Title: Impacts and interactions of biochar and biosolids on agricultural soil microbial communities during dry and wet-dry cycles

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Abstract:

Extreme hydrological processes, such as prolonged drought and frequent wet-dry cycles, are major consequences of climate change and can influence agroecosystems by altering soil microbial communities and associated processes. Two increasingly popular soil amendments, biosolids and biochar, may reduce the negative impacts of extreme hydrological processes as well as provide a low-cost carbon sequestration strategy, increase nutrient and soil water retention, and increase nutrient availability. We measured the response of microbial communities to amendments of biochar (walnut shell, 900 °C) and biosolids amendments, separate and mixed, under different water regimes. We used phospholipid fatty acid analysis (PLFA) to monitor microbial communities during a 12-week incubation experiment in which we applied different levels of biochar (0, 0.5 and 1% dry wt. added to dry soil) and biosolids (0 and 0.5% air-dry wt. added to dry soil) to agricultural soils under different soil moisture conditions (wet, drought, or wet-dry cycles). Biochar increased soil pH across the soil moisture and biosolids treatments at both 4-week and 12-week. Biosolids only significantly decreased soil pH in wet treatment and the change in soil pH was within 0.5 units. Biosolid increased soil mineral nitrogen. Biochar only increased microbial biomass at week 4 in the absence of biosolids, while biosolids increased microbial biomass regardless of biochar and water regime by 55% on average over 12 weeks. Drought and wet-dry cycles strongly influenced microbial communities in soil, reducing biomass and altering community composition. Biosolids amendment increased soil nutrient level, helped maintain soil microbial biomass and reduced impacts of soil moisture stress on soil microbial community. The effect of biochar on soil microbial community composition depended on its dose and soil nutrient conditions. The co-amendment of biosolids and biochar helped to reduce the changes in soil pH and mineral nitrogen. Amending soils with biosolids or biochar may provide an effective management tool to reduce the negative impact of drought and wet-dry cycles on microbial communities.

Keywords: biochar, biosolids, drought, wet-dry cycle, microbial community, Phospholipid fatty acid analysis (PLFA)

[1]1. Introduction

Extreme hydrologic processes, such as long-term drought, extreme precipitation, and frequent wet-dry cycles may be enhanced by climate change (Groisman et al., 2005; Lesk et al., 2016). These extreme hydrologic processes can strongly influence soil microbial biomass (Baldrian et al., 2010), soil microbial community structure (Nguyen et al., 2018), and processes mediated by soil microorganisms, including greenhouse gas production (Banerjee et al., 2016), pesticide degradation (Schroll et al., 2006), nutrient cycling, and carbon sequestration (Sardans and Peñuelas, 2005). Management strategies, including application of soil amendments, may be important for reducing detrimental changes to soil microbial communities and maintaining soil health and functioning in the face of climate change.

Biochar is a carbon-rich byproduct of biomass pyrolysis under limited oxygen conditions (Lehmann and Joseph, 2009). Biochar soil application is sometimes regarded as a low-cost carbon sequestration method to mitigate global climate change (Woolf et al., 2010) and can provide some agricultural benefits, such as increasing soil cation exchange capacity (Liang et al., 2006) and reducing nutrient leaching (Laird et al., 2010), and improving soil water retention in sandy soil (Karhu et al., 2011; Novak et al., 2012). The outcomes of previous research on the impact of biochar on soil microbial communities is also inconsistent (Dempster et al., 2012; Luo et al., 2013). Biosolids are the processed solid organic byproducts of sewage treatment. Land application of biosolids is considered by some as one of the best ways to recycle nutrients and

[1] Abbreviations:
PLFA, phospholipid fatty acid analysis; CCA, canonical correspondence analysis; F:B, fungal:bacterial

65 organic matter contained in biosolids (Sánchez-Monedero et al., 2004) and have been shown to
66 provide nutrients for both microbes and plants (Prosser et al., 2014; Tian et al., 2009). Biosolids
67 can also increase soil carbon sequestration by supplying organic carbon and enhancing plant root
68 biomass (Bolan et al., 2013). However, some of the concerns of applying biosolids to soil include
69 the potential to increase nutrient runoff (Wallace et al., 2013), add pathogenic microbes to soil
70 (McCall et al., 2015), or introduce contaminants including heavy metals (Yang et al., 2014) and
71 pharmaceuticals (Al-Rajab et al., 2015). However, co-application of biochar with biosolids may
72 help mitigate some of the negative impacts of biosolids, such as by reducing the nutrient pulses
73 and nutrient leaching induced by biosolids amendment (Knowles et al., 2011) and enhancing
74 sorption of heavy metal (Zhang et al., 2013) and organic contaminants (Wang et al., 2010).

75 We investigated the potential for biosolids and biochar amendments, separately and mixed,
76 to mitigate the impacts of extreme hydrological processes on soil microbial communities. While
77 previous studies have examined the impacts of biosolids or biochar on microbial communities,
78 little attention has been given to how these inputs may mitigate effects of moisture stress in soil.
79 In laboratory incubations of an agricultural soil, we measured responses of the soil microbial
80 biomass and community composition to biochar and biosolids amendments under different water
81 regimes: wet, drought, and wet-dry cycles. We hypothesized that (1) drought and wet-dry cycle
82 treatments would reduce total microbial biomass and alter microbial community structure with
83 respect to the wet treatment; (2) biosolids would increase microbial biomass and reduce moisture
84 stress on microbial communities; (3) biochar could provide a supplemental carbon source for
85 microbial community and induce chemical stress on the soil microbial community by altering

soil chemical properties, such as soil pH, nitrogen availability; (4) co-amendment of biosolids and biochar can mitigate the change in soil chemical properties due to biochar amendment.

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2. Materials and methods

2.1. Soil, biochar and biosolids

Soil was collected from the top 15 cm of a conventionally managed, irrigated, unfertilized wheat/fallow plot (plot 6-1) at the Russell Ranch Sustainable Agricultural Research Facility (http://asi.ucdavis.edu/rr). The soil is a Yolo silt loam soil (fine-silty, mixed, nonacid, thermic Typic Xerorthent), which has been previously characterized (Wang et al., 2016). The soil texture is a silt loam (42.75% sand, 35.20% silt and 22.05% clay) with 10.18 g organic C kg^{-1}, 20.6 cmol kg^{-1} cation exchange capacity and pH of 6.7. The soil samples were homogenized, air-dried, and sieved to pass through a 2 mm mesh.

The walnut shell biochar was selected from 12 kinds of commercially or locally available biochar. We characterized the available biochars from different feedstocks and produced under different pyrolysis conditions (Mukome et al., 2013). The surface characteristics of this walnut shell biochar was better than most of other material and information about the biochar production was available. This biochar was produced at a local walnut farm and using the locally produced biochar can reduce the carbon footprint of the biochar application. The biochar was produced from walnut shells at a pyrolysis temperature of 900 $^{\circ}$C, with 227.1 m^2 g^{-1} surface area, 40% ash content, 33.4 cmol g^{-1} cation exchange capacity and pH of 9.7. Biochar was oven-dried at 105 $^{\circ}$C and sieved through a 2 mm sieve prior to use.

