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Influence of coal-derived humic substances on soil and plant properties

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Influence of coal-derived humic substances on soil and plant properties

by

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ABSTRACT

Maintaining and improving soil quality is of the utmost importance to the sustainability of production agriculture and for feeding the people of our planet. In the present study, coal-derived humic substances (CDHS) are investigated to understand their influence on soil and plant properties. Coal-derived humic substances are currently not widely used as soil amendments because of high manufacturing costs associated with methods described in research publications. In this study, two CDHS products were prepared using a simplified, low cost, manufacturing method. CDHS-1 was prepared from coal using a solution pH-adjusted with KOH to 9.5; CDHS-2 was prepared using a solution adjusted to pH 7.0. After pH adjustment, both were oven dried and sieved to <0.50 mm before being incorporated into a Hargreave fine sandy loam soil at a rate of 1 g kg^{-1} of soil. Results showed lower soil bulk densities associated with the CDHS-1 treatment compared to the CDHS-2 treatment and controls during an initial 30-day consolidation period. During this same period, CDHS-2-treated soil showed a lower capacity to hold water compared to CDHS-1 and the control soil. After consolidation, corn was grown in the columns and subjected to simulated drought conditions. The corn (*Zea mays* L.) showed greater drought-stress resistance in the CDHS-1 treatment compared to the CDHS-2 treatment and controls during a final 40-day bioassay period. However, no differences in plant heights and dry biomass yields were detected during the bioassay. Combining merits of CDHS to improve soil quality and plant growth with a more cost-effective production method will potentially

attract more growers to conduct on-farm field trials to test the effectiveness of CDHS products.

INTRODUCTION

Growing crops for human food consumption can be traced back thousands of years and is an absolute necessity for human survival on this planet. The collapse of many ancient civilizations such as the Sumerians and Mayans can be linked to crop failures and the inability of civilizations to feed their people (Johnston et al., 2009). In the past and in modern times, a decrease in a soil's suitability to produce crops productivity often can be linked to soil degradation of some type: erosion, depletion of soil organic matter (SOM), salinization or acidification. Globally, 33% of the earth's land surface is estimated to be affected by some type of soil degradation (Lal 2015). Hence, maintaining and improving soil quality is of the utmost importance to the sustainability of production agriculture and for feeding the people of our planet.

Soil quality can be defined in different ways. Doran and Parkin (1994) defined soil quality as "the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health." For the purposes of this research, we are concerned with soil quality as it impacts the harvestable yields of crops grown for food, feed, fiber or fuel. One of the foremost indicators of soil quality and crop yields in agriculture is soil organic matter (SOM) content (Bezdicek et al., 1996; Overstreet and Dejong-Hughes, 2018; Reeves, 1997).

SOM plays many roles in terms of the chemical, physical and biological properties that influence soil quality and ultimately productivity (Laird and Chang, 2013). According to Brady and Weil (2008) the entirety of SOM can be divided into three fractions: living organisms (biomass), identifiable dead tissues (detritus) and nonliving-nonidentifiable tissue (humus).

Living organisms and detritus, mostly plant residues, are composed of cellulose, lignin, hemicellulose, proteins, sugars, starches and fats and waxes (Stevenson, 1994). These organic materials undergo decomposition processes by soil microbes and extracellular enzymes, producing fragments of these biopolymers and ultimately CO_2. The decomposition products of residue can be adsorbed on particle surfaces and recombined in various ways to form humus. Humus is composed of many different humic substances (HS) and makes up the majority of SOM (Tan, 2003). HS can be chemically fractionated into humin, humic acid (HA) and fulvic acid (FA) based on their solubilities in alkaline and acid conditions: humin is insoluble in alkali; HA is soluble only in alkali and precipitates in acid; FA is soluble in alkali and does not precipitate in acid (Stevenson, 1994). HS can also be extracted from various organic materials such as manure, compost, peat, coal, etc. (Zandonadi et al., 2013). Compared to other sources of organic materials, Coal-derived humic substances (CDHS) are of interest due to high concentrations of HS, being recalcitrant to microbial breakdown, and relatively low cost to produce (Ahmad et al., 2015; Piccolo et al., 1996; Piccolo et al., 1997).

Scientific literature provides many examples of the potential agricultural benefits of HS, but benefits can vary depending on the source of the organic material. In this study, the focus is exclusively on CDHS, which may or may not be relevant for HS derived from other sources (composts etc.,) because of potential chemical differences. Dr. Alessandro Piccolo at the University of Naples Federico has documented the potential agricultural benefits of CDHS. With regard to soil quality, Piccolo et al. (1989, 1997) showed statistically significant increases in aggregate stability, soil water content at field capacity, permanent wilting point and the capacity of soils to retain plant-available water Piccolo et al. (1996) when CDHS are added to

soils. Regarding plant growth, Piccolo et al. (1993) showed statistically significant increases in total dry biomass weight for lettuce (*Lactuga sativa*) and tomato (*Lycopersicum esculentum*) seedlings and showed statistically significant increased nitrate uptake for barley *(Hordeum vulgare)* seedlings (Piccolo et al., 1992) when CDHS were added to growth solutions.

In the aforementioned studies, humics were first extracted from the source coal using 1M NaOH (for extracting the total soluble fraction) at 100 °C for seven h under N_2 purge conditions. Non-soluble components were removed from the extracts by centrifuging, then the HA fraction was precipitated by adding 6M HCl to lower the solution pH to one. Finally, the precipitated HA were separated from the solution and soluble components by centrifugation, dialyzed against distilled water until chloride free and then vacuum dried at 70 °C for seven hours (Piccolo et al., 1997). Although CDHS produced by Piccolo's procedure have been proven to enhance soil quality and promote plant growth, the procedure is prohibitively costly for commercially producing CDHS for agriculture applications.

Piccolo et al. (1996) and Yamaguchi et al. (2004), have shown that CDHS can increase soil plant-available water, but there is no direct evidence on the effect of CDHS on soil bulk density. Soil bulk density is an important soil quality characteristic and affects water infiltration, rooting depth, available water-holding capacity, soil porosity, plant nutrient availability, and soil microorganism activity, which influence key soil processes that influence crop productivity (USDA-NRCS, 2019). Because of the link between bulk density and available water-holding capacity, soil bulk density may also influence the response of plants to drought stress.

Drought stress can severely restrict growth and productivity of agricultural crops and ultimately food supplies. In 2012, US agricultural drought losses were in excess of 30 billion dollars. It was estimated that nearly 90% of the US corn- and soybean-producing areas were located within an area experiencing drought (Rippey, 2015). No applicable research was found on the use of soil-applied CDHS to reduce drought stress; however, significant improvements with HS in other research scenarios have been reported. Wheat plants (*Triticum aestivum* L.) grown under water-stress conditions showed significant improvements in drought resistance with foliar applications of FA extracted and purified from coal (Xudan, 1986). In hydroponic research, rice plants (*Oryza sativa* L.) grown under water-stress conditions showed significant improvements in drought resistance when HA extracted and purified from vermicomposted cattle manure was added to the nutrient-growth solutions (Garcia et al., 2012; Garcia et al., 2014). These studies are evidence supporting the potential of HS for to mitigate the effects of drought, but more research needs to be done specifically with CDHS in soil applications.

The goal of the present study is to test a simplified, hence low cost, extraction procedure compared to that used by Piccolo et al. (1997), for the production of CDHS for use in production agriculture. Furthermore, the study addresses the influence of CDHS on soil bulk density and the impact of CDHS soil amendments on response of plants grown under drought conditions. The hypotheses being tested in this greenhouse soil-column study are: 1) that amending a fine sandy loam soil with CDHS will reduce soil bulk density, increase soil water retention, and reduce plant drought stress during a simulated in-season drought; and 2) that pH of the extracting solution influences the quality and hence the efficacy of CDHS for improving soil quality and reducing plant drought stress. In total, this study is designed to increase

understanding of the commercial potential of CDHS to be used as soil amendments for improving soil quality and enhancing crop resilience to drought stress.

MATERIALS AND METHODS

Soil Preparation and Analysis

The soil used in this study was obtained from a fallow field in Boulder County, Colorado, and was mapped as a Hargreave fine sandy loam (USDA, 2018). The top 2 to 30 cm of soil was carefully excavated, placed in several bins and taken to the greenhouse. In the greenhouse, the soil was spread out on a woven polyethylene tarp for drying. The soil was stirred daily for one week to facilitate drying evenly. The air-dried soil was sieved to <2 mm and put in a plastic 5-gallon bucket and sealed.

