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Rhizosphere bacterial communities of wheat vary across the growing season and among dryland farming systems.

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Abstract

Despite knowledge that seasonality and plant phenology impact soil microbiota, farming system effects on soil microbiota are not often evaluated across the growing season. We assessed the bacterial diversity in wheat rhizosphere soil through the spring and summer of 2016 in winter wheat (*Triticum aestivium* L.) in Montana, USA, from three contrasting farming systems: a chemically-managed no-tillage system, and two USDA-certified organic systems in their fourth year, one including tillage and one where sheep grazing partially offsets tillage frequency. Bacterial richness (range 605 – 1174 OTUs) and evenness (range 0.80 – 0.92) peaked in early June and dropped by late July (range 92 – 1190, 0.62-0.92, respectively), but was not different by farming systems. Organic tilled plots contained more putative nitrogen-fixing bacterial genera than the other two systems. Bacterial community similarities were significantly altered by sampling date, minimum and maximum temperature at sampling, bacterial abundance at date of sampling, total weed richness, and coverage of *Taraxacum officinale*, *Lamium ampleuxicaule,* and *Thlaspi arvense*. This study highlights that weed diversity, season, and farming management system all influence rhizosphere soil microbial communities. Local environmental conditions will strongly affect any practical applications aimed at improving soil diversity and functionality, especially in semi-arid regions where abiotic stress and seasonal variability in temperature and water availability drive primary production.

49
50   **1 Introduction**

51   Microbial communities in agricultural soil are influenced by myriad factors,

52   broadly including seasonality and local environmental conditions, and management

53   practices, and the interaction of these influences is understudied. In a dryland agricultural

54   system, we investigated the interaction of farming system and environmental variables,

55   such as soil moisture and temperature, on the bacterial community associated with the roots

56   of winter wheat (*Triticum aestivum* L.) at various points along a growing season.

57   Seasonality may result in variations in temperature, precipitation, soil moisture, and

58   solar radiation, all of which drive rates of soil microbial metabolism and respiration, as

59   well as taxonomic composition (Koranda et al., 2013; Orr et al., 2012; Prevost-Boure et al.,

60   2010; Wu et al., 2016). Likewise, environmental conditions alter plant phenology which,

61   in turn, can impact changes in soil microbial communities within a growing season (Donn

62   et al., 2015; Kumar et al., 2018; Sayer et al., 2017). Moreover, management practices,

63   particularly those in agricultural systems, are strongly tied to seasonality (Morrison-

64   Whittle and Goddard, 2015), yet the interplay of time and management on microbial

65   community dynamics in soil has not been well described (Bossio et al., 1998).

66   Farming practices such as use of pesticides and fertilizers, tillage, crop rotation,

67   irrigation, and the integration of crop and livestock operations directly select for specific

68   microbial communities in soil (Chaudhry et al., 2012; Ishaq, 2017; Lori et al., 2017).

69   Farming practices also indirectly influence soil microbial communities by influencing

70   weed diversity and biomass, soil compaction, soil moisture and permeability, and

71   arthropod populations (Cregger et al., 2012; Lennon et al., 2012; Ponce et al., 2011; Roger-

72   Estrade et al., 2010). Root-associated soil microbial communities can dramatically affect

73   trophic interactions in natural and agricultural settings, by cycling organic material or by

74   interfacing directly with plants to modulate plant growth, root exudates, and health status

75   (Ishaq, 2017; Mariotte et al., 2017).   Greater soil microbial phylogenetic diversity is

76   generally considered beneficial for soil and plant health, as it allows for functional

77   redundancy in the provision of ecosystem services, and for the stability and resiliency to

78   disturbances (Bérard et al., 2011; Kuan et al., 2006; Orwin and Wardle, 2004).

79   Previous studies determined that in comparison with conventional (chemically-

80   managed) no-till and conventional till systems, organic systems have higher soil bacterial

81   cell density and total taxonomic diversity (Chaudhry et al., 2012; Ishaq et al., 2017;

82   Pershina et al., 2015). Yet, many organic systems rely on mechanical tactics like tillage to

83   control weeds. However, tillage use in dryland systems could result in soil erosion,

84   moisture loss, and a change in the community structure of soil microbiota (Ishaq, 2017;

85   Lehnhoff et al., 2017). While there are benefits and limitations of integrated crop-animal

86   production systems (reviewed in (Thiessen Martens and Entz, 2011)), little has been

87   reported on the effect of livestock grazing on soil microbiota. Futher, much of that research

88   has been in pasture-based systems where light stocking increases carbon and nitrogen, and

89   sometimes increases bacterial but not fungal biomass (reviewed in (Ishaq, 2017)).

90   In the northern portion of the Great Plains of North America, wheat (*Triticum*

91   *aestivum* L.) is the most widely-planted crop, but production is threatened by insects,

92   climate change, and herbicide-resistant weeds (Keren et al., 2015; Lanning et al., 2010;

93   Menalled et al., 2016). Begininng in 2012, a study has been conducted at the Montana

94   State University Fort Ellis Research and Teaching Center, 3 km east of Bozeman, MT, to

95   compare wheat production challenges across three dryland farming systems: 1) a

96   chemically-managed no-till, 2) an USDA-certified organic system utilizing tillage to

97   manage weeds and terminate cover crops, and 3) an USDA-certified organic system where

98   sheep (*Ovis aries*) grazing is used to manage weeds and terminate cover crops with the

99   goal of reuducing tillage intensity.

100   In this study, we evaluated patterns in bacterial diversity across the three farming

101   systems described above to elucidate the relative effects of management systems, soil

102   moisture, nutrient content, wheat yield, as well as weed abunandace and diversity on the

103   wheat rhizosphere soil bacterial communities. We hypothesized that 1) date within the

104   growing season be the strongest determinant of bacterial diversity and community structure,

105   2) farming systems would select for different bacterial communities over the entire

106   growing season, and that 3) farming system would modulate the response of the bacterial

107   community to environmental variables such as low moisture, high temperature, and plant

108   senescence.

