BELL PEPPER GROWTH RESPONSES AND SOIL ENVIRONMENTAL CHANGES TO HUMIC SUBSTANCES AND DEFICIT IRRIGATION

A Thesis

by

KUAN QIN

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Chair of Committee,	Daniel I. Leskovar
Co-Chair of Committee,	Kevin M. Crosby
Committee Members,	Xuejun Dong
Head of Department,	Dan Lineberger

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ABSTRACT

Organic matter-derived soil amendments containing humic substances (HS) play a functional role in improving plant root growth and soil quality, but their interaction with water deficit levels is unknown. In this study, lignite-derived HS were mixed with pot soil in controlled environments (growth chamber and greenhouse) and field soil in two different environmental locations (clay in Uvalde and sandy in Weslaco), then subjected to four irrigation levels -- severe deficit (20%), mild deficit (40%), moderate deficit (60%) and well-watered (80%) on bell pepper (*Capsicum annuum* L.) plants based on water-holding capacity in controlled environment and evapotranspiration requirement in field conditions. Plant morphology and physiology were assessed in different growth environments. Root traits, soil chemical properties and microbial activities were measured and analyzed at the end of the study.

HS application significantly increased plant height and stem diameter during seedling development and early vegetative growth period, and decreased plant transpiration rates during early growth while maintaining photosynthesis at the same level as the control (increased water use efficiency), especially under severe or mild deficit levels. HS increased plant biomass accumulation in controlled environments and increased early yield in field conditions. HS increased root length, surface area and dry weight in controlled environments. These root promotion effects were consistent in field conditions although not statistically significant. HS also increased soil organic carbon as well as soil respiration and microbial population in both soil types. Plant growth performance was significantly decreased in severe and mild deficit stress, but was similar in moderate deficit and well-watered treatments.

These results suggest that HS have the ability to ameliorate severe or mild stress in the short-term, which can reduce water loss in plants exposed transiently to water deficit conditions. In addition, this study provides evidence that the application of HS might be considered for long-term agricultural use due to their capacity to improve crop early yield, soil nutrient cycling, organic carbon retention, microbial enrichment and activity under moderate stress or well-watered conditions.

DEDICATION

To my father

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Contributors

Part 1, faculty committee recognition

This work was supervised by a thesis committee consisting of Professor Daniel I. Leskovar as advisor and Professor Kevin M. Crosby as co-advisor of the Department of Horticultural Sciences and Professor Xuejun Dong of the Department of Soil and Crop Sciences.

Part 2, student/advisor contributions

All work for the thesis completed by the student, under the advisement of Daniel I. Leskovar of the Department of Horticultural Sciences.

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NOMENCLATURE

HS	Humic substances
OM	Organic matter
SA	Soil amendment
IR	Irrigation
WUE	Water use efficiency
WHC	Water-holding capacity
ET	Evapotranspiration

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Intense human activities in agricultural practices such as excessive irrigation, fertilization and the use of various agrochemicals cause soil degradation (*Diacono and Montemurro*, 2010), increase the soil nutritional imbalance and the susceptibility of plants to abiotic stress, which will become a threat for human health due to the decreasing crop yield and food quality (*Lal*, 2009). Besides, maximizing yields to feed the increasing human population under decreasing soil fertility and limited water resources is also a major challenge. It is expected that significant improvements in soil properties will also improve plant growth by changing water and nutrient uptake efficiency, and shoot and root morphological and physiological responses, which will ultimately enhance crop yield and quality, as well as economic benefits.

Organic soil amendments

Using functional soil amendments is an effective method to improve soil quality. Organic-matter derived from plant decomposition leads to a mixture of substrate that contains more than 70% lignite in the surface layer (0-5 cm) of soil (*Rumpel* et al., 1998), which provides a good source for organic matter derived-amendments. The amount of soil organic matter and N content can be significantly increased with the application of organic amendments along with inorganic fertilizers (*Goyal* et al., 1999), which makes organic amendments promising for the development of sustainable agriculture, high-yields and profitable production systems. Therefore, organic amendments have been studied in depth and used in practice (*Bulluck* et al., 2002; *Diacono and Montemurro*, 2010). Other than lignites, animal manure, crop residues and organic waste are also good sources to make organic amendments, such as compost and humic substances. Compost refers to the biological decomposition of organic matter under well managed and aerobic conditions into stabilized organic product (*Epstein*, 1996); Humic substances (HS) refer to a mixture of soil organic matter resulting from the decay of plant and animal residues. They have large impacts on soil composition and overall soil improvement, resulting in beneficial effects on soil biological and physiochemical environment changes as well as plant growth improvement.

Humic substances

HS can be applied as solid or liquid products extracted by various methods from a wide variety of raw materials including lignites, peat, composts and organic wastes, which determine the different physicochemical properties and application effects of HS (*Rose* et al., 2014). HS typically contain three fractions on the basis of their solubility in water under different pH conditions: fulvic acid, humic acid and humin (*MacCarthy* et al., 1990): 1) fulvic acid (FA): the fraction of HS that is soluble in water under all pH values; 2) humic acid (HA): the fraction of HS that is insoluble in water under acidic conditions ($pH < 2$) but soluble at higher pH values ($pH > 2$); 3) humin: the fraction of HS that is insoluble in water under any pH values. Based on the sequence of classification, they are increasing in color intensity: light yellow and yellow brown for fulvic acid, dark brown and grey black for humic acid, and black for humin; they are also increasing in molecular weight (in Daltons, Da) and carbon content but decreasing in oxygen content, degree of solubility and exchange acidity (the amount of total CEC occupied by the acidic cations H^+ and Al^{3+}) (*Rumpel* et al., 1998). A study by *Lobartini* et al. (1997) showed that humic acid fractions with different molecular weights (Da) had minor differences in elemental composition of C and N, with carbon elements accounting for 49% to 58% of the total composition, and nitrogen elements for 2.6% to 3.8%. By using infrared analysis, they also found humic acids had similar functional groups and bonds regardless of the different Da fractions. These evidences indicate that humic acid is composed of homogeneous fractions, which can ensure uniformity in its application. Based on that, humic acid became the most widely studied material for soil applications of HS, while only a few studies focused on fulvic acids.

Different raw materials can provide different HS components and properties, including various beneficial effects on plant growth and soil properties (*Chen and Aviad*, 1990). They can be summarized as follow:

a. Improve seed germination rates

HS application increased water absorption and enhanced overall enzyme activities in seeds, which led to an increase in respiration rate, and the energy released by respiration could be utilized in embryo development and rapid germination (*Chen and Aviad*, 1990). A study (*Piccolo* et al., 1993) tested the effects of coal-derived humic substances on lettuce and tomato seed germination, and found the fresh weight of seedlings increased with HS application in both crops, which was due to cell elongation, increased water absorption and the uptake of HS in seedlings.

b. Stimulate root initiation and growth

These are the most significant impacts of HS. *Canellas* et al. (2002) investigated the effects of HS isolated from earthworm compost and found that HS enhanced maize (*Zea mays* L.) seedling root elongation and lateral root initiation. *Nardi* et al. (1994) showed the roots of *Nicotiana plumbaginifolia* leaf explants were promoted when treated with HS or IAA alone, but were inhibited when adding an auxin inhibitor (TIBA) with HS or IAA. These studies concluded the root growth promotion was contributed by the auxin-like activity of HS. It is important to note that the hormone-like fraction of HS mostly comes from the low molecular weight (LMW) parts (< 5000 Da), which can easily reach and interact with plasma membranes of plant cells (*Nardi* et al., 2002; *Varanini* et al., 1993). By summarizing the outcomes of previous studies about the uptake of radioactive labeled ^{14}C-HS in plants, it showed that HS were absorbed by roots quickly in the initial timeframe, then absorption slowed down. The activity of uptake was promoted by increasing plant physiological metabolism and the solubility of HS (*Nardi* et al., 2002). After being absorbed in plant cells, LMW-HS was also shown to stimulate H^+-ATPase (proton-pumping ATPase) activity in isolated oat plasma membrane (*Varanini* et al., 1993). This generated the proton electrochemical gradient across the plasma membrane which was essential to activate most of the ion and metabolite transport (*Morsomme and Boutry*, 2000) and ultimately, enhance nutrient acquisition in roots, and therefore generate positive functions for root growth.

c. Improve shoot growth and affect plant physiological performance

Promotion of root growth also leads to improvements in shoot growth, *Tahir* et al. (2011) tested the effects of lignite-derived HS application on wheat (*Triticum aestivum* L.) growth and nutrient uptake under greenhouse conditions; they found an improvement in plant height, shoot weight and nitrogen uptake efficiency (NUE) at an optimal HS concentration. Another group (*Rose* et al., 2014) summarized previous research by using a random-effects meta-analysis and concluded that plant shoot and root dry weight increased 22.4% and 21.6%, respectively due to HS application. The increase of shoot dry weight appears to be due to increased mineral nutrient supply and uptake in HS treated soil (*Sharif* et al., 2002). In addition to increasing plant biomass accumulation, HS had been shown to affect some plant physiological performances like enzymes activity associated with photosynthetic (*Ferretti* et al., 1991) and carbohydrate metabolism of maize seedling leaves (*Merlo* et al., 1991). However, the effects of HS on plant gas exchange have not been widely explored (*Nardi* et al., 2002), which gives a potential aspect to emphasize in this study.

d. Improve soil structure and soil quality

The mechanisms by which HS change soil environment and quality are controversial and not clearly understood. HS have been shown to have positive effects on soil permeability, water-holding capacity and aeration (*Chen and Aviad*, 1990). The presence of functional groups such as cellulose and polyols in HS (*Vlčková* et al., 2009) alter the water uptake and holding capacity of soil particles (*Brooks* et al., 2004), resulting in higher water retention. These functional properties suggest that HS application may be a promising strategy for mitigating water-limited conditions. HS have also been reported to increase cation exchange capacity (CEC), organic carbon content, N and P concentration, and solubility of some micronutrients like Fe in the soil (*Sharif* et al., 2002). HS can also enhance the ability of phytoremediation for some heavy metals in contaminated soils like Cd, Cu and Pb. This response was due to the temporary biological activity of metal-humic complexes, decreasing their transferable toxic effects in the soil environment (*Halim* et al., 2003).

e. Enhance soil biological activity

In the soil microbial environment, studies by *Gryndler* et al. (2005) showed that HS stimulated root colonization and production of extra-radical mycelium induced by mycorrhizal fungi. The slow decomposition ability of HS can provide carbon and nitrogen resources to microbes especially with limited nutrient levels; some microbes can even use HS as electron acceptors for their oxidation to provide energy (*Lovley* et al., 1996). Additionally, the long-term interaction between HS and microorganisms may generate new HS (*Kulikova* et al., 2005), which provide extended benefits in soil quality changes.