Various properties of the soil were analyzed. Midwest Labs in Omaha, Nebraska performed the texture, percent organic matter (OM %), and total N analyses. Starting soil moisture %, total carbon (TC), total organic carbon (TOC) and total inorganic carbon (TIC) analyses were performed by Horizon Ag-Products in Louisville, Colorado. The TOC was determined by thermal combustion after reacting the soil with 37% reagent-grade HCl to remove carbonates. The TIC was determined by difference between TC and TOC. Soil characteristics are listed in Table 1 along with the methods used for the analyses.

Table 1. Attributes of the Hargreave fine sandy loam soil used in the study.

Parameter	Result	Method
Sand	64%	Hydrometer
Silt	22%	Hydrometer
Clay	14%	Hydrometer
Organic matter	0.80%	Loss on ignition (ASTM 2974)
Total carbon	0.38%	Thermal combustion (Shimadzu carbon analyzer)
Total organic carbon	0.32%	Thermal combustion after treatment with 37% HCl (Shimadzu carbon analyzer)
Total inorganic carbon	0.06%	Total organic carbon – Total carbon
Total nitrogen	415 mg kg^{-1}	LECO total N analyzer
Soil air dry moisture	0.92%	Moisture analyzer (130 °C)

Coal-Derived Humic Substances Preparation and Analysis

Coal was sourced from Horizon Ag-Products in Cuba, New Mexico. The chemical properties of the coal are listed in Table 2. The coal was ground in a mortar and pestle and sieved to <0.50 mm. Two materials (CDHS-1 and CDHS-2) were prepared for this study by adjusting the pH of the coal to increase the solubility of the humic components. For CDHS-1, 40 g of sieved coal and 400 g of DI water were placed in a beaker with a stir bar and the pH was raised by the gradual addition of 45% KOH until the pH equilibrated at 9.50 (initial pH of the solution was 3.76). For CDHS-2, 40 g of sieved coal and 400 g of DI water were placed in a beaker with a stir bar and the pH was raised by the gradual addition of 45% KOH until the pH equilibrated at 7.00. After the pH equilibrated, the samples in their entirety were placed in a laboratory oven to dry at 65 °C. After the material was visibly dry in the beaker, it was stirred and left in the oven for another 72 h. Dry, pH-adjusted CDHS-1 and CDHS-2 samples were ground in a mortar and pestle, sieved to <0.50 mm, placed in plastic bottles and capped.

Table 2. Chemical properties of coal used in the study.

Parameter	Result	Method
Total Nitrogen	9800 mg kg^{-1}	AOAC 993.13
Phosphorus	n.d.	Extracted by EPA 3050B and analyzed by ICAP†
Potassium	n.d.	Extracted by EPA 3050B and analyzed by ICAP†
Sulfur	6700 mg kg^{-1}	Extracted by EPA 3050B and analyzed by ICAP†
Calcium	9500 mg kg^{-1}	Extracted by EPA 3050B and analyzed by ICAP†
Magnesium	700 mg kg^{-1}	Extracted by EPA 3050B and analyzed by ICAP†
Iron	7830 mg kg^{-1}	Extracted by EPA 3050B and analyzed by ICAP†
Manganese	70 mg kg^{-1}	Extracted by EPA 3050B and analyzed by ICAP†
Sodium	4000 mg kg^{-1}	Extracted by EPA 3050B and analyzed by ICAP†
Boron	35 mg kg^{-1}	Extracted by EPA 3050B and analyzed by ICAP†
Zinc	13.3 mg kg^{-1}	Extracted by EPA 3050B and analyzed by ICAP†
Total carbon	41.96%	Thermal combustion (Shimadzu carbon analyzer)
Total organic carbon	38.38%	Thermal combustion after treatment with 37% HCl (Shimadzu carbon analyzer)
Total inorganic carbon	3.58%	Total organic carbon - Total carbon
pH	3.76	pH meter
Humic acid	50.21%	New Standard Method (Lamar et al., 2014)

†The coal sample was prepared for metals analysis by following EPA method 3050B (acid digestion of sediments, sludges and soils). The sample was then analyzed by ICAP (inductively coupled argon plasma) spectroscopy following EPA method 6010A.

Soil Columns

Soil columns were prepared using a design similar to that used by Laird et al. (2010). Schedule 40, 7.70 cm (i.d.) PVC tubing was cut to 27.30-cm lengths. A 3 mm hole was drilled

through schedule 40, 7.70 cm (i.d.) PVC endcaps for drainage. Any PVC burrs on the inside of the endcap were removed to prevent interference with drainage. The PVC pipe was put into the endcaps and gently tapped with a rubber mallet to achieve a uniform finished height of 23.50 cm from the top of the endcap to the top of the column, or 28.42 cm from the bottom of the endcap to the top of the column. On the inside of the columns, a mark was made with a permanent marker 3.20 cm down from the top of column. A square piece of plastic insect screening (5.50 cm X 5.50 cm) was inserted at the bottom of each endcap to prevent the drain hole from clogging and loss of soil. On top of the insect screen, 100 g of coarse sand (2.00 – 4.76 mm) was placed in the column. After the coarse sand was placed in the column, it was lightly tamped with a round wooden disk about 7.00 cm in diameter to level the coarse sand.

Three treatments were used in the study: soil only (control), CDHS-1, and CDHS-2. For the CDHS-1 and CDHS-2 treatments, 1.40 g of CDHS-1 or CDHS-2 was mixed with 1420.00 g of air-dry soil in a small plastic tub (30.50 cm L X 20.30 cm W X 15.25 cm H) by hand with a small spatula for 30 s. Mixing of the CDHS material and soil was done separately for each column to ensure accuracy of the CDHS treatment dosage. For the control treatments, columns received only soil, which was poured directly into the columns. For all treatments in the study, after pouring the soil into the column, the soil was lightly tamped with a round wooden disk about 7.00 cm in diameter and lightly tapped on a table until the soil height was close to the 3.20 cm line and fairly level. Each column was weighed after filling to get the total finished (air-dried) weight. Total finished weight = column (g) + cap (g) + plastic screen (g) + coarse sand (g) + air-dried soil. The gravimetric moisture content of the soil was determined using a Mettler Toledo

infrared moisture analyzer at 130 °C. Soil dry weight = mass of air-dry soil (g) - soil water content (g).

Determining Saturated Water Content at Field Capacity and Soil Bulk Density

Each column was placed in a water-filled tub to wet the columns from the bottom up. The tubs were filled to about 2/3 the height of the column (21.00 cm) with a 0.005 M $CaCl_2$ solution. Within one hour, all columns were visibly moist on the surface of the soil. The columns were kept in the 0.005 M $CaCl_2$ solution for a total of 48 h. Although 4.22 cm of soil in each column protruded above the water level, for purposes of this study we define water content measured this way as "saturated." After 48 h of being saturated, the columns were removed from the tub and weighed. After removal from the tub, the columns were allowed to drain for 10 s before being placed on the scale, to limit excess water on the outside of the column. Saturated water content % was calculated from the weight that was measured. Saturated gravimetric moisture % = (wet column weight - dry column weight)/dry weight of soil. The columns were then arranged in a completely randomized design on a table in the greenhouse for the next phase of the study.

To calculate soil bulk density, the distance from the soil surface to the top of the column was measured at four places around the column. The average distance was used to determine the amount of headspace in each column, which was deducted from the total column volume to estimate soil volume. Soil bulk density was calculated from the initial soil oven-dry weight and the soil volume after deducting the volume of coarse sand for each column.

To determine moisture content at field capacity, plastic wrap was secured with a rubber band over each column to limit water loss due to evaporation from the soil surface. Next, the

columns were allowed to drain freely for 48 h. After 48 h, the plastic wrap was removed, and each column was weighed. Then the distance from the soil surface to the top of the column was recorded to determine soil bulk density. The plastic wrap and rubber band were put back over each column after each weighing and measuring event. After 48 h, there was no visible dripping below the columns when they were sitting on the wire mesh table. However, when the columns were moved to the scale, a drop or two of water was observed, and this continued to happen for eight more days.

Consolidation Period

Every three to four days, the columns were weighed (starting 7 June) and the distance from the top of the column to the soil surface was recorded to determine bulk density. After weighing and measuring the soil column heights on 12 July, 100 mL of 0.005 M $CaCl_2$ solution was added to each column to facilitate soil consolidation. The $CaCl_2$ solution was poured into a water distribution cup, a small tapered plastic cup with 15 - 0.16 cm holes in the bottom of the cup. The distribution cup was placed about 0.60 cm above the soil surface and rested on the top of the columns to evenly and lightly distribute the water on the surface and avoid any significant displacement of soil. The addition of 100 mL of 0.005 M $CaCl_2$ brought soil moisture content back to approximately saturation. After each irrigation event, 25-40% of the applied volume would drain out. The consolidation period was 30 days and included eight irrigation events. On 6 July, 100 mL of fertilizer solution (Peters Excel 15-5-15 Cal-Mg-Special 120 g L^{-1}) was added instead of the 100 mL of $CaCl_2$ solution in preparation for planting. The 100-mg kg^{-1} N solution is a standard solution used in greenhouse corn experiments.