107 The biosolids used was a commercially available Milorganite biosolids produced by
108 Milwaukee Metropolitan Sewerage District, Milwaukee, WI. The biosolids were added as a
109 nutrient source to investigate how the soil microbial community respond to biochar amendment
110 and/or soil moisture stresses, such as drought and wet-dry cycles, under different nutrient level
111 (with or without biosolids amendment). The biosolids had been previously characterized
112 (Anderson et al., 2017) with 0.997% total nitrogen, 500.35 mg kg^{-1} ammoniacal nitrogen, 6.84
113 mg kg^{-1} nitrate nitrogen, 176 mg kg^{-1} Olsen-P and 612 mg kg^{-1} exchangeable potassium.
114 Biosolids were air-dried to reduce and homogenize its moisture content prior to use.
115
116 2.2. Incubation experiment setup
117 Mixtures of soil and various amounts of biochar and/or biosolids totaling 200 g were placed
118 in 500 mL Mason jars. Biochar doses were 0, 0.5, and 1% dry wt. added to dry soil (equivalent to
119 approximately 0, 10, and 20 t ha^{-1}). Biosolids doses were 0 and 0.5% air-dried matter to dry soil
120 (equivalent to approximately 0 and 10 t ha^{-1}).
121 The field capacity and permanent wilting point of Yolo soil were determined by measuring
122 the water retention at -33 and -1500 kPa, respectively, using pressure plate apparatus (Dane and
123 Hopmans, 2002). After soil moisture was adjusted to 80% of water holding capacity, jars were
124 equilibrated for two weeks before initiating the incubation at 23 ± 1 °C. The jars were kept in
125 dark and randomly distributed in the incubator during the incubation. Each treatment had 3
126 replicates.
127 Soil with/without biochar and biosolids mixture were well mixed with a spatula after

desired amount of water was added to make sure moisture was evenly distributed in the whole sample. After equilibration, incubations were run for 12 weeks during which time soil moisture was monitored by weighing the jars and adjusted weekly. The mason jars were loosely capped to let air flow during the incubation. As shown in Fig. 1, the wet treatment was maintained at a constant moisture content of approximately 80% of field capacity throughout the entire incubation. The drought treatment began at 80% of field capacity but received no water during the incubation and moisture content declined steadily throughout the experiment to a minimum of permanent wilting point. The moisture content of the wet-dry treatment was monitored and adjusted to the wet or drought condition every two weeks. The jars were opened and oven-dried at 30 °C to reduce soil moisture content or rewetted by spraying distilled water to reach desired soil moisture content. The soil moisture content in each of the jar was adjusted weekly by spreading water evenly on the top each jar without further disturbing the whole sample.

Destructive sampling was conducted in triplicate at 4 and 12 weeks. For wet-dry cycle treatments, sampling occurred at the end of each dry period before rewetting events.

2.3. Soil chemical properties

Soil samples (8 g) were extracted with 40 mL of 0.5 mol L^{-1} potassium sulfate in 50 mL polypropylene tubes and placed on an orbital shaker (250 rev min^{-1}, 1 h), then centrifuged (relative centrifugal force of 7969 × g for 15 min) to remove suspended solids. Dissolved organic carbon concentrations in supernatant solutions were determined by UV-persulfate oxidation (Teledyne-Tekmar Phoenix 8000). Soil pH were determined with a Mettler SevenGo Duo™

pH/Conductivity meter SG23 (Mettler Toledo, Switzerland). Spectrophotometric methods were used to determine nitrate and ammonia concentrations in the supernatant solutions (Doane and Horwáth, 2003; Verdouw et al., 1978). Soil mineral nitrogen was calculated as the sum of soil nitrate and ammonia nitrogen concentration.

2.4. Phospholipid-derived fatty acid (PLFA) analysis

Microbial community composition was measured using PLFA analysis following methods reported previously (Bartelt-Ryser et al., 2005; Buyer and Sasser, 2012). Briefly, a representative soil sample was taken at the end of the incubation, frozen (-80 °C), and then freeze-dried, yielding 8 g of dry material for lipid extraction. After initial extraction, solvents of increasing polarity were used to separate the phospholipid fraction from the neutral lipid and glycolipid fractions using solid phase extraction columns (0.58 Si; Supelco, Bellefonte, PA, USA). Fatty acids were then dried under N_2 gas, transesterified, and methylated. After methylation, the samples were dried again with N_2 gas and redissolved in hexane containing a known concentration of the internal standard 19:0. Fatty acids were then identified using the Sherlock software from Microbial Identification Systems (Microbial ID, Newark, DE, USA). Fatty acids were summed into biomarker groups as described by Bossio and Scow (1998), briefly: Gram-positive bacteria, iso and anteiso saturated branched fatty acids; Gram-negative bacteria, monounsaturated fatty acids and cyclopropyl 17:0 and 19:0; fungi, 18:2 ω6 cis and ratio of saturated to unsaturated PLFA. Total soil microbial biomass was calculated from total soil PLFAs according to the method described by Frostegard and Baath (1996)

170

171 2.5. Statistical Analyses

172 All statistical analyses were conducted with the XLSTAT Version 2018.7 add-in for
173 Microsoft Excel for Windows (Addinsoft, 2019). Statistically significant differences between
174 treatments were analyzed using analysis of variance (ANOVA) and Tukey's range test at 5%
175 significance level. One-way ANOVA was performed to test differences among biochar
176 treatments under each soil moisture and biosolids treatment at the same time point. The effect of
177 biosolids amendment and different soil moisture treatments under each biochar treatment at the
178 same time point were tested by two-way ANOVA. All reported differences were statistically
179 significant at a $P < 0.05$ level.

180 Canonical Correspondence Analysis (CCA) was applied also using conducted with the
181 XLSTAT Version 2018.7 add-in (Addinsoft, 2019) to evaluate the relationship between soil
182 properties (e.g. pH, nitrate concentration, ammonium concentration, soil type, soil aggregate
183 fractions, and biochar dose) and soil microbial community structure (all the PLFA lipids
184 detected). Monte Carlo permutation tests (999 random permutations used in the test) were
185 applied using the R package Vegan to explore multivariate differences in the PLFA data and
186 understand which factors best contributed to explaining variation in microbial communities (Jari
187 Oksanen, 2018).

188

189 3. Results

190 3.1. Soil pH and mineral nitrogen.

191 Drought and wet-dry cycle soil moisture treatments significantly increased soil pH after 12
192 weeks of incubation. The impact of biochar on soil pH depended on biochar application rate and
193 presence of biosolids. Biochar significantly increased soil pH, proportional to amount added
194 (Table 1). Biosolids amendment alone significantly reduced soil pH in the wet treatment.
195 Wet-dry cycles raised the soil pH by 1.2 units without biosolids, but only 0.6 units when
196 amended with biosolids. In combination with biochar, biosolids offset the increase in pH
197 triggered by biochar amendments and soil moisture fluctuations.

198 Biosolids significantly increased soil mineral nitrogen concentrations in all treatments,
199 while impacts of biochar were inconsistent. Biosolids always increased soil N by 275% to 628%,
200 regardless of the water regime. At week 4, biochar significantly increased soil mineral N in all
201 no-biosolids treatments, while biochar had no impact on mineral N with biosolids (Table 2). At
202 week 12, biochar only increased soil N in wet and drought treatments at low rates with no
203 biosolids, while 1% biochar reduced soil N in wet-dry cycle treatment when co-amended with
204 biosolids.

