Comparison of microbial community structures in soils with woody organic amendments and soils with traditional local organic amendments in Ningxia of Northern China

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ABSTRACT

Background: Addition of organic amendments has been commonly adopted as a means to restore degraded soils globally. More recently, the use of woody organic amendments has been recognized as a viable method of capturing and retaining water and restoring degraded and desertified soil, especially in semi-arid regions. However, the impacts of woody amendments on soil microbial community structure, versus other traditional organic supplements is less understood.

Methods: Three locally available natural organic materials of different qualities, i.e. cow manure (CM), corn straw (CS), and chipped poplar branches (PB) were selected as treatments in Ningxia, Northern China and compared with control soils. Four microcosms served as replicates for each treatment. All treatments contained desertified soil; treatments with amendments were mixed with 3% (w/w) of one of the above organic materials. After 7 and 15 months from the start of the experiment, soil samples were analyzed for chemical and physical properties, along with biological properties, which included microbial α-diversity, community structure, and relative abundance of microbial phyla.

Results: Both bacterial and fungal α-diversity indices were weakly affected by amendments throughout the experimental period. All amendments yielded different microbial community compositions than the Control soils. The microbial community composition in the CS and PB treatments also were different than the CM treatment. After 15 months of the experiment CS and PB exhibited similar microbial community composition, which was consistent with their similar soil physical and chemical properties. Moreover, CS and PB also appeared to exert similar effects on the abundance of some microbial taxa, and both of these treatments yield different abundances of microbial taxa than the CM treatment.

Conclusion: New local organic amendment with PB tended to affect the microbial community in a similar way to the traditional local organic amendment with CS, but different from the most traditional local organic amendment with CM in Ningxia, Northern China. Moreover, the high C/N-sensitive, and lignin and cellulose decompose-related microbial phyla increased in CS and PB have benefits in decomposing those incorporated organic materials and improving soil properties. Therefore, we recommend that PB should also be considered as a viable soil organic amendment for future not in Ningxia, but also in other places.

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INTRODUCTION

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68 The incorporation of locally available organic materials into degraded soil has been a widely

69 adopted practice. Organic amendments are recognized for their roles in increasing soil water

70 holding capacity, soil porosity, and water infiltration and percolation, while decreasing soil

71 crusting and bulk density (*Thangarajan et al., 2013*). Moreover, organic amendments stimulate

72 soil microbial community growth and activity in degraded soils, resulting in mineralization of

73 nutrients available to plants, and increasing soil fertility and quality (*Luna et al., 2016*). Based on

74 their benefits of improving water status and soil fertility, organic amendments are extensively

75 used in dry and desertified soils globally. In the arid and semi-arid regions of China and

76 elsewhere, retaining and returning crop residues (e.g. wheat straw and corn straw) and adding

77 livestock manure (e.g. cow manure, chicken manure, pig manure) to the soil have long been

78 regarded as a practical and effective method for improving soil quality and crop productivity

79 (*Fan et al., 2014*). However, a seldom employed strategy in these regions is the use of woody

80 materials as a soil amendment technique, although there is much available materials that could

81 be readily collected from more than 2.2 million km^2 of shelterbelt forests extending across China

82 (*Dai & Chu, 2010*). More specifically, rapidly growing poplars (*Populus alba* and other

83 members of the genus) are the most widespread and available tree throughout China (*Jia et al.,*

84 *2013*). Moreover, in the highly degraded and desertified areas of Northern China, three-quarters

85 of the poplar trees are planted as farmland shelterbelts (*Jing et al., 2015*). Within the seriously

86 desertified Ningxia Autonomous Region, poplar species rank first among all trees and distribute

87 through an area of 47,000 km^2 (*Sun et al., 2009*).

88 Notably, our previous research has demonstrated that incorporation of as little as 2% wood

89 chips (w/w) into the surface soil can effectively capture and retain more precipitation and

90 improve soil physicochemical properties in Ningxia, China (*Li et al., 2018*). Additionally, we

91 reported that wood chips presented several comparable benefits to traditional local amendments,

92 such as crop straw and cow manure, in improving soil health and enhancing crop growth.

93 Importantly, in contrast to rapidly decomposing compost materials, the incorporation of woody

94 material in severely degraded soils can have valuable and long-lasting benefits due to its low

95 decomposition rate (*Weedon et al., 2009*). However, the effects of wood chips as well as other

96 traditional local organic amendments on soil microbial communities are insufficiently

97 understood. Microbial communities are important for the functioning of the ecosystem, both in

98 relation to direct interactions with plants, and with regard to nutrient and organic matter cycling

99 (*Adak & Sachan, 2009*). Moreover, microbial abundance, activity, and composition largely

100 determine the sustainable productivity of agricultural land (*van der Heijden et al., 2008*), since

101 microbes play an integral and essential role in all soil processes (*Barrios, 2007*). In particular,

102 soil microbial communities play an important role in the C cycle and the metabolism of other