Corn Bioassay

Two corn seeds were planted approximately 3.50 cm deep in each column on 8 June. The corn variety used in this experiment was Syngenta's NK N45P-GTA. Immediately after planting, 50 mL of 100-mg kg-1 N fertilizer solution (15-5-15) was added to each column to settle the soil around the seed. Subsequently, 50 mL of the same fertilizer solution was added to each column on 10, 13, 17, and 20 July. At the V2 growth stage (17 July), columns were thinned to one healthy plant of about the same height in each column. Up until that time, heights for both plants were recorded; after that time only the height of the remaining plant was recorded. Plant heights were measured in mm with a Swanson aluminum meter stick. On 20 July (nine days after emergence), watering was stopped. From the date of emergence (11 July) to the date of termination (25 August), plants heights were measured from the soil surface to the top of the highest leaf (by pulling the upper two leaves up). When the plants started to show water stress on 24 July, drought observations began and continued until 15 August. Drought observations (Table 3) were recorded by a relative numeric index adapted from Rukundo et al. (2014) on a scale of 1-10. On 9 and 13 August, the columns were watered with 100 mL of fertilizer solution to observe differences in plant recovery from drought stress. On 22 August (40 days after emergence) final plant heights and plant V-stage observations were made, and the study was terminated.

Table 3. Visual corn drought-stress index

Score	Description of the symptoms
1	All leaves turgid. No signs of leaf wilting.
2-3	Minor leaf wilting appearing in upper leaves, lower leaves showing chlorosis.
4-5	Leaf wilting more evident, leaf folding towards the midrib (V-shape) beginning to appear, upper leaf-tip chlorosis, lower leaf chlorosis more severe.
6-7	Leaf folding more severe, leaf rolling appearing, upper leaf-tip chlorosis turning to necrosis, lower-leaf chlorosis turning to necrosis.
8-9	Almost all leaves have leaf rolled, upper leaf-tip necrosis more severe, more severe lower-leaf necrosis.
10	Total necrosis – physiological death

Harvesting occurred as follows. The corn stem was cut approximately 1.25 cm above the soil line and the harvested above-ground biomass was placed in a marked paper bag. The soil and roots were removed from the columns by striking the bottom of each column repeatedly. Roots were carefully washed to remove all soil. After the roots were clean, another cut was made right above where the root system starts, in order to get the roughly 1.25 cm part of the stem that was left above ground that was not able to be cut when the plant was in the column. This piece was placed in the bag with the rest of the above- ground biomass and the root system was placed in a separate bag. Both were dried in a fan-forced air oven for four days at 60 °C. Plant samples were allowed to cool for 24 h and then weighed.

Statistical Analysis

The study was set up as a completely randomized design. The study consisted of three treatments: untreated soil and the two CDHS treatments (CDHS-1 and CDHS-2). Each treatment was replicated seven times, producing a total of 21 study experimental units for the saturated moisture content, field capacity content and soil consolidation data. At planting, only six columns were utilized to keep one soil-only control column for each treatment. Therefore, for

the plant growth data there were three treatments, six replicates for a total 18 experimental units. The statistical analysis was performed using R with the help of staff at Horizon Ag-Products. One-way analysis of variance (ANOVA) models were used with a confidence level of 0.95 for the analysis of soil field capacity and for the biomass measurements. Two-way repeated measures ANOVA models were used, with time as the dependent variable and treatment as the independent variable, with a confidence level of 0.95 for bulk density and soil water contents over the 30-day consolidation period; and they were also used for corn plant heights over the 40-day corn bioassay. If significance was detected in the ANOVA models, Tukey's HSD was performed to determine significant differences among treatment means.

RESULTS AND DISCUSSION

Saturated Water Content

The mean gravimetric water content at saturation in the soil columns was similar among the three treatments. Soil treated with CDHS-1 and CDHS-2 showed no statistically significant difference (alpha < 0.05) in gravimetric water content at saturation compared to the control soil (Fig. 1).

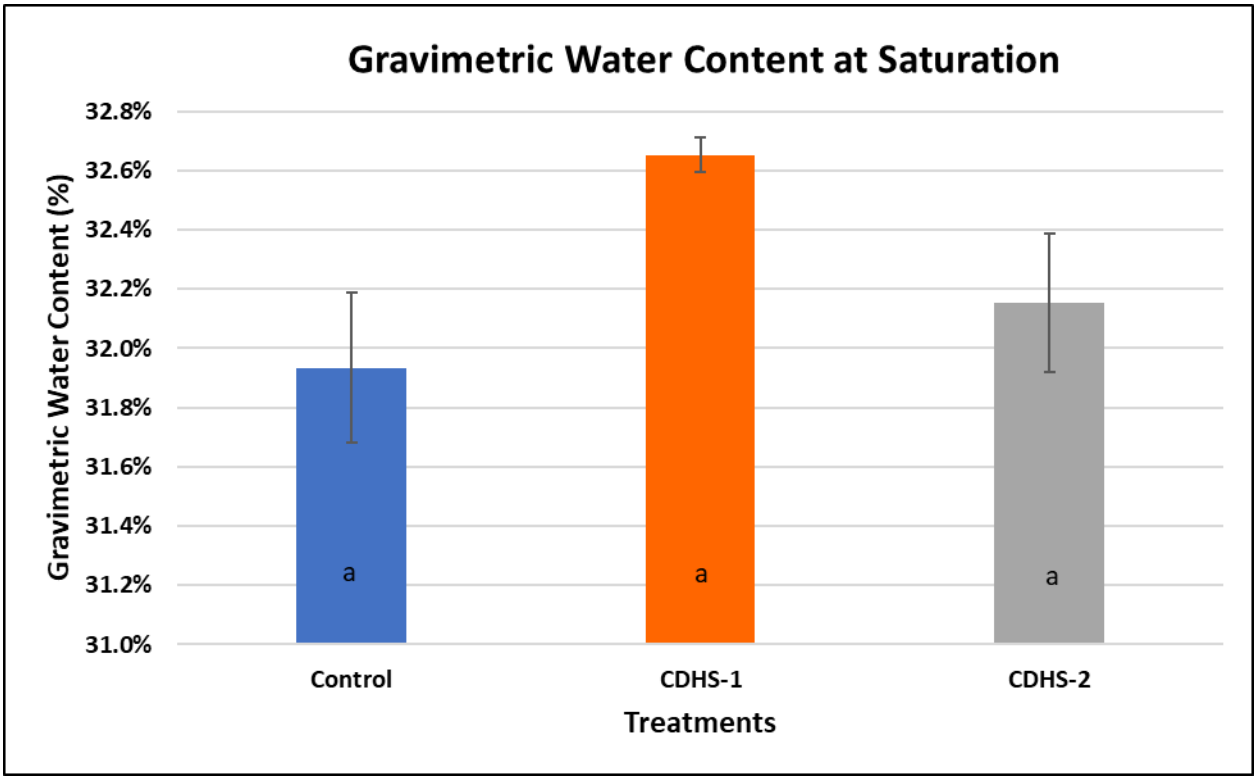

Fig. 1. Gravimetric water content at saturation of the soil columns treated with CDHS-1 and CDHS-2 and the control. Error bars are standard errors and treatment means with different letters are significantly different (alpha 0.05) by Tukey's HSD.

Water Content at Field Capacity

The mean gravimetric water content at field capacity in the soil columns was similar among the three treatments. Soil treated with CDHS-1 and CDHS-2 showed no statistically significant difference (alpha < 0.05) in gravimetric water content at field capacity compared to the control soil (Fig. 2).