In summary, HS have positive impacts on plant-soil interactions, and the effects on promoting root growth are more obvious than shoot growth (*Chen and Aviad*, 1990). Besides, conventional soil tillage management that causes organic matter degradation has negative effects on HS formation (*Shepherd* et al., 2001). So adding exogenous HS as a soil amendment can reverse the degradation along with soil tillage while improving soil properties (*Rose* et al., 2014). With these benefits and concerns, HS as soil amendments have been widely studied on agronomic crops like wheat, maize, oat (*Canellas* et al., 2002; *Varanini* et al., 1993; *Tahir* et al., 2011; *Sharif* et al., 2002; *Dunstone* et al., 1988; *Ferretti* et al., 1991) and vegetable crops like tomato, celery and lettuce (*David* et al., 1994; *Ciarkowska* et al., 2017; *Hartz and Bottoms*, 2010). However, higher HS application rates can easily exacerbate the plight of micronutrient deficiency by depleting the available pool for plant uptake (*Rose* et al., 2014), and finally inhibit plant growth. Therefore, a suitable rate of HS application into soils is critically important.

Water stress and deficit irrigation

Drought stress has cost $200 billion in losses from 1980 to 2013 in the U.S., which accounted for about 20% of total loss due to all other weather and climate disasters (*Smith and Matthews*, 2015). Agriculture is a major activity that heavily depends on water demand, and therefore has suffered the greatest damage from water stress. Due to insufficient precipitation (available water from outside the soil) and low soil water-holding capacity (available water from inside the soil), water deficit stress can seriously affect plant morphology and physiology and therefore overall growth and productivity. In order to decrease water losses, plants have unique mechanisms to cope with deficit stress. For example, plant cell water potential is reduced and abscisic acid (ABA) is accumulated, which induce stomatal closure, thereby decreasing transpiration rate (*Taiz* et al., 2015). However, stomatal closure also decreases CO_2 uptake, which will inhibit leaf photosynthesis, cause the imbalance of free electrons and $NADP^+$

dehydrogenase and leads to the generation of reactive oxygen species (ROS), which will impede plant growth by oxidizing and damaging the normal functional cellular constituents in plant cells such as proteins, DNA, RNA and lipids (*Taiz* et al., 2015).

Improving plant water use efficiency (WUE) as a potential solution to alleviate worldwide water shortages, has become a paradigm for scientists. Deficit irrigation, the application of water below sufficient crop-water requirements, without causing significantly economic losses, is an important method to cope with limited water supply and improve WUE, especially in arid or semi-arid regions of the world (*Fereres and Soriano*, 2006). For example, based on a watermelon study of *Leskovar* et al. (2016), suitable water stress achieved by deficit irrigation maintained plant performance and improved plant water use efficiency, as well as forcing a surplus of vegetative growth to be transformed into economic fruit growth, while severe stress significantly decreased plant yield. It showed that appropriate deficit irrigation could become an essential tool in agricultural production. But a successful deficit irrigation procedure is not easy to accomplish, as it depends on accurate soil water content and proper irrigation scheduling methods (*Jones,* 2004). In this study, in order to test the effects of deficit irrigation on bell pepper growth, we implemented deficit irrigation in controlled environments (growth chamber and greenhouse) based on soil water-holding capacity, and uncontrolled environments (field) based on crop evapotranspiration (ET) demand. We hope these studies will provide additional useful information when considering deficit irrigation in agriculture.

Research questions

A large body of research reviewed the biological effects of HS on biota growth (*Kulikova* et al., 2005), and concluded that HS were ideal modulators in adverse soil environments such as drought, salinity and other abiotic stress conditions due to the detoxifying ability of HS. However, there is a lack of understanding of short- or long-term effects of HS on alleviating plant biological responses in water deficit scenarios, such as the modification of leaf gas exchange and WUE. Within this framework, this research is intended to address the following five questions, using bell pepper as a model plant system:

a. Will HS added to soils at optimal rates promote plant morphological and physiological responses and increase growth compared to control?

b. Do HS regulate the activity and solubility of major nutrients in soil?

c. Do HS applications improve the concentration and activity of soil microorganisms, such as bacteria and fungi?

d. Can HS mitigate potential crop losses under water deficit conditions?

e. Do HS applied to different soil textures (sandy and clay) uniquely affect soil properties, water content, nutrient levels, and plant growth?

CHAPTER II

BELL PEPPER GROWTH RESPONSES AND SOIL PROPERTY CHANGES TO HUMIC SUBSTANCES AND DEFICIT IRRIGATION IN CONTROLLED ENVIRONMENTS

Introduction

Peppers, which originated in Mexico and South America, are becoming popular in people's diet due to their various colors, flavor, spice and nutritional values (*Villalón*, 1981). Bell green pepper (*Capsicum annuum* L.), as one of the pepper types, has a high economic value in agriculture. In 2014, the yield of green peppers is 16.7 t/ha with the total production 32 million tons on a global basis, and the gross production value is more than $30 billion all around the world (*FAO*, 2014). Due to the benefit prospects, the potential application of HS on vegetable crops like bell pepper is becoming promising and important.

HS can be applied as commercial solid or liquid products derived from soil and water (*Malcolm and MacCarthy*, 1986). For practical field applications, the solid form seems to be better than liquid due to less cost and potentially less leaching losses. In this study, we focus on lignite-derived solid HS, and then use bell pepper as a model vegetable crop and controlled environments as suitable growth conditions, to access and test the HS effects on bell pepper morphology and physiology responses and soil environmental changes at different irrigation levels. We hypothesize that through the alteration in plant gas exchange, root growth and soil microbial activity, HS will mitigate potential crop losses under water deficit conditions.

Approach

a. Growth environments and soil materials

Two studies were conducted in controlled environments growth chamber (Conviron PGR15, Manitoba, Canada) and greenhouse at the Texas A&M AgriLife Research Center at Uvalde, TX (29.21°N, 99.79°W). The growth chamber was set with a ramping temperature from 20°C to 28°C and a ramping light intensity up to 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the daytime for 16h, followed by constant 20°C without light during the night for 8h. The environmental parameters inside the greenhouse were monitored by a control system Wadsworth (Arvada, CO) and recorded by a weather station WatchDog (Spectrum Technologies Inc., Aurora, IL). During the greenhouse experiment, the average daily light integral and temperature were 11.0 $mol \cdot m^{-2} \cdot day^{-1}$ and 29.4°C, respectively.

In the first growth chamber experiment, the short-term impacts of HS application on plant seedlings were assessed in a sandy soil. A following greenhouse experiment was conducted to assess the mid-term effects of HS on plants as well as soil among two types, sandy and clay. The basic soil properties are shown in Table 1. Lignite-derived solid HS (Novihum Co., Dresden, Germany, Table 2) was used as a soil amendment mixed with soil at a rate of 0.5 $kg \cdot m^{-2}$.

b. Plant material, soil amendment and irrigation treatments

Bell pepper (*Capsicum annuum* cv. Revolution) seeds were directly sown into 2.5 L pots (15 cm diameter, 15 cm height) filled with sandy soil previously amended with 8.8 $g{\cdot}pot^{-1}$ HS at the beginning of the growth chamber experiment. At the initial stage of the greenhouse experiment, 8-week old bell pepper seedlings grown in 200-cell trays ($2.7 \times 2.7 \times 7.2$ cm^3 per cell) were transplanted into 10 L pots (25 cm diameter, 20 cm height) filled with sandy and clay soil previously amended with 24.5 $g{\cdot}pot^{-1}$ HS. Control pots filled with soil without HS amendment were included.

Three deficit irrigation levels -- severe stress (20% soil water-holding capacity, WHC), mild stress (40% WHC) and moderate stress (60% WHC), with a well-watered (80% WHC as no stress) treatments were initiated 4 weeks after direct seeding in the growth chamber and 1 week after transplanting in the greenhouse. Pots were weighed daily and irrigation management was conducted at different times and frequencies based on daily soil moisture loss in each treatment. Plants were fertilized using macro-fertilizer 3N-1P-2K (CNS17, Botanicare, Chandler, AZ) and micronutrients (Valagro Brexil Multi, Italy) during the growth period. Standard bell pepper management practices and pest control were applied in both environments.

c. Plant growth response measurements

During the growing period, plant morphological and physiological measurements were conducted after imposing water-deficit stress. Plant height and stem diameter were measured using ruler and digital caliper (VWR, Radnor, PA); rate of photosynthesis (*Pn*), stomatal conductance (g_s) and transpiration (*E*) were measured with a portable photosynthesis system (LI-6400 XT, LI-COR Biosciences, NE) and chlorophyll content index (SPAD) with a chlorophyll meter (SPAD-502 Plus, Minolta, Japan). These parameters were collected at biweekly intervals in the growth chamber for 4 weeks which represented the bell pepper seedling stage, and the experiment ended 10 weeks after direct seeding; measurements were taken at monthly intervals in the greenhouse for 3 months which represented the bell pepper vegetative stage, flowering stage and mid-harvest stage, respectively, and the experiment ended 4 months after transplanting.

At the end of the experiment, plant leaf, shoot and fruit dry weight were determined as above-ground biomass after oven drying at 75°C for 48 hours. For root measurements, in the growth chamber, whole roots were carefully washed and collected; while in the greenhouse, partial root samples were taken out with a soil auger (0-15 cm depth), and carefully collected. Root length, surface area and average diameter were scanned using an EPSON V700 scanner (Epson, Japan) and measured with a WinRHIZO software (V5.0, Regent Instruments, Canada), while root dry weight was determined after oven drying at 75°C.

d. Soil chemical and biological analysis

At the end of the growing period, in the greenhouse experiment, a soil auger with diameter 2.5 cm was used to collect soil cores within 0-15 cm depth in the pots. About 50% of the fresh soil samples were split into two parts. The first part was immediately shipped to Earthfort Lab (Corvallis, OR) for microbial activity analysis, and the rest part was used for soil respiration (Soil CO_2-Burst) (*Haney* et al., 2008) test with SOLVITA soil respiration box (Woods End Laboratories, Mt Vernon, ME). Total and active bacteria, total and active fungi were obtained by following staining procedures (*Stamatiadis* et al., 1990), direct microscope observation and other procedures provided by *Babiuk and Paul* (1970), *Van Veen and Paul* (1979), and *Ingham and Klein* (1984).