205

206 3.2. Total PLFA

207 Fifty-seven PLFAs were detected and thirty-five PLFAs (detected in more than 5% samples)
208 were used in the analysis of soil communities. Microbial biomass remained stable or decreased
209 in all treatments between 4 and 12 weeks (Table 3). Under most soil amendment combinations,
210 drought and wet-dry treatments did not significantly change microbial biomass. However, in
211 soils amended with 0.5% biochar and biosolids, the wet-dry treatment reduced microbial biomass

212 with respect to the wet treatment at 4 weeks. By 12 weeks, in soils amended with 1% biochar,
213 microbial biomass was reduced in both drought and wet-dry treatments.

214 Biochar amendment had inconsistent effects on microbial biomass, depending on biosolids
215 amendment and time, while biosolids always significantly increased microbial biomass (Table 3).
216 Without biosolids, biochar significantly increased biomass in most treatment at week 4 except
217 for 1% biochar under wet-dry treatment. However, biochar's beneficial impacts on biomass
218 diminished over time and 1% biochar decreased biomass in drought treatment at week 12.
219 Biosolids increased microbial biomass regardless of water regime at both times by 55% on
220 average.

221

222 3.3. Fungal and bacterial PLFA

223 Biochar often increases fungal:bacterial (F:B) ratio and its impact also depend on soil
224 moisture condition and biosolids amendment (Table 4). Biosolids along did not impact F:B ratio
225 under all water regimes, while increased F:B ratio in most water regimes at both times when
226 co-amended with biochar. After 4-week incubation, 0.5% biochar increased F:B ratio under
227 drought condition. Meanwhile, both 0.5% and 1% biochar increased F:B in wet-dry treatment. In
228 wet treatment, both 0.5% and 1% biochar increased F:B ratio after 12 weeks. In drought
229 treatment, 0.5% biochar increased F:B ratio, while 1% biochar significantly reduced F:B ratio
230 after 12 weeks incubation. In biosolids amended treatments, 0.5% biochar amendment
231 significantly increased the F:B ratio in all moisture treatments, and 1% biochar amendment
232 significantly increased F:B ratio in drought and wet-dry treatments after 4 weeks. Both rates of

biochar amendment significantly increased the F:B ratio after 12-week incubation in all treatments, except for the 1% biochar wet treatment.

3.4. Gram-positive to Gram-negative bacteria ratio

The ratio of Gram-positive-bacteria-associated PLFA to Gram-negative-bacteria-associated PLFA was influenced by biosolids amendment and soil moisture conditions (Table 5). Biosolids amendment significantly reduced the Gram-positive:Gram-negative PLFA ratio at both 4 and 12 weeks. In non-biosolids treatments, drought treatment significantly increased the Gram-positive:Gram-negative PLFA ratio after 4 weeks in 0 and 0.5% biochar treatments. The wet-dry cycle treatment significantly increased the Gram-positive:Gram-negative PLFA ratio after 4 weeks in 0.5% biochar treatment. However, the wet-dry cycle treatment reduced Gram-positive:Gram-negative PLFA ratio in no-biochar amendment treatment after 12-week incubation. Drought treatment significantly increased the Gram-positive:Gram-negative PLFA ratio in 1% biochar treatment after 12 weeks. In the treatments with biosolids amendment, wet-dry treatment significantly increased the Gram-positive:Gram-negative PLFA ratio in 1% biochar treatment after 4 weeks. The drought treatment increased the ratio in 1% biochar treatments after 12 weeks.

3.5. Saturated to unsaturated PLFA ratio

Saturated to unsaturated PLFA ratio, used as an indicator of microbial stress (Guckert et al., 1986; Kieft et al., 1994; Rice and Oliver, 1992), was influenced by biosolids and soil moisture

condition (Table 6). Biosolids significantly reduced saturated to unsaturated PLFA ratio at both 4 and 12 weeks. In non-biosolids treatments, wet-dry treatments significantly reduced saturated : unsaturated PLFA ratio in 0.5 and 1% biochar treatments after 4 weeks incubation. Wet-dry treatment also reduced the ratio in no biochar treatment after 12 weeks. Presence of biosolids in the wet-dry cycle treatment increased the ratio in both 0.5 and 1% biochar treatments. Biochar amendment generally had no impact or reduced the ratio of saturated:unsaturated PLFAs.

3.5 Actinomycete PLFA

Actinomycete PLFA was influenced by soil moisture conditions in several treatments. In non-biosolids and biochar amended treatments, significantly reduced actinomycete-associated PLFA was observed in wet-dry and drought treatments with 1% biochar after 12 weeks (Table S1). In non-biosolids and biochar-amended treatments, significantly lower actinomycete PLFA was observed in dry and wet-dry cycle treatments after 4 weeks. In biosolids-amended treatments with no biochar added, actinomycete-associated PLFA was significantly higher in the drought treatment after 4 weeks.

3.6. Microbial community composition by canonical correspondence analysis

Canonical correspondence analysis (CCA) provided more detailed analysis for how the overall community composition responded to different inputs, water treatments and time (Fig. 2 and 3). The first two CCA axes explained 50.4% and 24.2% of the variation in composition at

week 4 and explained 46.9% and 28.4% of the variation in composition at week 12. Based on the Monte Carlo permutation test, community composition was most strongly influenced by rate of biosolids amendment, followed by nitrate concentration, soil pH, dissolved organic carbon, biochar rate, ammonium concentration and water treatments. All treatments receiving biosolids clearly separated from those without biosolids, independent of biochar and water treatment, along axis 1. Treatments without biosolids clearly separated based on whether or not they received biochar at 4 weeks, while this was not the case at week 12. Water regime was weakly associated with some groupings of treatments along axis 2. Biosolids treatments showed patterns similar to the no-biosolids treatments, though with much greater scatter among biochar treatments along axis 2. Treatments with different soil moisture conditions separated better when comparing within the same biochar treatment.

4. Discussion

4.1. Impact of soil moisture treatments on microbial community

Soil moisture stresses imposed in the form of simulated drought and repeated wet-dry cycles often caused insignificant changes in microbial biomass but altered soil microbial community composition. Impacts of drought on soil microorganisms include direct effects through physical osmotic stress and changes in microbial secondary metabolite production, as well as indirect effects through altered nutrient availability, changes in redox and pH (Naylor and Coleman-Derr, 2018). Frequent wet-dry cycles also alter soil properties, such as disrupt soil aggregate structure and release more organic C and other nutrients (Denef et al., 2001; Miller et

296 al., 2005), which in turn can benefit microbial communities and allow for their stabilization.
297 After multiple wet-dry cycles, the microbes can primarily metabolize the released C to
298 synthesize cell polymers, including cell wall constituents, to accommodate to the moisture
299 situation, and communities can shift to favor microbes best adapted to alternating moisture
300 conditions (Borken and Matzner, 2009; Manzoni et al., 2012; Schimel et al., 2007). Thus, we
301 speculate after multiple wet-dry cycles, the microbial community will stabilize and be dominated
302 by certain species resistant to both desiccation and rewet stresses, and newly available nutrients
303 released by physical disturbance of soil aggregates may provide extra nutrients essential for
304 sustaining the metabolic demands of moisture stress adaptations. Previous research also reported
305 that with repeated exposure to wet-dry cycles, soil microbial community composition became
306 stable and adapted to such moisture condition (Evans and Wallenstein, 2012; Fierer et al., 2003;
307 Lundquist et al., 1999), supporting our observations and hypotheses. However, to confirm the
308 establishment of a stable microbial community would require a longer period of measurement
309 which was not possible in our study.