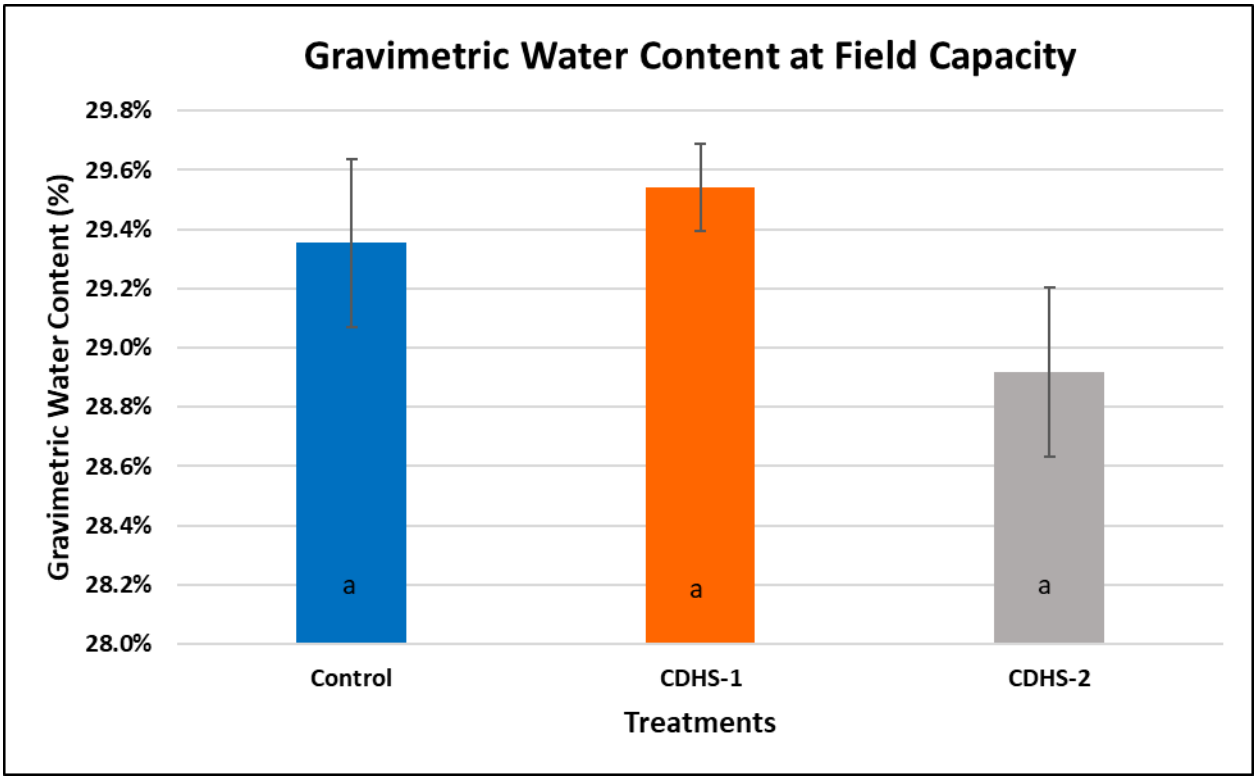

Fig. 2. Gravimetric water content at field capacity of the soil columns treated with CDHS-1, CDHS-2 and the control. Error bars are standard errors and treatment means with different letters are significantly different (alpha 0.05) by Tukey's HSD.

Consolidation Period—Bulk Density

Soil bulk density measurements were taken 11 times over a 30-day consolidation period. Consolidation is the process by which soils change in volume over time, which is reported here as changes in bulk density. Wetting and drying cycles cause consolidation

(increase in bulk density) as soil aggregates becoming smaller and more tightly packed causing a reduction in total pore space between aggregates. A trend of increasing bulk density during the consolidation period was observed for all three treatments; however, CDHS-1 had consistently lower bulk density during the consolidation period compared to the other treatments (Fig. 3). Analysis at each time point revealed two statistically significant (alpha < 0.05) trends. First, at nine of the 11 time points, soil treated with CDHS-1 had a lesser bulk density than soil treated with CDHS-2. Second, at six of the 11 time points, soil treated with CDHS-1 had a lesser bulk density than both soils treated with CDHS-2 and the control soil. Over the course of the consolidation period, all three treatments significantly (alpha < 0.05) increased in bulk density from the starting value of 1.35 g/cm^3. When the data from each time point of bulk density measure were pooled over the course of the consolidation period (Table 4), the treatments and days of measure were individually significant (alpha < 0.05) but their interaction between the two was not. When treatment means were analyzed by Tukey's HSD (Table 5), soil treated with CDHS-1 had a statistically significant (alpha < 0.05) lesser bulk density than soil treated with CDHS-2 and the control soil over the consolidation period, and CDHS-2 and the control soil did not differ from each other.

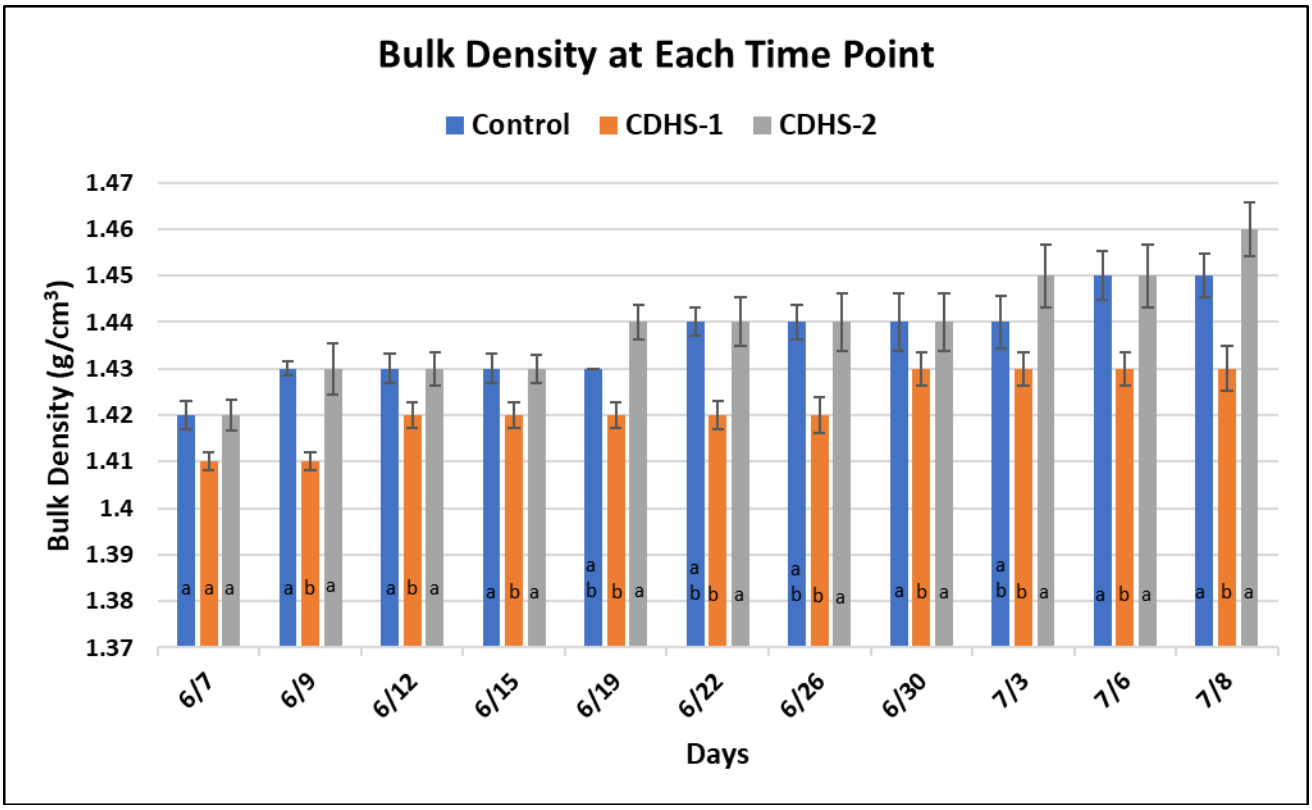

Fig. 3. Changes in soil bulk density for soil columns treated with CDHS-1, CDHS-2 and the control during the consolidation period. Error bars are standard errors and treatment means on a given day with different letters are significantly different (alpha 0.05) by Tukey's HSD.

Table 4. ANOVA table for bulk density measures when mean daily measures are analyzed over the course of the study.

Source	DF	SS	MS	F-Value	P-Value
Treatment	2	0.012	0.006	52.496	< 0.001
Date	11	0.150	0.014	117.500	< 0.001
Treatment × Date	22	0.002	0.000	0.945	0.536

Table 5. Summary of all pairwise comparisons for treatment means of bulk density over the course of the study by Tukey's HSD.

Treatment	Means
soil only	1.436a†
CDHS-2	1.439a
CDHS-1	1.421b

† Means followed by different letters are significantly different (alpha 0.05) by Tukey's HSD.