109

110   **2 Materials and methods**

111   **2.1 Site description**

112   Beginning in July 2012, a long-term agricultural field experiment was located at the

113   Montana State University Fort Ellis Research and Teaching Center (45.652664056 N -

114   110.97249611 W) to assess agronomic and ecological challenges of chemically-managed

115   (i.e. 'conventional') and USDA-organic farming systems, as well as the integration of

116   livestock into organic farming systems. Soils at the Fort Ellis site are a Blackmore silt loam

117   (a fine-silty, mixed, superactive, frigid Typic Arguistoll) with 0 to 4% slopes and consistent

118   ratio of 1 part sand, 2 parts silt, 1 part clay by weight (Miller and Menalled, 2015). Monthly

119   air temperature in Bozeman in 2016 was higher than historic maximum and minimums

120   from 1981 – 2010, and mean monthly precipitation (Table S1) was lower by 18 mm in

121   May, 16 mm June, and 14 mm in July ("PRISM Climate Group," 2018).

122   The experiment followed a randomized split-plot design; using farming system as

123   the main plot (90 x 75m) with three field replicates per farming system, and crop identity

124   as the split-plot (90 x 13 m). Farming systems consisted of 1) chemical no-till system

125   (CNT), in which synthetic inputs were used in the form of fertilizers, herbicides, and

126   fungicides, 2) USDA-certified till organic (OT), and 3) USDA-certified organic with

127   grazing (OG), which integrates sheep grazing to terminate cover crops and manage weeds,

128   with the overall goal of minimizing tillage intensity in organic production. Split-plots were

129   randomly assigned to a starting crop of a 5 yr crop rotation: year 1 – safflower (*Carthamus*

130   *tinctorius* L.) under-sown to yellow sweet clover (*Melilotus oficinalis* (L.) Lam.), year 2 –

131   sweet clover cover crop, year 3 – winter wheat (*Triticum aestivum* L.), year 4 – lentil (*Lens*

132   *culinaris* Medik.), and year 5 – winter wheat (Lehnhoff et al., 2017).

133   Chemical inputs utilized in the CNT system included 2,4-D, bromoxynil, dicamba,

134   fluroxypyr, glyphosate, MCPA, pinoxaden, and urea for winter wheat rotations [see Tables

135   2.7 and 2.8 in (Johnson, 2015)], which are reflective of typical farm management practices

136   in the Northern Great Plains region. Both organic treatments began the organic transition

137   process in July 2012, making crops harvested as of 2015 USDA-certified as organic. In

138   the OT system, tillage was accomplished using a chisel plow, tandem disk, or field

139   cultivator, as needed for weed control, seedbed preparation, and to incorporate cover crops

140   and crop residues. Weed control was enhanced with a rotary harrow. In the OG system,

141   targeted sheep grazing was used to reduce tillage intensity for pre-seeding and post-harvest

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142   weed control and to terminate the cover crops, with duration and intensity of grazing based

143   on weed biomass (Lehnhoff et al., 2017). Grazing was supplemented with tillage as

144   necessary, based on soil conditions and weed pressure. Seeding was done with a low-

145   disturbance no-till double-disk seeder. Further details of the management practices used

146   within each system and farming history prior to planting of these crops can be found

147   elsewhere (Barroso et al., 2015; Johnson, 2015; Lehnhoff et al., 2017). Outside of normal

148   farm management activities, soil disturbance and compaction was minimized during

149   sampling procedures.

150

151   **2.2 Soil measurements and collection**

152   In the present study, soil was sampled in the year 3 winter wheat split plots of each

153   faming systems. Soil moisture was measured weekly using gypsum blocks (Friis Dela,

154   2001) buried in the center of each sample area to a depth of 7.5 cm and a Delmhorst soil

155   moisture tester (Model KS-D1, Delmhorst Instrument Co.). Every four hours between

156   April 14, 2016 (one week prior to the first sampling) and July 25, 2016 (final sampling

157   date), soil temperature was recorded within sample areas using an iButton (Maxim

158   Integrated) buried at 7.5 cm.

159   To characterize soil microbial communities, rhizosphere soil cores were obtained

160   from each one of the nine year 3 winter wheat split-plots, within an 0.75 m^2 area, situated

161   randomly along the length of each 90-m split-plot. Three soil cores were obtained to a

162   depth of 15 cm using a 2-cm diameter core sampler, which was sterilized with 70%

163   isopropanol and air-dried between sample areas. and from each sample area. Soil (50 mL)

164   was homogenized and placed on ice until transport back to Montana State University,

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165   where they were stored at -20°C until analysis. Each area was repeatedly sampled five

166   times during the 2016 growing season: April 21, May 12, June 1, June 22, and July 25

167   (prior to wheat harvesting). Additional soil samples were obtained on July 25, and stored

168   at 4°C until shipped to an independent laboratory (Agvise Laboratories, Northwood, North

169   Dakota, US) for analysis of organic matter, nitrate, phosphorous (Olsen), potassium, and

170   pH (Table S2).

171

172   **2.3 Plant community measurements and collection**

173   Aboveground biomass of all weed species present within sampled areas was

174   harvested by hand in late June, when most weeds and wheat had matured. Weeds were

175   visually identified and separated by species, dried in an oven at 55° C for two weeks, and

176   weighed. Total wheat biomass was harvested from sampled areas by hand from 1.5 row

177   meters on July 25, 2016, immediately after soil samples had been collected. Wheat biomass

178   was dried in an oven for a week at 55° C, weighed, and then mechanically threshed to

179   remove grain. The grain was submitted for protein analysis to the Montana State University

180   Grain Quality Lab (Bozeman, MT).

181

182   **2.4 DNA extraction and sequencing**

183   DNA was extracted using 0.25 g of a 15 -30 g soil sample homogenized from at

184   least 3 soil cores within the sample area and processed following protocols described in

185   Ishaq et al. (2017) using the PowerSoil 96-well Soil DNA Isolation Kit (MoBio

186   Laboratories, Inc.). Following extraction, an additional cleaning step was added: a 10%

187   volume of 2M sodium acetate was added to each sample, followed by a 200% volume of

188   100% ethanol. Samples were vortexed and refrigerated overnight at -20°C to precipitate

189   DNA, after which they were centrifuged at 16,000 x G for 5 min, supernatant was poured

190   off, and sample tubes were air-dried. Pellets were washed with 100% ethanol, allowed to

191   air dry again, and eluted into 100 µl of molecular-grade water. The V3-V4 region of the

192   16S rRNA gene was PCR amplified using the KAPA HotStart PCR Kit (Kapa Biosystems,

193   Wilmington, MA) with 10 µL Kappa HotStart Mastermix, 6 µL molecular-grade water, 1

194   µL of each forward and reverse primer at 10 mM concentration, and 2 µL sample DNA.