103 nutrients, such as nitrogen for energy acquisition (*Falkowski et al., 2018*). Some scientists have
104 reported that C addition seems to select for specific microbial groups that feed primarily on
105 organic compounds, changing the composition of the microbial community (*Zhong et al., 2010;*
106 *Hu et al., 2012*). But other studies also indicated that a change in microbial community structure
107 does not always involve a change in microbial community function or an increase in availability
108 of plant nutrients and crop productivity (*Franco-Otero et al., 2012*; *Lazcano et al., 2013*).
109 Nevertheless, a series of studies have focused on exploring the functions of microorganism in
110 soil process, and they have reported the ecological roles some microorganisms are playing in
111 soils, including C, N and other nutrient cycling (*Fierer et al., 2007, 2012; Hartmann et al., 2015;*
112 *Zhang et al., 2018*). These previous studies are useful for us to speculate what ecological roles
113 microorganisms may play in our soil organic amendments, because wood decomposition
114 processes are related to microorganisms in the soil (*Sariyildiz et al., 2005*) and those
115 microorganisms, in turn, affect soil nutrient cycling (*Wutzler et al., 2017*). In addition, soil
116 microbial parameters can react rapidly to changes in soil management (*Gil-Sotres et al., 2005*),
117 and shortly after application of organic amendments, fast increases in soil microbial biomass and
118 activity have been observed (*Dinesh et al., 2010*). For all of these reasons, it is important for us
119 to assess short-term effects of our soil organic amendments via the reaction of microbial
120 communities.
121   Previous studies also have indicated that pH and organic C were two major soil properties
122 that drive soil microbial communities (*Hollister et al., 2010; Liu et al., 2014*). In this context, we
123 assume that exogenous C input, such as organic amendments, would substantially influence
124 microbial communities in soils. Moreover, woody materials and crop straw have more lignin and
125 carbon (C) content, and a higher C: nitrogen (N) ratio than livestock manures, which makes them
126 more difficult to decompose, so we hypothesize that they also differently affect soil microbes
127 relative to manure, and other short-lived organic amendments. This contention is supported in
128 other studies that indicated that organic amendments with a high C:N ratio resulted in higher
129 microbial biomass and activity, and also displayed differences in microbial composition,
130 compared to materials with low C:N ratio (*Heijboer et al., 2016*). Nevertheless, we know little
131 about the effects of all local organic amendments, such as cow manure, corn straw, and poplar
132 branches, on soil microbiota in degraded and desertified soils such as those in Ningxia, China.
133 Better understanding of the microbial processes that take place in soils that have been treated
134 with organic amendments could help identify the main drivers determining nutrient availability
135 in order to improve soil health and facilitate crop growth. Therefore, in this context, in pursuit of
136 the overall goal of our study, we employed an Illumina sequencing approach of bacterial and
137 fungal ribosomal markers to compare the effects on soil microbiota of the addition of new woody
138 amendments and the addition of traditional local amendments. The detailed objectives of this

139 study were to (a) compare soil amendments of poplar chips versus traditional organic
140 amendments, including cow manure and corn straw, and their impacts on soil microbial α-
141 diversity and community composition,; (b) reveal the different effects of local organic
142 amendments on specific microbial taxa; (c) determine the relationships between soil properties
143 and soil microbial α-diversity, community composition and specific microbial taxa, respectively.
144

145 **MATERIAL & METHODS**

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147 Study Site
148 A microcosm experiment was conducted for 15 months from April 2015 to July 2016 in a
149 greenhouse in the Yinchuan Belly Desert, which is located at the eastern foot of the Helan
150 Mountains in Ningxia Hui Autonomous Region, China (106°08′~107°22′ E, 38°28′~38°42′ N).
151 The characteristics of the study site are representative of those associated with the ongoing
152 desertification processes throughout Northern China. Historically, the native landscape was
153 grasslands, but it has been desertified after centuries of agriculture. Currently, the region is
154 characterized by shifting dunes with scattered farmland shelterbelt forests, which along with the
155 farmlands are supported by extensive irrigation. The elevation is 1,115 m above mean sea level
156 and the region is characterized by a temperate continental climate, with 181.2 mm average
157 annual precipitation and 1882.5 mm mean annual evaporation. The annual average temperature
158 is 10.1 ℃, with a maximum of 37.2 ℃ in July, and a minimum of -27.9 ℃ in January. The
159 average wind speed is 1.6 m s^{-1} and the frostless period is 160-170 days each year. The particle
160 size distribution of soils is 92.5% sand (size < 2 mm), 5.5% silt, and 2.0% clay. The soil
161 chemical properties are listed in **Table S1**.
162

163 Material Collection and Preparation
164 The base soils for this experiment were collected at the study site. After collection, soils were
165 sifted through a sieve with a mesh diameter of 2 mm and were air-dried before mixing with the
166 selected organic materials or being used as control soils. Woody materials were obtained from
167 pruned branches from a locally abundant poplar species (*Populus alba* L.), and herbaceous
168 materials consisted of straw residues collected from local corn (*Zea mays* L.) after harvesting.
169 Poplar branches and corn straw were air-dried, then ground to lengths of about 0.5 cm. Cow
170 manure, collected from a local dairy, was also air-dried and sieved through a screen with a mesh
171 diameter of 0.5 cm to remove coarse material. The chemical properties of organic materials are
172 also listed in **Table S1**.

173

Experimental Design

175 Four treatments were tested in this study, including 1) the Control with desertified soil only, and
176 soil treatments with incorporated organic material (3% w/w), including: 2) 3% cow manure
177 (CM); 3) 3% corn straw (CS); and 4) 3% poplar branch (PB). Organic materials were added to
178 the base soil and then mixed fully. For example, the soil mixture of 3% corn straw was created
179 by adding 300 g corn straw (dry weight) to 9700 g base soil (dry weight) and mixing completely.
180 Within the continuum of amendments, these proportions represented the minimum requirements
181 for soil water retention, which was tested in a previous set of our experiments (*Li et al., 2018*).
182   Control soil and soil mixtures were placed in buckets with top and bottom diameter of 31.5
183 cm and 26.0 cm, respectively, a height of 31.5 cm. All buckets had small drainage holes at the
184 bottom. Twelve kilograms of each soil mixture (base soil + 3% incorporated material) was
185 packed in each bucket, after which 5 g of urea ($CO(NH_2)_2$) and 2 g of monopotassium phosphate
186 (KH_2PO_4), dissolved into solution, were added to each bucket prior to the experiment. In order to
187 supply basic nutrient requirement for alfalfa growth, the required amounts of urea and
188 monopotassium phosphate added to the bucket were based on the results of our previous studies
189 (*Li et al., 2018*). Initially, 12 plump alfalfa seeds were sowed in each bucket, but after the first
190 month, only the six most robust seedlings were kept growing for the remainder of the
191 experiment. Alfalfa was selected because it is an indicator plant in our study region. In all, 4
192 treatments × 4 replicates (buckets) × 2 times (7 months and 15 months) = 32 buckets were set up
193 and arranged in a randomized block design in a greenhouse. Throughout the whole experiment,
194 soil moisture of all buckets was monitored based on weight. All buckets were watered once
195 every ten days and were maintained at an approximately constant water content equal to about
196 70% of the water holding capacity of the Control. The indoor greenhouse temperature was

