1 Title: Impact of biochar amendment on soil water soluble carbon in the context of
2 extreme hydrological events
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4 Authors: Daoyuan Wang^{a, b}, Deirdre E. Griffin^a, Sanjai J. Parikh^a and Kate M. Scow^a
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6 ^a Department of Land, Air and Water Resources, University of California, Davis, 95616,
7 United States

8 ^b College of Environmental Science & Engineering, Donghua University, Shanghai,
9 201620, China
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Abstract:

Biochar amendments to soil have been promoted as a low cost carbon (C) sequestration strategy as well as a way to increase nutrient retention and remediate contaminants. If biochar is to become part of a long-term management strategy, it is important to consider its positive and negative impacts, and their trade-offs, on soil organic matter (SOM) and soluble C under different hydrological conditions such as prolonged drought or frequent wet-dry cycles. A 52-week incubation experiment measuring influence of biochar on soil water soluble C under different soil moisture conditions (wet, dry, or wet-dry cycles) indicated that, in general, dry and wet-dry cycles increased water soluble C, and biochar addition further increased release of water soluble C from native SOM. Biochar amendment appeared to increase transformation of native SOM to water soluble C, based on specific ultraviolet absorption (SUVA) and C stable isotope composition; however, the increased amount of water soluble C from native SOM is less than 1% of total biochar C. The impacts of biochar on water soluble C need to be carefully considered when applying biochar to agricultural soil.

Keywords: biochar, water soluble C, drought, wet-dry cycles

1. Introduction

As a major challenge of our time, climate change is expected to have long-term impacts on soil hydrological processes (Vrochidou et al., 2013). Extreme hydrological events, such as extended periods of drought, increased flooding, and more frequent wet-dry cycles, will in turn influence soil nutrient leaching (Bloor and Bardgett, 2012), the stability of soil organic matter (SOM) (Schmitt and Glaser, 2011) and soil microbial activities (Hueso et al., 2012).

Soil water soluble C molecules make up a small part of total SOM, but they play important roles in many soil microbial activities (Hennion, 2000; Marshall Clark and Kenna, 2001; Smolander and Kitunen, 2002; Peterson et al., 2013). Drought events have been shown to destabilize soil C stocks (Fenner and Freeman, 2011) and increase soluble C (Acero et al., 2009). Rapid wetting events enhance microbial activity in soils through what is known as the “Birch effect” (Birch, 1958; Bottner, 1985). Increased soil microbial activities may also increase production of water soluble molecules (Guggenberger and Zech, 1994; Guggenberger et al., 1994).

Biochar is a mostly recalcitrant, carbonaceous product obtained from pyrolysis of biomass under limited or no oxygen (Lehmann et al., 2011). Some biochars are byproducts of energy acquisition processes, such as the synthesis of bio-oil (Özçimen and Karaosmanoğlu, 2004) and syngas (Gaunt and Lehmann, 2008). Previous researches indicated that biochar soil amendment can increase soil water holding capacity of sandy soil (Karhu et al., 2011), influence soil cation exchange capacity (Van Zwieten et al.,

2010), increase crop yields (Asai et al., 2009; Vaccari et al., 2011) and alter greenhouse emissions from soil (Spokas and Reicosky, 2009; Roberts et al., 2010).

The potential degradation of biochar after application to soil is a concern if it is to be used as a C sequestration tool. Biological and abiotic processes are both involved in biochar degradation (Bruun et al., 2011; Kasin and Ohlson, 2013; Mukome et al., 2014), though the mechanisms involved are not well understood (Kuzyakov et al., 2009; Zimmerman, 2010). Biochar amendment can increase the priming effect of native soil C, but the interaction between biochar and native soil C varies across different systems and also depends on biochar C composition (Cross and Sohi, 2011). It is also unclear how soil hydrological events impact the biochar itself and its interactions with native organic C.

The objective of this study was to investigate the impacts of biochar amendment on the amount and composition of soluble C in an agricultural soil under conditions of dry and wet-dry cycles. We hypothesized that (1) biochar can influence soil chemical properties including water soluble C concentration and composition, and (2) both dry and wet-dry cycles treatments can increase water soluble C and change its properties.