195   PCR protocol was as follows: 95º C for 3 min; 5 cycles of denaturation at 98º C for 20 sec,

196   annealing at 52º C for 30 sec, elongation at 72º C for 45 sec; 25 cycles of denaturation at

197   98º C for 20 sec, annealing at 60º C for 30 sec, elongation at 72º C for 45 sec. Primers

198   included the MiSeq adaptors (A for forward, B for reverse), the sample index/barcodes, the

199   two-nucleotide linker, and primers 341F (5'-CCTACGGGAGGCAGCAG-3') and 806R

200   (5'-GGACTACHVGGGTWTCTAAT-3') (Caporaso et al., 2011). High-throughput

201   sequencing was performed using an Illumina MiSeq (Illumina, San Diego, CA) and a 500-

202   cycle V2 kit, with PhiX used as a positive control at a 10% spike-in, and molecular-grade

203   sterilized water as a negative control. Sequencing output data can be found in the Sequence

204   Read Archive (SRA) at NCBI under BioProject PRJNA383161.

205

206   **2.5 DNA data processing and analysis**

207   Bioinformatics were performed similar to previously described protocols (Ishaq et

208   al., 2017) with a few exceptions. Forward and reverse DNA sequence fragments were

209   assembled into contigs using PANDAseq (Masella et al., 2012) with > 15 nucleotide

210   overlap and default quality parameters, then processed using mothur ver. 1.38 (Schloss et

211   al., 2009). Sequences that contained ambiguous bases, homopolymers > 8 nt, < 300 nt or

212   > 580 nt, were discarded. Sequences were aligned to the Silva nr 119 database (Quast et

213   al., 2013) using Needleman-Wunsch alignment (Needleman and Wunsch, 1970), after

214   which all-blank columns and unaligned sequences were removed. Samples with > 50,000

215   sequences were subsampled down to 50,000 to improve analysis time. Chimeras were

216   identified and removed using mother-integrated UCHIME (Edgar et al., 2011), and then

217   taxonomically classified with the Wang algorithm/Ribosomal Database Project (RDP)

218   Classifier (Wang et al., 2007) and Silva reference database. Sequences identified as

219   unknown, mitochondria, chloroplast, or Eukaryotic (< 500 combined) were removed.

220   Sequences which were identified to genera known to contain nitrogen-fixing species in soil

221   were identified from previous literature. The number of seqences per sample which passed

222   QA ranged from 1,305 – 157,761.

223   Sequences were subsampled (normalized) to the size of the smallest sample (n =

224   3232 sequences per sample), removing nine samples with fewer sequences each from

225   downstream analysis. Distance was calculated considering consecutive gaps to be one

226   event, and sequences were clustered into presumptive species-level operational taxonomic

227   units (OTUs) using the nearest neighbor algorithm at a 0.03 cutoff. OTUs which only

228   contained singletons or doubletons were removed. One sample sampled in July in OT

229   (OT_725_114) contained a dramatically different microbial community than its replicates

230   as it had more Firmicutes and Bacteroidetes and far fewer of any other phyla represented

231   than other samples (included in Fig 4, S2), and was removed from all group-based

232   statistical analysis.

233   Diversity was assessed using the mothur-integrated versions of Shannon Diversity

234   (Segata et al., 2011), and multivariate analysis and data visualization was performed in R

235   (RCoreTeam, 2018). Diversity data did not meet normality distribution assumptions via

236   Shapiro-Wilks test, thus comparisons of means were conducted with non-parametric

237   Conover tests (Dinno, 2017) using Bonferrroni p value correction for multiple comparisons.

238   Classification-based random forest trees with permuational analysis (rfPermute) were used

239   to identify discriminant taxa, based on "mean decrease accuracy" or the importance of a

240   factor in predicting the microbial community, as measured by randomly re-assigning factor

241   levels and comparing to the original tree of microbial community distribution. Regression-

242   based random forest trees were used to identify important factors for Shannon diversity of

243   abundance of specific taxa (Breiman et al., 2018). The number of trees which minimized

244   the out-of-box error rate was used, typically ntree = 500.

245   Unweighted Jaccard distance (uJ) and Bray-Curtis Dissimilarity (BC) were

246   calculated to compare community membership (presence/absence) and community

247   structure (presence/absence and abundance), respectively, and assessed using

248   permutational analysis of variance (PERMANOVA) with the Adonis function in the vegan

249   package (Oksanen et al., 2012), or pairwise in the RVAideMemoire package (Herve, 2019),

250   with Block to stratify data, split-plot as a repeated measure, 1,000 permuations, and

251   Bonferroni p-value correction. There was heterogeneity in samples by replicate block,

252   indicating a spatial difference in the soil conditions, which significantly affected bacterial

253   communities (PERMANOVA, uJ, $F = 4.498$, $p = 0.001$; Bray-Curtis, $F = 3.6676$, $p = 0.001$),

254   thus, all permutational calculations of distance included field replicate block as a

255   stratification. Non-Metric Multidimensional Scaling Plots (NMDS) based off Bray-Curtis

256   Dissimilarity were calculated in mothur and were visualized using ggplot2 (Wickham,

257   2009). A heatmap of significant Pearson's correlations between treatment parameters and

258   OTU abundance was created using corrplot (Wei et al., 2017), which generated correlations

259   and tested significance.

260   Distance-based redundancy analysis (dbRDA) was conducted using Helligner-

261   transformed community data (Legendre and Gallagher, 2001) and the capscale function of

262   the vegan package, using Block as a condition. The environmental data included

263   categorical treatment levels; soil moisture at soil sampling; the minimum, maximum, and

264   mean soil temperature on the day of soil sampling; the minimum, maximum and mean soil

265   temperature averaged over 3 and 7 days prior to sampling. Biotic and abiotic (meta-) data

266   also included bacterial Shannon diversity; total weed species, total weed coverage,

267   individual weed species coverage, and wheat coverage the previous fall (October 25, 2015),

268   early spring (April 8, 2016), and mid-summer (June 14, 2016), as well as weed biomass in

269   mid-summer (June 14, 2016) and wheat biomass in late-summer (July 25, 2016).

