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1 Response of soil microbial communities to site preparation before
2 afforestation

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Abstract:

Site preparation is a common practice before afforestation, which can increase soil carbon (C) release by changing the soil microbial community. This study examined changes in soil microbial communities at two different times (the 7th and 98th days, D7 and D98) after site clearing (brush clearing vs. controlled burning), followed by soil preparation (overall soil preparation, OP; spot soil preparation, SP; and no soil preparation, NP). Results showed that the total, bacterial, fungal, and actinomycetic phospholipid fatty acids (PLFAs) in controlled burning plots increased compared to that in the brush clearing plots at the two sampling times. Within brush clearing plots, OP significantly reduced the total, bacterial, fungal, and actinomycetic PLFAs, whereas SP showed a significant increase in these groups in comparison to NP. In addition, soil microbial community showed obvious seasonal variation in brush clearing plots. Within controlled burning plots, OP significantly decreased the total, bacterial, fungal, and actinomycetic PLFAs. The variations in microbial community composition significantly correlated with soil organic C, total nitrogen (N), dissolved organic C and C:N ratios. Our results suggest that controlled burning and spot soil preparation can create some soil condition more conducive to soil microbial communities in short-term, but the long-term effects merit further investigation.

Keywords Brush clearing; Controlled burning; Overall soil preparation; Spot soil preparation; Subtropical China

42 **Introduction**

43 China has the largest forest plantation area in the world, which is approximately 69.33
44 million ha, based on the most recent national 8th forest inventory. The plantations play
45 an increasingly important role in forest carbon sequestration and mitigation of CO₂
46 emission (Huang et al. 2007). However, some studies have indicated that plantations
47 do not increase the soil C at their initial stage (Kaye et al. 2000; Huang et al. 2009).
48 This may occur because the site preparation before afforestation increases the soil C
49 release (Wang et al. 2016). The rapid increase in microbial activity following site
50 preparation is inherent in determining the C release (Schmidt et al. 2011). On the
51 other hand, soil microbial activity also influences the soil nutrient cycling, which may
52 directly or indirectly influence the plant productivity and C sequestration (Van Der
53 Heijden et al. 2008). Therefore, a complete understanding of the soil microbial
54 dynamics would help explain the variation in soil C release and predict plant
55 productivity after site preparation.

56 In order to improve the survival and growth of planted trees, site preparation is a
57 common silvicultural practice before planting. Generally, site preparation includes site
58 clearing and soil preparation; in which site clearing can be further divided into brush
59 clearing and controlled burning, while soil preparation can be divided into overall soil
60 preparation and spot soil preparation (Wang et al. 2016). Site preparation removes or
61 inverts the forest floor materials, causing changes in bulk density, moisture, and
62 thermal characteristics of mineral soils (Van Der Heijden et al. 2008). Such changes
63 may further affect soil microbial community composition. Brush clearing has been

64 shown to reduce the abundance of soil fungi (Bååth 1980), bacteria, and
65 actinomycetes (Haque et al. 2012), due to a decline in soil C availability induced by
66 the cessation of root growth and exudation. However, the remaining detritus after
67 brush clearing may increase nutrient availability and improve the abundance of soil
68 microorganisms in the short-term (Harmon et al. 2009). Controlled burning may
69 affect soil microorganisms via both direct and indirect mechanisms (Dooley and
70 Treseder 2012). High temperature during controlled burning may lead to heat-induced
71 microbial mortality (Hart et al. 2005). In addition, controlled burning may alter the
72 soil microbial community composition due to the differences in microbial sensitivity
73 to temperature (Cairney and Bastias 2007). Controlled burning also alters soil
74 physicochemical properties, such as soil temperature, moisture and nutrients (Certini
75 2005). These changes may have positive or negative consequences for microbes (Peay
76 et al. 2009; Dooley and Treseder 2012). Although numerous studies have measured
77 the changes in soil microbial community following brush clearing and controlled
78 burning, the results are inconsistent.

79 Soil preparation is a combination of plowing and raking. Overall soil preparation
80 and spot soil preparation refer to soil preparation that cover the total area and only
81 parts of the area, respectively. Soil preparation mixing organic materials with mineral
82 soils may stimulate microbial growth and a shift in the microbial community structure,
83 due to the improved soil aeration and substrate quality (Mallik and Hu 1997). For
84 example, aerobic microorganisms may become dominant in these plots because soil
85 preparation decreases the soil moisture and bulk density but promotes the diffusion of

86 oxygen (Mathew et al. 2012). Although many studies have investigated the effects of
87 soil preparation on soil microbial communities, the impact of soil preparation
88 intensity on the soil microbial community structure is not well understood. In addition,
89 soil preparation following site clearing would result in the mixing of detritus or ash
90 with mineral soil, which may have interactive effects on soil microbial communities.