310 The composition of soil microbial communities responds faster than does the total microbial
311 biomass to soil moisture stress, due to selective enrichment or repression of specific groups
312 under those conditions resulting from differential moisture stress responses of different types of
313 microbes. For instance, Gram-positive bacteria often grow slower than Gram-negative bacteria
314 (Cescutti, 2010), while Gram-positive bacteria were more tolerant to environmental stresses,
315 including drought, than gram-negative bacteria (Higgins and Dworkin, 2012; Uhlířová et al.,
316 2005). Some microbes, Gram-positive bacteria, actinomycetes (Zenova et al., 2007) and some

317 fungi (Kogej et al., 2007) for instance, were more tolerant of moisture stress among microbes,
318 while other microbes, such as Gram-negative bacteria (Fierer et al., 2003), are more sensitive to
319 soil moisture stress. Bastida et al. (2017) also found that the diversity and composition of the
320 bacterial community, together with enzyme activities, quickly responded to drought stress, while
321 soil microbial biomass remained the same. In addition, changes in soil chemical and physical
322 characteristics resulting from different soil moisture conditions may favor microorganisms
323 occupying specific functional niches. For example, reduced substrate availability under drought
324 conditions likely favors specialists and oligotrophs and reduced anaerobic conditions may be
325 detrimental to fermenters and other anaerobes (Naylor and Coleman-Derr, 2018).

326 We expected to see consistent changes in biomarkers which indicated water stress-tolerant
327 microorganisms in water stresses implemented treatments. However, while nearly all treatments
328 did showed significant changes in at least one of the measured biomarkers (fungal:bacterial
329 PLFA, Gram-positive:Gram-negative PLFA, actinomycete PLFA, and saturated:unsaturated
330 PLFA), only the wet-dry with biosolids and 0.5% and 1% biochar treatments at 4 weeks showed
331 a change consistent across multiple groups. In these treatments, all biomarkers except
332 actinomycete PLFA (no change, Table S1) increased. This may be that the higher nutrient
333 availability due to biosolids amendment allowed relatively rapid growth of microbial groups
334 suited to drought conditions. While the PLFA biomarkers we examined didn't show consistent
335 changes, the CCA showed clear clustering by moisture, biochar, and biosolids treatments (Fig. 2
336 and 3). It may be that the broad biomarkers we considered lacked sufficient resolution and thus
337 couldn't detect changes that may have occurred in small groups. This is consistent with a

small-subunit rRNA sequencing study of California grassland soils, which showed that changes in community composition in response to drought were dominated by a small number of phyla (Barnard et al., 2013).

4.2. Impact of biochar amendment on microbial community

The impact of biochar on microbial communities depends on the soil nutrient availability, controlled by biosolids amendment in this experiment, and on dose. Our results indicated that when soil nutrient availability was low, the dominant impact of low rate biochar amendment on soil microbial community was positive, while the dominant impact of high-rate biochar amendment on soil microbial community was negative. However, our results also showed that impacts of biochar on microbial community were negative regardless of biochar dose when soil nutrient level was high. Low biochar amendment may benefit both fungi and bacteria, while higher amount of biochar can inhibit microbes. We speculate that low biochar amendment in nutrient-poor soil can reduce microbial nutrient stress, while at higher levels, biochar amendment can also induce chemical stresses to the microbial community. For example, biochar can be a source of nutrients (Biederman and Harpole, 2013; Xu et al., 2013; Yuan et al., 2016), but polycyclic aromatic hydrocarbon in biochar can be toxic (Sun et al., 2012), while the biochar elevated soil pH (Rousk et al., 2010) and soil ionic strength (Rath and Rousk, 2015) can also modify the microbial community (Khodadad et al., 2011; Mitchell et al., 2015; Wang et al., 2015). In soils with high nutrient availability, these sources of stress from biochar may be more pronounced because there are no nutrient limitations to be lifted by the biochar. The walnut shell

biochar was a high pH and high potassium content biochar produced at high temperature (Mukome et al., 2013). Thus, its impacts on soil microbial community might not be common among other biochars.

4.3. Impact of biosolids on microbial community

Presence of biosolids appeared to offset some of the effects of moisture stress and biochar on microbial communities, as evidenced by the lack of difference in total microbial biomass in biosolids-amended soils, regardless of water and biochar treatment. The influence of biosolids on soil nutrient level also depended on soil moisture condition, as severe soil moisture stress can also diminish nutrient availability (Dungait et al., 2012). Manzoni et al. (2012) reported that under decreasing soil moisture conditions, solute diffusion in soil may become the most limiting factor of microbial activities.

The positive effect of biosolids might be due to relief of nutrient stress, which becomes more pronounced at low moisture conditions, allowing microbial communities to invest in drought adaptations, which are resource-intensive (Manzoni et al., 2012). While greater nutrient availability may help to reduce the negative impacts associated with the high metabolic demands of adaptation to moisture stress, different adaptation strategies employed by different organisms still incur different costs and may favor the competitiveness of some organisms over others, causing shifts in community composition (Borken and Matzner, 2009; Schimel et al., 2007).

Co-amendment of biochar and biosolids can help maintain the stability of soil microbial communities by mitigating the abrupt change in soil chemical properties. Biosolids helped

reduce the change in soil pH induced by biochar (Table 1), which helped avoid the negative impact of abrupt change in soil pH on soil microbial biomass (Table 3) and community composition (Fig. 2 and 3). Biochar also retained mineral N released from biosolids (Table 2) and become slow-release N source for microbial communities (Ding et al., 2016; Hagemann et al., 2017) Knowles et al. (2011) also reported that biochar reduced soil N leaching in biosolids amended two silt loam soils under field condition.

5. Conclusion

More frequent and intense drought and wet-dry cycles in soil induced by global change are known to impact microbial biomass and microbial community composition. Organic amendments that provide C and nutrients, such as biosolids, may reduce the negative consequences of frequent and intense extreme hydrologic conditions by helping maintain a high and active soil microbial biomass. The organic inputs of biosolids help to offset nutrient diffusion limitations and provide substrate for microbes to meet the higher metabolic demands required for adapting to moisture stress.

Benefits of adding biochar were less clear; under some conditions, there may be benefits to microbial communities, but in our study, it was dependent on biochar dose and soil nutrient status. The higher dose decreased microbial biomass, possibly due to its own toxicity to the microbial community. The co-amendment of biochar and biosolids can mitigate the alternation in soil chemical properties, such as soil pH and mineral nitrogen, so as to maintain soil microbial community stability. A co-benefit of using these amendments, particularly biosolids, is that it

creates a beneficial use of what might otherwise pose a waste disposal challenge; however, potential impacts of co-contaminants in biosolids must be considered before adopting these practices particularly in agricultural soils (Godlewska et al., 2017). To conclude, we found that both biosolids and biochar amendments hold promise for mitigating extreme hydrologic events exacerbated by climate change, but further research is needed to identify specific conditions and management practices for implementation.