270   Additional significant variables were removed from the original model to create a reduced

271   model, as they were co-linear aliases of included variables, including the biomass in June

272   2016 of *C. pastoris*, *C. arvense*, *Melilotus* spp., and *T. arvense*, the plot coverage in April

273   2016 of *C. album*, *G. aparine*, *L. serriola*, and *T. arvense* plot coverage the previous fall in

274   October 2015.

275   Each farming system contained a distinct weed community profile, and collectively

276   the dominant weed species identified over all systems and time points included *Bromus*

277   *tectorum* L*., Capsella bursa-pastoris* L*., Chenopodium album* L*., Cirsium arvense* L*.,*

278   *Galium aparine* L*., Lactuca serriola* L*., Lamium amplexicaule* L*., Lens culinaris*

279   (Medikus)*, Malva neglecta* (Wallr.)*, Melilotus officinalis* L.*, Monolepsis nuttalliana*

280   (Schult.)*, Taraxacum officinale* L.*, Thlaspi arvense* L.*, Tragopogon dubius* (Scop.)*,*

281   *Trifolium hybridum* L.*,* and *Trifolium pratense* L. Factors for which only one time point

282   was present, such as wheat biomass, soil pH, and soil nutrients, all collected at harvest,

283   were assessed for the July time point only. Additional information and analysis on wheat

284   and weed data are provided elsewhere (Lehnhoff et al., 2017; Seipel et al., 2018). Across

285   all tests, significance was determined at $P < 0.05$

286

287   **3 Results**

288   The bacterial community in soil was comprised of Actinobacteria, Proteobacteria,

289   and Acidobacteria, followed by Bacteroidetes, Firmicutes, Gemmatimonadates,

290   Verrucomicrobia, and Planctomycetes, and several low-relative-abundance phyla (Fig. 1).

291   Within each sampling date, the relative abundance of Acidobacteria, Actinobacteria,

292   Armatimonadetes, Chlorobi, Chloroflexi, Cynaobacteria, Deinococcus-Thermus,

293   Fibrobacteres, Firmicutes, Proteobacteria, and Verrucomicrobia, as well as the candidate

294   phyla BD1-5, BRC1, SHA-109, SM2F11, TM7, WCHB1-60, and WS6 differed among

295   farming systems (Fig. 1; Table S3, $p < 0.05$). There was an increase in the mean coefficient

296   of variation for the relative abundance of each phylum over the growing season (Fig. S1),

297   indicating that there was more variation in field replicates as bacterial abundance was

298   divergent across individual sample areas when soil was hottest and driest in July.

299   A total of 8,547 OTUs (97% cutoff) were identified, including many common soil

300   genera which were abundant throughout the growing season in all plots, including

301   *Blastococcus*, *Arthrobacter*, *Skermanella*, *Sphingomonas*, as well as unclassified genera

13

302   from several different families (Fig. S2). Other OTUs were specific to system, including

303   *Bacteroides* which was prominent in OT sample 202 during July, and the insect-associated

304   *Wolbachia* which was abundant only in a single CNT sampled area in May (Fig. S2).

305

3.1 Seasonal effect

307   The effect of sampling date was manifested by the unimodal trend of soil bacterial

308   richness over the 2016 growing season. Across the three farming systems, observed OTU

309   (97% cutoff) richness peaked in early June, when it was greater than in April, May, or July

310   (Fig. 2 A, B; Conover, $p < 0.05$ each, Bonferroni corrected (BF)). Richness was also

311   greater in late June than in July (Fig. 2 A, B; Conover, $p < 0.05$ each, BF). Similarly,

312   Shannon-Weiner diversity index of soil bacteria peaked in early June when it was

313   significantly higher than in April, May, late June, and July (Fig. 2C; Conover, $p < 0.05$

314   each, BF). This change in Shannon diversity over the growing season was driven by

315   changing richness, rather than changes in evenness (Fig. 2D) which was only significantly

316   increased in early June as compared to April (Conover, $p = 0.004$, BF).

317   Sampling date significantly affected bacterial community similarity based on

318   member presence/absence collectively across the growing season (Table 1, uJ), but was

319   not significantly altered in any pairwise sampling date comparison (uJ, $p > 0.05$ BF),

320   contrary to our first hypothesis. Sampling date similarly affected bacterial community

321   similarity in relation to relative abundance (Fig. 3; Table 1, BC, $p = 0.0001$), and was driven

322   by significant differences between June and July (Table S4) when richness was low: early

323   June - July ($p = 0.014$), and late June-July ($p = 0.022$).

324

14

3.2 Farming system

Farming system had an equivocal effect on community membership to sampling date during the growing season, and significantly affected both OTU presence/absence (Table 1, uJ), and membership with respect to relative abundance (Table 1, BC). The collective effects that farming system exerted upon soil bacterial communities was observed between CNT and OG plots (Table S5), both in presence/absence (uJ, F = 1.387, p = 0.01), and weighted community structure (BC, F = 1.676, p = 0.014), indicating a difference in presence/absence as well as relative abundance, respectively. OG and OT contained significantly different communities by presence/absence (uJ, F = 1.41, p = 0.011). CNT and OT had only equivicoally different presence/absence composition (uJ, p = 0.051).

Richness, evenness, and Shannon Diversity were not significantly different between farming systems at any time point, or averaged across the growing season (Conover, $p > 0.05$, Bonferroni corrected (BF)). Thus, there was no significant difference on a pairwise basis between each farming system within each time point (Fig. 3; PERMANOVA uJ and BC, $p > 0.05$). However, there was more variability in soil bacterial communities at the end of the growing season (betadisp, uJ and BC, $p < 0.01$), there was no significant interaction between sampling date and farming system (PERMANOVA, uJ and BC, $p > 0.05$; Table 1). There was no significant interaction between sampling date and farming system in soil microbial community clustering (PERMANOVA, BC and uJ, p = 1 BF).

Within the CNT and OG farming systems, a large number of OTUs were commonly sampled across the growing season: CNT samples shared 4,530 (53.0% of total OTUs), OG shared 4,536 (53.1% of total OTUs), and OT shared 4,207 OTUs (49.2% of total

348   OTUs). All farming systems shared 3,555 OTUs in April (54.3% of total), 3,086 OTUs in

349   May (47.2% of total), 2,973 OTUs in early June (45.4% of total), 3,025 in late June (46.2%

350   of total), and 2,043 in July (23.9% of total).

351   Random forest classification was not able to predict bacterial communities based

352   on farming system (Fig 4; OOB estimate of error rate: 48%) or time point (not shown;

353   OOB estimate of error rate: 69%) with reliably high accuracy. OT plots contained more

354   putative nitrogen-fixing bacterial genera than OG or CNT (Fig. 5A). *Arthrobacter* was

355   more abundant in organic plots across all time points, and particularly OT plots, though

356   CNT plots contained more *Flavobacterium* and an unclassified 97%-cutoff OTU in the

357   Bradyrhizobiaceae family (Fig. 5A). All but the least abundant genera were significantly

358   ($p < 0.05$) differential for farming system (Fig. 5B). Soil moisture, soil temperature, and

359   total and individual weed species biomas were important predictors of *Arthrobacter*

360   abundance (Fig. 5C).