91 Phospholipid fatty acids (PLFAs) analysis is a method to examine the soil
92 microbial community composition, and the phospholipids are rapidly degraded
93 following cell death. Therefore, PLFAs are a reliable indicator of living
94 microorganisms (Bardgett et al. 1999). The main objective of the present study was to
95 assess the effects of different site preparations on soil microbial communities in
96 subtropical China. We hypothesized that (1) compared with brush clearing, controlled
97 burning would increase the PLFAs abundance in the short-term by alleviating nutrient
98 limitations, but decrease the fungal PLFAs, as fungi are more heat sensitive than
99 bacteria, (2) the PLFAs abundance would increase after soil preparation due to the
100 increase in the amount of bioavailable substances after the mixing of the organic and
101 mineral layers, and (3) site preparation would affect the seasonal variation in soil
102 microbial community composition.

103 **2. Materials and methods**

104 *2.1. Study area*

105 The experimental site is located at the Qianyanzhou Ecological Research Station
106 (26°44'39"N, 115°03'33"E) in subtropical China. The climate is a typical subtropical

monsoon with a significant seasonal air temperature variation and an uneven distribution of rainfall. The mean annual temperature is 17.9 °C and the precipitation is 1485 mm. The temperature and precipitation of the experiment period are shown in Fig. S1. The soil is classified as Ultisols according to the World Reference Base Soil Taxonomy (1998). The plots were laid out on an abandoned orange orchard, which had a slope of less than 5°. The vegetation of the plots was dominated by Cogon grass (*Imperata cylindrica* (Linn.) Beauv.) and Canadian fleabane (*Conyza canadensis* (L.) Cronq.). Additional information about the soil physicochemical and microbial community properties are provided in Table S1.

2.2. Experimental design

A split-plot design was used for this experiment. The type of site clearing, i.e., brush clearing (BC) and controlled burning (CB), make up the primary treatment, while soil preparation, i.e., overall soil preparation (OP), spot soil preparation (SP), and no soil preparation (NP), comprise the secondary treatment (Fig. S2), thus six treatments were established. There were three pairs of 14 m × 9 m (each pair formed a block). In each pair, plants growing in the plots were removed by brush clearing and controlled burning, respectively. The brush clearing or controlled burning plots were divided into three 4 m × 4 m subplots and then treated with overall soil preparation, spot soil preparation (four spots in each subplot, each spot was distributed approximately 2 m apart with size of 50 cm × 50 cm × 40 cm (Length × Width × Depth)) or no soil preparation. Further details of the site preparations are listed in Table S2.

128 2.3. Soil sampling and analysis

129 Site preparation was undertaken on 24 March 2011. After 7 and 98 days of site
130 preparation (D7 and D98), surface (0–10 cm) soil samples were collected after
131 removing the litter and organic horizon. In NP and OP, five soil samples from two
132 diagonal lines through each plot were collected and pooled in one composite sample.
133 In SP, we collected soil samples from the center of the spots. A total of 36 soil
134 samples (two sampling times) were collected for analyzing the soil physicochemical
135 properties and the microbial community composition. Soil samples were divided into
136 two parts after being passed through a 2-mm sieve. One was used to determine the
137 total organic carbon (TOC), total nitrogen (TN), dissolved organic carbon (DOC) and
138 pH. The other was stored at -20 °C until microbial community analysis. The soil bulk
139 densities were measured using ringer samplers. The TOC and TN contents were
140 measured using the CN-element analyzer (Elementar Vario Max CN, Mt. Laurel, NJ).
141 The DOC was extracted with 0.5 mol L⁻¹ K₂SO₄ solution, and determined by a total
142 organic carbon analyzer (Shimadzu TOC-5050A, Kyoto, Japan). The pH was
143 measured potentiometrically in soil-water suspension (soil:water, 1:2.5).