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582 Table 1. Soil pH after 4 and 12 weeks incubation with and without biosolids, with and
583 without the addition of walnut shell biochar at varying application rates (doses). In each
584 column, the numbers to the right of each value represent the standard error about the
585 mean. Significant differences ($P < 0.05$) between treatments are indicated by different
586 letters in parentheses to the right of each value. The letter by each value indicated
587 significant differences between soil moisture by biosolids treatment within the same time
588 point and the letter in the brackets indicated significant differences between biochar doses
589 under each soil moisture and biosolids treatment.
590

Time	Biosolids application rate	Soil moisture	Biochar application rate		
			0%	0.5%	1%
4 week	0%	wet	6.61 ± 0.070 b (a)	7.79 ± 0.060 c (b)	7.94 ± 0.055 b (b)
		dry	6.88 ± 0.023 c (a)	7.69 ± 0.026 bc (b)	8.07 ± 0.035 c (c)
		wet-dry cycle	6.75 ± 0.060 bc (a)	7.73 ± 0.085 c (b)	8.16 ± 0.045 c (c)
	0.5%	wet	6.17 ± 0.0058 a (a)	7.49 ± 0.038 ab (b)	7.68 ± 0.021 a (c)
		dry	6.25 ± 0.067 a (a)	7.45 ± 0.095 a (b)	7.78 ± 0.065 a (c)
		wet-dry cycle	6.14 ± 0.056 a (a)	7.65 ± 0.11 abc (b)	8.14 ± 0.020 c (c)
12 week	0%	wet	6.19 ± 0.032 b (a)	7.26 ± 0.055 a (b)	7.84 ± 0.072 ab (c)
		dry	7.10 ± 0.045 d (a)	7.57 ± 0.081 bc (b)	8.15 ± 0.070 c (c)
		wet-dry cycle	7.40 ± 0.046 e (a)	7.64 ± 0.040 c (b)	8.07 ± 0.025 c (c)
	0.5%	wet	5.93 ± 0.031 a (a)	7.17 ± 0.025 a (b)	7.69 ± 0.032 a (c)
		dry	6.47 ± 0.045 c (a)	7.49 ± 0.050 b (b)	7.84 ± 0.040 b (c)
		wet-dry cycle	6.48 ± 0.031 c (a)	7.46 ± 0.036 b (b)	7.85 ± 0.064 b (c)

591

Table 2. Soil mineral nitrogen (in mg-N/kg soil) after 4 and 12 weeks incubation with and without biosolids, with and without the addition of walnut shell biochar at varying application rates (doses). In each column, the numbers to the right of each value represent the standard error about the mean. Significant differences ($P < 0.05$) between treatments are indicated by different letters in parentheses to the right of each value. The letter by each value indicated significant differences between soil moisture by biosolids treatment within the same time point and the letter in the brackets indicated significant differences between biochar doses under each soil moisture and biosolids treatment.

Time	Biosolids application rate	Soil moisture	Biochar application rate		
			0%	0.5%	1%
4 week	0%	wet	10.18 ± 0.47 a (a)	16.89 ± 1.14 a (b)	15.35 ± 0.89 a (b)
		dry	12.14 ± 0.99 a (a)	17.71 ± 0.30 a (c)	14.24 ± 0.46 a (b)
		wet-dry cycle	8.91 ± 1.71 a (a)	16.94 ± 0.17 a (b)	16.19 ± 1.34 a (b)
	0.5%	wet	50.19 ± 9.66 b (a)	50.73 ± 5.99 b (a)	55.19 ± 1.38 b (a)
		dry	50.16 ± 5.86 b (a)	62.67 ± 4.08 bc (b)	58.39 ± 4.07 b (ab)
		wet-dry cycle	64.79 ± 8.30 b (a)	75.12 ± 11.64 c (a)	84.39 ± 0.90 c (a)
12 week	0%	wet	25.13 ± 0.83 a (a)	30.85 ± 2.87 a (b)	29.29 ± 0.88 a (ab)
		dry	24.62 ± 1.10 a (a)	29.97 ± 1.77 a (b)	22.08 ± 0.33 a (a)
		wet-dry cycle	24.17 ± 0.83 a (a)	29.96 ± 1.96 a (a)	27.81 ± 8.02 a (a)
	0.5%	wet	108.76 ± 42.08 b (a)	115.58 ± 2.18 b (a)	104.24 ± 16.91 b (a)
		dry	128.95 ± 14.06 b (a)	123.14 ± 3.13 c (a)	135.69 ± 10.11 c (a)
		wet-dry cycle	125.67 ± 6.74 b (b)	117.42 ± 2.56 bc (ab)	104.52 ± 7.12 b (a)

602 Table 3. Total PLFA (in nmol g^{-1}) at 4 and 12 weeks incubation with and without
603 biosolids, with and without the addition of walnut shell biochar at varying application
604 rates (doses). In each column, the numbers to the right of each value represent the
605 standard error about the mean. Significant differences ($P < 0.05$) between treatments are
606 indicated by different letters in parentheses to the right of each value. The letter by each
607 value indicated significant differences between soil moisture by biosolids treatment
608 within the same time point and the letter in the brackets indicated significant differences
609 between biochar doses under each soil moisture and biosolids treatment.
610

Time	Biosolids application rate	Soil moisture	Biochar application rate		
			0%	0.5%	1%
4 week	0%	wet	23.74 ± 0.85 a (a)	29.14 ± 2.18 a (b)	28.15 ± 1.53 ab (b)
		dry	22.35 ± 0.51 a (a)	31.11 ± 1.28 a (c)	25.10 ± 0.15 ab (b)
		wet-dry cycle	19.61 ± 0.57 a (a)	27.69 ± 2.65 a (b)	22.60 ± 1.07 a (a)
	0.5%	wet	39.61 ± 2.33 b (a)	39.30 ± 1.22 b (a)	34.58 ± 3.79 b (a)
		dry	43.56 ± 3.21 b (a)	38.28 ± 2.01 b (a)	33.81 ± 8.99 ab (a)
		wet-dry cycle	42.49 ± 4.77 b (b)	32.35 ± 0.61 a (a)	34.44 ± 1.77 b (a)
12 week	0%	wet	22.84 ± 3.79 a (a)	27.86 ± 3.49 ab (a)	26.94 ± 1.84 b (a)
		dry	23.41 ± 1.63 a (b)	23.44 ± 0.015 a (b)	18.94 ± 1.41 a (a)
		wet-dry cycle	21.68 ± 2.29 a (a)	20.22 ± 3.23 a (a)	21.03 ± 0.98 a (a)
	0.5%	wet	38.96 ± 4.62 b (a)	39.17 ± 1.55 c (a)	31.51 ± 2.96 b (a)
		dry	38.46 ± 1.50 b (b)	34.65 ± 2.62 bc (b)	29.52 ± 1.25 b (a)
		wet-dry cycle	39.88 ± 1.64 b (b)	33.15 ± 5.01 bc (ab)	31.49 ± 1.16 b (a)

611

Table 4. Fungal PLFA to bacterial PLFA ratio at 4 and 12 weeks incubation with and without biosolids, with and without the addition of walnut shell biochar at varying application rates (doses). In each column, the numbers to the right of each value represent the standard error about the mean. Significant differences ($P < 0.05$) between treatments are indicated by different letters in parentheses to the right of each value. The letter by each value indicated significant differences between soil moisture by biosolids treatment within the same time point and the letter in the brackets indicated significant differences between biochar doses under each soil moisture and biosolids treatment.