Consolidation Period—Water Content

Gravimetric water content of soil in the columns was measured eight times during the 30-day consolidation period (Fig. 4). Gravimetric water content was measured immediately before an irrigation event, or two to four days after the previous irrigation event. During the consolidation period, the soil columns were allowed to freely drain and were uncovered. Thus, the gravimetric water content measured during the consolidation period provided a relative assessment of the treatment effects on soil water retention for each date. Differences in gravimetric water content between dates, however, were influenced by differences in length of the drying periods that preceded the measurements. In general, soils treated with CDHS-1 had the highest water content, followed by the control soil; soil treated with CDHS-2 had the least water content. At six of the eight time points, soil treated with CDHS-2 had significantly (alpha < 0.05) lower gravimetric water content compared to soils treated with CDHS-1, and at two of the eight time points gravimetric water content of CDHS-2 was significantly (alpha <0.05) less than the control soil. Soil treated with CDHS-1 did not show any significant difference in gravimetric water content at any time point compared to the control soil. When the data from each time point of water-content determination were pooled over the course of the consolidation period (Table 6), the treatments and days of measure were individually significant (alpha < 0.05) but the interaction was not. When treatment means are analyzed by Tukey's HSD (Table 7), soil treated with CDHS-2 had a statistically significant (alpha < 0.05) lower water content than soil treated with CDHS-1 and the control soil over the consolidation period. CDHS-1 and the control soil did not differ significantly from each other.

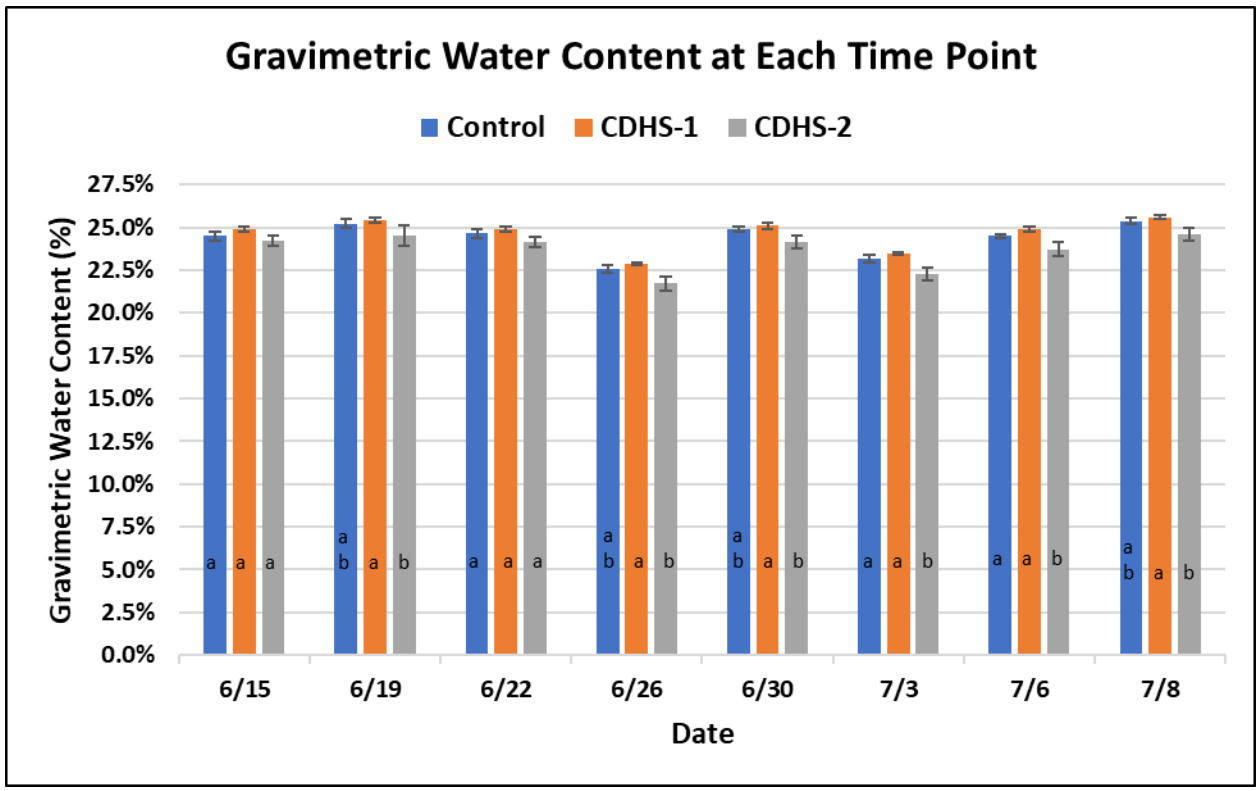

Fig. 4. Changes in gravimetric water content of the soil columns treated with CDHS compared to the control during the consolidation period at each time point. Error bars are standard errors and treatment means on a given day with different letters are significantly different (alpha 0.05) by Tukey's HSD.

Table 6. ANOVA table for mean daily gravimetric water content analyzed over the course of the study.

Source	DF	SS	MS	F-Value	Pr>F
Treatment	2	0.003	0.001	31.171	<0.001
Date	7	0.015	0.002	47.629	< 0.001
Treatment × Date	14	0.000	0.000	0.200	0.999

Table 7. Summary of all pairwise comparisons for treatment means of gravimetric water content over the course of the study by Tukey's HSD.

Treatments	Means
soil only	24.345%a†
CDHS-1	24.625%a
CDHS-2	23.660%b

† Means followed by different letters are significantly different (alpha 0.05) by Tukey's HSD.

Bioassay—Plant Heights

Plant heights were measured 17 times during the 40-day bioassay period (Fig. 5). The mean plant heights at any one date in the soil columns were similar among the three treatments. Soil treated with CDHS-1 and CDHS-2 showed no statistically significant difference (alpha < 0.05) in plant heights compared to the control soil. Furthermore, no statistical differences in plant heights were observed during the recovery phase (13-22 August) of the bioassay (Fig. 5). When the data from each time point of plant height measure is pooled over the course of the bioassay period (Tables 8 and 9), the soil treatments were not significant, but the day of measure was significant (alpha <0.05) and the interaction was not.

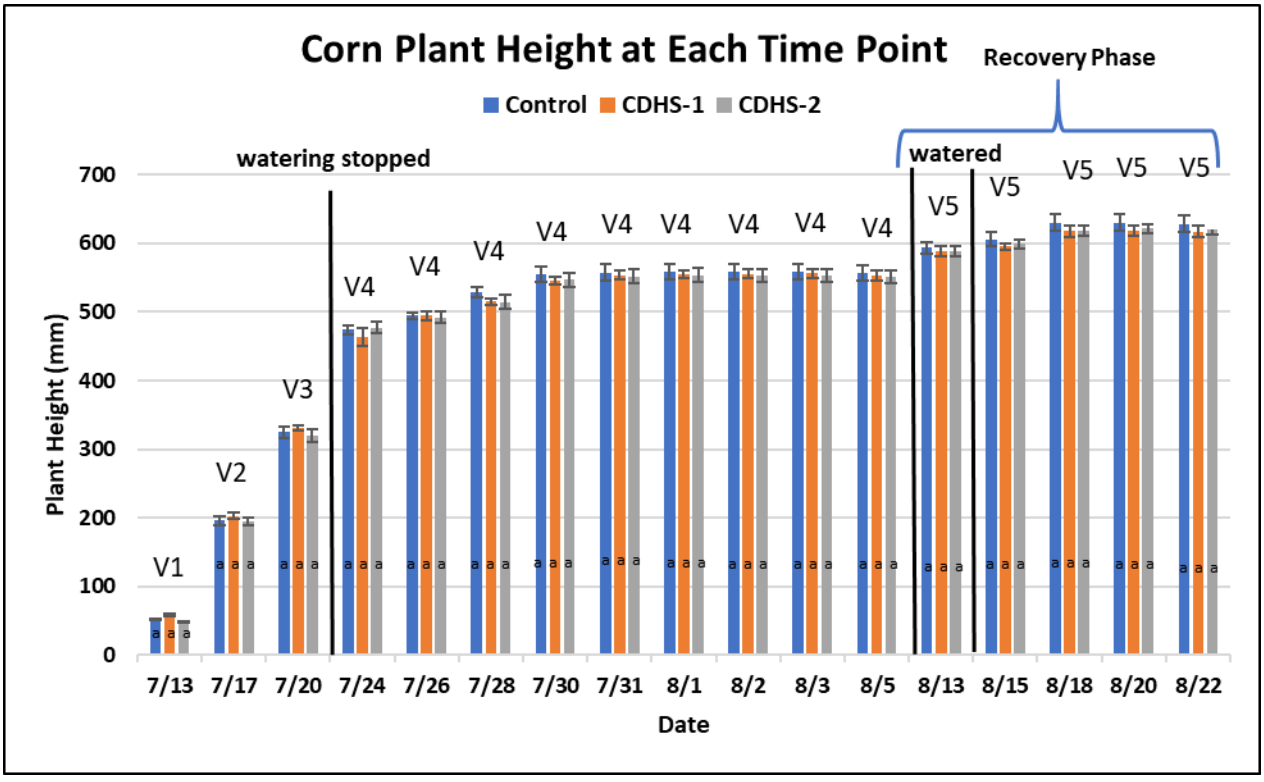

Fig. 5. Plant heights for the soil columns with the CDHS-1, CDHS-2, and control treatments during the bioassay. Error bars are standard errors and treatment means on a given day with different letters are significantly different (alpha 0.05) by Tukey's HSD.