361

362   **3.2 Plant community and environmental variables**

363   Biotic and abiotic variables affecting the soil bacterial communities included

364   farming system, sampling date, minimum and maximum temperature on the day of

365   sampling, total weed diversity on both April and June 2016, *Taraxacum officinale* percent

366   coverage the previous fall in October 2015, *Lamium ampleuxicaule* percent coverage in

367   April 2016, and *T. arvense* percent coverage in April 2016 (ANOVA, $p < 0.05$; Fig. 6).

368   Only weak (-0.4 to 0.4) Pearson's correlations existed between bacterial Shannon

369   diversity and various bacterial and weed species (Fig. S3, $p < 0.05$), likely because most

370   samples maintained a high Shannon Index (range 5.2 - 6.6). When separated out by

371   sampling date, the strength of the correlations between the top soil bacterial OTUs in early

372   June (Fig. S4) and late June (Fig. S5, $p < 0.05$) increased. Total Shannon diversity and

373   abundance of many of the most-abundant bacterial OTUs were negatively correlated with

374   increased soil temperature at respective date of sampling (Fig. S6, $p < 0.05$). However,

375   *Arthrobacter*, *Skermanella*, *Sphingomonas*, Comamonadaceae, *Bacteroides*, *Arenimonas*,

376   and *Microvirga* were positively correlated with soil temperature (Fig. S6, $p < 0.05$). Soil

377   moisture on the day of sampling was positively correlated with the most-abundant bacterial

378   OTUs, but showed a weakly-positive correlation with Shannon diversity (Fig. S6, $p < 0.05$).

379   Soil pH was negatively associated with a number of bacterial genera, as was wheat

380   protein (Fig. S7, Pearson's corelations, $p < 0.05$). Soil pH was lower in CNT than OG

381   (ANOVA, $p < 0.05$); CNT (mean 5.9), OG (mean 7.00), OT (mean 6.77). Nitrate was

382   negatively associated with the putative genus 480-2, an unnamed clade in the

383   Actinobacteria phylum (Fig. S7). Organic matter was not correlated with any of the most-

384   abundant bacterial OTUs identified in July soils, but nitate was strongly negatively

385   associated with the putative genus 480-2 (order Solirubrobacterales, phylum

386   Actinobacteria) (Fig. S7).

387

388   **4 Discussion**

389   Agricultural production in the Great Plains of North America is strongly tied to

390   seasonality, which alters temperature, moisture, solar radiation, and plant phenology.

391   Wheat (*Triticum aestivum* L.) is the most widely-planted crop, and understanding the

392   dynamics between production, soil microbial communities, and farming system, separately

393   and with respect to seasonality, is important to continued sustainability. This research

394   assessed the impact of management systems and time within the growing season on the

395   status of bacterial communities in three contrasting dryland farming systems: a chemically-

396   managed no-till system and two organic farming systems that were maintained for four

397   years under USDA-organic prescribed conditions.

398

399   **4.1 Farming system over the growing season**

400   Farming systems selected for different bacterial communities, which has been well-

401   established at single time-points, however we did not find significant farming system x

402   time effects. Transitioning a farm to a USDA organic-certified system takes just three

403   years to complete legally, yet it has been noted that soil microbial systems may take years

404   to decades to transition to a new, stable community (Chaudhry et al., 2012; Hartmann et

405   al., 2015; Sayer et al., 2017; Stagnari et al., 2014). Thus, the studied soil bacterial

406   communities can be considered to be in transition, but, to our knowledge, the changes in

407   soil microbial communities occurring during that transition or in recently certified organic

408   system are unknown. However, the relevance of our study resides in the fact that the

409   sampled organic fields represent certified systems from which farmers could perceive a

410   significant economic premium (Lawrence et al., 2018; Miller et al., 2007). Variations in

411   the soil bacterial community, including a reduction in total taxonomic diversity and a shift

412   in abundance towards particular taxa, resulting from a change in management system

413   occurs on a shorter time scale, (Lupwayi et al., 2004; Stagnari et al., 2014) compared with

414   soil fungal communities (Stagnari et al., 2014). Nevertheless neither soil bacterial and

415   fungal communities may reach a steady state for a long time, if ever, depending on the

416   specific disturbance regime associated with in the agricultural system where they occur.

417   Further, even after just four years under these management practices, we saw changes in

418   the soil bacterial community.

419   In this study, soil from the OG system exhibited less variation between plots in

420   bacterial species richness and moisture later into the growing season than the plots from

421   the OT system, and a neutral pH compared to the CNT systems. In July, more dispersion

422   was observed in community clustering, and in particular, OT plots had highly variable

423   mean evenness and richness between field replicates in July. This suggests a random

424   divergence of the bacterial community rather than a selective pressure in those plots at that

425   time. These differences may suggest that farming system may mediate the effects of the

426   environment, per our third hypothesis. Yet, the lack of strong community divergence by

427   farming system would suggest that the effects of environment may supersede the selective

428   effects of farming, or else that the farming systems, though past USDA certification, had

429   not been implemented long enough to significantly alter bacterial communities in the soil.

430   However, many of our time x farming system comparisons lacked significiance, possibly

431   due to the variability in OT plots, or too few replicates. The effect of time within the

432   growing season on soil microbial community dynamics is not well studied (DeBruyn et al.,

433   2011; Marine et al., 2015).

434

435   **4.3 Plant community and environmental effects over the growing season**

436   July in Montana is very hot and dry, so much so that all three farming systems

437   exhibited similarly low moisture levels. The unimodal nature of bacterial richness and

438   eveness in all systems coincides with peak crop growth, and the reduction is July

439   corresponds with both the weather and crop senescence, both of which would affect

19

440   microbial communities. Bacterial community structure in soil varies seasonally, reflecting

441   seasonal changes in plant phenology, solar radiation, moisture or temperature, all strong

442   drivers of bacterial species richness (Prevost-Boure et al., 2010; Wu et al., 2016). Bacterial

443   activity, such as nitrogen fixation, is also strongly tied to soil moisture (Koranda et al.,

444   2013; Orr et al., 2012). In our study, soil moisture was not a significant factor structuring

445   the entire soil bacterial community, which may reflect the prevalence of relic DNA in soils

446   (Carini et al., 2017). However, moisture was an important predictor of the abundance of

447   the genus *Arthrobacter*, some members of which are nitrogen-fixers (Westerberg et al.,

448   2000), and which are reported to thrive in adverse conditions, including low moisture and

449   high radiation conditions (Mongodin et al., 2006; SantaCruz-Calvo et al., 2013). Futher,

450   numerous *Arthobacter* species are adept at using various carbon sources, including

451   pesticides (Hagedorn and Holt, 1975). This is in contrast to other nitrogen-fixers, which

452   are susceptible to pesticide toxicity (Orr et al., 2012).