144 The microbial community composition was determined by PLFAs analysis.
145 Before the PLFAs extraction, soil moisture was measured using gravimetric methods
146 and then measured after drying to a constant weight at 105 °C. The PLFAs profile was
147 determined for each sample by extraction as described by Sun et al. (2011). Fatty
148 acids were expressed in nmol g⁻¹ dry soil. Bacteria were represented by the PLFAs
149 i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, 17:0, cy17:0, 18:1ω7, and

cy19:0, fungi were represented by 18:2 ω 6, 9, and 18:1 ω 9, and actinomycetes were represented by 10Me16:0, 10Me17:0, and 10:Me18:0 (Bossio and Scow 1998; Frostegård and Bååth 1996; Frostegård et al. 2011; Sun et al. 2011).

2.4. Statistical analysis

We used nested analysis of variance (ANOVA) to test the effects of site clearing and soil preparation, as well as their interactions on soil physicochemical properties and soil microbial community using SPSS Version 16.0 (SPSSInc., Chicago, Illinois, USA). The treatments were set as between-subject factors, and the measurement dates were selected as within-subjects variable. Principal component analysis (PCA) was performed to evaluate the changes in the microbial community composition under different site preparation treatments using CANOCO 4.5 (Ithaca, NY, USA). Redundancy analysis was used to determine which factors were related to the variation in the soil microbial community composition among the various experimental treatments. The significance of the RDA results was tested by a permutation test (999 permutations). Statistically significant differences were identified at $P < 0.05$.

3. Results

3.1. Responses of soil microbial community to site preparation

Significant effects of site preparation on soil microorganisms were identified (Fig. 1 and Table 1). Compared with brush clearing (NP), controlled burning (NP)

170 significantly increased the total PLFAs (totPLFAs), bacterial PLFAs (bacPLFAs),
171 fungal PLFAs (funPLFAs), and actinomycic PLFAs (actPLFAs), on average (based
172 on two sampling times), by 44%, 54%, 33%, and 57%, respectively. Furthermore,
173 controlled burning significantly reduced the funPLFAs:bacPLFAs ratio by 16% at D7.

174 Soil preparation also significantly affected the soil microbial community. Within
175 brushing clearing plots, over two sampling times, OP significantly decreased the
176 totPLFAs (33.5%), bacPLFAs (33.3%), funPLFAs (39.9%), and actPLFAs (43.9%),
177 whereas SP significantly increased them by 26.0%, 27.8%, 26.8%, and 35.2%,
178 respectively, compared with NP. Within controlled burning plots, much lower values
179 of totPLFAs, bacPLFAs, funPLFAs and actPLFAs were observed in OP. Compared
180 with NP, on an average, they were decreased by 67.5%, 69.1% 74.0%, and 70.9%,
181 respectively. In contrast, SP significantly increased them by 26.0%, 27.8%, 26.8%,
182 and 35.2%, respectively.

183 Sampling times significantly affected the soil microbial community (Table 1).
184 Across the 18 plots, totPLFAs, bacPLFAs, funPLFAs, and actPLFAs, were 15%, 14%,
185 17%, and 34% higher at D7 than those at D98, respectively. In addition, the results
186 also revealed significant interactive effects of site clearing and sampling time on soil
187 microbial community (Table 1). For example, totPLFAs was significantly decreased
188 by 29.8% at D98 than that at D7 in brush clearing plots (NP). However, it was only
189 decreased slightly (10.1%) in the controlled burning plots (NP).

190 3.2. Main factors affecting soil microbial community

Principal component analysis (PCA) of the PLFAs data showed that the shift in microbial community composition was due to the site preparation treatment and the sampling time (Fig. 2). PC1 explained 72.1% (D7, Fig. 2a) and 50.3% (D98, Fig. 2b) of the variation and discriminated OP from NP and SP (along PC1, $P < 0.01$ from ANOVA). OP was clustered to the right, and SP and NP to the left of the origin. Specifically, OP was more abundant in i14:0, i17:0, and i18:0, whereas SP and NP were more abundant in 16:1w5c, 18:1w7c, 18:1w9c, and cy19:0. PC2 separated the BC from the CB, but accounted for only 7.8% of the variation at D7 (along PC2, $P < 0.01$ from ANOVA). Higher values in the BC along PC2 were the 16:0 and i18:0, while the CB primarily composed of 15:0, i15:0, and a17:0. By contrast, there were no major differences in the microbial community composition between BC and CB plots at D98.

