337   soil OM in this soil layer (Table S6). Kielak et al. (2016) proposed that not all *Acidobacteria*

338   possess the same ecological strategy, with a high variation of physiological characteristics within

339   this phylum.

340   Extensive studies have suggested that there are strong interrelationships between

341   aboveground vegetation and soil microbial communities (Hart et al., 2005; Nielsen et al., 2010;

342   Dangi et al., 2010). A larger microbial biomass was found in a forest of greater aboveground

343   diversity than in a homogeneous plant cover environment (Fioretto et al., 2009). Hart et al. (2005)

344   considered that changes in vegetation community structure in the years following a fire might

345   have a greater effect on soil microbial communities than the direct influence of the fire

346   disturbance itself. For example, the soil nutrient and carbon content could be influenced by the

347   inputs of litter (leaves, stems, roots) and exudate from newly established plants after burning

348   (Knelman et al., 2015). In this study, the Shannon diversity and canopy density of aboveground

349   vegetation were found to have significant effects on the acidobacterial community structure in

350   the topsoil (Fig. 4a). We hypothesize that an important reason for the higher diversity of the

351   acidobacterial community in the topsoil of the high-severity area is the increase in aboveground

352   vegetation diversity (Han et al., 2007; Zhang et al., 2014) and we also found $H'_{Vegetation}$ play an

353   important in driving the change of acidobacterial community in this soil layer (Fig. 4a). Further,

354   changes in plant community structure, such as the decline in canopy density, increase the light

355   availability and soil temperature in the forest, which are beneficial for the bacterial communities

356   (Arunachalam & Arunachalam, 2000; Bolat, 2014). However, the result of the VPA showed that

357   a large amount of the variation remained unexplained in this study, especially in the topsoil (Fig.

358   4b; Fig. 4d). Other studies show that climate change (Maestre et al., 2015), soil metal elements

359   (Navarrete et al., 2015), and soil enzyme activities (Zhang et al., 2014) also play important roles

360   in determining microbial community structures. Accordingly, further comprehensive studies of

361   *Acidobacteria* are necessary.

362   **CONCLUSION**

363   In summary, this study conducted the response of soil *Acidobacteria* to fire disturbance by

364   the Illumina MiSeq sequencing technique. We found no significant differences in acidobacterial

365 α-diversity in the topsoil or subsoil across different fire severities. However, the community

366 structure of soil *Acidobacteria* in the topsoil was significantly different from that in the subsoil.

367 We found that soil pH was a key factor in driving acidobacterial community structure in both soil

368 layers while several other factors (e.g. total nitrogen, NH4+-N, H'Vegetation and organic matter)

369 also have an important effect. The *Acidobacteria* subgroups were found to show different

370 variations after a wildfire. The differential responses of the *Acidobacteria* subgroups to specific

371 environmental factors can help to reveal what drives their population changes in forest soils

372 recently suffering a fire disturbance and open the possibilities to explore acidobacterial

373 subgroups as bioindicators for ecosystem restoration in *Pinus tabulaeformis* stands.

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Figure 1

Photographs depicting different fire severity areas.

(a) high severity area (b) moderate severity area (c) low severity area (d) unburnt control area.

Figure 2

Relative abundance of dominant acidobacterial subgroups in areas affected by differing fire severity.