453   Changes in weed diversity has been found to correlate with soil bacterial diversity

454   throughout the year (Aguilera et al., 2017; Cardinale et al., 2015; Grayston et al., 1998;

455   Ishaq et al., 2017; Whiting et al., 2001). Contrary to previous studies, (Flohre et al., 2011;

456   Kubota et al., 2015) though, bacterial richness was positively correlated with total weed

457   diversity only in April and weakly negatively correlated with total weed diversity in June,

458   which was driven by the interaction between bacteria and several plant species. In

459   agreement with the present study, *T. arvense* has been found to be associated with low

460   bacterial diversity in soil (Whiting et al., 2001), possibly because members of the

461   Brassicaceae family of plants produce a number of glucosinolates and other antimicrobial

462   compounds (Pal Vig et al., 2009), which may inhibit microbial growth in the rhizosphere.

463   Similarly, and in accordance with (Cardinale et al., 2015), *L. serricola* was associated with

464   low soil bacterial relative abundance.

465   Previous studies have shown that some weed species are better at recruiting

466   beneficial microbiota than domesticated crops, allowing them to be more competitive

467   (Aguilera et al., 2017; Massenssini et al., 2015; Trognitz et al., 2016). Recent discussions

468   have examined the possibility that weed species are more dependent on plant-microbial

469   connections, while crops only need them under non-ideal growing conditions (Trognitz et

470   al., 2016). Also, it has been shown that plants will reprioritize microbial interactions under

471   stressful conditions (Fuchslueger et al., 2014). However, seasonal changes in moisture or

472   nutrient availability, as well as plant phenology, dictate the need for plants to form

473   symbiotic relationships with soil microbiota (Fuchslueger et al., 2014; Kumar et al., 2018),

474   thus the interaction between plant species and microbial species may be uncoupled at

475   certain points of the year, i.e. at plant senesence.

476

477   **4.4 Summary**

478   In the context of these receent studies, our results raise speculative but interesting

479   questions for the sustainability of managed systems; how does soil bacterial diversity

480   impact crop-weed competitive interactions under stressed and ideal environmental

481   situations? Does the degree of these dependeces vary across farming systems (Johnson et

482   al., 2017)? What are the mechanisms driving the impact of soil microbial communities on

483   plant growth and multi-trophic interactions? Local environmental conditions strongly

484   affect any practical applications aimed at improving soil diversity and functionality,

485   especially in semi-arid regions where abiotic stress and seasonal variability in temperature

21

486   and water availability drive primary production. Thus, it is imperative to incorporate

487   seasonality into studies on the microbial ecology of agricultural systems.

488

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726

727   **Tables**

728

729   **Table 1 PERMANOVA model output of the effects of farming systems, date of**
730   **sampling and their interactions on soil microbial communities for OTUs at a 97%**
731   **genetic cutoff.**

732

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
		Jaccard Simmilarity (unweighted)					
Farming	2	0.8421	0.42103	1.36298	0.06524	0.0001	***
Date	4	1.4228	0.3557	1.15148	0.11024	0.0009	***
Farming:Date	8	2.3009	0.28761	0.93107	0.17828	0.5954	
Residuals	27	8.3404	0.3089		0.64624		
Total	41	12.9061			1		
		Bray-Curtis Dissimilarity (weighted)					
	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Farming	2	0.6692	0.33458	1.66846	0.07503	0.0002	**
Date	4	1.2354	0.30886	1.54019	0.13852	0.0001	**
Farming:Date	8	1.5995	0.19994	0.99705	0.17935	0.477	
Residuals	27	5.4144	0.20053		0.60709		
Total	41	8.9185			1		

733

734   **Figure Legends**

735

736   **Figure 1 Relative abundance of bacterial phyla in soil from conventional (CNT),**

737   **organic grazed (OG), and organic tilled (OT) systems during the 2016 growing season.**

738   **Samples are grouped along the x-axis by farming system, and sorted by sampling date**

739   **within the growing season.**

740

741   **Figure 2 Soil bacterial communities' A) richness, B) evenness and C) diversity indices**

742   **for from conventional (CNT, red), organic tilled (OT, blue) systems , and organic**

743   **grazed (OG, green). Error bars show Standard Error of Means (SEM) for n = 3**

744   **samples per time point and farming system.**

745

746   **Figure 3 Non-Metric Multidimensional Scaling (NMDS) of Bray-Curtis dissimilarity**

747   **for soil bacterial communities from conventional (CNT), organic tilled (OT), and**

748   **organic grazed (OG) systems, over the 2016 growing season.**

749

750   **Figure 4 Relative abundance of 97% cutoff OTUs discriminatory to farming system**

751   **[conventional (CNT), organic grazed (OG), and organic tilled (OT)], over the 2016**

752   **growing season. Samples are sorted along the x-axis by farming system and then by**
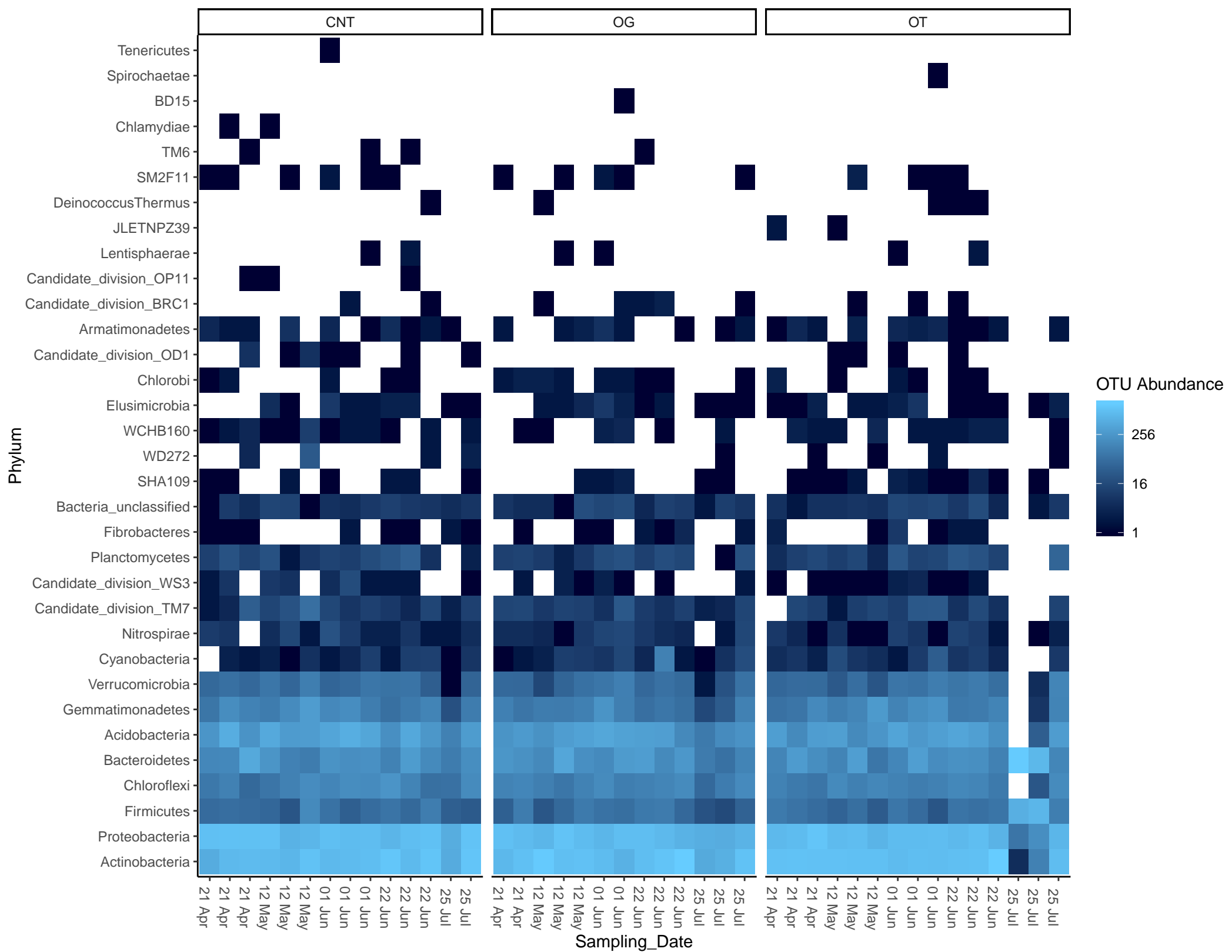
753   **time.**