3.3. Correlations between microbial community and soil properties

The site preparation significantly affected the soil physicochemical properties, especially after soil preparation (Table 2 and Table S3). Redundancy analysis (RDA) revealed that soil physicochemical properties have significant effects on soil microbial community composition (Fig. 3). Soil physicochemical properties explained 64.9% and 64.1% of the variation in soil microbial community structure at D7 and D98, respectively. The first axis (RD1) explained 53.9% (Fig. 3a) and 40.0% (Fig. 3b) of the total variance in the PLFA profiles and was most strongly correlated with TOC, C/N, DOC and TN (Table S3). The second axis (RD2) accounted for only 5.4% and

212 15.5% of the variance at D7 and D98, respectively.

213 To better understand how each soil property variable influenced the soil
214 microbial community structure, variation partitioning analysis (VPA) was performed
215 using the selected variables. Three important soil properties (TOC, TN and DOC)
216 were selected by the BioEnv procedure and variance inflation factor (VIF) in
217 cluster-cluster aggregation (CCA) model. A total of 37.2% (D7) and 41.4% (D98)
218 variation in the microbial communities could be explained by the three soil properties
219 and their interactions. In D7, TOC was able to independently explain 14.6% of the
220 variation, whereas TN explained 7.3%, and DOC explained 10.7% of the variation. In
221 D98, TOC, TN, and DOC independently explained 14.3%, 6.1%, and 14.2% of the
222 variation, respectively. These results suggest that the variables of DOC over time
223 appeared to have more influence in shaping the microbial community functional
224 structures.

225 **4. Discussion**

226 *4.1. Site clearing effects on the microbial community composition*

227 Compared with brush clearing, controlled burning significantly increased the soil
228 microbial PLFAs abundance and altered the microbial community structure. These
229 results support our first hypothesis, and are consistent with the results of Rutigliano et
230 al. (2007) and Okonkwo (2010), but are inconsistent with those of Dooley and
231 Treseder (2012) and Wang et al. (2012). Differential responses in the microbial
232 community to controlled burning could be related to the differences in the burning

intensity. Pourreza et al. (2014) observed a significant decrease in soil microbial biomass (assessed by substrate-induced respiration method) in high severity burning, but not in low severity burning. In our experiment, large shrubs were removed, and the controlled burning generally lasted for less than 20 minutes. Therefore, the direct effect of burning heat on microorganisms may not be too serious. In addition, the changes in soil properties after controlled burning may increase the soil microbial biomass. Bárcenas-Moreno et al. (2011) found that large amounts of DOC were released, and Wan et al. (2001) found the soil inorganic nitrogen increased immediately after burning. These pulse of soil nutrients could stimulate the growth of surviving microorganisms. However, in our study, we lack the measurements for soil inorganic nitrogen, but we found that the DOC significantly increased by 40.0% immediately after controlled burning. Furthermore, controlled burning may change the soil microclimate. Previous studies have concluded that burning increases the soil temperature and decreases the soil moisture (Neary et al. 1999; Kim and Tanaka 2003). In the present study, compared with brush clearing plots, controlled burning had no significant effects on soil temperature, but it significantly decreased the soil moisture by 14.1% and 9.8% at D7 and D98, respectively. In this region, the rainfall occurred mainly (approximately 60%) from March to June (Wang et al. 2012), and thus, this excess precipitation might have limited the microbial growth. Therefore, the differences in DOC and soil moisture might help explain the differences between two site clearing methods.

In our study, the funPLFAs:bacPLFAs ratio was lower in controlled burning plots

255 than in brush clearing plots. Many previous studies have noted that fungi may be
256 more severely affected by burning, as they are more sensitive to heat than bacteria
257 (Dooley and Treseder 2012). However, compared with brush clearing, there was no
258 significant effect in funPLFAs, but the bacPLFAs was significantly increased in
259 controlled burning. It is assumed that changes in soil physical-chemical properties
260 after controlled burning may favor the growth of bacteria. Bárcenas-Moreno et al.
261 (2011) found that fungi are the dominant species in the soils with low pH, as they are
262 more tolerant towards acidic soils. However, ash deposition followed by controlled
263 burning increases the soil pH, which may favor the growth of bacteria (Okonkwo
264 2010). In addition, once the bacteria recolonize the soil substrate, they could exhibit
265 an antagonistic effect on fungal growth (Bárcenas-Moreno and Bååth 2009). In this
266 study, we found that soil pH was slightly increased after controlled burning, which
267 could favor an increase in bacterial activity, while negatively affecting fungal growth.