The remaining soil samples were dried at 75°C in the oven, then ground and sieved to 2 mm, and shipped to the Soil, Water and Forage Testing Laboratory (Texas AgriLife Extension Service, College Station, TX) for chemical analysis. Soil pH and electrical conductivity were measured in a 1:2 soil: water ratio extract (*Rhoades*, 1982); nitrate-nitrogen (NO_3-N) was extracted using 1 M KCl solution (*Keeney and Nelson*, 1982) and determined by spectrophotometry. Soil P and K were extracted using an extractant evaluated by *Mehlich* (1978) and then determined by an ICP-MS.

e. Statistical analysis

A two-way factorial completely randomized design with two soil amendments (Control and HS) and four irrigation levels (20%, 40%, 60% and 80% WHC) replicated six times was used in growth chamber experiment. The same experimental design, with two soil types (sandy and clay) was used in the greenhouse experiment. Plant morphology and physiology performances were analyzed by repeated measures analysis of variance using Proc Mixed in SAS (Version 9.4, SAS Institute, Cary, NC); plant biomass accumulation, root and soil traits were analyzed using the ANOVA; while multiple comparisons of means were analyzed using the least significant difference (LSD) at $\alpha = 0.05$. A principal component analysis (PCA) was used to evaluate the relationship between selected variables and treatments using the *prcomp* function in R (Version 3.4.0).

Results

a. Plant growth responses to humic substances and deficit irrigation

Based on the results of repeated measures analysis of variance (Table 3, as the main purpose is to test the time effects, the *P*-values for factor soil amendment, irrigation and their interaction were not shown), different irrigation levels had significantly different effects on plant morphological and physiological traits within different growth period in both controlled environments. In terms of soil amendment, it had significantly different effects on plant stomatal conductance and transpiration among different seedling stage in the growth chamber, and the rate of photosynthesis and transpiration in different growth period in the greenhouse. In addition, time significantly affected the effects of soil amendment under different irrigation levels on stomatal conductance (*P*-value = 0.030) in greenhouse sandy soil, and transpiration (*P*-value = 0.024) in clay soil.

In the seedling or vegetative period, HS significantly increased plant height and stem diameter, and also promoted plant chlorophyll content (SPAD) in sandy soil, but the results were opposite in clay soil (Table 4). Interestingly, during the seedling stage in growth chamber, HS application significantly decreased plant stomatal conductance while maintaining photosynthesis the same level as control; but in the greenhouse, stomatal conductance as well as plant photosynthesis were both decreased in plant vegetative stage regardless of the soil types as a result of HS application, and these effects lasted even longer in clay soil (flowering stage). Leaf gas exchange decreased under severe and mild water stress, and that reaction was further accelerated with HS application especially in sandy soil (data not shown), yet the HS-related reduction in stomatal conductance and photosynthesis appeared less evident in late plant growth. Reduced gas exchange resulted in higher plant morphological performances in sandy soil but lower performances in clay soil, indicating that HS had different behaviors in different soil types.

In the growth chamber experiment, HS increased plant above-ground dry weight (Figure 1 a) compared to the control, especially significant in mild, moderate water stress and well-watered conditions. In addition, HS treated pots showed a set of small fruits, indicating an early promotion of the reproductive development. Despite differences in early plant growth between HS and control, no significant difference was found in the final dry matter accumulation of plants grown in greenhouse for 4 months (Figure 1 b, c); there was only a slight increase due to HS application occurring in sandy and clay soil, under moderate water stress and well-watered conditions. Under severe and mild water stress, HS slightly decreased plant biomass in sandy soil. Besides the relatively insignificant effects on bell pepper above-ground biomass, HS showed remarkable promotions in root parameters (Figure 2), especially in root length, surface area and root dry weight under moderate water stress (60%) and well-watered (80%) conditions in both soil types. HS also increased root average diameter under severe water stress (20%) in the growth chamber sandy soil and under well-watered in the greenhouse clay soil environments.

Water deficit treatments had significant effects on plant morpho-physiological traits after irrigation treatment started. Severe and mild water stress significantly decreased plant shoot and root performance, biomass accumulation regardless of soil types. Moderate water stress maintained and even promoted plant growth compared to well-watered treatment. Irrigation levels also differentially impacted plant shoot and root growth on different soil types in greenhouse. For example, well-watered treatment decreased plant above-ground biomass accumulation in sandy soil but increased it in clay soil compared to deficit stress (Figure 1 b, c); similar effects were observed for root growth, whereas well-watered treatment decreased root parameters in sandy soil but increased them in clay soil especially compared to severe and mild deficit water stress (Figure 2 b, c).

b. Soil environmental changes humic substances and deficit irrigation

In the greenhouse under clay soil condition, HS slightly decreased soil pH, but significantly increased soil electrical conductivity (EC) by 25.6% and NO_3-N content by 68.7%, and decreased K content by 10.0% (Tables 5). In sandy soil, HS significantly increased soil EC in severe and mild water stress (20%, 40%), and also increased K content regardless of irrigation levels. The K content change indicating the response of K to HS depends on soil type. HS significantly increased soil total bacteria by 11.8% in sandy soil and by 43.8% in clay soil, but decreased total fungi by 30.9% in clay soil. There were no significant differences between HS and control in soil respiration, active bacteria and fungi population. Severe and mild deficit irrigations significantly decreased soil pH but they increased soil electrical conductivity and N, P, K content in both soil types. The increase in nutrient retention might be due to the reduced irrigation frequency and less nutrient leaching. In addition, low irrigation (20%, 40%) decreased soil respiration and soil active bacteria population especially in clay soil; furthermore, these two parameters were significantly positively correlated (r = 0.58 with *P*-value 0.003).

Table 1. Sandy and clay soil basic properties

Soil type	Sand %	Clay %	Silt %	Density $g \cdot cm^{-3}$	WHC [a] $g \cdot cm^{-3}$	pH	EC [b] $\mu mhos \cdot cm^{-1}$	Nitrate-N mg/kg	Phosphorus mg/kg	Potassium mg/kg
Sandy	94	4	2	1.61	0.48	5.9	81	0.3	17	62
Clay	33	37	30	1.26	0.73	7.9	384	21	59	900

[a] WHC: water holding capacity; [b] EC: electrical conductivity

Table 2. Composition of humic substances (HS) soil amendment

pH	Density $g \cdot cm^{-3}$	Carbon %	Nitrogen %	Ash %	Fulvic acid %	Humic acid %	Humin %
7.7	0.6	65.8[a]	5.78	5.2	0.7	56.7	24.1

[a] All percentages are in relation to dry matter of the HS material

Table 3. Time affected source of variations and *P*-values from repeated measures analysis of variance on plant height (PH), stem diameter (SD), chlorophyll content index (SPAD), rate of photosynthesis (*Pn*), stomatal conductance (g_s) and transpiration (*E*) of bell pepper grown in growth chamber (GC) and greenhouse (GH) environments

Environment	Source of variation [a]	PH cm	SD mm	SPAD	*Pn* $\mu mol \cdot m^{-2} \cdot s^{-1}$	g_s $mol \cdot m^{-2} \cdot s^{-1}$	*E* $mmol \cdot m^{-2} \cdot s^{-1}$
GC sandy soil	SA × T	0.285	0.466	0.002	0.958	0.015	0.038
	IR × T	< 0.001	0.002	< 0.001	< 0.001	< 0.001	< 0.001
	SA × IR × T	0.362	0.751	0.118	0.751	0.693	0.403
GH sandy soil	SA × T	0.015	0.379	0.347	0.023	0.170	0.036
	IR × T	< 0.001	< 0.001	0.007	< 0.001	< 0.001	< 0.001
	SA × IR × T	0.677	0.229	0.156	0.121	0.030	0.559
GH clay soil	SA × T	0.021	0.134	0.152	0.001	0.101	0.044
	IR × T	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	SA × IR × T	0.884	0.180	0.882	0.123	0.071	0.024

[a] SA: soil amendment; IR: Irrigation; T: time effects of biweekly interval in growth chamber and monthly in greenhouse.

Table 4. Time-course effects (*P*-values) of HS on plant height (PH), stem diameter (SD), chlorophyll content index (SPAD), rate of photosynthesis (*Pn*), stomatal conductance (g_s) and transpiration (*E*) under different controlled environments

Environment	Time [a]	PH	SD	SPAD	Pn	gs	E
		cm	Mm		μmol·m^{-2}·s^{-1}	mol·m^{-2}·s^{-1}	mmol·m^{-2}·s^{-1}
GC sandy soil	Week 2	< 0.001 + [b]	< 0.001 +	< 0.001 +	0.742	0.047 - [c]	0.059
	Week 4	< 0.001 +	< 0.001 +	0.092	0.811	0.079	0.309
GH sandy soil	Month 1	0.002 +	0.015 +	0.353	0.001 -	< 0.001 -	0.006 -
	Month 2	0.696	0.193	0.284	0.767	0.755	0.522
	Month 3	0.725	0.021 +	0.738	0.710	0.283	0.704
GH clay soil	Month 1	0.071	0.027 -	0.008 -	0.011 -	0.037 -	0.077
	Month 2	0.065	0.633	0.223	0.016 -	0.036 -	0.030 -
	Month 3	0.336	0.576	0.843	0.537	0.679	0.864

[a] Time was shown biweekly interval in growth chamber and monthly in greenhouse after imposing different irrigation treatments; [b] + indicated significantly increased, [c] - indicated significantly decreased compare HS to control at $\alpha = 0.05$ according to LSD test.