754

755   **Figure 5 (A) Rarefied abundance of putative nitrogen-fixing bacterial genera in soil**

756   **from conventional (CNT), organic grazed (OG), and organic tilled (OT) farming**

757   **systems, over the 2016 growing season. (B) Importance of putative-nitrogen fixing**

758   **species in discriminating between farming system. (C) Importance of factors in**

759   **explaining abundance of *Arthrobacter* species in agricultural soil, including biomass**

760   **or plot coverage (cov) of plants at different times, as well as minimum or maximim**
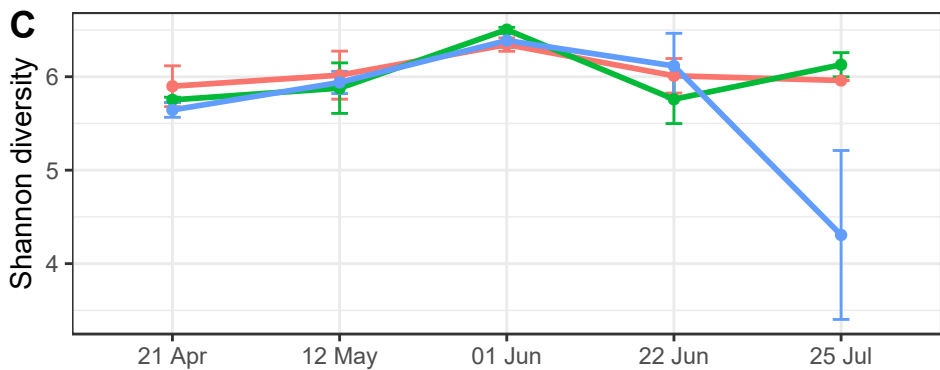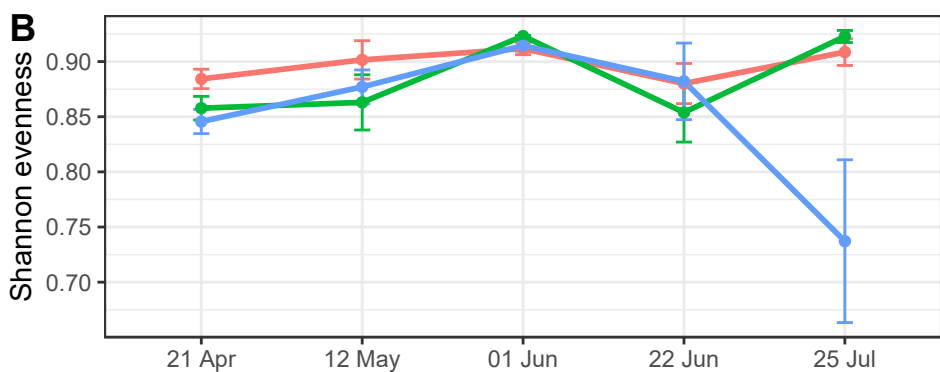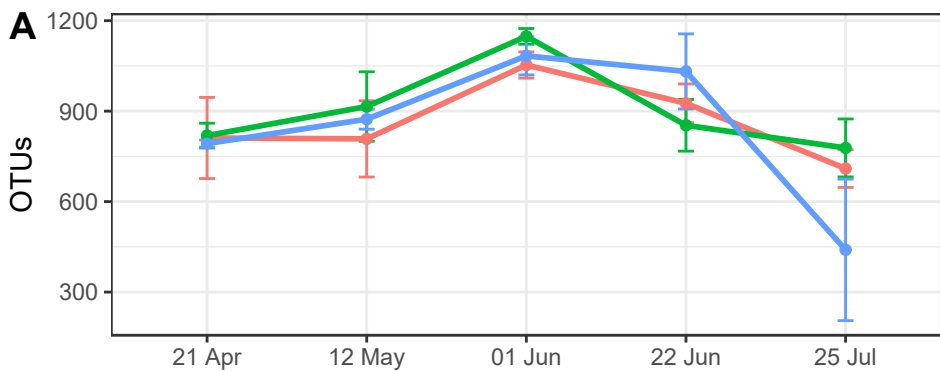
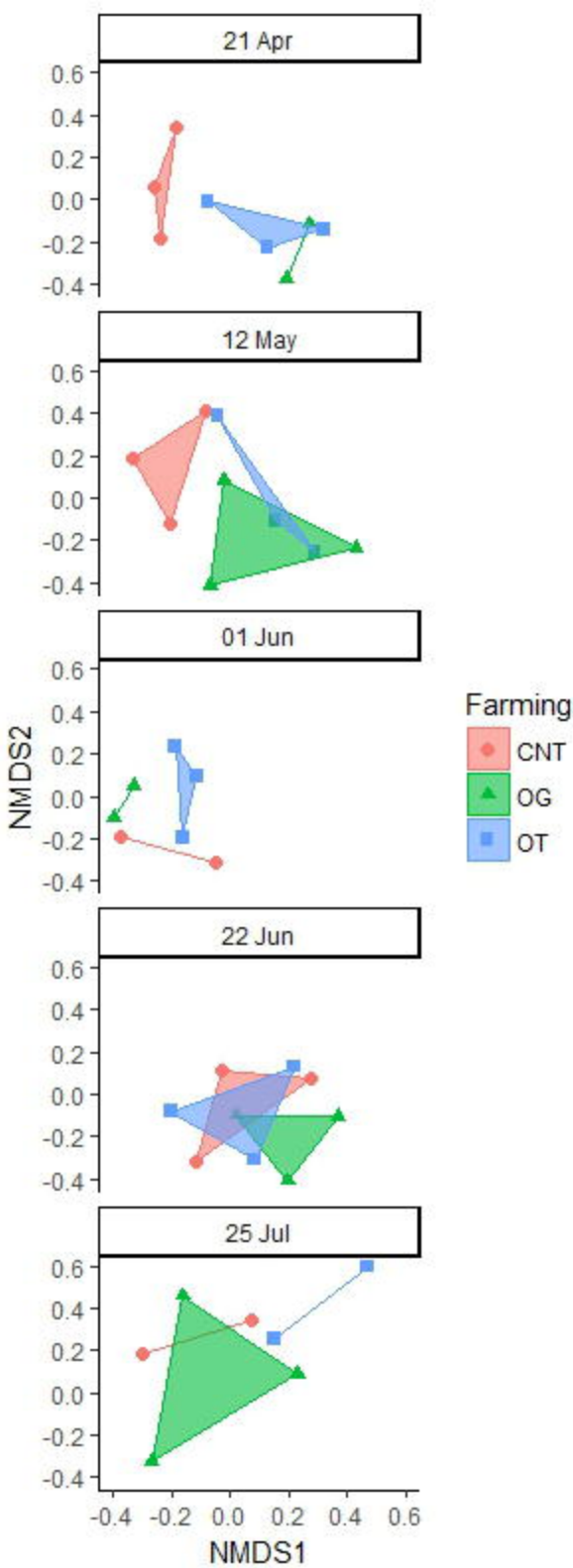761   **soil temperature (T).**