2. Materials and methods

2.1. Soil and biochar

In January 2012, we sampled the top 15 cm of a Yolo silt loam soil (fine-silty, mixed, nonacid, thermic Typic Xerorthent) from Plot 6-1 (conventionally managed, irrigated, unfertilized wheat/fallow treatment) at the Russell Ranch Sustainable Agricultural

Research Facility (<http://asi.ucdavis.edu/rr>), University of California, Davis. The soil samples were air dried, sieved to pass through a 2 mm mesh, sealed in glass bottles, and stored at room temperature until use. The soil was a silt loam (42.75% sand, 35.20% silt and 22.05% clay) with 10.18 g organic C kg⁻¹, 1.15 g kg⁻¹ total N content, 11.0 mg kg⁻¹ Olsen-P content, 20.6 cmol kg⁻¹ cation exchange capacity and pH of 6.7. The soil measurements were performed by the Analytical Lab, University of California, Davis.

The biochar used was a walnut shell (WS) biochar that is commercially available, produced by Dixon Ridge Farms in Winters, CA. Detailed information on biochar characteristics and methods of analysis have been presented elsewhere (Mukome et al., 2013). The WS biochar was produced from walnut shells at a pyrolysis temperature of 900 °C, with 227.1 m² g⁻¹ surface area, 40% ash content, 33.4 cmol g⁻¹ cation exchange capacity and pH of 9.7. WS biochar was similarly processed as soil before use.

2.2 Incubation experiment setup

A laboratory incubation experiment was conducted from February 2012 to February 2013. Soil or biochar and soil mixtures totaling 200 g were placed in 500 mL Mason jars. Soils were equilibrated before the experiment began to avoid interference with measurements while the system was redistributing the added water. To do so, soil with different amounts of biochar were well mixed with water at the beginning of the experiment and pre-incubated for two weeks. The incubation experiment was conducted at 23 ± 1 °C.

The wet, dry and wet-dry cycles were conducted based on the water retention capacity of the soil (the soil moisture of different jars was adjusted as shown in Figure. 1). Based on soil water retention properties, we chose 80% of field capacity as the wet condition to keep the soil moist and have good aggregate structure. Dry and wet-dry cycle conditions were designed based on wet conditions and permanent wilting point. The field capacity and permanent wilting point of the Yolo silt loam soil were determined by measuring water retention using a pressure plate apparatus at 33 kPa and 1500 kPa, respectively (Dane and Hopmans, 2002).

The soil moisture content was adjusted weekly by oven-drying at 30°C or rewetting by watering with distilled water gently and evenly to the desired soil moisture content. Soil moisture was monitored by weighing the jars. Biochar doses were 0, 0.5, and 1 g per 100 g dry soil (equivalent to approximately 0, 10, and 20 t ha⁻¹). These biochar amendment rates are feasible amounts of material to add in agricultural practice and still be affordable for farmers (Major, 2010).

Destructive sampling was conducted in triplicate at 4, 12, and 52 weeks. For wet-dry cycle treatments, sampling points were at the end of each dry period, before rewetting events.

2.3 Water soluble C characteristic and ion analysis

At each time point, 8 g of a representative soil sample was mixed with 40 mL of water in 50 mL polypropylene tubes and placed on an orbital shaker (250 rev min⁻¹, 1 h).

Water ($>18.2 \text{ M}\Omega\cdot\text{cm}$) was supplied by a Barnstead Nanopure water system (Thermo Scientific, OH). After shaking, samples were centrifuged to remove suspended solids. Supernatant solutions were retained for water soluble C concentrations (mg L^{-1}) measurement by UV-persulfate oxidation (Teledyne-Tekmar Phoenix 8000). Specific UV absorbance (SUVA_{254} , $\text{L g}^{-1} \text{ cm}^{-1}$) of water soluble C was determined by measuring absorbance at 254 nm using an UV/VIS spectrophotometer (UV mini-1240, Shimadzu, Japan). SUVA_{254} index of each sample is defined as the UV absorbance at 254 nm measured in inverse meters (m^{-1}) divided by the dissolved organic matter concentration measured in milligrams per liter (mg L^{-1}). SUVA_{254} was found to be strongly correlated with the aromaticity of organic matter of a water soluble C sample (Weishaar et al., 2003). A weighted average calculation was done based on aromaticity of water soluble C from soil and biochar to identify the source of water soluble C after incubation.

Major anions (Cl^- , SO_4^{2-} , Br^- and NO_3^- , Dionex Ion Pac AS18 column) and cations (Na^+ , K^+ , Ca^{2+} , and Mg^{2+} , Dionex Ion Pac CS17 column) were analyzed using a Dionex ICS-2000 Ion Chromatography System (Dionex Corp., Sunnyvale, CA).

2.4 Stable isotope analysis and calculation

Isotopic composition of C in fresh soil and biochar solid samples were analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Water soluble C samples were prepared as described in 2.3 from all the treatments, and fresh biochar and Yolo soil

were analyzed with an O.I. Analytical Model 1030 TOC Analyzer (OI Analytical, College Station, TX) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) utilizing a GD-100 Gas Trap Interface (Graden Instruments). These analyses were conducted by the Stable Isotope Facility at the University of California, Davis.