Time	Biosolids application rate	Soil moisture	Biochar application rate		
			0%	0.5%	1%
4 week	0%	wet	0.066 ± 0.0071 ab (a)	0.077 ± 0.0090 a (a)	0.083 ± 0.0043 ab (a)
		dry	0.066 ± 0.0070 ab (a)	0.097 ± 0.013 ab (b)	0.088 ± 0.0073 b (ab)
		wet-dry cycle	0.077 ± 0.0037 b (a)	0.090 ± 0.0038 ab (b)	0.088 ± 0.0035 b (b)
	0.5%	wet	0.057 ± 0.0025 a (a)	0.11 ± 0.014 b (b)	0.067 ± 0.0026 a (a)
		dry	0.063 ± 0.0035 ab (a)	0.11 ± 0.0060 b (c)	0.081 ± 0.010 ab (b)
		wet-dry cycle	0.076 ± 0.0075 b (a)	0.14 ± 0.012 c (c)	0.11 ± 0.011 c (b)
12 week	0%	wet	0.058 ± 0.0040 a (a)	0.075 ± 0.0017 a (b)	0.078 ± 0.0020 b (b)
		dry	0.061 ± 0.0017 ab (b)	0.074 ± 0.0010 a (c)	0.052 ± 0.0043 a (a)
		wet-dry cycle	0.076 ± 0.0035 b (a)	0.073 ± 0.0034 a (a)	0.069 ± 0.0084 ab (a)
	0.5%	wet	0.056 ± 0.0082 a (a)	0.082 ± 0.0054 a (b)	0.067 ± 0.0074 ab (ab)
		dry	0.056 ± 0.0037 a (a)	0.078 ± 0.0034 a (b)	0.087 ± 0.0092 b (b)
		wet-dry cycle	0.064 ± 0.0084 ab (a)	0.10 ± 0.0074 b (b)	0.14 ± 0.015 c (c)

Table 5. Gram$^+$ bacteria to Gram$^-$ bacteria ratio at 4 and 12 weeks incubation with and without biosolids, with and without the addition of walnut shell biochar at varying application rates (doses). In each column, the numbers to the right of each value represent the standard error about the mean. Significant differences ($P < 0.05$) between treatments are indicated by different letters in parentheses to the right of each value. The letter by each value indicated significant differences between soil moisture by biosolids treatment within the same time point and the letter in the brackets indicated significant differences between biochar doses under each soil moisture and biosolids treatment.

Time	Biosolids application rate	Soil moisture	Biochar application rate		
			0%	0.5%	1%
4 week	0%	wet	1.26 ± 0.091 b (a)	1.22 ± 0.033 c (a)	1.22 ± 0.053 bc (a)
		dry	1.37 ± 0.0087 a (c)	1.30 ± 0.015 a (b)	1.18 ± 0.0075 a (a)
		wet-dry cycle	1.29 ± 0.017 c (a)	1.28 ± 0.0072 d (a)	1.27 ± 0.020 d (a)
	0.5%	wet	1.07 ± 0.034 a (ab)	1.01 ± 0.021 a (a)	1.10 ± 0.034 b (b)
		dry	1.08 ± 0.015 ab (b)	1.01 ± 0.0020 d (a)	1.11 ± 0.0056 c (b)
		wet-dry cycle	1.08 ± 0.017 a (a)	1.11 ± 0.025 b (a)	1.21 ± 0.0066 a (b)
12 week	0%	wet	1.51 ± 0.066 c (a)	1.50 ± 0.0091 c (a)	1.45 ± 0.012 bc (a)
		dry	1.48 ± 0.0076 a (ab)	1.48 ± 0.018 a (a)	1.56 ± 0.054 a (b)
		wet-dry cycle	1.36 ± 0.027 d (a)	1.41 ± 0.0094 c (b)	1.49 ± 0.014 d (c)
	0.5%	wet	1.25 ± 0.081 a (a)	1.30 ± 0.028 ab (a)	1.33 ± 0.015 b (a)
		dry	1.35 ± 0.027 d (ab)	1.32 ± 0.037 bc (a)	1.41 ± 0.023 c (b)
		wet-dry cycle	1.32 ± 0.025 b (a)	1.30 ± 0.071 a (a)	1.30 ± 0.013 a (a)

Table 6. Ratio of saturated to unsaturated PLFA ratio after 4 and 12 weeks incubation with and without biosolids, with and without the addition of walnut shell biochar at varying application rates (doses). In each column, the numbers to the right of each value represent the standard error about the mean. Significant differences ($P < 0.05$) between treatments are indicated by different letters in parentheses to the right of each value. The letter by each value indicated significant differences between soil moisture by biosolids treatment within the same time point and the letter in the brackets indicated significant differences between biochar doses under each soil moisture and biosolids treatment.

Time	Biosolids application rate	Soil moisture	Biochar application rate		
			0%	0.5%	1%
4 week	0%	wet	0.48 ± 0.023 b (b)	0.42 ± 0.0089 b (a)	0.41 ± 0.0072 a (a)
		dry	0.49 ± 0.0063 b (c)	0.44 ± 0.0065 bc (b)	0.41 ± 0.0044 a (a)
		wet-dry cycle	0.50 ± 0.0065 b (b)	0.45 ± 0.011 c (a)	0.44 ± 0.012 b (a)
	0.5%	wet	0.43 ± 0.0014 a (b)	0.39 ± 0.0071 a (a)	0.40 ± 0.010 a (a)
		dry	0.42 ± 0.0038 a (b)	0.39 ± 0.00084 a (a)	0.39 ± 0.0041 a (a)
		wet-dry cycle	0.44 ± 0.0019 a (a)	0.43 ± 0.0060 b (a)	0.44 ± 0.011 b (a)
12 week	0%	wet	0.57 ± 0.021 c (a)	0.53 ± 0.0070 b (a)	0.53 ± 0.046 bc (a)
		dry	0.55 ± 0.0072 bc (b)	0.51 ± 0.0036 b (a)	0.54 ± 0.015 c (ab)
		wet-dry cycle	0.52 ± 0.0088 ab (a)	0.52 ± 0.014 b (a)	0.52 ± 0.010 abc (a)
	0.5%	wet	0.51 ± 0.026 ab (a)	0.49 ± 0.010 a (a)	0.48 ± 0.0073 ab (a)
		dry	0.50 ± 0.024 a (a)	0.48 ± 0.0061 a (a)	0.48 ± 0.0069 ab (a)
		wet-dry cycle	0.50 ± 0.0059 a (b)	0.48 ± 0.014 a (ab)	0.47 ± 0.0074 a (a)

Figure Captions:

Figure 1. Diagram of soil moisture change in different water treatments.

Figure 2. The impact of biochar, biosolids and environmental factors on soil microbial community composition after 4 weeks incubation, as determined by canonical-correlation analysis (abbreviations in the legends, w for wet, d for drought, c for wet-dry cycles, 0 for 0% biochar, 1 for 0.5% biochar, 2 for 1% biochar, - for 0% biosolids, + for 0.5% biosolids;). In the legends, color represents soil moisture treatments (blue for wet, yellow for wet-dry cycles, red for drought), shape represents biochar treatments (triangle for 0% biochar, square for 0.5% biochar, circle for 1% biochar), filling of symbols represents biosolids treatment (hollow for 0% biosolids, solid for 0.5% biosolids). Arrows represent the vectors for key soil chemical properties, soil amendments and soil moisture treatments.