197 maintained at 15–20℃ from November to the following March, and 20–35℃, from April to
198 October during the experimental period.
199

Soil sampling and nutrient measurements

201 All 32 buckets were divided into two successive batches with 16 buckets, including four
202 treatments with four replicates, in each batch. For each batch, the length of time before the
203 measurements was based on the precise alfalfa flowering stage. The analyses of the first batch of
204 16 buckets were conducted when the alfalfa first flowered and was harvested in October 2015
205 (seven months after the start of the experiment), while the second set of measurements were
206 conducted in July 2016 (15 months after the beginning of the experiment) when the alfalfa was
207 harvested after flowering for a second time. After plants were harvested, soil from each entire

208 bucket was mixed with a sterile shovel. Then a portion was collected, air dried, and sieved

209 through a sieve with the mesh diameter of 0.5 mm for chemical analysis, while another portion

210 was placed in a sterile plastic bag, transported to the laboratory on ice, and then sieved (2-mm-

211 mesh sieve) and stored at -80℃ for DNA extraction and later analysis of other biological

212 property.

213   Soil organic C (SOC) was measured with the $K_2Cr_2O_7–H_2SO_4$ oxidation method of Walkley-

214 Black (*Nelson et al., 1996*); total N (TN) was analyzed using the Kjeldahl procedure (*ISSCAS,*

215 *1978*); Alkaline-hydrolyzable (AN) was determined by an alkaline diffusion method (*ISSCAS*

216 *1978*); total P (TP) was determined using $H_2SO_4+HClO_4$ digestion (*Olsen et al., 1982*); available

217 P (AP) was extracted with 0.5 mol l^{-1} $NaHCO_3$ (pH 8.5) (*ISSCAS, 1978*); total K (TK) was

218 determined following the Cornfield method (*Kundsen et al., 1982*); and available K (AK) was

219 determined using a CH_3COONH_4 extraction method (*Tran & Simard, 1993*). Microbial C (MBC)

220 and N (MBN) were estimated by the fumigation-extraction method (*Vance et al., 1987*). Above

221 data were listed in **Table S2**.

222

223 DNA extraction, PCR amplification, and Illumina sequencing

224 Samples were separately tested at each sampling date. The total DNA was extracted from 0.5 g

225 homogenized soil per sample using a Power Soil^{TM} DNA Isolation Kit (MOBIO Laboratories,

226 Carlsbad, CA, USA) following the manufacturer's instructions. A total of 16 extracted DNA

227 samples, at each sampling date, were quantified using a Nanodrop-1000 spectrometer

228 (NanoDrop Technologies, Wilmington, DE, USA) and diluted to a final concentration of 10 ng

229 µL^{-1}. We used the universal primers 515F 5′- GTGCCAGCMGCCGCGGTAA -3′ and 909R 5′-

230 CCCCGYCAATTCMTTTRAGT -3′ that target the V4-V5 regions of the 16S rRNA bacterial

231 gene and produce accurate phylogenetic information (*Weisburg et al., 1991*). For fungi, the

232 ribosomal ITS region was amplified using primers ITS1F: 5′-

233 CTTGGTCATTAGAGGAAGTAA-3′ (*Gardes & Bruns, 1993*) and ITS2R: 5′-

234 GCTGCGTTCTTCATCGATGC-3′ (*White et al., 1990*). To simultaneously analyze several

235 samples in a single sequencing run, the 5' end of the forward primer was fused with a 12 bp

236 different barcode sequence to a single sample. Each 25 µL PCR reaction contained 10 ng

237 extracted DNA as a template, Mg^{2+}-free PCR buffer, 3 mM $MgCl_2$, 200 mM dNTP, 200 nM

238 forward primer, 200 nM reverse primer, and 1 unit of PrimeSTAR Max DNA Polymerase

239 (Takara, Dalian, China). The thermal cycling protocol was 95 °C for 2 min as the first step,

240 followed by 30 cycles of PCR at 95 °C for 15 s, at 57 °C for 15 s, and at 72 °C for 40 s, and a

241 final 10-min extension at 72 °C. All the amplicons were run in a 1% agarose gel (w/v), and the

242 separated bands of the appropriate length were excised from the gel and purified using a

243 QIAquick Gel Extraction kit (QIAGEN GmbH, Hilden, Germany). Finally, high-throughput

244 sequencing of the 16S rDNA and ITS rDNA genes were conducted using Illumina MiSeq, and

245 300 bp paired-end reads were generated (Nanjing Tree & Cloud InfoTech Ltd., Nanjing, China).

246 All the sequences have been deposited in the Sequence Read Archive (SRA) of NCBI under the

247 accession number of SRP149652 for 16S rDNA and the accession number of PRJNA503750 for

248 ITS rDNA.

249

250 Post-run analysis for 16S rDNA

251 The raw data were processed using the UPARSE pipeline with default parameters for each of the

252 following steps: (1) to sort those exactly matching the specific barcodes into different samples;

253 (2) to merge the paired-end reads with FLASH; (3) to trim the adapters, barcodes, and primers,

254 and to remove sequences shorter than 200 bp; (4) to perform a quality filter using E (the sum of

255 the error probabilities) > 1; and (5) to cluster operational taxonomic units (OTUs) using a 97%

256 identity threshold, discard singleton reads, and perform chimera filtering

257 (http://drive5.com/uparse/) (*Edgar*, 2013). However, in order to compare relative difference

258 between samples in downstream analyses, a randomly selected subset of 10,510 sequences were

259 considered per bacteria community sample and 7,786 sequences per fungal community sample.