H, high severity; M, moderate severity; L, low severity; C, unburnt control; 10, topsoil (0-10 cm); 20, subsoil (10-20 cm).
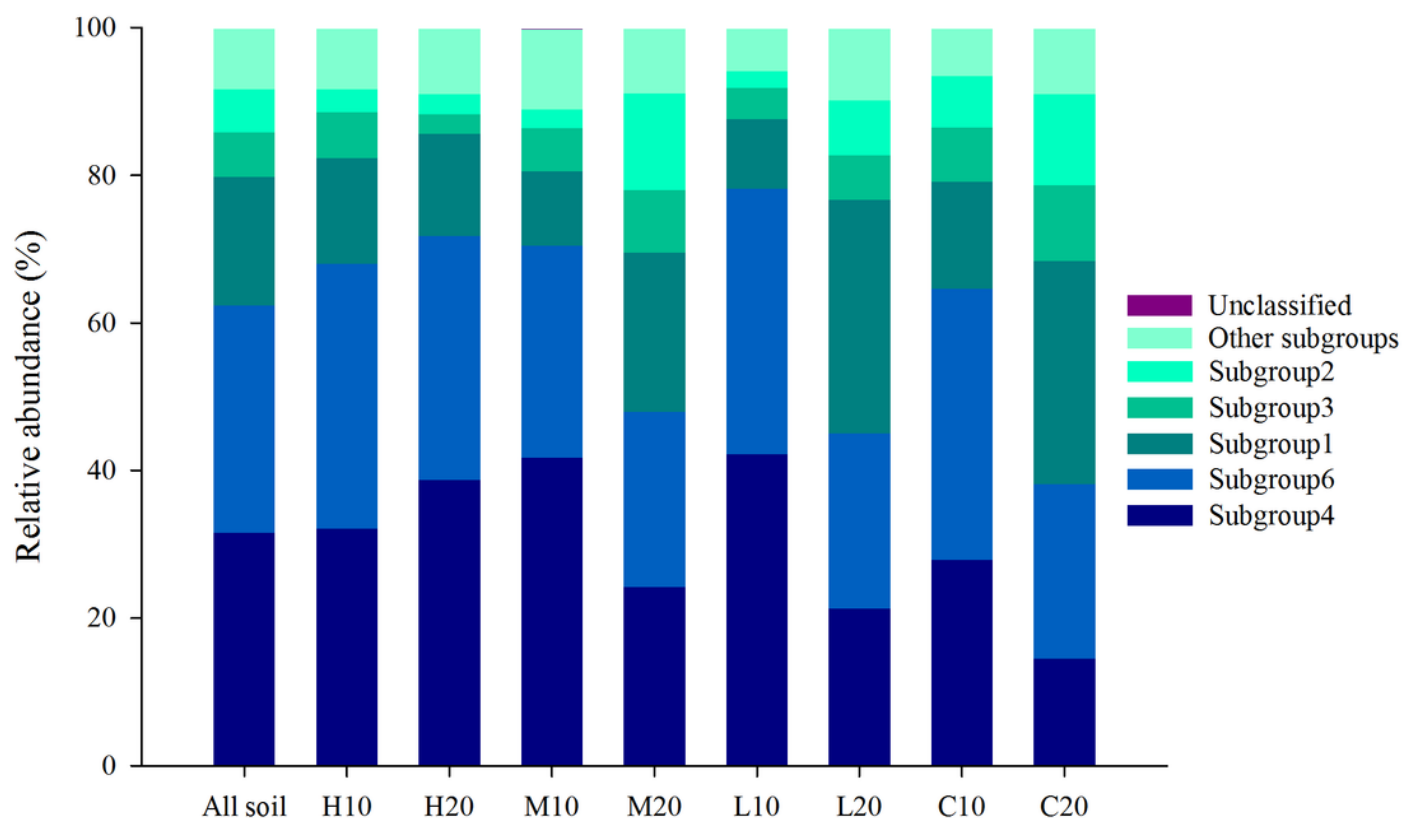