Figure 3. The impact of biochar, biosolids and environmental factors on soil microbial community composition after 12 weeks incubation, as determined by canonical-correlation analysis (abbreviations in the legends, w for wet, d for drought, c for wet-dry cycles, 0 for 0% biochar, 1 for 0.5% biochar, 2 for 1% biochar, - for 0% biosolids, + for 0.5% biosolids;). In the legends, color represents soil moisture treatments (blue for wet, yellow for wet-dry cycles, red for drought), shape represents biochar treatments (triangle for 0% biochar, square for 0.5% biochar, circle for 1% biochar), filling of symbols represents biosolids treatment (hollow for 0% biosolids, solid for 0.5% biosolids). Arrows represent the vectors for key soil chemical properties, soil amendments and soil moisture treatments.

Supplemental material:

Table S1. Actinomycete PLFA (in nmol g^{-1}) at 4 and 12 weeks incubation with and without biosolids, with and without the addition of walnut shell biochar at varying application rates (doses). In each column, the numbers to the right of each value represent the standard error about the mean. Significant differences ($P < 0.05$) between treatments are indicated by different letters in parentheses to the right of each value. The letter by each value indicated significant differences between soil moisture by biosolids treatment within the same time point and the letter in the brackets indicated significant differences between biochar doses under each soil moisture and biosolids treatment.

Time	Biosolids application rate	Soil moisture	Biochar application rate		
			0%	0.5%	1%
4 week	0%	wet	1.91 ± 0.11 a (a)	2.26 ± 0.12 a (b)	2.27 ± 0.17 a (b)
		dry	1.84 ± 0.044 a (a)	2.39 ± 0.089 a (c)	2.08 ± 0.0080 a (b)
		wet-dry cycle	1.61 ± 0.034 a (a)	2.34 ± 0.19 a (b)	2.06 ± 0.084 a (b)
	0.5%	wet	2.51 ± 0.077 b (a)	2.51 ± 0.037 a (a)	2.59 ± 0.25 a (a)
		dry	2.93 ± 0.16 c (a)	2.46 ± 0.090 a (a)	2.60 ± 0.72 a (a)
		wet-dry cycle	2.82 ± 0.25 bc (b)	2.28 ± 0.019 a (a)	2.59 ± 0.073 a (ab)
12 week	0%	wet	1.93 ± 0.29 a (a)	2.38 ± 0.29 ab (a)	2.38 ± 0.13 b (a)
		dry	2.00 ± 0.13 a (a)	2.12 ± 0.024 ab (a)	1.93 ± 0.13 a (a)
		wet-dry cycle	1.81 ± 0.20 a (a)	1.75 ± 0.25 a (a)	2.00 ± 0.076 a (a)
	0.5%	wet	2.76 ± 0.32 b (a)	3.06 ± 0.16 c (a)	2.68 ± 0.18 b (a)
		dry	3.03 ± 0.11 b (a)	2.68 ± 0.27 bc (a)	2.66 ± 0.076 b (a)
		wet-dry cycle	3.07 ± 0.075 b (b)	2.53 ± 0.34 bc (a)	2.67 ± 0.11 b (ab)

Table S2. Gram^{+} bacteria PLFA (in nmol g^{-1}) after 4 and 12 weeks incubation with and without biosolids, with and without the addition of walnut shell biochar at varying application rates (doses). In each column, the numbers to the right of each value represent the standard error about the mean. Significant differences ($P < 0.05$) between treatments are indicated by different letters in parentheses to the right of each value. The letter by each value indicated significant differences between soil moisture by biosolids treatment within the same time point and the letter in the brackets indicated significant differences between biochar doses under each soil moisture and biosolids treatment.

Time	Biosolids application rate	Soil moisture	Biochar application rate		
			0%	0.5%	1%
4 week	0%	wet	7.2 ± 0.2 a (a)	8.6 ± 0.8 a (b)	8.2 ± 0.3 ab (ab)
		drought	6.9 ± 0.1 a (a)	9.3 ± 0.3 abc (b)	7.3 ± 0.01 ab (a)
		wet-dry cycle	5.7 ± 0.1 a (a)	8.2 ± 0.8 a (b)	6.7 ± 0.3 a (a)
	0.5%	wet	11.2 ± 0.6 b (a)	10.6 ± 0.2 c (a)	10.0 ± 1.3 b (a)
		drought	12.2 ± 0.8 b (a)	10.3 ± 0.5 bc (a)	9.7 ± 2.5 ab (a)
		wet-dry cycle	11.7 ± 1.5 b (b)	9.0 ± 0.06 ab (a)	10.1 ± 0.5 b (ab)
12 week	0%	wet	7.2 ± 1.1 a (a)	8.8 ± 1.1 bc (a)	8.4 ± 0.5 b (a)
		drought	7.2 ± 0.5 a (ab)	7.2 ± 0.06 ab (b)	6.2 ± 0.5 a (a)
		wet-dry cycle	6.4 ± 0.7 a (a)	6.2 ± 1.0 a (a)	6.7 ± 0.3 a (a)
	0.5%	wet	11.2 ± 1.3 b (a)	11.7 ± 0.4 d (a)	9.7 ± 0.8 b (a)
		drought	11.5 ± 0.4 b (b)	10.4 ± 0.9 cd (ab)	9.2 ± 0.4 b (a)
		wet-dry cycle	11.8 ± 0.3 b (b)	9.7 ± 1.2 cd (a)	9.3 ± 0.3 b (a)

693 Table S3. Gram^{-} bacteria PLFA (in nmol g^{-1}) after 4 and 12 weeks incubation with and
694 without biosolids, with and without the addition of walnut shell biochar at varying
695 application rates (doses). In each column, the numbers to the right of each value represent
696 the standard error about the mean. Significant differences ($P < 0.05$) between treatments
697 are indicated by different letters in parentheses to the right of each value. The letter by
698 each value indicated significant differences between soil moisture by biosolids treatment
699 within the same time point and the letter in the brackets indicated significant differences
700 between biochar doses under each soil moisture and biosolids treatment.
701

Time	Biosolids application rate	Soil moisture	Biochar application rate		
			0%	0.5%	1%
4 week	0%	wet	5.7 ± 0.4 a (a)	7.1 ± 0.5 ab (b)	6.8 ± 0.5 abc (ab)
		drought	5.1 ± 0.1 a (a)	7.2 ± 0.3 ab (c)	6.2 ± 0.03 ab (b)
		wet-dry cycle	4.4 ± 0.1 a (a)	6.4 ± 0.6 a (b)	5.3 ± 0.2 a (a)
	0.5%	wet	10.5 ± 0.7 b (a)	10.5 ± 0.4 c (a)	9.1 ± 0.8 c (a)
		drought	11.3 ± 0.9 b (a)	10.2 ± 0.5 c (a)	8.8 ± 2.2 bc (a)
		wet-dry cycle	10.8 ± 1.3 b (b)	8.1 ± 0.2 b (a)	8.4 ± 0.5 bc (a)
12 week	0%	wet	4.8 ± 0.9 a (a)	5.8 ± 0.7 ab (a)	5.8 ± 0.3 b (a)
		drought	4.8 ± 0.4 a (b)	4.9 ± 0.02 a (b)	4.0 ± 0.3 a (a)
		wet-dry cycle	4.7 ± 0.5 a (a)	4.4 ± 0.7 a (a)	4.5 ± 0.2 a (a)
	0.5%	wet	9.0 ± 1.0 b (a)	9.0 ± 0.2 c (a)	7.3 ± 0.6 c (a)
		drought	8.5 ± 0.4 b (b)	7.9 ± 0.5 c (b)	6.5 ± 0.2 bc (a)
		wet-dry cycle	8.9 ± 0.4 b (a)	7.6 ± 1.2 bc (a)	7.2 ± 0.2 c (a)

702

Figure 1. Diagram of soil moisture change in different water treatments.