260

261 Statistical analysis

262 Indices of α-diversity analysis (Shannon and Simpson indices) were carried out in Quantitative

263 Insights Into Microbial Ecology (QIIME) on rarefied OTU tables (**Dataset S1**), which were

264 obtained from the results of the high-throughput sequencing. Then, all data analyses were

265 computed using the Vegan package in R. In all tests, a *p*-value < 0.05 was considered statistically

266 significant. The differences of soil properties, microbial α-diversity, and relative abundances of

267 microbial taxa among amendments were tested using one-way ANOVA, followed by Tukey's

268 post-hoc test. The statistical significances of the dissimilarities in the microbial community

269 composition between all pairs of amendments were analyzed with permutational multivariate

270 analysis of variance. Principal coordinate analysis (PCoA) based on the Bray-Curtis dissimilarity

271 was used to visualize the distribution patterns of the microbial community. Distance-based

272 redundancy analysis (dbRDA) (based on the Bray-Curtis dissimilarity) was used to estimate the

273 proportion of variability in the microbial community composition caused by each of the selected

274 soil properties, and marginal tests were performed to test the significance of each test. Pearson's

275 correlation analysis was used to detect the relationships between soil properties and the means of

276 α-diversity, as well as soil properties and relative abundance of microbial taxa.

277

278 **RESULTS AND DISCUSSION**

279

280 Effects of local organic amendments on microbial α-diversity

281 It seems that previous studies have not achieved an agreement on the effect of organic

282 amendments on soil microbial α-diversity. Some studies reported an increase (*Francioli et al.,*

283 *2016; Sharma et al., 2017*), some studies found a decrease (*Montiel-Rozas et al., 2018*), and

284 some others found no effect on soil microbial α-diversity after organic amendment (*Zhang et al.,*

285 *2018*). Our study appears similar to the latter, in that we found organic amendments had weak

286 effects on microbial α-diversity, although there were some significant differences observed for in

287 Shannon index values for fungi after 7 months (**Table 1**). Specifically, only the CM treatment

288 had significantly lower fungal Shannon index values than CS after 7 months; there were no

289 significant differences between other treatments. All other bacterial and fungal α-diversity

290 indices were not significantly influenced by amendment both after 7 months and after 15 months.

291 Other researchers have concluded that it's difficult to draw robust inferences from the effect of

292 organic amendments on soil microbial α-diversity, in part because these metrics often have little

293 power in explaining differences in community structure (*Hartmann & Widmer, 2006*). Other

294 studies also confirmed that microbial diversity is a highly complex parameter, and its

295 measurement by diversity indices is usually less informative than qualitative community

296 structure analysis (*Bonilla et al., 2012*). Our studies support those studies.

297   Similarly, the correlation analysis, between soil properties and microbial α-diversity, also

298 indicated that organic amendments had weak effects on α-diversity, since there were only few

299 soil properties that were significantly correlated based on Shannon or Simpson index values

300 (**Table 2**). Nevertheless, we should acknowledge that some other soil properties that we did not

301 determine in this study might impact α-diversity as well. Meanwhile, we should also

302 acknowledge that alfalfa growth affected microbial α-diversity.

303

304 Effects of local organic amendments on microbial community composition

305 Previous studies have indicated that even when microbial α-diversity was unaffected, organic

306 amendments have shown a strong influence on the soil microbial community composition

307 (*Bonilla et al., 2012*). Similarly in this study, all local organic amendments exhibited different

308 microbial community compositions than the Control at after both 7 and 15 months of study ($p <$

309 0.05) (**Table 3**). In addition, both CS and PB treatments had different bacterial and fungal

310 community compositions than CM at both sampling times, which was probably linked to the

311 different soil properties among these treatments (**Table S2**). However, the CS treatment

312 exhibited different bacterial and fungal community composition than the PB treatment after

313   seven months, but they had similar microbial community compositions after 15 months ($p >$
314   0.05). Reinforcing the link with soil properties, treatments CS and PB had dissimilar soil
315   properties after seven months, but they became more similar after 15 months. For example, AK
316   was higher in the CS treatment than in PB after seven months, but showed no significant
317   difference by the time of the second sample. Similar findings were also documented by others
318   (*Zhang et al., 2018*).
319   In our study, PCoA analysis also showed that microbial communities in all treatments were
320   only weakly separated from each other after seven months (**Figure 1A and Figure 1C**).
321   However, after 15 months, all communities in amendment treatments were clearly separated
322   from the Controls, and CS and PB were clustered together for both the bacterial and fungal
323   communities and separate from the CM and Control treatments (**Figure 1B and Figure 1D**).
324   In all, compared with the Control, all local organic amendments altered the microbial
325   community composition, but in the non-traditional PB organic amendment, microbial community
326   composition became similar to the traditional CS amendment only at a later stage. Notably, both
327   CS and PB amendments had different effects on microbial community composition than the most
328   common traditional organic amendment, CM. These differences were evident throughout the
329   whole experimental period.
330   Distance-based RDA analysis showed that bacterial and fungal community compositions
331   also were affected by different soil properties (**Table 4**). After seven months, pH, SOC, and TN
332   were the factors that drove changes in both soil bacterial community composition (explaining
333   20.4, 9.7, and 14.0% of the total variance, respectively) and fungal community composition
334   (explaining 13.1, 9.2, 11.4%, respectively). This result was similar to *Hartmann et al*. (*2015*).
335   However, AK also exhibited a strong effect on fungal community composition, accounting for
336   9.3% of the total variance. But among all soil parameters, pH was the strongest factor that drove
337   variation in both bacterial and fungal community compositions in the soil; this was like the
338   findings of other studies (*Lauber et al., 2009; Rutigliano et al., 2014*). Even after 15 months, pH,
339   SOC, TN, and TP affected both bacterial (explained 12.8, 10.1, 13.7, and 14.3%, respectively)
340   and fungal community composition (explained 9.0, 9.8, 9.5, 10.5, and 15.1%, respectively) in the
341   soil. For fungal community composition, AK was the most influential factor, explaining 15.07%
342   of the total variance. Therefore, we conclude that the fungal community in the desertified soils in
343   our study were more sensitive to AK than the bacterial community. Moreover, we also noted that
344   TP became a stronger factor affecting soil microbial community composition at the later stage.
345   But, in the broadest perspective, pH, SOC and TN should be considered the most important
346   factors driving microbial communities in this study, since they affected both bacterial and fungal
347   communities throughout entire experimental period.
348