762

763   **Figure 6 Distance-based redundancy analysis (dbRDA) of significant factors**

764   **determining   Hellinger-transformed   soil   microbial   community   data   from**

765   **conventional (CNT, red), organic grazed (OG, green), and organic tilled (OT, blue)**

766   **systems**. **The model was significant: ANOVA, F = 1.4191, *p* = 0.001, as were the axes:**

767   **CAP1, F = 6.9957, *p* = 0.002; CAP2, F = 6.5696, *p* = 0.002. Significant factors**

768   **(ANOVA, *p* < 0.05) are listed in the main text.**
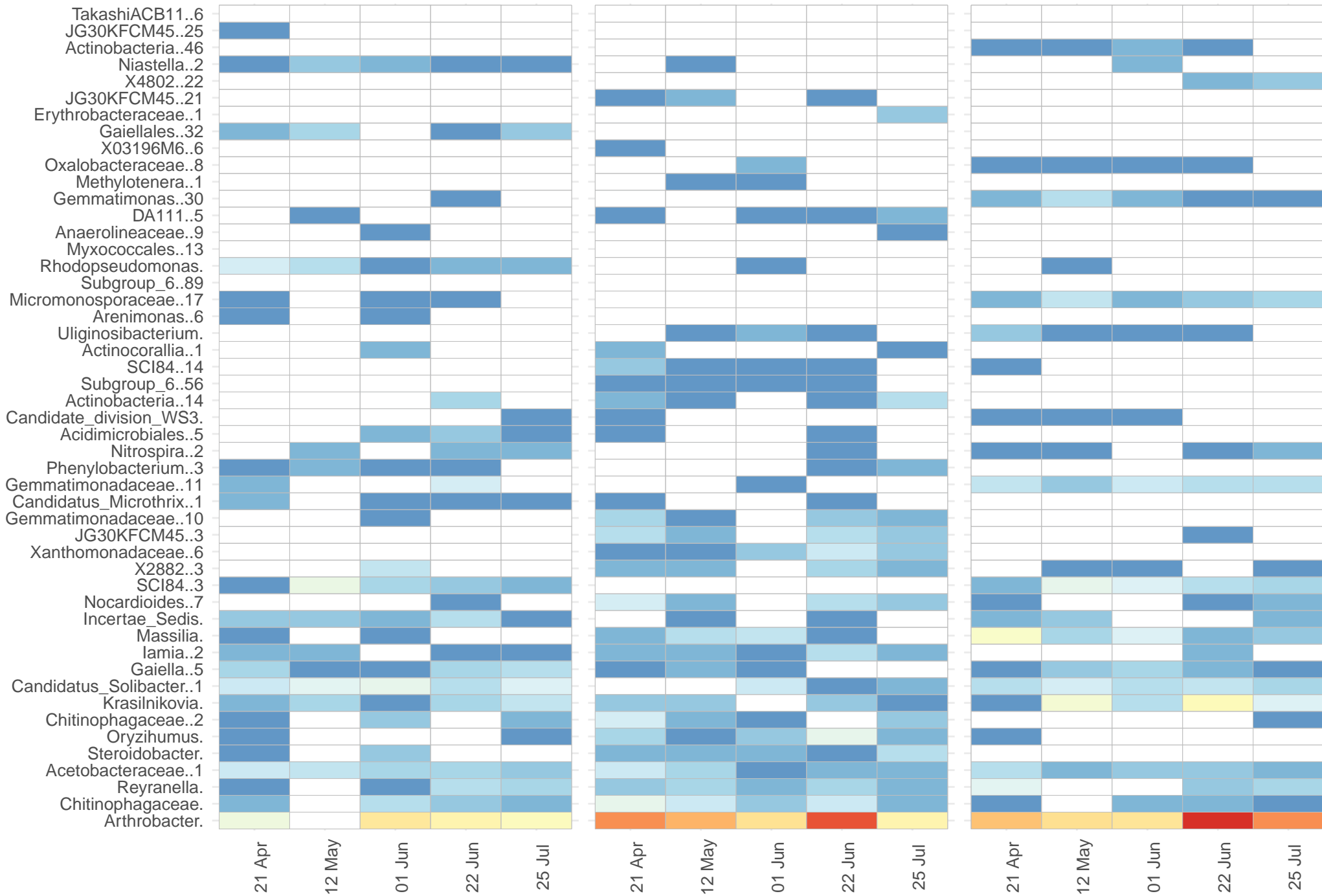
769