Table 5. Sandy and clay soil final chemical and microbial properties as affected by soil amendment (SA) and irrigation (IR) treatments in greenhouse (GH) environment. Soil chemical properties including pH, electrical conductivity (EC), nitrate-nitrogen (NO$_3$-N), phosphorus (P) and potassium (K); microbial properties including soil respiration (CO$_2$-C), active bacteria (AB), total bacteria (TB), active fungi (AF) and total fungi (TF)

Environment			pH	EC µmhos·cm^{-1}	NO$_3$-N mg/kg	P mg/kg	K mg/kg	CO$_2$-C mg/kg	AB µg·g^{-1}	TB µg·g^{-1}	AF µg·g^{-1}	TF µg·g^{-1}
GH sandy	SA	Control	8.1	271	15.0	264	41 b	4.17	27.12	1020 b *	6.71	433
		HS	7.9	265	10.2	259	50 a	4.86	28.68	1140 a	7.56	388
	IR	20%	7.6 c	396 a	38.1 a	314	66 a	2.72 b	26.43	1069	5.07	352
		40%	7.9 b	310 b	10.6 b	333	54 a	4.25 b	25.68	1098	8.48	352
		60%	8.3 a	191 c	1.2 b	213	31 b	4.17 b	30.22	1104	9.92	437
		80%	8.2 a	176 c	0.5 b	186	31 b	6.92 a	29.25	1048	5.08	500
	P-value	SA	0.074	0.796	0.263	0.923	0.047	0.357	0.565	0.046	0.577	0.483
		IR	< 0.001	< 0.001	< 0.001	0.152	< 0.001	0.007	0.580	0.881	0.082	0.299
		SA × IR	0.886	0.0188	0.062	0.268	0.517	0.104	0.197	0.202	0.869	0.649
GH clay	SA	Control	8.1	941 b	57.2 b	181	881 a	82.72	35.95	828 b	13.71	243 a
		HS	8.0	1182 a	96.5 a	172	792 b	80.01	33.33	1191 a	13.22	168 b
	IR	20%	8.0 b	1235 a	128.9 a	195 ab	1109 a	60.75 bc	31.38 bc	1112	10.67	159
		40%	7.9 b	1441 a	166.7 a	213 a	1126 a	54.97 c	30.53 c	1078	11.89	209
		60%	8.2 a	781 b	6.6 b	140 c	578 b	100.90 ab	37.57 ab	945	11.44	236
		80%	8.1 a	788 b	5.2 b	158 bc	534 b	108.83 a	39.07 a	904	19.87	218
	P-value	SA	0.207	0.004	0.015	0.554	0.031	0.849	0.261	0.005	0.880	0.014
		IR	< 0.001	< 0.001	< 0.001	0.011	< 0.001	0.031	0.035	0.513	0.177	0.260
		SA × IR	0.762	0.154	0.095	0.770	0.547	0.723	0.289	0.245	0.608	0.421

* Different letters within column from the same factor indicate significant differences at $\alpha = 0.05$ according to LSD test.

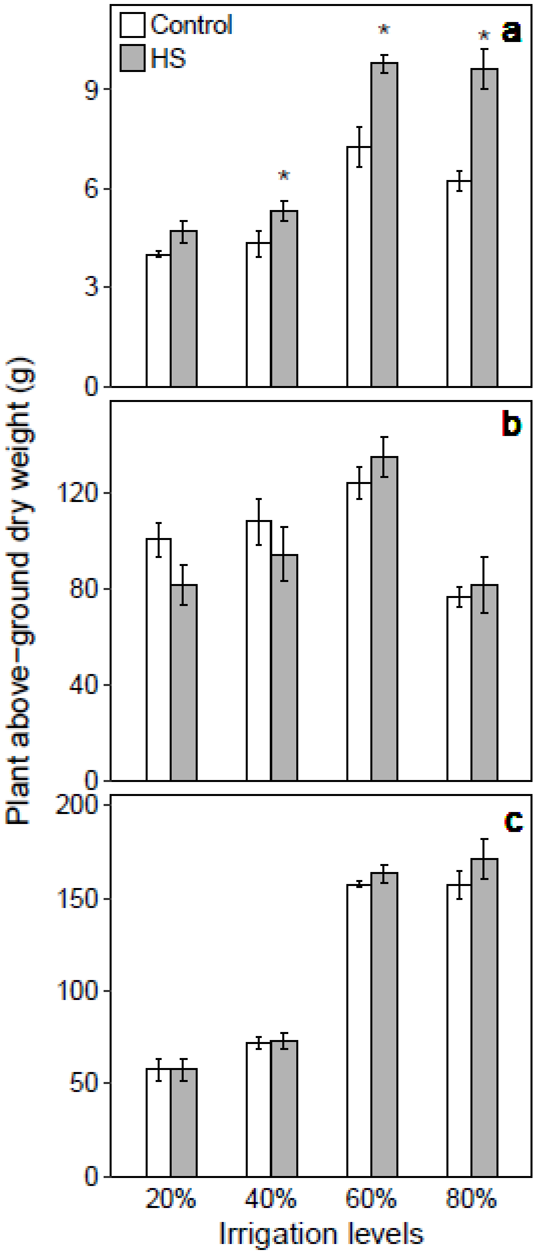

Figure 1. Bell pepper above-ground dry weight accumulation (± standard error) in the growth chamber sandy soil (a), greenhouse sandy soil (b) and clay soil (c) for soil amendment and different irrigation levels.

* indicated significantly differences between HS and control at α = 0.05 according to LSD test.

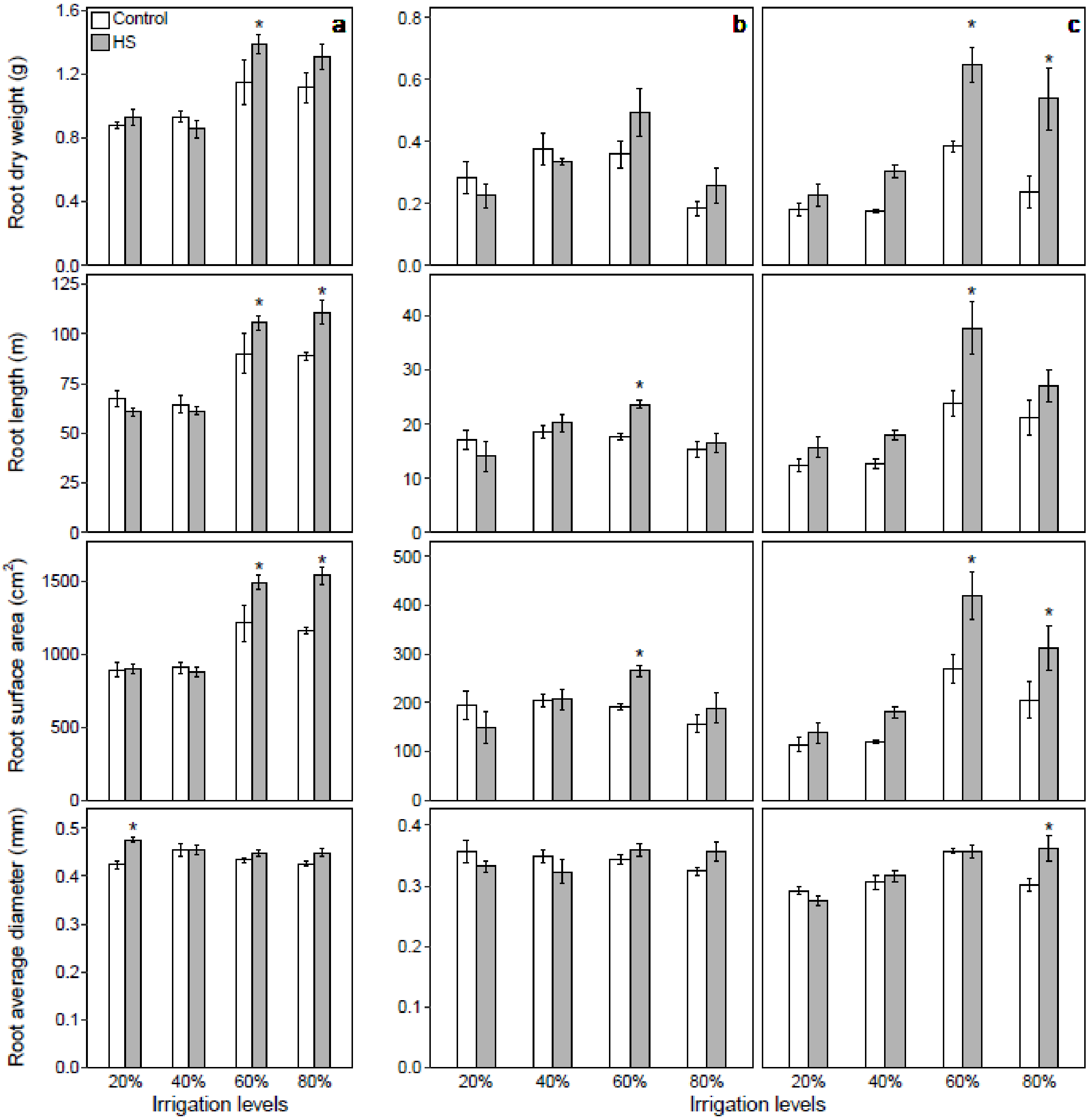

Figure 2. Bell pepper root dry weight, length, surface area and average diameter (± standard error) in the growth chamber sandy soil (a), greenhouse sandy soil (b) and clay soil (c) for soil amendment and different irrigation levels.
* indicated significantly differences between HS and control at α = 0.05 according to LSD test.

Discussion

The application of soil amendments is ultimately aiming to promote plant growth and minimize yield loss due to unsuitable environments. As organic matter derived soil amendment, HS have two positive influences: improve biota growth and mitigate some abiotic stress due to the detoxifying nature of HS (*Kulikova* et al., 2005). This discussion will combine the results from the growth chamber and greenhouse, and analyze the potential impacts of HS on plant and soil response variables under different environment conditions.

a. HS improved biota growth

In the growth chamber study, the promotive responses of HS in plant height, stem diameter, chlorophyll content (SPAD) and plant biomass during seedling growth, agree with the results of *Azcona* et al. (2011), who used sewage sludge derived HS on pepper plant (*Capsicum annuum* L. cv. Piquillo) in greenhouse conditions. The increase in plant chlorophyll content suggests that HS treated plants had an increased nutrient absorption ability, particularly in sandy soil during early growth. Although similar promotive effects were measured for some variables in the greenhouse, total plant above-ground biomass was minimally affected by HS. Instead, plant roots were highly affected by HS application regardless of the growth environments and soil types -- it has been reported that HS have auxin-like activity, reaching and interacting with the plasma membrane of plant cells (*Varanini* et al., 1993; *Nardi* et al., 2002), and therefore conferring functional benefits for root growth. HS were also shown to stimulate H^+-ATPase (proton-pumping ATPase) activity in plasma membrane of maize (*Canellas* et al., 2002) and isolated oat (*Avena sativa* L.) roots (*Varanini* et al., 1993), these ATPases generate a proton electrochemical gradient across plasma membrane that is essential to complete most ions and metabolite transport process for nutrient acquisition in roots (*Morsomme and Boutry*, 2000). Moreover, future understanding of the potential mechanism of auxin-like activity of HS and the study about how to transfer the beneficial outcomes from root to shoot growth is still needed.