Table 8. ANOVA table for plant height measures when mean daily measures are analyzed over the course of the study.

Source	DF	SS	MS	F-Value	Pr>F
Treatment	2	1805.067	902.533	2.242	0.109
Date	14	7125686.200	508977.586	1264.513	<0.001
Treatment x Date	28	2906.933	103.819	0.256	1.00

Table 9. Summary of all pairwise comparisons for treatment means of plant height measures over the course of the study by Tukey's HSD.

Treatment	Means
control	566.333a†
CDHS-2	559.893a
CDHS-1	559.107a

† Means followed by different letters are significantly different (alpha 0.05) by Tukey's HSD.

Bioassay—Drought Stress

Four days after watering was stopped on 20 July, the plants started to show drought-stress symptoms such as leaf wilting and lower leaf chlorosis. Each plant was rated according to the index described in the Materials and Methods at a total of 10 time points during the three phases (pre-drought, drought and drought recovery) of the bioassay (Fig. 6). In general, plants would first show leaf wilting, followed by leaf folding and lastly leaf curling. During the pre-drought phase (20 – 24 July) soil treated with CDHS-1 and CDHS-2 showed no statistically significant difference (alpha < 0.05) in drought stress compared to the soil-only control at individual time points. When the data is pooled for both time points in the pre-drought phase, the treatment and treatment by date interaction were not significant (alpha < 0.05), but the day of measure was significant (Table 10). During the drought phase (31 July to 9 August) the drought-stress index data revealed two statistically significant (alpha < 0.05) trends. First, for four of five time points, plants grown in soil treated with CDHS-1 showed less stress than plants grown in soil treated with CDHS-2. Second, for two of five time points, plants grown in soil

treated with CDHS-1 showed less drought stress than plants grown in the soil-only control. When the data is pooled across the five time points in the drought phase, the treatment and date of measure are significant (alpha < 0.05) but their interaction is not (Table 10). When treatment means are analyzed by Tukey's HSD (Table 11), soil treated with CDHS-1 had a significantly lower average drought-stress index during the drought phase compared to soil treated with CDHS-2 and the control soil. During the recovery phase (13 to 15 August) soil treated with CDHS-1 and CDHS-2 showed no statistically significant difference (alpha < 0.05) in drought stress compared to the control at individual time points. When the data is pooled for both time points in the recovery phase, soil treated with CDHS-1 and CDHS-2 showed no statistically significant difference (alpha < 0.05) in drought stress compared to the soil-only control.

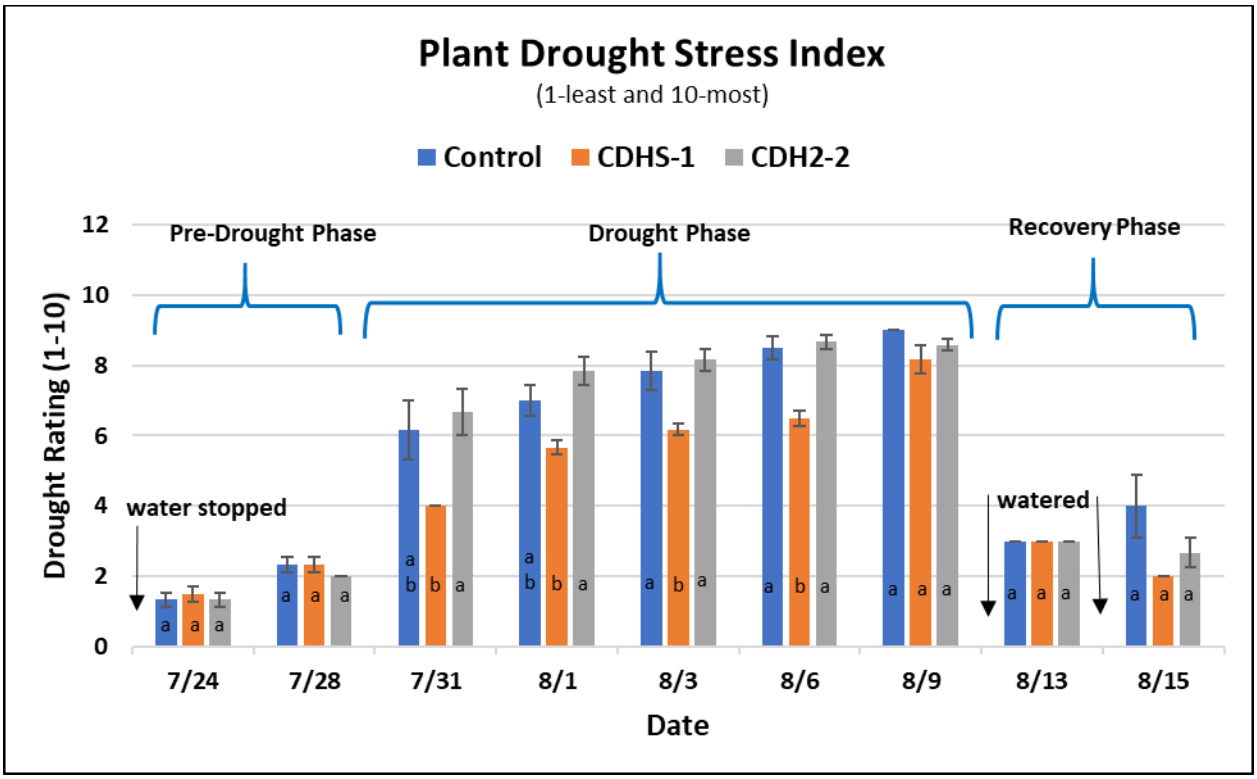

Fig. 6. Drought-stress index for plants grown in the soil columns given the CDHS-1, CDHS-2, and the control treatments during the bioassay. A greater drought rating (1-10) means that greater drought stress is visible. Error bars are standard errors and treatment means on a given day with different letters are significantly different (alpha 0.05) by Tukey's HSD.

Table 10. ANOVA table for plant drought-stress index when mean daily measures are analyzed over the course of the study.

Pre-Drought Phase					
Source	DF	SS	MS	F-Value	Pr>F
Treatment	2	0.389	0.194	0.854	0.436
Date	1	6.25	6.250	27.439	< 0.001
Treatment + Date	2	0.167	0.083	0.366	0.697
Drought Phase					
Treatment	2	64.089	32.044	33.929	< 0.001
Date	4	95.222	23.806	25.206	< 0.001
Treatment + Date	8	7.911	0.989	1.047	0.409
Recovery Phase					
Treatment	2	6.222	3.111	3.182	0.056
Date	1	0.111	0.111	0.114	0.738
Treatment + Date	2	6.222	3.111	3.182	0.056

Table 11. Summary of all pairwise comparisons for treatment means for the plant drought-stress index over the course of the study by Tukey's HSD.

Pre-Drought Phase		Drought Phase		Recovery Phase	
Treatment	Means	Treatment	Means	Treatment	Means
CDHS-1	1.917a†	CDHS-2	8.033a†	Control	3.500a†
Control	1.833a	Control	7.700a	CDHS-2	2.833a
CDHS-2	1.667a	CDHS-1	6.100b	CDHS-1	2.500a

† Means followed by different letters are significantly different (alpha 0.05) by Tukey's HSD.

Bioassay—Dry Biomass

At the end of the bioassay, the plants were harvested for shoot and root biomass (Fig. 7). In general, plants grown in soil treated with CDHS-1 and CDHS-2 had higher mean biomass compared to the soil-only control. However, there were no statistically significant differences ($\alpha < 0.05$) in shoot, root or total dry biomass among the treatments.