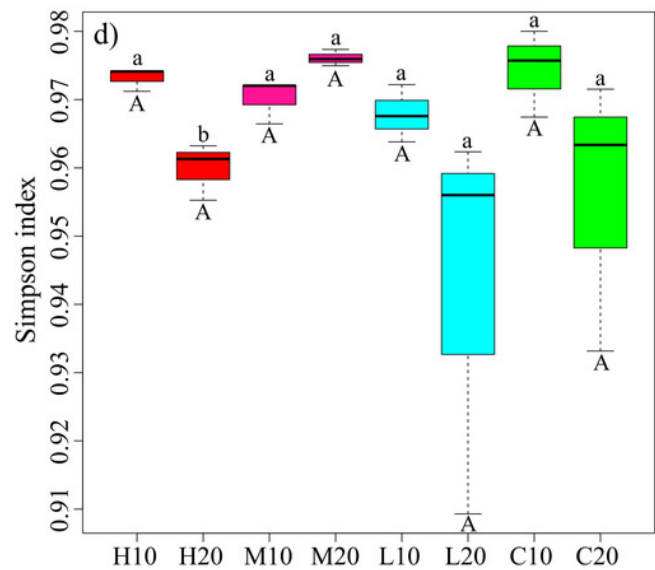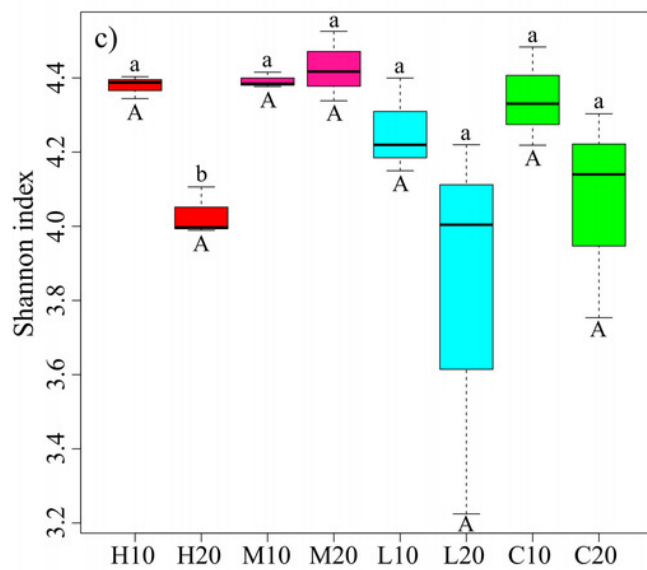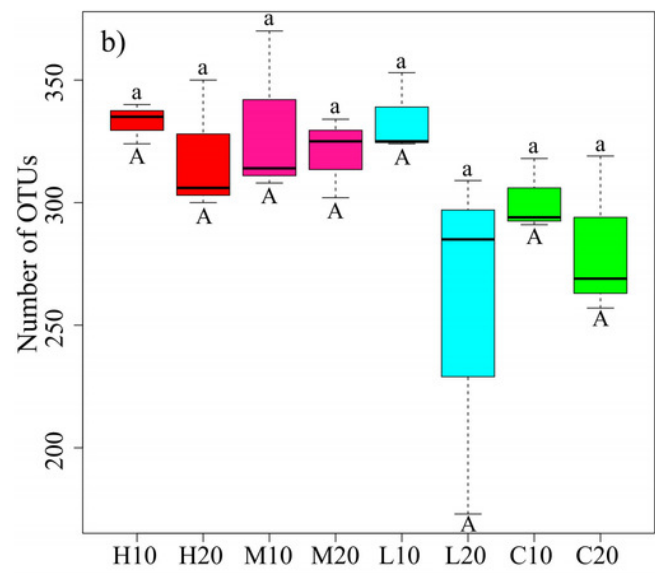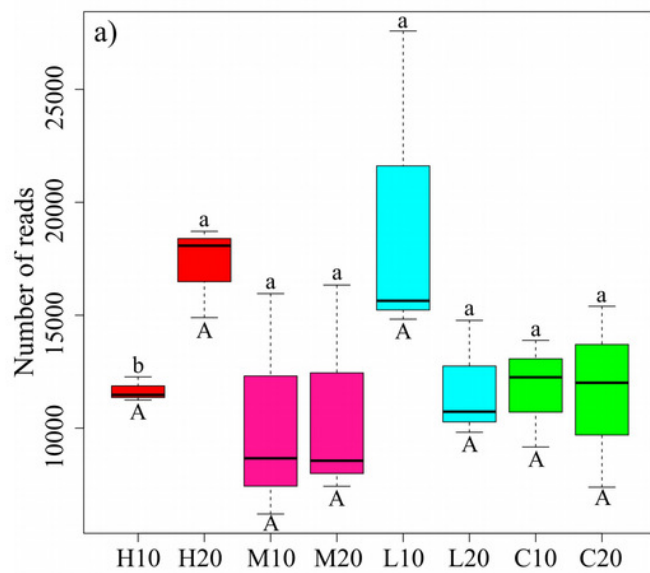
Figure 3