349   Effects of local organic amendments on relative abundance of microbial phyla

350   Although local organic amendments were applied for a relatively short time in this study, we

351   found, nevertheless, that distributions of microbial phyla in each treatment had changed

352   significantly (ANOVA, $p = 0.001$ or < 0.001) (**Table S3**). Moreover, we also found that all

353   amendments induced changes in the abundance of some dominant bacterial and fungal phyla,

354   compared to the Controls (**Figure 2A and Figure 2B**). In addition, changes in those dominant

355   bacterial and fungal phyla among treatments were different between the two sampling times.

356   This finding was also similar to *Zhang et al.* (*2018*), and we assume that these changes through

357   time were related to qualities of incorporated organic materials, changes in soil properties and

358   sampling seasons. Also, similar to other studies (*Zeng et al., 2016; Lladó et al., 2017; Zeng et*

359   *al., 2017*), the dominant bacterial phyla of Proteobacteria, Actinobacteria, Acidobacteria,

360   Bacteroidetes, and Firmicutes all were detected in our amended soil. Among all bacterial phyla,

361   Proteobacteria was the most dominant and had higher abundance in treatments CS and PB, than

362   in CM and the Control after 15 months ($p < 0.05$). This result might be due to the reason that

363   Proteobacteria is copiotrophic microorganism (*Fierer et al., 2012*) and a decomposer of lignin

364   (*Tian et al., 2015*). However, Proteobacteria was negatively correlated with TP and AN, but

365   positively correlated with SOC and MBC after 15 months (**Table 5**), which was probably

366   because CS and PB enhanced the microbial activity and fixed more nutrients resulting in

367   microbial growth in the microcosm buckets after 15 months. This was supported by the much

368   higher MBC we observed in CS and PB treatments than in CM (**Table S2**). Furthermore,

369   previous researchers also documented that organic amendments with a high C:N ratio resulted in

370   a higher microbial biomass and activity (*Heijboer et al., 2016*).

371   Similarly, Actinobacteria is also putatively identified as a group of copiotrophic taxawhich

372   thrive in the condition of high C availability, and exhibit relatively rapid growth rates (*Fierer et*

373   *al., 2012*). Our study supports this finding since the CM treatment, after 7 months, not only

374   increased SOC and other soil nutrient contents (**Table S2**), but also increased abundance of

375   Actinobacteria compared with the Control, (**Figure 2A**). In addition, after seven months

376   Actinobacteria was also positively correlated with AN, AP and AK, but negatively correlated

377   with pH, (**Table 5**). Bacteroidetes is regarded as another group of typically copiotrophic taxa

378   (*Fierer et al., 2007*), and have been found to be positively correlated with soil total P (*Tian et al.,*

379   *2015*) and soluble P (*Yashiro et al., 2016*). In our study, Bacteroidetes had higher abundance in

380   CM than in CS and PB treatments after 15 months (**Figure 2B**), but we did not find any soil

381   nutrient that was correlated with this group (**Table 5**). Hence, we speculate that Bacteroidetes is

382   slow-growing copiotrophic microorganism (*Fierer et al., 2007*), which reacted to soil nutrients

383   too slowly to detect in our short-term study.