268 4.2. Soil preparation effects on the microbial community composition

269 OP significantly decreased the abundance of PLFAs at the two sampling times.
270 These results are inconsistent with the second hypothesis and could be explained by
271 the followings. First, the soil preparation could directly affect the soil microorganisms.
272 For example, some groups of organisms, such as mycorrhizal fungi, are reduced by
273 the disruption of their hyphal networks (van der Heyde et al. 2017). In addition, soil
274 preparation could also directly affect soil microorganisms by altering the soil layers,
275 as the microbial biomass is concentrated in surface soils (Taylor et al. 2002). Second,

276 site preparation could also indirectly affect the soil microbial community by changing
277 the soil physicochemical properties (Dooley and Treseder 2012). In the same regions,
278 Gao et al. (2015) investigated the soil C, N, and P content for six different land use
279 types in the same region, and found that they all declined with increasing soil depth.
280 Soil preparation may homogenize the soil nutrients and stimulate the microbial
281 growth of subsoil. However, in this study, we just investigated the soil PLFAs
282 abundance in the surface soil (0–10 cm), and found that OP significantly decreased
283 them as well as the soil nutrients. The RDA results also indicated that the microbial
284 community structures were correlated with the TOC, C/N, TN, and DOC (Fig. 3). In
285 contrast with OP, SP increased the abundance of PLFAs. This result indicated that the
286 soil preparation intensity is an important factor that affects the composition of soil
287 microbial community. Compared with NP, SP had no significant effects on TOC, TN
288 and DOC, which ensured an adequate nutrient supply for soil microorganisms. In
289 addition, the surrounding no soil preparation patches might have acted as a biotic
290 source for microbial re-colonization.

291 A lower funPLFAs:bacPLFAs ratio was observed after OP on D7 (Fig. 1). The
292 change in the microbial community structure following site preparation suggests that
293 microbial species are differentially affected by disturbances and that fungi are more
294 sensitive to disturbance than bacteria. In OP, the treatments would disrupt the hyphal
295 networks of fungi, resulting in a decrease in the fungal biomass (Achatz et al. 2013).
296 As fungi typically exhibit a hyphal growth form, the hyphal network could transfer
297 the nutrients and resources to the sites where they are limiting (Frey et al. 2003). In

contrast, bacteria are present as individual cells, so soil preparation has a lesser impact on soil bacteria. SP had no effect on the funPLFAs:bacPLFAs ratio, because it created a mosaic of heterogeneous patches, wherein the fungal hyphae could explore a large soil volume and rapidly build up their hyphal networks (Herman et al. 2012). Thus, it is reasonable to expect that fungal hyphae can explore spots and rapidly recolonize.

4.3. Temporal variation in the soil community composition

Sampling time had a significant effect on the soil microbial community composition (Table 3). Compared with D7, the average PLFA abundances (among the six site preparation methods) were lower at D98. These results were consistent with those of Stres et al. (2008), who observed a slight increase in microbial biomass under wet and cold conditions (March and May). The soil temperature positively correlates with soil microbial activity. However, soil microbial biomass (measured by substrate-induced respiration) was higher in the soil exposed to cold than to warm conditions (Stres et al. 2008). The soil moisture was an important driver of microbial activity, and the high or low soil moisture would decrease microbial activity by reducing oxygen diffusion or soluble substrate diffusion. Dong et al. (2015) found that the soil moisture strongly limited the soil PLFA abundance in Chinese fir in the same area. In this study, soil temperature increased from March to June, and strong temporal fluctuations in soil moisture were observed (Fig. S4). On an average, the soil temperature was 10.34 °C higher, whereas the soil moisture was 21.31% lower at D98 than at D7. Therefore, it is not surprising that season had a large effect on microbial

319 community structure.

320 Other studies have found that the differences in microbial community between
321 sampling dates on the seasonal basis in change carbon availability (Griffiths et al.
322 2003; Rasche et al. 2011). Griffiths et al. (2003) has showed that seasonal changes in
323 microbial activity and biomass correlate with increases in C source availability from
324 root exudation. However, in order to exclude the effects that may arise from plants, no
325 tree was planted after site preparation in the present study. Therefore, there was little
326 C input through litter and root exudates, and the microbial growth was considered to
327 be limited by C availability. However, the decreases in PLFA abundances were found
328 only in the brush clearing plots. The abundance of total microbes, bacteria, fungi and
329 actinomycetes, on average (based on three soil preparation methods), were 29.1%,
330 28.9%, 25.9%, and 29.5% lower at D98 than at D7, respectively. In contrast, sampling
331 time had no effect on the controlled burning plots. Compared with brush clearing, an
332 immediate increase in DOC and other nutrients was found after controlled burning,
333 which could stimulate the microbial growth. Bárcenas-Moreno et al. (2011) found that
334 a large amount of DOC is released after burning, and that this increase lasts for 8
335 months and then decreases. Therefore, it is assumed that the improvement in nutrient
336 availability after controlled burning can regulate the effects of seasonal weather
337 changes on soil microorganisms in the experiment period.