HS have been considered to be a nutrient carrier, with beneficial impacts on soil nutrient supply and retention, which are the basis for plant growth. However, these effects appear to differ across soil types as reported by *Ciarkowska* et al. (2017). That study found HS increased shoot and root biomass of celery and leek due to increased soil available nutrients, and they also observed that the mean biomass was higher in medium and fine textured (silt to clay) soil compared to coarse textured (sandy) soil with poor nutrient retention capacity. In this study, although the above-ground biomass of bell pepper was not significantly affected by HS application, the root biomass was indeed improved by HS, possibly due to an increase in soil EC and NO_3-N content. The increase of root biomass by HS was only about 10% in sandy soil, which was much lower compared to 80% in clay soil. Moreover, roots grew better in the fine texture (clay) than coarse textured (sandy) soil, especially at moderate deficit and well-watered conditions. It is expected that HS might have an effective ability to enhance the existing poor fertile quality of sandy soil: *Valdrighi* et al. (1996) showed that compost derived HS significantly increased chicory biomass in sandy soil, especially when applied at a higher rate (4000 $mg \cdot kg^{-1}$) as in aqueous solutions. However, in this study, except for a notable biomass promotion in the growth chamber sandy soil experiment, the effects of HS on sandy soil were not significant in the mid-term greenhouse experiment. The lack of response may be explained by three possible scenarios: 1) the sandy soil we used could not provide sufficient nutrients to plant growth when compared to nutrient-rich clay soil; 2) the application rate of HS was not high enough to bring on significant growth changes in sandy soil especially for a mid- to long-term growth; however, we need to be careful for the application rate because extra HS beyond an optimal level may deplete soil nutrient by "stealing" the nutrient cations and made them unavailable for root uptake, and ultimately decreasing plant growth (*Chen and Aviad*, 1990); 3) the diverse nature of HS caused inconsistent effects on different soil conditions, as previously reported by *Kulikova* et al. (2005).

Soil microbial activity of bacteria and fungi is a key factor that influences plant productivity, changes plant-water relations, affects soil properties, and regulates decomposition of organic material and nutrient cycling (*Neher*, 1999). The total bacteria and fungi population represent soil nutrient cycling capacity, while active bacteria and fungi population represent the part that currently metabolize organic compounds to provide nutrients to plants. A large population of microbial community was reported to decompose HS as nutrient and energy resources (*Lovley* et al., 1996; *Kulikova* et al., 2005), which revealed a potential ability of HS to improve the long-term soil microbial population. The alteration of microbial growth from HS was a key response to reveal soil health, as well as plant-microbial interactions. In this study, HS increased soil total bacteria in the greenhouse environment regardless of soil types. HS also decreased total fungi population in clay soil, resulting in a lower fungi-to-bacteria (F/B) ratio (0.14) compared to control (0.29). It had been reported F/B ratio changed rapidly as a result of diverse soil managements, especially the soil with higher N inputs lowered F/B ratio (*De Vries* et al., 2006). In our study, a higher NO_3-N retention was observed in clay soil as a result of HS application, and it was negatively correlated with the F/B ratio ($r = -0.47$ with *P*-value 0.02). In addition, a survey study across forest, cultivated and livestock pasture soil showed bacteria population was highly associated with soil pH while fungi population was associated with current soil nutrient status (*Lauber* et al., 2008). Moreover, soil with higher F/B ratio was hypothesized to have more sustainability because the activity of fungi was positively correlated with soil C content (*Bailey* et al., 2002). However, in our study, as an organic-amendment, HS didn't show a positive effect on F/B ratio. Instead, an increase in total bacteria was observed with increased soil EC and NO_3-N content in clay soil. When *Hartz and Bottoms* (2010) tested five commercial HS, they found the effects of HS were not consistent, and most HS were ineffective in promoting soil nutrient retention as well as microbial activity. Therefore, although the results were contrary to expectations, the decline in F/B ratio doesn't imply a negative impact of HS on plant growth, since HS didn't reduce the plant above-ground development and even significantly promoted plant root growth; moreover, the results only showed a mid-term effects from HS, a long-term test is still required for a better understanding. The specific mechanism of HS impact on bacteria and fungi community is also needed to be examined, with the consideration of different raw materials derived

HS, application rate and diverse environments, in order to achieve the best beneficial effects of HS application on biota growth.

b. HS mitigated water limited stress

Due to the special structural features, HS are able to bind toxic organic and inorganic compounds that exist in polar and hydrophobic environments, thereby reducing their bio-availability and toxicity (*Kulikova* et al., 2005). As a result, HS were commonly used for pollutants detoxification (*Bollag and Myers*, 1992; *De Paolis and Kukkonen*, 1997). This is one of the key functions by which HS mitigate abiotic stress conditions. In the current study, we were interested in the time-course effects of HS on mitigating water stress, because water stress tends to occur over a short period in non-suitable environments. In order to decrease water loss, plants have unique responses to cope with deficit stress. For example, plant cell water potential is reduced and abscisic acid (ABA) is accumulated, which induce stomatal closure, thereby decreasing transpiration rate (*Taiz* et al., 2015). However, stomatal closure also decreases CO_2 uptake, which will inhibit photosynthesis and potentially cause significant crop yield losses. In our study, we found that HS can mitigate short-term severe or mild water stress by reducing plant transpiration and moisture loss (increase water use efficiency) in plant seedling and vegetative development stages, especially in the soil with a low water-holding capacity (sandy). Although these reductions were accompanied by reduced photosynthesis and showed inconsistent plant morphology performance in different soil types, the final above-ground biomass accumulation was not much different. HS seemed to cause plants become more sensitive to water stress, with a rapid and positive response from root growth -- HS had short- to mid-term influences for stimulating plant root growth under moderate water stress, which was critical for plant water and nutrient absorption, as well as for mid-term non-severe stress tolerance. Similarly, an early study by *Dunstone* et al. (1988) found HS reduced wheat stomatal conductance and transpiration. In that study, the conductance of wheat leaf sprayed with liquid fulvic acid solution was reduced by more than 50% compared to control, but the effects only lasted for a short period and was mainly occurring on well-watered plants, not on dry conditions. A study by *Azcona* et al. (2011) found HS increased bell pepper gas exchange, which is in contrast to the findings in our study. More likely, a possible reason for the differential response is associated with the nature and properties of HS, since they used compost sludge derived HS. In addition, they used soilless medium under optimal irrigation conditions, and didn't test the responses under water deficit stress. Therefore, these findings illustrate that HS-based soil amendment can differentially affected bell pepper plant performance based on specific soil moisture levels and soil textural types.

Besides the reduction in leaf gas exchange, water stress will also cause the imbalance of free electrons and NADP$^+$ dehydrogenase and leads to the generation of reactive oxygen species (ROS), which will impede plant growth by oxidizing and damaging the normal functional cellular constituents in plant cells such as proteins, DNA, RNA and lipids (*Taiz* et al., 2015). In addition to the toxin-binding property and the ability to alter leaf gas exchange, with the phenolic function group as electron donor, HS also have an antioxidant capacity (*Aeschbacher* et al., 2012) to scavenge ROS generated by plants under water stress, which provides a new scope for future studies about the effects of HS on ameliorating abiotic stress conditions.

c. Relationship between plant, soil responses and environmental factors

Principal component analysis (PCA) was used to explore the differences or associations between main environmental factors (soil amendment, deficit irrigations) by soil types (Figure 3). Since the most plant and soil responses from 80% and 60% WHC irrigation were similar, we combined these two irrigations as a high irrigation level, while those of 40% and 20% WHC were combined as a low irrigation level. In sandy soil, the first and second components explained 89% of the variability. PC1 accounted for 64% variance, which was more contributed by soil chemical variables, and less by soil microbial and plant biomass variables. It distinguished the difference between low and high irrigation -- low irrigation tended to associate with soil nutrient retention (N, P, K, EC), while high irrigation was highly related with plant growth and soil active microbial population, as well as soil pH; PC2 accounted for 25% variance, which was shaped by plant biomass, soil respiration, active fungi and total bacteria. It only differentiated the impacts of HS on low irrigation (HS-L versus C-L) -- HS was positively associated with soil biota activity but negatively associated with plant biomass accumulation, while there was no clear difference on high irrigation. In clay soil, the first and second components explained 92% of the variability. PC1 accounted for 76% variance, which was most attributed by every selected variable, except a slight contribution from soil total bacteria and fungi. It featured the effects of irrigation levels, which was similar to sandy soil. Additionally, the application of HS in high irrigation soil tended to negatively associate with total bacteria (TB) population; PC2 accounted for 16% variance, which was mainly shaped by soil total microbial population and plant root biomass. It differentiated the effects of HS regardless of irrigation levels -- HS was positively related with plant root growth and total bacteria but negatively related with total fungi (TF) population. A potential explanation for the negative relationship between HS and TB in PC1 and TF in PC2 especially under the high irrigation conditions is the soil with higher clay content and higher field water-holding capacity tends to have a lower decomposition rate of organic carbon (*Xu* et al., 2016), which provides less nutrient sources for fungi and bacteria populations to consume.

In both soil types, plant root biomass accumulation was highly positively correlated with soil pH but negative correlated with soil N, P, K content, soil respiration was positively correlated with active bacteria and fungi, as well as soil pH. There was a negative correlation between total or active fungi population with N content as previously mentioned in the results, additional N fertilization decreased diversity of fungi by altering plant carbon inputs and reduced microbial biomass, which caused declined soil CO_2 emissions (*Treseder*, 2008; *Allison* et al., 2007). This indicates that the balance between nitrogen input and the activity of microorganism community need to be carefully controlled. Interestingly, we found soil active microbial populations had profound influences on plant biomass accumulation especially the active bacteria (AB): AB had positive correlation with plant above-ground and root dry weight regardless of soil types, while active fungi performed differently in different soil types -- negative in sandy soil, positive in clay soil.

All the information from PCA provides three possible future directions when considering new HS research: 1) the decomposition rate of HS in different soil types and irrigation levels, and its relationship with soil total and active microbial population; 2) the activity of different species of soil fungi that are affected by HS and different soil nitrogen content, and their relationship with plant biomass or crop yield in different soil types; 3) the population of active bacteria that is affected by HS, and its relationship with plant biomass or crop yield under different abiotic stress conditions.