Sequencing results and acidobacterial α-diversity in different fire severity areas.

Different lowercase letters indicate a significant difference in different soil layers of the same fire severity plots at the $P<0.05$ level; different capital letters represent a significant difference in the same soil layer of different fire severity plots at the $P<0.05$ level. H, high severity; M, moderate severity; L, low severity; C, unburnt control; 10, topsoil (0-10 cm); 20, subsoil (10-20 cm).

Figure 4

Redundancy analysis (RDA) and variance partitioning analysis (VPA) show the relationship between acidobacterial community and environmental factors in the topsoil (a&b) and subsoil (c&d).

Circle diagrams indicate the independent and shared variance explained by environmental factors in the topsoil (b) and subsoil (d) respectively. I: edaphic factors; II: understory vegetation diversity factors; III: plant community structure factors. H, high severity; M, moderate severity; L, low severity; C, unburnt control; 10, topsoil (0-10 cm); 20, subsoil (10-20 cm).
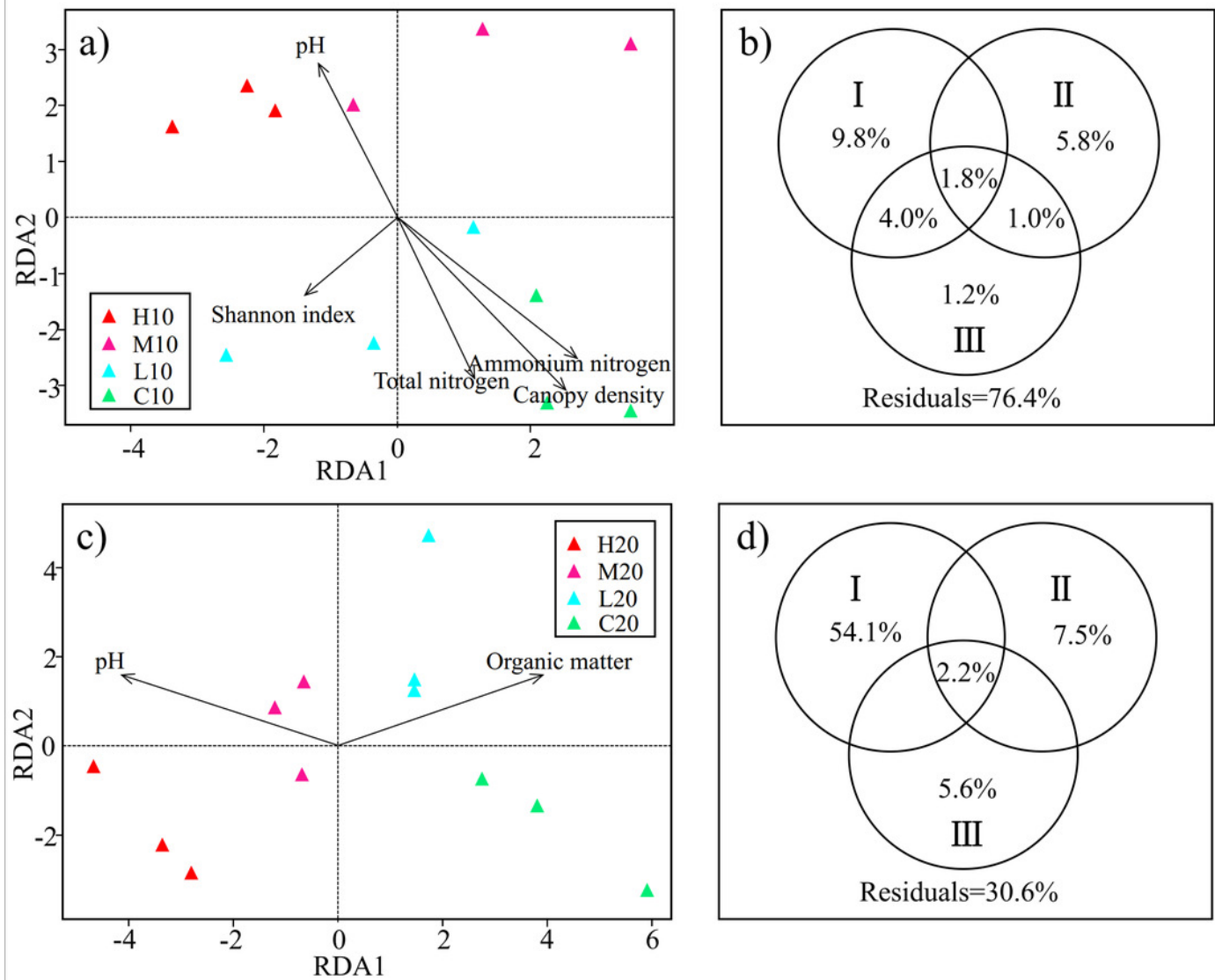
Table 1(on next page)