338 5. Conclusions

339 This study demonstrated that, in short-term, different site preparations had
340 significantly different effects on soil microbial community composition, which varied
341 with the type and intensity of disturbance. Controlled burning stimulated the growth
342 of microorganisms, due to the increase in availability of inorganic nutrients by ash
343 deposition in short-term. Soil microorganisms were significantly increased by spot
344 soil preparation, but were significantly decreased by overall soil preparation. In
345 addition, these effects varied with the site clearing methods. Seasonal variation in
346 microbial community composition was observed in brush clearing plots, but not in
347 controlled burning plots. This could be attributed to the differences in soil
348 microclimate (soil moisture) and soil nutrients between brush clearing and controlled
349 burning. Long-term studies are needed to examine the responses of microbial
350 community to site preparation and their feedbacks to plant communities.

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Table 1 Soil microbial variables in response to different site preparation methods. TotPLFAs, total PLFAs; BacPLFAs, bacterial PLFAs; FunPLFAs, fungal PLFAs; ActPLFAs, actinomycetic PLFAs; F: B ratios, fungal PLFAs:bacterial PLFAs ratios.

Time	Treatments	TotPLFAs (nmol g ⁻¹)	BacPLFAs (nmol g ⁻¹)	FunPLFAs (nmol g ⁻¹)	F: B ratios	ActPLFAs (nmol g ⁻¹)
	Site clearing (C)	***	***	ns	***	**
	Soil preparation (P)	***	***	***	***	**
	Sampling time (T)	***	**	**	ns	***
	C × P	***	***	***	ns	*
	C × T	***	**	**	ns	*
	P × T	ns	ns	*	ns	*
	C × P × T	ns	ns	ns	ns	**

ns indicates not significant. *, ** and *** represent $P < 0.05$, 0.01 and 0.001, respectively.

Table 2 Soil physicochemical properties as affected by the site preparation. TOC, soil total organic carbon; TN, total nitrogen; BD, soil bulk density; DOC, dissolved organic carbon.

Treatments	Moisture (cm ³ cm ⁻³)	BD (g cm ⁻³)	TOC (g kg ⁻¹)	TN (g kg ⁻¹)	C/N	DOC (mg kg ⁻¹)	pH
Site clearing (C)	ns	ns	ns	ns	**	ns	ns
Soil preparation (P)	***	***	***	***	***	***	ns
Sampling time (T)	*	***	***	***	*	ns	*
C × P	*	ns	**	ns	**	ns	ns
C × T	ns	**	ns	*	ns	*	ns
P × T	***	ns	ns	ns	ns	ns	*
C × P × T	ns	**	ns	ns	ns	ns	ns

ns indicates not significant. *,** and *** represent $P < 0.05$, 0.01 and 0.001 , respectively.

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486 **Fig. 1** Responses of the soil microbial PLFAs to the site preparation. The bar represents standard
487 error. Different letters indicate significant differences among the three soil preparation methods
488 within brush clearing and controlled burning plots ($P < 0.05$).

489 **Fig. 2** Principal component analyses (PCA) of PLFAs under different site preparation treatments
490 (a, D7; b, D98). The open circle, square and triangle represent the no, overall, and spot soil
491 preparation after brush clearing, while the closed circle, square and triangle represent the no,
492 overall, and spot soil preparation after controlled burning, respectively.

493 **Fig. 3** Redundancy analyses (RDA) of the soil microbial community composition under different
494 types of site preparation (a, D7; b, D98). Vectors represent environmental variables. TOC, total
495 organic carbon; TN, total nitrogen; C/N, C:N ratio; M, soil moisture; BD, soil bulk density; DOC,
496 dissolved organic carbon. The open circle, square and triangle represent no, overall and spot soil
497 preparation after brush clearing, while the closed circle, square and triangle represent no, overall
498 and spot soil preparation after controlled burning, respectively.

Fig. 1

Fig. 2

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506

507 Fig. 3