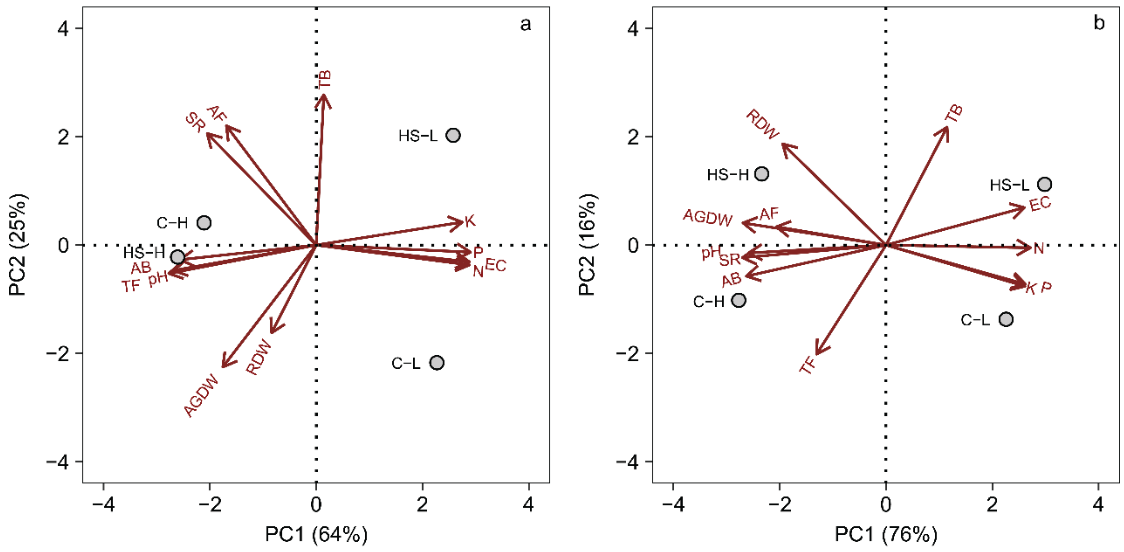

Figure 3. The principal component analysis (PCA) of the relationship between selected variables and different environmental factors in the greenhouse sandy (a) and clay soil (b) experiment. Selected variables were displayed by arrows and included plant biomass parameters: above-ground dry weight (AGDW) and root dry weight (RDW); and soil parameters: soil pH, electrical conductivity (EC), nitrate-nitrogen (N), available phosphorus (P), available potassium (K), soil respiration (SR), total bacteria (TB), active bacteria (AB), total fungi (TF), active fungi (AF). Environmental factors were displayed by filled grey circles and included two parts separated by dashes: the first part shows control (C) or humic substances (HS), the second part shows high irrigation level (H) or low irrigation level (L).

CHAPTER III

BELL PEPPER GROWTH RESPONSES AND SOIL PROPERTY CHANGES TO HUMIC SUBSTANCES AND DEFICIT IRRIGATION IN FIELD CONDITIONS

Introduction

In the studies under controlled environments, we found that HS have an ability to improve biota growth from plant and soil, and have potential to mitigate deficit stress. That study also found that soil texture greatly influenced the effects of HS application. However, since field environmental conditions are difficult to predict, we expect that plant responses to field application of HS will be significantly different from those in growth chamber and greenhouse experiments. In order to test the long-term effects and potential application of HS, field experiments were conducted for two years at two Texas locations with different soil types.

Unlike the controlled environment, in the field, water deficit stress was imposed based on weather conditions. Evapotranspiration (ET) based approach is a widely used technique in agricultural practices to quantify the water consumption by crops, and therefore this method was used in our water deficit studies. The basic parameters to determine a particular crop ET (ET_c) are reference ET (ET_0) and crop coefficient (K_c) (*Allen* et al., 1998). ET_0 is obtained from a reference grass growing surface with adequate irrigation, and the change of ET_0 is only affected by climatic factors; while K_c is changed and acquired according to different crop types, growth stages and other crop characteristics. By multiplying these two parameters we can obtain the standard ET_c or evapotranspiration rate for varied crops, which represents the daily water loss from soil as well as the water requirement by the crops (*Allen*, 2000). Therefore, water deficit treatments can be achieved by multiplying ET_c with a deficit percentage, and set the daily cumulative results as reference to regulate the irrigation schedule in field practices and compensate the deficit water required. The combination of HS application and ET-based deficit irrigation was used in two field studies, with adjustments based on precipitation and other field conditions such as percentage of covered soil with plastic mulch and the flow rate from drip tape.

Approach

a. Growth environments and soil materials

The field experiments were conducted in 2016 and 2017 for two growth seasons at two Texas A&M AgriLife Research and Extension Centers: Uvalde, Texas (29.21° N, 99.79° W) represented by a clay soil type; and Weslaco, Texas (26.16° N, 97.99° W) represented by a sandy soil type. The climatic conditions such as temperature, relative humidity, daily light integral and precipitation for both locations are shown in Figure 4. During the experimental period, the seasonal mean temperature, relative humidity, daily light integral and total precipitation were 23°C, 73%, 66 $mol \cdot m^{-2} \cdot day^{-1}$ and 426 mm, respectively in 2016 Uvalde; 26°C, 67%, 86 $mol \cdot m^{-2} \cdot day^{-1}$ and 210 mm, respectively in 2017 Uvalde; 26°C, 72%, 70 $mol \cdot m^{-2} \cdot day^{-1}$ and 155 mm, respectively in 2016 Weslaco; and 30°C, 72%, 88 $mol \cdot m^{-2} \cdot day^{-1}$ and 126 mm, respectively in 2017 Weslaco. The basic surface soil properties (0-20 cm depth) from Uvalde and Weslaco are also shown in

Table 6. Lignite-derived HS from the Novihum company (Germany) was used as soil amendment, by mixing with field soil at the rate of 5 t/ha.

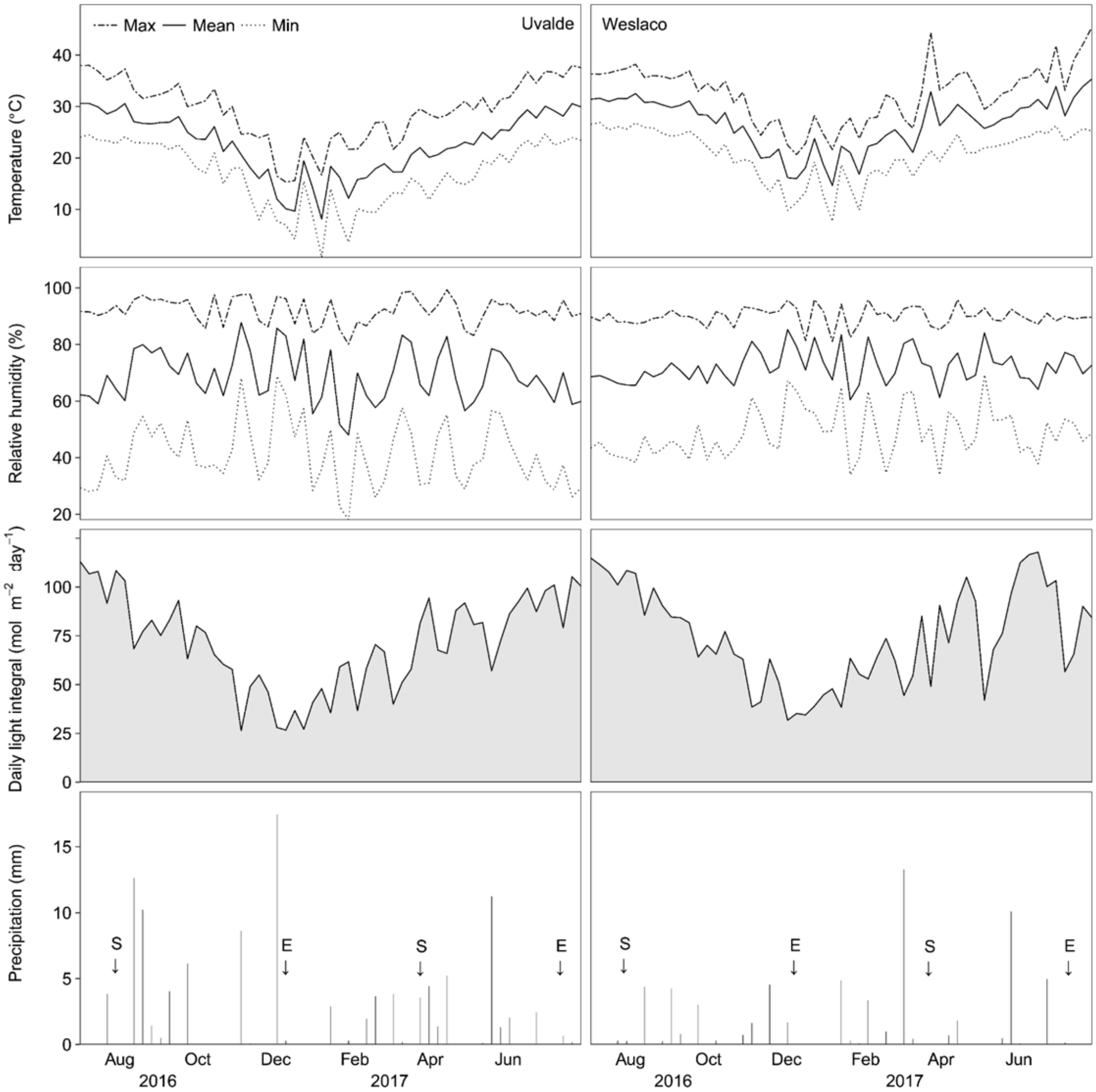

Figure 4. Weather data from Uvalde and Weslaco, TX from Aug 2016 to Aug 2017.
Letter "S" indicates the start time of the experiments (after transplanting), letter "E" indicates the end time of the experiments (after final harvest).

Table 6. Basic soil properties from Uvalde and Weslaco, TX

Soil properties	Sand %	Clay %	Silt %	pH	EC µmhos/cm	NO_3-N mg/kg	P mg/kg	K mg/kg	Ca^{2+} mg/kg	Mg^{2+} mg/kg
Uvalde	28	47	25	8.2	338	18	73	776	12378	335
Weslaco	63	25	12	7.9	480	42	29	320	3687	242

b. Plant material, soil amendment and irrigation treatments

Bell pepper seeds (*Capsicum annuum* cv. Revolution) were sowed into 200 cells trays (2.7 × 2.7 × 7.2 cm^3 per cell) for 7-8 weeks growth at a commercial nursery (Speedling, Alamo, TX). After transplants reached the mature standard size, 120 pepper transplants were established in a 6 m × 6 m block previously amended with HS at the rate of 5 t/ha. HS were first evenly sprayed on the surface of the soil, then were evenly mixed with the ground soil (0-20 cm) by using a tractor. Each block contained 3 rows (1 center row, 2 guard rows) with 2 lines per row and 4 replicates. The rows spaced 2 m apart, the plants grown in a row were 0.3 m apart in distance. Control in absence of HS was included. Drip irrigation system was installed 10-15 cm below the soil surface. Soil moisture sensors (EC5, Decagon devices, WA, USA) were installed at 15 and 30 cm depth to assess daily moisture variation. Standard bell pepper management practices were followed including fertilization, weeding, trellis, pest and disease control.