The total C and $\delta^{13}\text{C}$ were measured in the following treatments: (1) solid samples of fresh WS biochar and Yolo soil; (2) water soluble C of WS biochar and Yolo soil; (3) water soluble C of 0 and 1% WS biochar-amended incubated samples. The $\delta^{13}\text{C}$ ratio of samples from all the treatments were measured and calculated according to the following equations (Fry, 2007) to investigate the water soluble C composition:

$$\delta_{\text{sample}} = \delta_{\text{soil}} * f_{\text{soil}} + \delta_{\text{biochar}} * f_{\text{biochar}} \quad (1)$$

$$f_{\text{soil}} + f_{\text{biochar}} = 1 \quad (2)$$

where δ_{soil} (in ‰) is the $\delta^{13}\text{C}$ ratio of the soil sample, δ_{biochar} (in ‰) is the $\delta^{13}\text{C}$ ratio of the biochar sample, f_{soil} (in %) is the percentage by weight of soil in the sample or unknown percentage of water soluble C from SOM, f_{biochar} (in %) is the percentage by weight of biochar in the sample or the unknown percentage of water soluble C from biochar.

2.5 Statistical Analyses

All data were subjected to statistical analysis using Microsoft Excel for Windows 2010 add-ins with XLSTAT Version 2014.6 (XLSTAT, 2014). Statistically significant differences between treatments were analyzed using analysis of variance (ANOVA) and

Tukey's multiple comparison test at 5% significance level.

3 Results

3.1 Soil water soluble C

WS biochar can potentially release significantly more water soluble C than Yolo soil. In a preliminary experiment, we conducted a water soluble C extraction experiment using WS biochar and Yolo soil with a solid/liquid ratio of 1:400 to investigate the maximum amount of water soluble C that can be extracted from soil and biochar. The results indicated that WS biochar contains 2116 mg kg⁻¹ water soluble C while Yolo silt loam soil contains 135 mg kg⁻¹ water soluble C.

Biochar can significantly increase water soluble C during the 52 week incubation experiment (Table 1, 2). After 4 week incubation, biochar amendments increased water soluble C, while no significant difference observed between 0.5% and 1% biochar treatments. After 12 week and 52 week incubation, biochar amendments continued increased water soluble C and 1% biochar amendment was significantly higher than 0.5%. After 52 week incubation, interactions between biochar and soil moisture conditions observed.

Soil moisture condition can influence the amount of water soluble C and the impacts can change over time. After 4 week incubation, dry and wet-dry cycle treatments were not significantly different from wet treatments. After 12 week incubation, dry and

wet-dry cycle treatments significantly increased water soluble C and no significantly difference between dry and wet-dry cycle treatments. After 52 week incubation, dry and wet-dry cycle treatments can both significantly increase water soluble C and significantly more water soluble C observed in wet-dry cycle treatments than in dry treatments.

3.2. SUVA₂₅₄ results

The SUVA₂₅₄ of water soluble C from soil samples was measured after 52 weeks of incubation (Table 3), to provide a measure of the aromatic character of the dissolved organic matter. The SUVA₂₅₄ of water soluble C derived from WS biochar was consistent across all solid/liquid ratios (from 1:50 to 1:400, wt.). A significant linear correlation between SUVA₂₅₄ absorption and water soluble C concentration was observed ($R^2 = 0.96$) (Figure A.1). The SUVA₂₅₄ of water soluble C of the original WS biochar, before incubation, was significantly lower ($1.87 \text{ L mg-C}^{-1} \text{ cm}^{-1}$, standard error 0.07) than in the non-incubated Yolo soil ($2.35 \text{ L mg-C}^{-1} \text{ cm}^{-1}$, standard error 0.10) (Table 3). A statistically significant ($P < 0.01$) increase of SUVA₂₅₄ with biochar amendment was observed in wet and wet-dry cycle treatments. The SUVA₂₅₄ increased in order of fresh biochar (not incubated) < 0% biochar treatment < 0.5% biochar treatment < 1% biochar treatment. SUVA₂₅₄ indices of dry treatments were significantly higher than in the wet and wet-dry cycle treatments.