384   Though after 15 months, Cyanobacteria also showed comparatively higher abundance in the
385   treatment CM than CS and PB (but not significantly different from each other) (**Figure 2B**), this
386   group is regarded as a N$_2$-fixing microorganism common in soils and soil crusts (*Antoninka et*
387   *al., 2016*; *Rippin et al., 2018*). Therefore, we assume that the higher values of TN and AN in CM
388   relative to CS and PB could be the reason for higher abundance of Cyanobacteria found in CM
389   (**Table S2**), although our correlation analysis did not detect this (**Table 5**).Nevertheless,
390   Acidobacteria is regarded to be more adapted to a nutrient-limited soil environment (*Ward et al.,*
391   *2009; Fierer et al., 2012*) and some scientists also characterize them as generally preferring soil
392   environments with high carbon resource (*Větrovský & Baldrian, 2013*). Our study also reinforces
393   that viewpoint because after 15 months Acidobacteria was found in higher abundance in
394   treatments CS and PB (**Figure 2B**), which contained lower available N, P, and K at that
395   sampling time (**Table S2**). Furthermore, Acidobacteria was also negatively correlated with TP,
396   AN, and AP in this study (**Table 5**). Gemmatimonadetes also showed copiotrophic features in
397   this study, because a greater relative abundance was detected in the higher-nutrient treatment CM
398   after seven months (**Figure 2A**). Correlation analysis also revealed that the relative abundance of
399   Gemmatimonadetes was positively correlated with AN, AP, and AK (**Table 5**). Similarly, some
400   previous studies support our findings (*Bernard et al., 2007*; *Pfeiffer et al., 2013*), yet little is
401   known about the ecological roles of Gemmatimonadetes (*Whitman et al., 2016*). In contrast, both
402   Planctomycetes and Chloroflexi were more abundant in treatments CS and PB than in CM after 7
403   months (**Figure 2A**). Similarly, correlation analysis also indicated that those two bacterial phyla
404   were negatively correlated with some soil nutrients (**Table 5**). However, we assume that
405   Planctomycetes were related to decomposition of CS and PB in soils, since Planctomycetes are
406   regarded as the decomposers of organic matter, including cellulose and hemicellulose (*Chapman*
407   *et al., 2017*; *Ivanova et al., 2017*). The high abundance of Chloroflexi may be a result of the
408   accumulation of halogenated organic compounds generated during decomposition (*Chapman et*
409   *al., 2017*).
410   Fungal phyla compositions were also different among treatments between the two sampling
411   times (**Figure 2C and Figure 2D**). Though we know little about the ecological function of
412   detected fungal phyla, some studies reported that the cellobiohydrolase gene is unique to fungi,
413   broadly distributed in Ascomycota and Basidiomycota in forest soils, and encodes for an enzyme
414   which is critical to cellulose breakdown (*Edwards et al., 2011*). Therefore, we assume that the
415   higher relative abundance of Ascomycota (after 7 months), Basidiomycota (after both 7 and 15
416   months), and Zygomycota (after 7 months) detected in treatments PB and CS (**Figure 2C and**
417   **Figure 2D**) could be correlated with the process of wood and straw decomposition in our
418   treatments. Correlation analysis also showed that Ascomycota was positively correlated with
419   MBC, and Basidiomycota was positively correlated with MBC and MBN, but both were

420   negatively correlated with AN (**Table 6**). Hence, those fungal phyla might be accelerated to fix
421   more AN and to decompose incorporated PB and CS in soils.
422   In addition, we consider the above fungi to play an essential role in incorporated organic
423   material decomposition in the early stages, since some studies indicated that fungi were
424   dominant litter decomposers at an early stage (*Voříšková & Baldrian 2013; Žifčáková et al.,*
425   *2016*), while bacteria were dominant decomposers at later stages (*Berg, 2014*). However, it is
426   essential to acknowledge that we can only speculate on the ecological role of the detected taxa
427   based on what has been previously described in other studies. Also, we only identified several
428   organic amendment-sensitive bacteria and fungi to the level of phylum in this study. Therefore, a
429   more in-depth analysis should also be conducted based on our data to find more information
430   about specific microbial ecological roles. Moreover, long-term field studies should also be
431   considered as the next important step. Importantly, we should acknowledge that alfalfa growth
432   also likely affected the microbial community, although our main objective was to tease out the
433   effects of different organic materials as soil amendment strategies.
434

435 ## CONCLUSIONS

436   In this study, we found that soil microbial α-diversity reacted weakly to all local organic
437   amendments. However, bacterial and fungal community compositions changed significantly for
438   different amendments. Non-traditional organic amendments such as woodchips (treatment PB)
439   exhibited similar effects to corn straw treatment (CS). Both PB and CS treatments influenced soil
440   communities differently that the most traditional amendment using cow manure (CM). Soil
441   organic carbon, total nitrogen and pH were the most important factors driving microbial
442   community structure. In conclusion, woodchips should also be considered as a viable soil
443   amendment for future applications.
444

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448

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Effects of organic amendments on α-diversity of soil bacteria and fungi after durations of 7 and 15 months (mean ± SE).

CS = corn straw, CM = cow manure, PB = poplar branch. Means with different letters are significantly different with $p < 0.05$ assessed by Tukey's HSD test.

Time	Treatment	Bacteria		Fungi	
		Shannon	Simpson	Shannon	Simpson
7 months	Control	8.07±0.62a	0.94±0.03a	3.94±0.48ab	0.77±0.08a
	CM	7.96±0.24a	0.98±0.00a	3.58±0.06b	0.76±0.02a
	CS	8.96±0.12a	0.99±0.00a	4.83±0.23a	0.89±0.02a
	PB	8.22±0.22a	0.99±0.00a	4.25±0.05ab	0.89±0.01a
15 months	Control	8.97±0.14a	0.99±0.00a	4.38±0.34a	0.90±0.02a
	CM	8.75±0.35a	0.99±0.01a	5.46±0.26a	0.95±0.01a
	CS	9.17±0.11a	0.99±0.00a	3.32±0.82a	0.68±0.14a
	PB	9.11±0.10a	0.99±0.00a	4.77±0.61a	0.91±0.03a

Correlations between microbial α-diversity and soil properties

p < 0.05, ***p* < 0.01

Time	Microbiome	α-diversity	pH	SOC	TN	TP	TK	AN	AP	AK	MBC	MBN
7 months	Bacteria	Shannon	0.273	0.160	-0.121	-0.083	-0.111	-0.421	-0.247	-0.014	0.353	0.358
		Simpson	0.232	0.164	-0.261	-0.446	-0.314	-0.564*	-0.449	-0.232	0.634**	0.430
	Fungi	Shannon	0.164	0.340	-0.064	-0.287	-0.184	-0.348	-0.325	-0.185	0.649**	0.480
		Simpson	0.302	-0.198	-0.160	-0.350	0.055	-0.293	-0.378	-0.229	0.256	-0.111
15 months	Bacteria	Shannon	-0.026	-0.108	0.156	0.467	-0.248	0.418	0.464	0.609*	-0.284	-0.188
		Simpson	0.273	0.160	-0.121	-0.083	-0.111	-0.421	-0.247	-0.014	0.353	0.358
	Fungi	Shannon	0.231	0.627**	0.367	0.184	0.319	-0.266	0.114	0.294	0.362	0.522*
		Simpson	0.164	0.340	-0.064	-0.287	-0.184	-0.348	-0.325	-0.185	0.649**	0.480