In both locations, two different experiments were conducted. The first was aimed to test the residual long-term effects of HS application on plant-soil relationships, so a two-years trial was conducted without replenishing HS in the field. The second experiment was aimed to verify the first-year effects of HS application, so the repeat one-year trial was also conducted in the same environmental location, but in a new field and with a new HS application. We separately named the experiments as year-1 (2016), year-2 (2017, same field location as year-1) and new year-1 (2017).

Bell peppers were transplanted to the field and subjected to four irrigation levels: a well-watered, 100% of evapotranspiration (ET) demands and three water deficit irrigation treatments, moderate stress 75% ET, mild stress 50% ET and severe stress 25% ET demands. The irrigation rate was determined by dynamically changing the K_c of green bell pepper following specific phenological stages: the initial growth and development stages was 60 days with a $K_{c\ ini}$ of 0.6; the middle growth stage was 40 days with a $K_{c\ mid}$ of 1.15; the final growth stage was 20 days with a $K_{c\ end}$ of 0.9 (*Allen* et al., 1998). Irrigation was provided based on calculated volumes of application, using the respective proportion of 100% ET (base reference). Different volumes of applications for four irrigation levels were adjusted by time, for example 12 hours irrigation for the well-watered, 3 hours for the severe deficit treatment. These were reflected by dynamic soil moisture changes, with the percentage of volumetric soil moisture from high irrigation treatment (100% ET) having higher values but changing rapidly, especially in 15 cm depth as compared to 25%ET (Figure 5).

For year-1 experiments in both locations Uvalde and Weslaco in 2016, all four irrigation levels were conducted. Since we found similar responses between W1 and W2, W3 and W4 in year-1, for year-2 and new year-1 experiments in 2017, we decided to use only two irrigation levels, a well-watered (100% ET demands) and a mild deficit (50% ET demands) irrigation treatments.

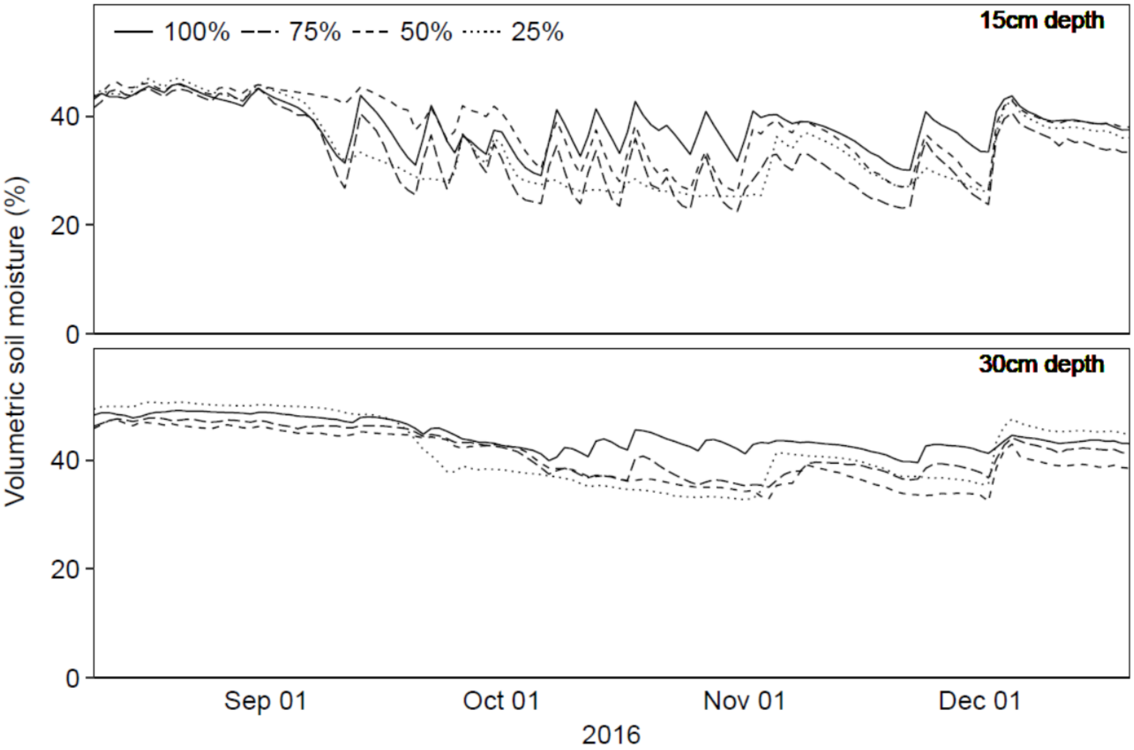

Figure 5. Volumetric soil moisture data in Uvalde, TX from Aug to Dec 2016. Irrigation levels were imposed based on crop-evapotranspiration (ET) demand.

c. Plant growth response measurements

Regular measurements were conducted on pepper plants after imposing water-deficit stress: plant height and stem diameter were measured using ruler and digital caliper (VWR, Radnor, PA); photosynthesis rate (*Pn*), stomatal conductance (g_s) and transpiration (*E*) were measured with a portable photosynthesis system (LI-6400 XT, LI-COR Biosciences, NE); and chlorophyll content index (SPAD) was measured using a chlorophyll meter (SPAD-502 Plus, Minolta, Japan). The parameters were collected on monthly intervals for 2 months after irrigation treatment started, which in bell pepper represented the flowering and fruiting stage. From the beginning of harvesting to the end of the experimental period, bell pepper marketable yield, total yield and fruit quality were measured following the USDA standard grading system (*USDA*, 2005). Bell pepper sizes include US Fancy (diameter > 3 inches or 7.6 cm, length > 3.5 inches or 8.9 cm), US No.1 (both diameter and length > 2.5 inches or 6.4 cm) and unmarketable cull fruit (sun-burn, decay, misshapen, etc.). Measurements of bell pepper quality included soluble sugar content (^{o} Brix) (ATAGO, PR-32α digital refractometer, Japan), firmness (digital force gauge, DFS II, Chatillon Inc., Largo, FL) and wall thickness (digital caliper).

All the experiments ended 4 months after transplanting. At the end point, part of the plant root system (0-20 cm depth) was collected by a soil auger and carefully washed. Root length, surface area and average diameter were then scanned and measured using an EPSON V700 scanner (Epson, Japan) and analyzed with a WinRHIZO software (V5.0, Regent Instruments, Canada), while root dry weight was measured after oven drying at 75°C for 2 days.

d. Soil chemical and biological analysis

At the end of the experiment, a soil auger was also used to collect soil cores within 0-20 cm depth in the field close to the plant rhizosphere. Part of the fresh soil samples collected from field was immediately shipped to Earthfort Lab (Corvallis, OR) for microbial activity analysis and the rest were used for the soil respiration (Soil CO_2-Burst) (*Haney* et al., 2008) test by using the SOLVITA soil respiration box (Woods End Laboratories, Mt Vernon, ME). Total and active bacteria, total and active fungi were obtained by following staining procedures (*Stamatiadis* et al., 1990), direct microscope observation and other procedures provided by *Babiuk* and *Paul* (1970), *Ingham and Klein* (1984) and *Van Veen and Paul* (1979).

Another part of the soil samples was dried at 75°C in the oven, then grinded and sieved the soil to 2 mm, and shipped for chemical analysis to the Soil, Water and Forage Testing Laboratory (Texas AgriLife Extension Service, CS, TX). Soil pH and electrical conductivity were obtained in a 1:2 soil: water ratio extract (*Rhoades*, 1982); and nitrate-nitrogen (NO_3-N) was extracted by 1 M KCl solution (*Keeney and Nelson*, 1982) and determined by spectrophotometry. Soil P, K, Ca, Mg, S and Na were extracted and evaluated following the method of *Mehlich* (1978) and then determined by an ICP-MS.

e. Statistical analysis

A two-way randomized complete block design with soil amendment and irrigation levels was used in the studies. Regular measurements of plant morphology and physiology, crop yield and quality, root and soil traits were analyzed by the analysis of variance (ANOVA) in SAS (Version 9.4, SAS Institute, Cary, NC); and multiple comparisons of the means were analyzed by the least significant difference (LSD) at $\alpha = 0.05$.

f. Timeline

Table 7 shows the main experimental periods for the field experiments in 2016 and 2017.

Table 7. Timeline for the field experiments in Uvalde and Weslaco in 2016 (year-1) and 2017 (year-2, new year-1)

Location	Season	2-Aug	8-Sep	13-Oct	14-Nov	14-Dec
Uvalde	Year-1	Seedling transplant	ITS [a]	PM [b]	PM	End experiment
		1-April	2-May	5-Jun	5-Jul	30-Jul
	Year-2, New Year-1	Seedling transplant	ITS	PM	PM	End experiment
Location	Season	2-Aug	4-Oct	1-Nov	6-Dec	13-Dec
Weslaco	Year-1	Seedling transplant	ITS	PM	PM	End experiment
		1-April	15-May	14-Jun	20-Jul	30-Jul
	Year-2, New Year-1	Seedling transplant	ITS	PM	PM	End experiment

[a] ITS: Irrigation treatment start; [b] PM: plant measurement.

Results - Uvalde

a. Year-1 plant (2016)

Growth and physiology **-** Based on the results from table 8, after irrigation treatment started, HS significantly decreased plant height regardless of irrigation levels. HS did not exhibit significant effects on plant physiology. Deficit irrigation increased leaf chlorophyll content (SPAD) but decreased leaf gas exchange during the flowering period compared to the well-watered treatment.

Yield and quality - Table 9 shows the lack of significant interaction between soil amendment and irrigation levels. Therefore, data was combined to investigate the effects of soil amendment and irrigation separately. HS significantly increased pepper early marketable yield by 28.9% and total yield by 32.8% as compared to control. However, total harvest yield was similar to the control (Figure 6). In terms of irrigation, severe (25% ET) and mild (50% ET) deficit significantly decreased yield from early and total harvest, especially Fancy size fruit yield as compared to moderate deficit (75% ET) and well-watered (100% ET). The marketable and total yields were not different between severe and mild deficit irrigations; moderate deficit (75% ET) and well-watered irrigations also had similar yields regardless of early or total harvest (Figure 7). Table 10 shows the bell pepper quality from the Uvalde year-1 experiment. Overall, HS did not affect fruit quality, while severe and mild deficit irrigation had an increase in sugar content (^{o} Brix) with a *P*-value = 0.05, and a numerical, but not statistical increase in ascorbic acid concentration.

Root development - Root parameters are shown in Table 11. In general, root parameters were not affected by HS or irrigation, except for a slight increase in root length by HS and a decrease in root average diameter by the combination of HS and severe deficit irrigation.

b. Year-1 soil (2016)

At the end of the experiment, soil amendment and deficit irrigation treatments did not affect soil environmental changes, since soil chemical properties and soil microbial activity were similar (Tables 12, 13).