3.3. ¹³C stable isotope analysis and calculation

The $\delta^{13}\text{C}$ ratios of samples after incubated for 52 weeks in 0 and 1% biochar treatments were measured and no significant difference observed between biochar treatments and soil moisture treatments (as presented in Table 4). The $\delta^{13}\text{C}$ ratio of water soluble C from WS biochar was -19.97‰ (standard error 0.07‰) and was a significantly higher value than that of water soluble C from Yolo soil in 0% biochar treatments (Table 4). The standard errors are presented in Table 4. Composition of water soluble C in each treatment were based on $\delta^{13}\text{C}$ ratio of water soluble C from WS biochar and $\delta^{13}\text{C}$ ratio of water soluble C from non-biochar treatment with same moisture condition (Figure 2).

3.4 Ionic strength

The ionic strength of soil samples from all the incubated samples increased with biochar amendment by about 73 - 100% (Fig. 3). WS biochar contained a higher concentration of water extractable ions, especially potassium, compared to Yolo silt loam soil, (Table A.2). WS biochar amendment can significantly increase the ionic strength of the soil solution and change the relative composition of soil ions. Ionic strength in all water treatments increased in the sequence of 0% biochar treatment < 0.5% biochar treatment < 1% biochar treatment. In the dry and wet-dry cycle treatments, ionic strength was approximately 20% that in the wet treatment. Similar reductions in ionic strength were observed in both non-biochar and biochar-amended treatments.

4. Discussion

4.1 Amounts and sources of water soluble C in biochar amended treatments

Biochar can increase water soluble C release from soil native organic matter in the context of drought and wet-dry cycles. After a 52 week incubation, both SUVA₂₅₄ and stable isotope analysis were explored to identify sources of water soluble C, while we were not able to identify water soluble C contribution by different materials at week 4 and 12. Although both biochar and soil native organic matter can be sources of water soluble C, soil native organic matter contributed more to water soluble C. SUVA₂₅₄ results indicated that aromaticity of increased water soluble C due to biochar amendment and soil moisture condition was similar to that of soil native C. Stable isotopic results confirmed that only minor portion (0 – 5.6%) of water soluble C was derived from biochar C (Figure. 2).

4.2. Potential mechanisms of how biochar facilitates release of water soluble C from native SOM

Increases in soil pH due to biochar amendment, in our case from pH 6.6 to 8.2 (Table A.1), can potentially increase the release of water soluble C from native SOM. Increases in soil pH can lead to dispersion of clay and SOM (Dungait et al., 2012; Mavi et al., 2012), increase the amount of negatively-charged groups on the humus colloids and thus increase solubility of native SOM. Andersson and Nilsson (2001) reported that increasing soil pH (from pH 4.2 to 6.2) resulted in an increase in dissolved organic C leaching under different temperatures in a laboratory experiment in soil from a coniferous

forest.

Another potential mechanism for the release of water soluble C by biochar is addition of salts, particularly potassium (K) in our case, by the biochar. Salt content in biochar varies as a function of feedstock and pyrolysis conditions and the WS biochar contains 9.32% (wt.) K (Mukome et al., 2013). Addition of K⁺ ions can lead to occupation of the soil's cation exchange sites and dispersion of clay and SOM (Kalbitz et al., 2000) resulting, in turn, to an increase of soluble organic C. Li et al. (2013) observed more C released from native SOM in soils with higher salt content.

4.3. Combined influence of biochar and soil moisture on water soluble C release

Biochar application in the context of wet-dry cycles may lead to more water soluble C from native SOM than wet-dry cycles alone. The physical disturbance caused by wet-dry cycles can increase the availability and accessibility of SOM to microbial communities (Kemmitt et al., 2008) and increase mineralization of SOM (Peinemann et al., 2005). Microbial communities under long term nutrient stress are more likely to metabolize aromatic compounds than organisms not under nutrient stress (Warnock et al., 2007; Major et al., 2010). Thus C, that otherwise would have not be utilized under less nutrient-stressed conditions, may have been mobilized from the SOM pool into microbial biomass. Because microbial cell walls and other cellular constituents are major precursors to SOM (Fontaine et al., 2003; Kuzyakov, 2010), the C transferred into microbial biomass may have eventually then be converted to SOM and be susceptible to release as water

soluble C.

Differences among the biochar treatments were not as evident in the dry compared to other water treatments. Microbial biomass and metabolites are feedstocks for soil organic matter formation. Microbial-derived aliphatic compounds, especially those from cell walls (Schurig et al., 2013) and cell wall envelopes of bacteria (Kindler et al., 2009) and fungi are stabilized in soil and contribute significantly to small-particulate SOM formation (Miltner et al., 2012). Under the harsh biological conditions of the dry treatment, the soil microbial biomass likely declined, and nutrient turnover and enzyme activities certainly were reduced. Thus following rewetting of the soil, the microbial community was not able to quickly respond to chemical stress from biochar and therefore water soluble C properties in dry treatment with different biochar amendment were similar to each other.