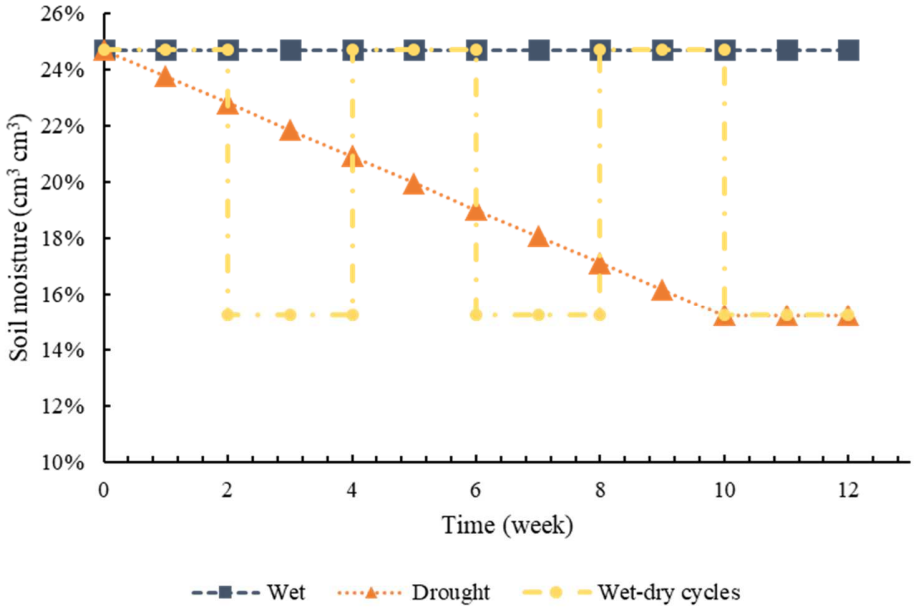

Figure 2. The impact of biochar, biosolids and environmental factors on soil microbial community composition after 4 weeks incubation, as determined by canonical-correlation analysis (abbreviations in the legends, w for wet, d for drought, c for wet-dry cycles, 0 for 0% biochar, 1 for 0.5% biochar, 2 for 1% biochar, - for 0% biosolids, + for 0.5% biosolids). In the legends, color represents soil moisture treatments (blue for wet, yellow for wet-dry cycles, red for drought), shape represents biochar treatments (triangle for 0% biochar, square for 0.5% biochar, circle for 1% biochar), filling of symbols represents biosolids treatment (hollow for 0% biosolids, solid for 0.5% biosolids). Arrows represent the vectors for key soil chemical properties, soil amendments and soil moisture treatments.

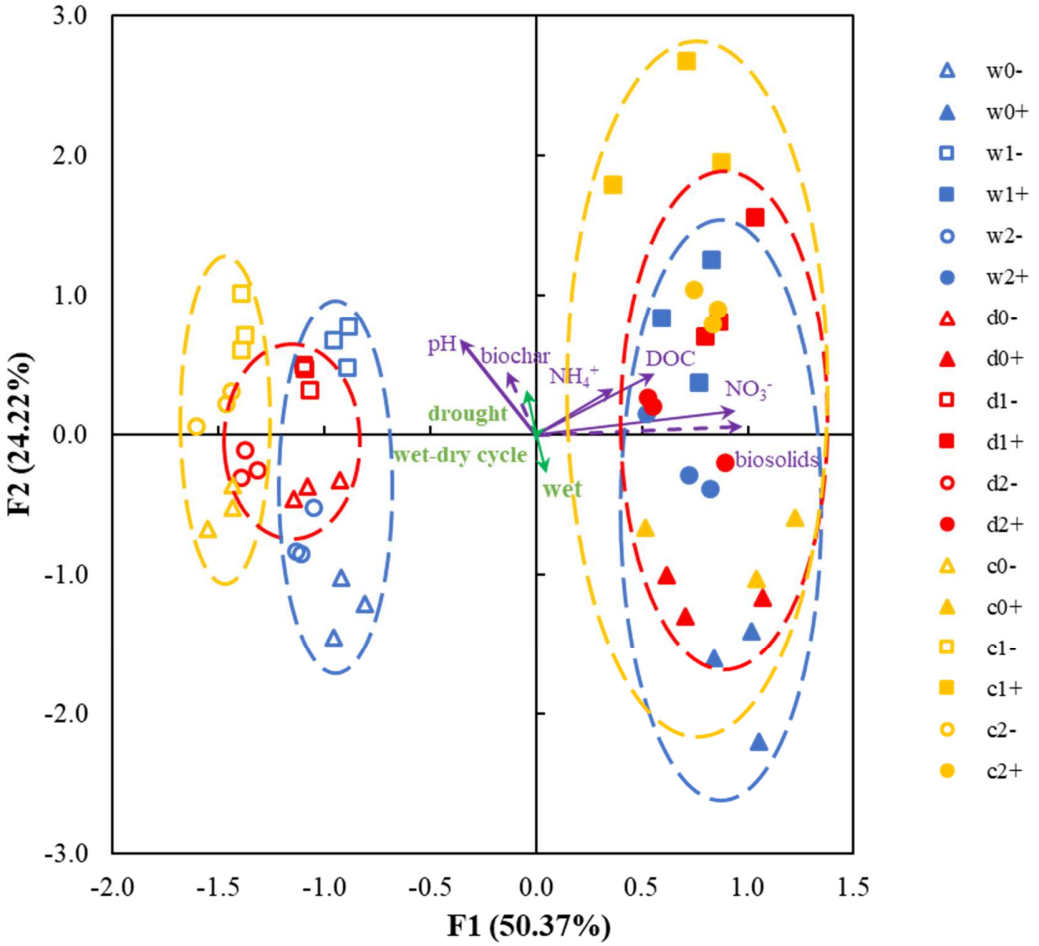

Figure 3. The impact of biochar, biosolids and environmental factors on soil microbial community composition after 12 weeks incubation, as determined by canonical-correlation analysis (in the legends, w for wet, d for drought, c for wet-dry cycles, 0 for 0% biochar, 1 for 0.5% biochar, 2 for 1% biochar, - for 0% biosolids, + for 0.5% biosolids). In the legends, color represents soil moisture treatments (blue for wet, yellow for wet-dry cycles, red for drought), shape represents biochar treatments (triangle for 0% biochar, square for 0.5% biochar, circle for 1% biochar), filling of symbols represents biosolids treatment (hollow for 0% biosolids, solid for 0.5% biosolids). Arrows represent the vectors for key soil chemical properties, soil amendments and soil moisture treatments.