Pairwise comparison of soil microbial community composition under different organic amendments at different times

Pairwise comparisons were analyzed by multivariate permutational analysis of variance (PERMANOVA). Values represent the pseudo-F ratio (*F*) and the level of significance (*p*)

Pairwise comparison	7 months				15 months			
	Bacterial		Fungi		Bacterial		Fungi	
	F	p	F	p	F	p	F	p
Control vs CM	3.21	0.030	2.99	0.035	3.81	0.032	4.55	0.034
Control vs PB	3.24	0.031	3.64	0.032	2.04	0.033	3.79	0.024
Control vs CS	2.58	0.030	4.14	0.029	2.60	0.033	4.01	0.023
CM vs PB	7.97	0.024	3.57	0.023	3.17	0.031	6.77	0.025
CM vs CS	7.83	0.038	2.91	0.028	3.57	0.034	3.96	0.032
PB vs CS	2.87	0.037	3.36	0.036	1.42	0.185	1.69	0.062

Correlations between soil microbial community composition and soil properties

Values represent the estimation variance component (VC) that explained the distribution of microbial community composition are shown out of the bracket, and the corresponding levels of significance (p) are shown in the bracket. Values at $p < 0.05$ are shown in bold.

Soil	7 months		15 months	
properties	Bacteria	Fungi	Bacteria	Fungi
pH	**20.45(0.001)**	**13.05(0.005)**	**12.83(0.008)**	**8.96(0.021)**
SOC	**9.73(0.024)**	**9.20(0.038)**	**10.12(0.012)**	**9.84(0.040)**
TN	**13.98(0.012)**	**11.44(0.012)**	**13.74(0.002)**	**9.53(0.041)**
TP	5.49(0.195)	5.31(0.420)	**14.30(0.003)**	**10.49(0.009)**
TK	3.42(0.554)	4.38(0.648)	6.86(0.138)	4.43(0.538)
AN	6.18(0.152)	6.62(0.209)	5.34(0.221)	5.07(0.374)
AP	6.07(0.139)	5.37(0.413)	3.72(0.572)	4.01(0.712)
AK	6.43(0.130)	**9.34(0.038)**	3.86(0.579)	**15.07(0.011)**
MBC	3.93(0.419)	4.30(0.681)	3.85(0.550)	3.58(0.737)
MBN	3.92(0.496)	4.52(0.670)	3.19(0.733)	4.21(0.625)

Correlations between relative abundance of major bacterial phyla and soil properties

Bolded bacterial phyla were detected significantly different in relative abundance among treatments. *$p < 0.05$, **$p < 0.01$.

Time	Bacterial phyla	pH	SOC	TN	TP	TK	AN	AP	AK	MBC	MBN
7 months	Proteobacteria	0.442	-0.047	-0.260	-0.336	-0.291	-0.325	**-0.515***	-0.432	0.461	0.334
	Actinobacteria	**-0.544***	0.198	0.409	0.349	0.009	**0.448**	**0.589***	**0.629****	-0.449	-0.187
	Firmicutes	**-0.530***	0.202	0.400	0.432	0.221	**0.540***	**0.673****	0.411	-0.453	-0.407
	Bacteroidetes	0.393	0.326	0.363	0.360	0.457	0.154	0.304	**0.604***	0.036	0.256
	Cyanobacteria	-0.129	-0.325	-0.222	-0.001	0.181	0.032	-0.089	-0.298	-0.198	-0.308
	Acidobacteria	**0.554***	-0.017	-0.321	**-0.616***	-0.239	**-0.800****	**-0.718****	-0.396	**0.680****	**0.571***
	Planctomycetes	0.352	-0.192	-0.452	**-0.540***	-0.352	**-0.566***	**-0.670****	**-0.501***	0.416	0.348
	Chloroflexi	0.354	0.051	-0.282	-0.319	-0.372	**-0.566***	**-0.576***	-0.333	0.459	0.400
	Gemmatimonadetes	-0.483	-0.130	0.135	0.343	0.063	**0.648****	**0.615***	**0.498***	-0.495	**-0.601***
15 months	**Proteobacteria**	-0.406	**0.700****	0.342	**-0.563***	0.218	**-0.466**	-0.469	-0.072	**0.680****	0.000
	Actinobacteria	0.259	**-0.587***	-0.412	0.302	0.011	0.247	0.248	-0.078	**-0.566***	-0.031
	Firmicutes	-0.158	-0.123	0.232	**0.823****	0.217	**0.817****	**0.819****	0.491	-0.400	0.255
	Bacteroidetes	0.238	-0.235	-0.060	0.440	-0.404	0.356	0.393	0.181	-0.304	-0.135
	Cyanobacteria	**0.578***	-0.400	-0.467	0.172	**-0.569***	-0.048	0.022	-0.216	**-0.585***	0.015
	Acidobacteria	-0.031	0.164	-0.119	**-0.814****	0.104	**-0.718****	**-0.753****	-0.419	0.478	-0.003
	Planctomycetes	0.089	0.052	0.163	-0.149	0.137	-0.104	-0.164	0.048	0.303	0.111
	Chloroflexi	-0.329	0.178	0.179	**-0.502***	0.299	-0.243	-0.339	-0.218	0.489	-0.199
	Gemmatimonadetes	**0.652****	**-0.644****	**-0.646****	-0.125	**-0.665****	-0.307	-0.270	-0.393	-0.480	-0.438

Correlations between relative abundance of fungal phyla and soil properties

Bolded fungal phyla were detected significantly different in relative abundance among treatments. *$p < 0.05$, **$p < 0.01$.