5. Conclusions

Both drought and wet-dry cycles increase water soluble C quantities and, in particular, wet-dry cycles were associated with an increase in aromatic C. The release of water soluble C from native SOM by wet-dry cycles is enhanced with biochar amendment. Our results suggest that application of biochar to agricultural soil may lead to more C release from native SOM in the context of wet-dry cycles. All impacts of biochar amendments on agricultural soil need to be carefully considered when developing management strategies (e.g. for climate change mitigation or nutrient management) that

289 use biochar.

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291 Acknowledgements:

292 This publication was made possible by grant number 5 P42 ES004699 from the
293 National Institute of Environmental Health Sciences (NIEHS), NIH and the contents are
294 solely the responsibility of the authors and do not necessarily represent the official views
295 of the NIEHS, NIH. Additional funding was provided by the United States Department of
296 Agriculture (USDA) National Institute of Food and Agriculture (NIFA) through Hatch
297 Formula Funding CA 2122-H and multistate regional project W-2082.

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TABLES

Table 1. Amount of water soluble C in different treatments (mg-C kg⁻¹ dry soil). The numbers to the right of each value represent the standard error about the mean.

Time (week)	Biochar (%)	Wet	Dry	Wet-dry cycle
4	0	24.7 ± 11.3	26.5 ± 10.4	27.2 ± 0.8
	0.5	32.3 ± 3.1	31.7 ± 1.0	37.8 ± 0.3
	1	32.3 ± 3.8	38.3 ± 2.1	46.8 ± 4.9
12	0	22.0 ± 1.0	29.8 ± 6.3	27.2 ± 0.8
	0.5	33.2 ± 3.3	37.8 ± 1.0	37.7 ± 1.9
	1	42.0 ± 5.1	48.7 ± 1.0	47.5 ± 2.3
52	0	19.3 ± 0.9	28.4 ± 2.4	33.9 ± 0.5
	0.5	27.1 ± 2.0	38.7 ± 2.4	45.7 ± 3.5
	1	32.0 ± 2.2	55.6 ± 1.0	54.9 ± 3.3

Table 2. ANOVA results of water soluble C ($\alpha = 0.05$).

Source of variance	<i>p</i> Values		
	4 weeks	12 weeks	52 weeks
biochar	0	< 0.0001	< 0.0001
Water	0.033	0.001	< 0.0001
biochar * water	0.415	0.922	0

Table 3. Specific ultraviolet absorption (SUVA₂₅₄) of soluble C from incubated soil samples (52 week, in L mg⁻¹ m⁻¹). The numbers to the right of each value represent the standard error about the mean. Significant differences between treatments are indicated by different letters in parentheses to the right of each value.

Biochar (%)	Wet (L mg⁻¹ m⁻¹)	Dry (L mg⁻¹ m⁻¹)	Wet-dry cycle (L mg⁻¹ m⁻¹)
0	2.2 ± 0.4 (ab)	3.2 ± 0.8 (ac)	2.1 ± 0.3 (b)
0.5	3.3 ± 0.1 (c)	3.3 ± 0.1 (c)	3.4 ± 0.3 (c)
1	2.9 ± 0.2 (abc)	3.5 ± 0.1 (c)	3.5 ± 0.1 (c)

Table 4. The $\delta^{13}\text{C}$ ratios of water extractable organic matter from incubated soil samples (52 week, ‰). The numbers to the right of each value represent the standard error about the mean. Significant differences between treatments are indicated by different letters in parentheses to the right of each value.

Biochar (%)	Wet (‰)	Dry (‰)	Wet-dry cycle (‰)
0	-26.8 ± 0.4 (a)	-27.0 ± 0.1 (a)	-27.2 ± 0.2 (a)
1	-26.8 ± 0.08 (a)	-26.7 ± 0.4 (a)	-26.8 ± 0.2 (a)

Figure 1

FIGURES

Figure 2

Figure 3

1 Figure captions:

2 Figure 1. Diagrammatic sketch of soil moisture change in different water treatments.

3

4 Figure 2. Calculated water extractable organic matter composition based on $\delta^{13}\text{C}$ ratios
5 (52 week and 20 t ha⁻¹ biochar treatments). The error bars represent standard errors of
6 total amount of water soluble C and bars with different letters indicate statistically
7 significant ($P < 0.05$) differences.

8

9 Figure 3. Ionic strength of soil samples (52 week). The error bars represent standard
10 errors and bars with different letters indicate statistically significant ($P < 0.05$)
11 differences.