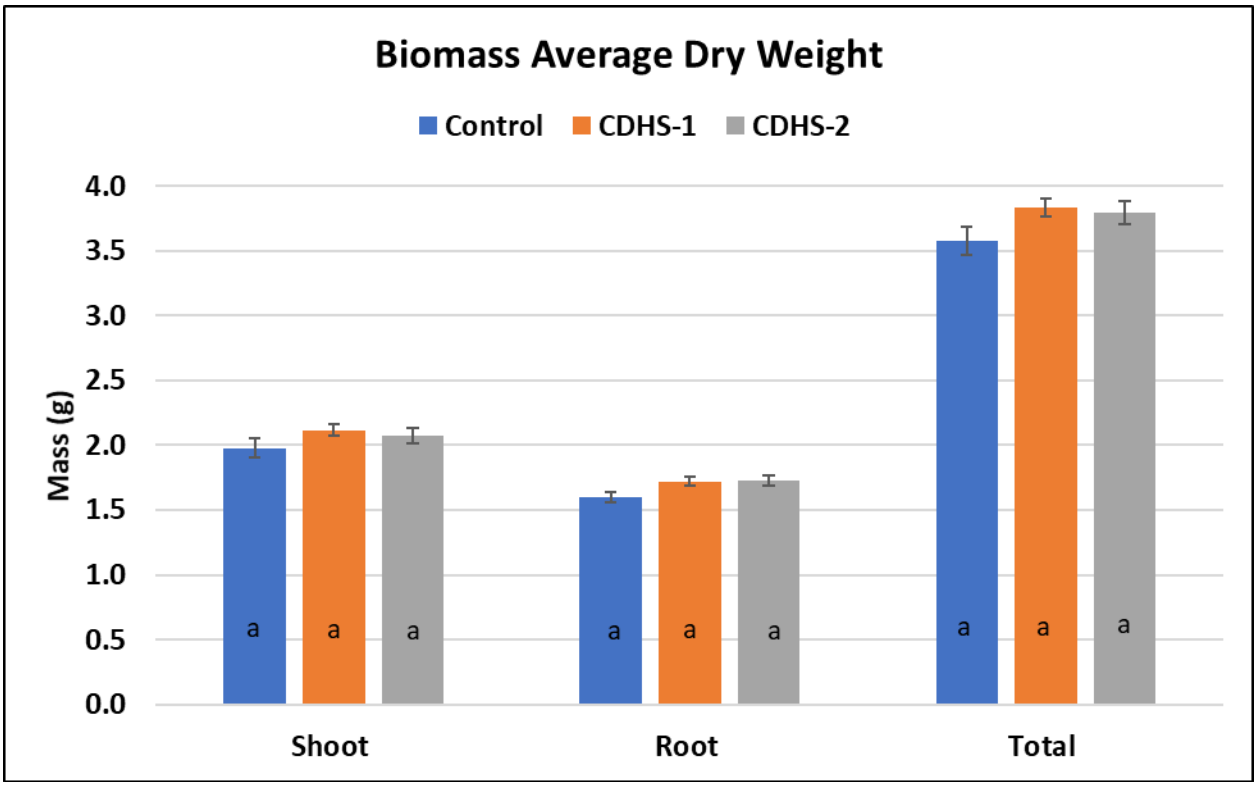

Fig. 7. Dry biomass of plants grown in soil columns given the CDHS-1, CDHS-2 and control treatments. Error bars are standard errors and treatment means with different letters are significantly different (alpha 0.05) by Tukey's HSD.

Consolidation Period—Soil Quality Characteristics

No significant changes in soil gravimetric water content at saturation or field capacity due to the addition of CDHS were observed at the start of the study. During the 30-day consolidation period, the drained gravimetric water content of CDHS-1 treated soils were never significantly different from the control. However, soil columns receiving the CDHS-2 treatment had significantly less gravimetric water content than the control soils and CDHS-1 treated soils on two and six of eight measurement dates, respectively, during the consolidation period. Table 12 shows the average percent increase or decrease in saturation, field capacity, and consolidation period soil gravimetric water content for the CDHS-1 and CDHS-2 treatments

relative to the controls. These results are in contrast, though not unexpectedly, to the significant increases in soil water-holding capacity following addition of coal-derived HA to soil reported by Piccolo et al. (1996) from their laboratory studies. Piccolo used a different process to extract and purify HA from coal than was used in this study. To our knowledge, there is no other relevant research regarding the influence of CDHS extraction procedures as related to water-holding capacity was found in published literature. Chen and Schnitzer (1976) suggest that significant chemical changes can occur in the molecular structure of HS based on the pH of the environment they are in. Therefore, extraction procedures may influence the molecular structure of CDHS and hence the impact of CDHS on the water-holding capacity of soils. In this study the CDHS-2 treatment significantly decreased gravimetric water content during the consolidation period relative to CDHS-1 and the control. This result supports the argument that HS have surfactant-like properties that influence soil structure and the movement and retention of water through soils and hence soil water-holding capacity (Quagliotto et al., 2006).

Table 12. Average change in gravimetric water content for soils given the CDHS-1 and CDHS-2 treatments relative to the controls. * indicates significant differences from the controls.

Treatment	Saturation	Field Capacity	Consolidation Period
CDHS-1	2.65%	0.63%	0.88%
CDHS-2	0.69%	-1.49%	-2.67*

Significant increases in soil bulk density for all treatments occurred during the consolidation period due to settling and consolidation of the soil as the columns were watered and leached 10 times during 30-day period. Soil columns given the CDHS-1 treatment consistently had significantly lower bulk densities relative to the controls, whereas soils given the CDHS-2 treatment were never significantly different from the controls (Fig. 3). Table 13

shows the percent increase in soil bulk density during the consolidation period and the percent increase or decrease compared to the control to elucidate the numeric trends in the study. The results from columns treated with CDHS-1 in this study agree with Piccolo et al. (1989, 1997), who showed significant increases in aggregate stability in response to CDHS. Such increases in aggregate stability should slow the rate of soil consolidation, resulting in less bulk densities. Our results suggest that HS from CDHS-1 create humic coatings around soil aggregates and/or provide substrate for soil microorganisms whose activity stabilizes soil structure (Piccolo et al., 1996; Laird et al., 2001). The different responses to CDHS-1 and CDHS-2 are attributed to the differences in manufacturing each of those materials. These results indicate that concentrations of KOH, and pHs of the extracting solutions can influence the properties of HS extracted from coal and that HS properties can influence their ability to stabilize soil structure (Chotzen et al., 2016).

Table 13. Average percent increase in soil bulk density in the soil columns from the starting bulk density until the end of the consolidation period and percent increases or decreases in bulk density for the CDHS-1 and CDHS-2 soils relative to the control soils. Different letters indicate significantly different (alpha <0.05) changes in bulk density.

Treatment	Bulk Density Increase Over the Consolidation Period	Increase or Decrease From the Control Over the Consolidation Period
Control	7.41% a	0.00%
CDHS-2	8.15% a	+9.09%
CDHS-1	5.93% b	-24.95%

Soil bulk density is inversely related to soil porosity, which strongly influences the ability of soils to retain plant-available water (Easton and Bock, 2016). Thus, lower bulk density values for a given soil tend to increase the amount of plant-available water (USDA, 1998). For example, at the end of the consolidation period on 8 August, CDHS-1 had the lowest bulk density

measure and the highest gravimetric water content (Fig. 8). However, in this study, soil columns treated with CDHS-1 had significantly lower mean bulk density values which did not lead to a significant increase in gravimetric water content.