Table 1 Criteria for the classification of forest fire severity (Zheng et al., 2012).

1   Table 1 Criteria for the classification of forest fire severity (Zheng et al., 2012).

Fire severity	Criteria for the classification
High severity	Trees more than 80% burns or burned to death, the undergrowth shrubs all consumed, char height of trunks were more than 5 m. As a result of the ground organic matter all burned, mineral soil color and structure are changed.
Moderate severity	10% to 80% of the trees were burned and the char height of trunks was between 2 to 5 m. The upper part of forest litter was burned.
Low severity	Trees were burned less than 10%, part of the undergrowth shrubs was consumed (below 40%), char height of trunks were below 2 m.
Control	Unburned

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Table 2(on next page)

Soil physical and chemical properties

Notes: OM, organic matter; TN, total nitrogen; NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; SM, soil moisture. Different lowercase letters after the entries indicate significant differences at the same soil depth of different sample plots at $P<0.05$. Values are means ±standard deviations ($n=3$).

1   Table 2 Soil physical and chemical properties

Soil properties	Soil layers	Fire severity				One-way ANOVA	
		High severity	Moderate severity	Low severity	Unburned control	f-values	p-values
OM (mg·kg^{-1})	topsoil	9190±90b	17130±760a	17460±1230a	16160±550a	76.11	0.000
	subsoil	4000±490b	6910±590a	7390±1180a	7920±440a	16.89	0.001
TN (mg·kg^{-1})	topsoil	310±10c	560±40b	850±30a	880±50a	171.71	0.000
	subsoil	180±10d	270±40c	400±10b	660±40a	169.47	0.000
NH$_4^+$-N (mg·kg^{-1})	topsoil	1.72±0.03c	1.45±0.37c	3.70±0.62b	19.49±0.90a	674.98	0.000
	subsoil	1.25±0.30c	3.01±0.68c	11.78±0.67b	16.32±1.07a	285.64	0.000
NO$_3^-$-N (mg·kg^{-1})	topsoil	1.64±0.43b	2.45±0.84a	3.56±0.36a	2.64±0.06a	7.29	0.011
	subsoil	2.10±0.62b	2.10±0.44b	3.87±0.72a	3.16±0.54a	6.49	0.015
SM (%)	topsoil	17.23±1.20a	10.22±1.44c	11.04±1.31c	13.43±0.68b	20.72	0.000
	subsoil	11.39±0.10b	7.21±0.68c	11.25±0.99b	15.41±0.66a	71.59	0.000
pH	topsoil	6.90±0.27a	6.13±0.12b	5.68±0.38b	5.65±0.25b	13.89	0.002
	subsoil	6.19±0.03a	5.67±0.14a	5.62±0.49a	5.61±0.44a	2.07	0.183

2   Notes: OM, organic matter; TN, total nitrogen; NH$_4^+$-N, ammonium nitrogen; NO$_3^-$-N, nitrate nitrogen; SM,

3   soil moisture. Different lowercase letters after the entries indicate significant differences at the same soil depth

4   of different sample plots at $P<0.05$. Values are means ±standard deviations ($n=3$).