Time	Fungal phyla	pH	SOC	TN	TP	TK	AN	AP	AK	MBC	MBN
7 months	**Ascomycota**	0.425	0.279	-0.205	-0.284	0.025	**-0.515***	-0.483	-0.454	**0.665****	0.382
	Basidiomycota	0.479	0.323	0.020	-0.090	-0.013	**-0.588***	-0.384	0.055	**0.674****	**0.610***
	Zygomycota	0.322	0.214	-0.090	-0.225	-0.052	-0.421	-0.342	-0.383	0.408	0.458
	Glomeromycota	0.257	**-0.527***	-0.411	-0.214	-0.146	-0.320	-0.421	-0.427	-0.107	-0.172
	Chytridiomycota	0.284	-0.028	-0.260	-0.284	0.025	**-0.636****	-0.483	-0.454	0.394	0.494
15 months	Ascomycota	0.096	0.183	-0.137	-0.408	-0.023	-0.463	-0.492	**-0.598***	0.234	0.200
	Basidiomycota	-0.155	0.423	0.153	-0.231	-0.172	-0.223	-0.241	0.062	0.331	-0.167
	Zygomycota	-0.140	0.065	0.133	-0.013	-0.081	0.070	0.083	**0.541***	0.070	-0.217
	Glomeromycota	0.356	**-0.725****	**-0.634****	-0.129	-0.179	-0.238	-0.216	-0.412	**-0.509***	-0.321
	Chytridiomycota	0.175	-0.188	-0.243	-0.332	-0.441	-0.300	-0.280	0.142	-0.026	-0.482

Figure 1

Principal coordinate analysis (PCoA) of microbial community composition.

(A)bacterial community after 7 months, (B) bacterial community after 15 months, (C) fungal community after 7 months, and (D) fungal community after 15 months. CS = corn straw, CM = cow manure, PB = poplar branch.
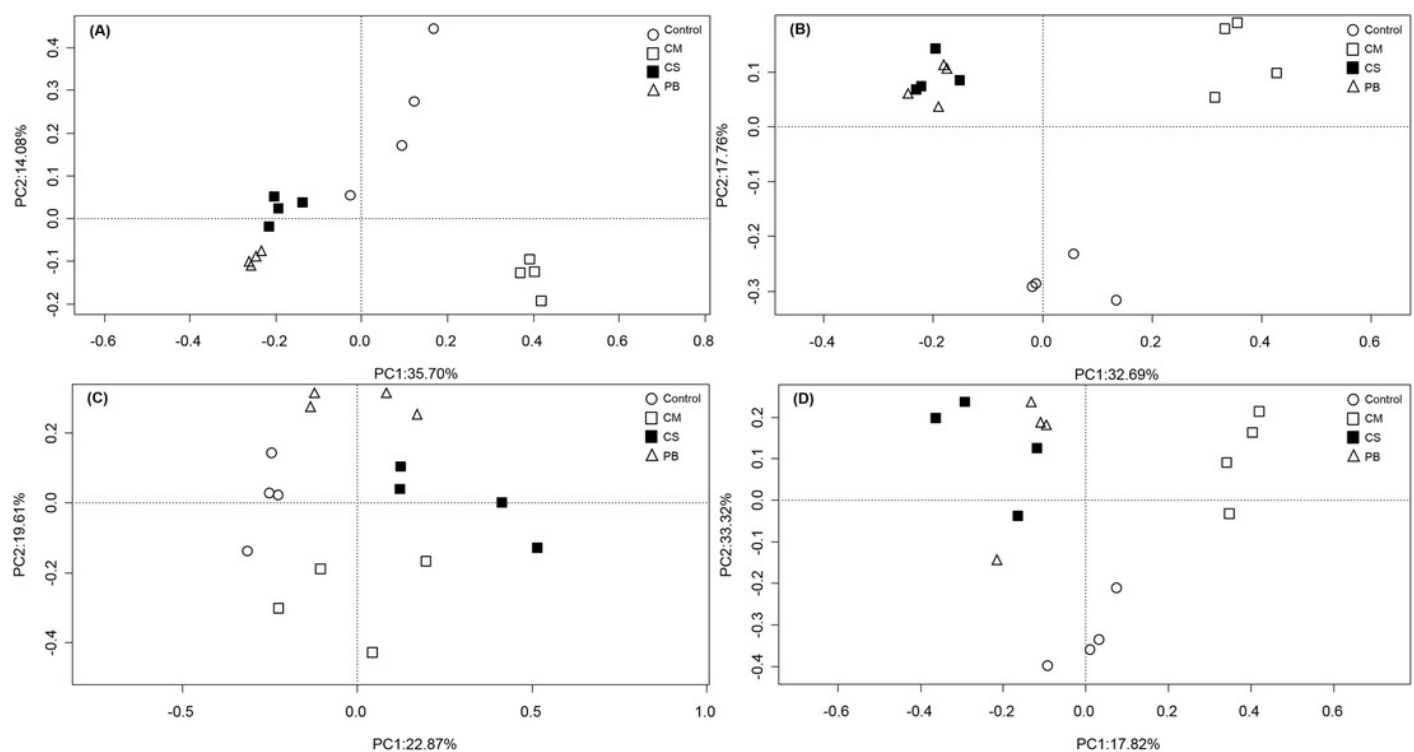
Figure 2

Relative abundance of the dominant microbial phyla.

(A)bacterial phyla after 7 months, (B) bacterial phyla after 15 months, (C) fungal phyla after 7 months, and (D) fungal phyla after 15 months. Different letters indicate significant differences based on Tukey's HSD test ($p < 0.05$). CS = corn straw, CM = cow manure, PB = poplar branch. Only the phyla with significantly different relative abundance among amendments were labelled with letters. Error bars represent standard error (n = 4).