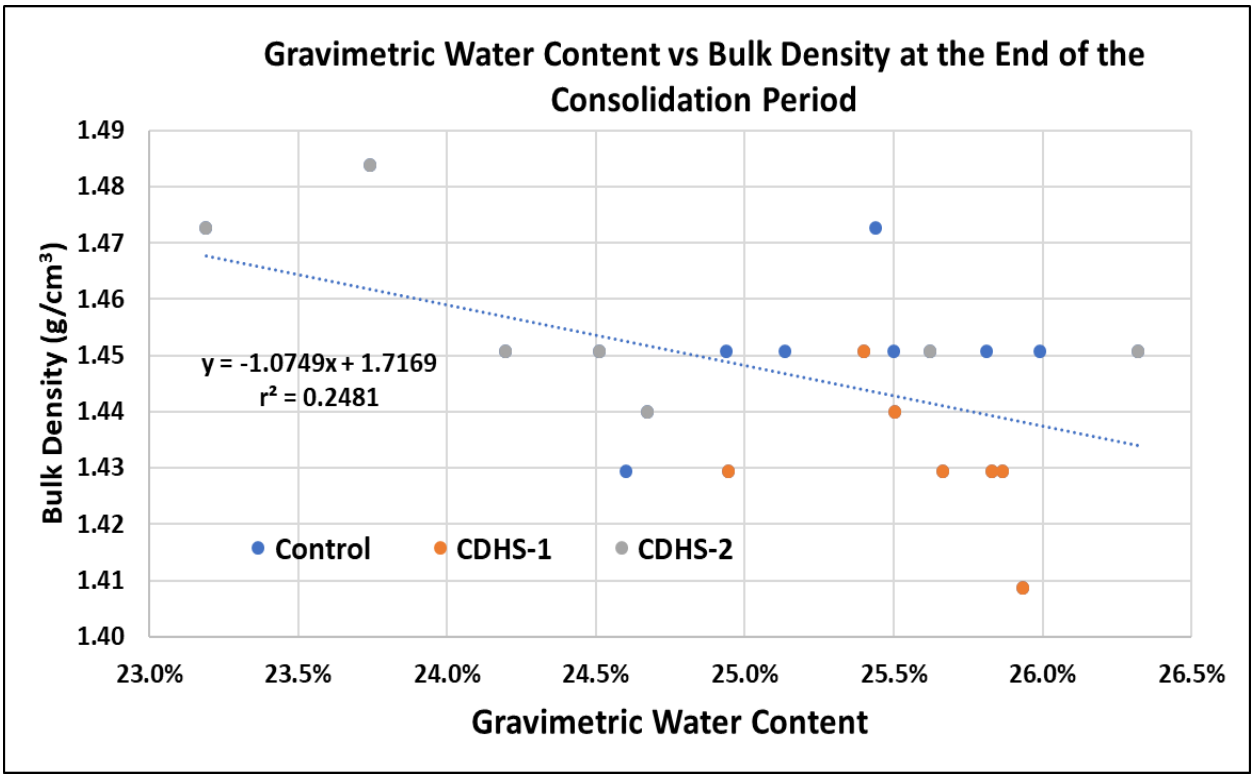

Fig. 8. Relationship between the average gravimetric water content and average bulk density of the soil columns treated with CDHS and the control at the end of the consolidation period.

Bioassay—Plant Growth

None of the treatments significantly affected plant heights during the 40-day bioassay period. All the plants in the bioassay followed the same general growth pattern: rapid growth from 13 July to 24 July, slow growth from 24 July to 31 July, no growth from 31 July to 5 August (drought), followed by a small burst of growth after watering. In general, plant heights for CDHS-1 and CDHS-2 were similar throughout the drought and recovery phases. These results

are in contrast to the significantly greater wheat (*Triticum aestivum* L.) plant heights reported by Tahir et al. (2011) in greenhouse pot studies for plants grown in soils treated with CDHS relative to controls. However, Tahir et al. (2011) used a different procedure to extract HA from coal in their study than was used in the present study, and their wheat plants were not subjected to drought stress during their 30-day (from emergence) growing period.

In contrast to plant height, the drought-stress index responded strongly to the treatments. Plants grown in soil given the CDHS-1 treatment had significantly less drought-stress index values during the drought phase than the plants grown in the control or CDHS-2 treated soils. After the last watering on 20 July, the plants started to show drought stress symptoms. At the early onset of drought stress (24 to 28 July) there were no significant differences. However, from 31 July to 6 August, plants grown in soil given the CDHS-1 treatment showed significantly less drought stress than plants grown in CDHS-2 treated soils, and from 3 to 9 August, significantly less drought stress than plants grown in the control soil. Furthermore, the results from the drought-stress index in relation to the gravimetric soil water contents during the consolidation period are mixed in response to the different CDHS treatments. For example, the CDHS-2 treated soils had significantly lower gravimetric water contents compared to both the CDHS-1 and control soils during the consolidation period, but plants grown in the CDHS-2 soil only showed significantly greater drought stress relative to plants grown in the CDHS-1 soils during the bioassay. Also, soils receiving the CDHS-1 treatment did not contain significantly more water when compared to the control soils during the consolidation period, but plants grown in the CDHS-1 soils did show significantly less drought stress during the bioassay compared to the controls. Limited literature is available on the effects of CDHS soil

treatments on plant drought stress. Zhang and Ervin (2004) investigated the effects of CDHS treatments on drought stress with creeping bentgrass (*Agrostis palustris* Huds. A). They reported the presence of cytokinin-like compounds in the coal and greater levels of cytokinin in the plants after application of CDHS. They theorized the cytokinin-like compounds increased drought-stress resistance of the bentgrass. Cytokinins are thought to aid plants by counteracting many deleterious drought-stress responses such as stomatal closure and the acceleration of plant senescence (Novakova et al., 2007).

The different CDHS treatments did not significantly affect root and shoot dry biomass. These results contrast with reports showing statistically significant increases in dry biomass production with the use of CDHS (Piccolo et al., 1993; Mbagwu and Piccolo, 1997). Piccolo et al. (1993) and Mbagwu and Piccolo (1997), used different processes to extract and purify HA from coal than were used in this study. Specifically, from the source coal they did an alkaline extraction with NaOH, removed non-soluble components by centrifuging, acidified with HCl to precipitate the HA, removed soluble components from the precipitated HA by centrifuging, dialyzed against distilled water until chloride free and then vacuum dried the sample. In the present study from the source coal, we only did an alkaline extraction with KOH and oven dried the material. The different extraction procedures may influence the chemical composition of CDHS and hence the impact of CDHS treatments on soil moisture retention and plant growth.

Overall, the dry biomass results were not consistent with the soil moisture and soil bulk density results. First, the CDHS-2 treated soils held significantly less water during the consolidation period relative to the CDHS-1 treated and control soils, but this decreased in soil water retention did not significantly impact dry biomass production during the bioassay.

Second, the CDHS-1 treatment significantly decreased soil bulk density during the consolidation period, which should provide a soil with better plant growth potential, but the lower bulk densities of the CDHS-1 treated soils did not translate to significantly greater biomass production during the subsequent bioassay. Third, the significant reduction in drought-stress index for plants grown in CDHS-1 treated soils compared to plants grown in control and CDHS-2 treated soils did not result in significantly greater biomass. We have no clear explanation for the dry biomass results not being consistent with the gravimetric soil moisture and soil bulk density results over the consolidation period in this study.

Overall, the evidence in this study points toward the importance of optimizing an extraction procedure for the HS contained in the coal from which they are derived. The point of the extraction procedure is to solubilize or “free up” the HS contained in the coal, and it was evident that when the source coal was pH adjusted with KOH to 9.5 (CDHS-1), it was more effective than when pH adjusted to 7.0 (CDHS-2) in this study. The exact chemical ramifications of the differences between the two extraction procedures can only be speculated upon without in-depth chemical analysis. The procedure used by Piccolo et al. (1997) is a more thorough procedure for the extraction and purification of HA, but at the same time is cost prohibitive for industry unless substantial yield increases are realized. Conversely, the results of this study that utilized a more simplistic approach to treating coal did not produce some of the positive effects that Piccolo et al. (1989, 1993, 1996) and others have observed with more purified extracts. Clearly more basic research is needed to determine the effects of different extraction procedures on the chemical properties of CDHS. Furthermore, more applied research is needed to determine the optimum methods for producing CDHS that minimize production costs while

maximizing the positive impacts of CDHS in varied agricultural production scenarios. Not investigated in the present study were the effects of different source coals on the properties of CDHS. This is another variable that may influence the properties and efficacy of CDHS.

CONCLUSIONS

Overall, the results from this greenhouse soil column study indicate that CDHS can reduce soil bulk density and enhance resilience of corn to drought stress. Statistically significant reductions in soil bulk density and drought stress symptoms were achieved with the addition of CDHS to a fine sandy loam soil at a rate 1 g kg^{-1} of soil. Decreases in soil bulk density during the consolidation period were attributed to the ability of CDHS to create humic coatings on soil aggregates and/or provide a substrate for soil microorganisms whose activity stabilizes soil structure. However, the enhanced resilience of corn grown in CDHS-1 treated soil to drought was not consistent with the observed effect of CHDS-1 on soil water holding capacity. Therefore some other factor, possibly releases of cytokinins from CDHS-1, may have influenced corn plant response to drought stress. The usefulness of this study is that reducing soil bulk density and enhancing resilience of corn to drought stress are important agronomic topics in production agriculture.

The results from this study indicated two other important findings. First, CDHS extracted from coal using KOH solutions adjusted to pH 9.5 resulted in significantly lower soil bulk densities and greater corn drought-stress resilience than CDHS extracted from coal with a solution adjusted to pH 7.0. This suggests a qualitative difference between the two CDHS materials; however, the effects of extraction procedures on properties of CDHS were not specifically investigated in this study. Also, this study suggests that a simple pH adjustment of the extracting solution can be used to improve the quality of CDHS products and their impact on soil properties and plant growth without performing more complex chemical procedures as done by previous researchers.

Improving and sustaining soil quality in crop production will require both knowledge and materials for farmers. Combining merits of CDHS to improve soil quality and plant growth with a more cost-effective production system will potentially attract more growers to conduct on-farm field trials to test the effectiveness of CDHS in an agricultural setting. Future research should be carried out to better understand the influence of different rates of CDHS, on different soil textures, with different crops and under different cropping systems. In summary, CDHS were proven to reduce soil bulk density and enhance resilience of corn to drought stress. More research is needed to understand the mechanisms of CDHS action in soils and to optimize production procedures.

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