Table 8. Summary of morphological and physiological traits of bell pepper grown in year-1 experiment at Uvalde

Time [a]	Source		PH cm	SD mm	SPAD	*Pn* μmol m^{-2} s^{-1}	g_s mol m^{-2} s^{-1}	*E* mmol m^{-2} s^{-1}
1	SA	Control	52.73 a *	14.64	61.76	21.66	0.40	3.50
		HS	50.20 b	14.72	60.22	19.64	0.41	3.28
	IR	25%	51.78	14.52	63.28 a	16.37	0.24 b	2.39 b
		50%	50.66	14.90	59.47 b	19.09	0.34 b	3.15 b
		75%	51.44	14.51	61.76 ab	24.89	0.54 a	4.09 a
		100%	52.00	14.79	59.45 b	22.23	0.50 a	3.92 a
	P-value	SA	0.049	0.869	0.159	0.570	0.843	0.381
		IR	0.871	0.903	0.045	0.362	< 0.001	< 0.001
		SA × IR	0.951	0.896	0.716	0.899	0.887	0.817
2	SA	Control	52.30 a	15.14	66.53	12.47	0.17	2.91
		HS	48.55 b	15.06	65.03	12.00	0.16	2.53
	IR	25%	48.78	14.77	67.10	12.82	0.18	2.92
		50%	51.13	15.06	66.18	12.15	0.14	2.50
		75%	50.97	15.23	65.19	11.70	0.16	2.63
		100%	50.81	15.35	64.65	12.27	0.18	2.83
	P-value	SA	0.014	0.839	0.380	0.610	0.592	0.120
		IR	0.611	0.755	0.740	0.850	0.634	0.577
		SA × IR	0.722	0.650	0.167	0.971	0.714	0.596

[a] Time is shown as monthly interval after irrigation treatment started for 2 months. PH: Plant height, SD: stem diameter, SPAD: chlorophyll content index, *Pn*: photosynthesis, g_s: stomatal conductance, *E*: transpiration. * Different letters within column from the same factor indicate significant differences at α = 0.05 according to LSD test.

Table 9. Source of variations and *P*-values from ANOVA on bell pepper year-1 yield in Uvalde

Source of variation	Early harvest					Total harvest				
	Fancy	No.1	MY	Cull	TY	Fancy	No.1	MY	Cull	TY
SA	0.082	0.147	0.033	0.022	0.019	0.601	0.525	0.932	0.524	0.943
IR	0.014	0.783	0.021	0.883	0.048	< 0.001	0.089	< 0.001	0.537	< 0.001
SA × IR	0.856	0.808	0.834	0.824	0.795	0.453	0.728	0.436	0.930	0.476

SA: soil amendment; IR: deficit irrigation; Early harvest: first 3 harvests; Total harvest: 6 harvests; MY: marketable yield, sum of US Fancy and No.1; TY: total yield, sum of marketable and cull yield.

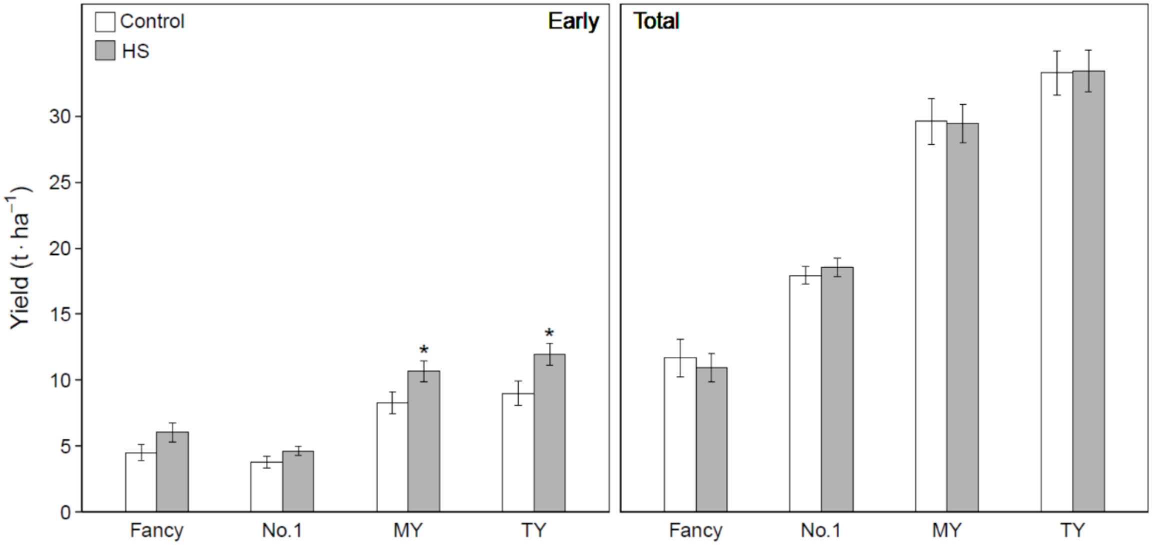

Figure 6. Bell pepper year-1 early and total harvest yield in response to soil amendment treatment in Uvalde.
MY: marketable yield, sum of US Fancy and No.1; TY: total yield, sum of marketable and cull yield. * indicated significant difference between HS and control at α = 0.05 based on LSD test.

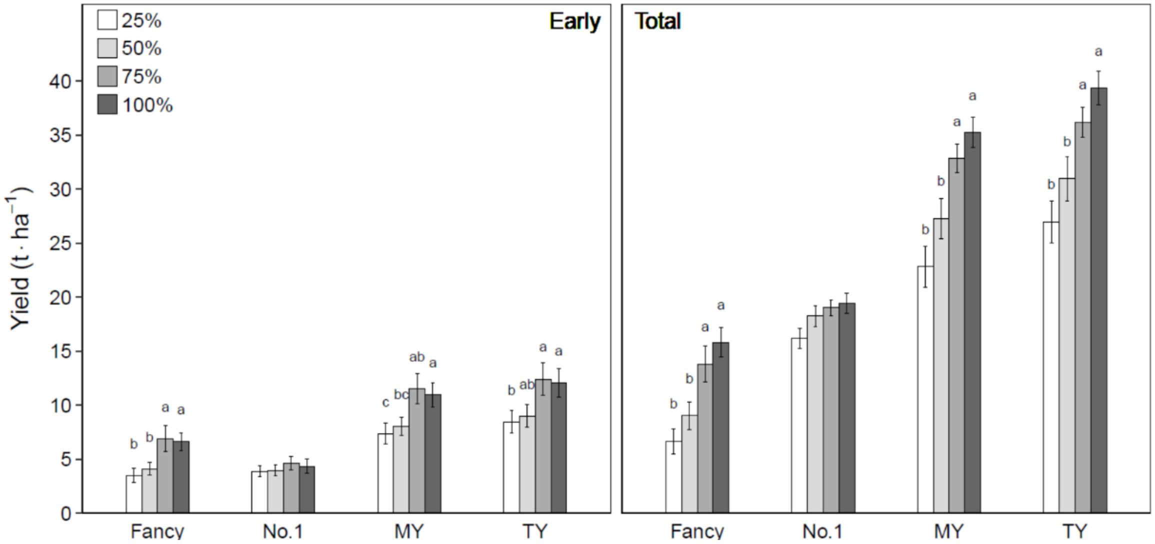

Figure 7. Bell pepper year-1 early and total harvest yield in response to different irrigation levels in Uvalde.
The percentage of irrigation levels is based on bell pepper evapotranspiration (ET) demands. MY: marketable yield, sum of US Fancy and No.1; TY: total yield, sum of marketable and cull yield. Different letters among irrigation levels indicate significantly different at α = 0.05 based on LSD test.

Table 10. Bell pepper year-1 quality in Uvalde

Source		° Brix	Firmness KgF	Wall thickness mm	Ascorbic acid µg/g FW
SA	Control	3.64	1.51	6.44	828.3
	HS	3.64	1.52	6.19	837.3
IR	25%	3.92 a *	1.57	6.51	949.2
	50%	3.71 a	1.48	6.07	832.6
	75%	3.64 ab	1.45	6.00	749.8
	100%	3.29 b	1.54	6.68	800.8
P-value	SA	0.979	0.834	0.248	0.918
	IR	0.050	0.590	0.082	0.353
	SA × IR	0.759	0.949	0.899	0.870

FW: fresh weight. * Different letters within column from the same factor indicate significant differences at α = 0.05 according to LSD test.

Table 11. Summary of plant root traits under soil amendment (SA) and irrigation (IR) treatments in year-1 field at Uvalde

Source		RL m	RSA cm^2	RAD mm	RDW g
SA	Control	9.75	116.59	0.38 a *	0.26
	HS	10.16	116.37	0.36 b	0.26
IR	25%	10.12	113.22	0.35 b	0.21
	50%	9.30	116.01	0.39 a	0.30
	75%	9.59	109.39	0.36 b	0.24
	100%	10.82	127.29	0.38 ab	0.28
P-value	SA	0.781	0.990	0.049	0.923
	IR	0.888	0.903	0.044	0.639
	SA × IR	0.751	0.823	0.294	0.947

RL: root length; RSA: root surface area; RAD: root average diameter; RDW: root dry weight. * Different letters within column from the same factor indicate significant differences at α = 0.05 according to LSD test.

Table 12. Summary of soil pH, electrical conductivity (EC), chemical component analysis under soil amendment (SA) and irrigation (IR) treatments in year-1 field at Uvalde

Source		pH	EC umhos/cm	NO_3-N mg/kg	P mg/kg	K mg/kg	Ca mg/kg	Mg mg/kg	S mg/kg	Na mg/kg
SA	Control	8.1	576	10.5	69	891	16750	415	14	29
	HS	8.0	592	9.4	68	879	14989	393	12	30
IR	25%	8.0	570 b *	9.3	64	857	15867	394	12	26
	50%	8.1	597 ab	11.0	71	927	15881	411	14	28
	75%	8.0	610 a	10.8	70	880	16191	409	13	31
	100%	8.1	560 b	8.7	68	876	15539	402	13	33
P-value	SA	0.076	0.219	0.364	0.755	0.663	0.151	0.273	0.316	0.802
	IR	0.599	0.044	0.445	0.290	0.314	0.985	0.925	0.602	0.164
	SA × IR	0.647	0.076	0.513	0.935	0.677	0.978	0.942	0.838	0.975

* Different letters within column from the same factor indicate significant differences at $\alpha = 0.05$ according to LSD test.

Table 13. Summary of soil respiration (SR), active bacteria (AB), total bacteria (TB), active fungi (AF) and total fungi (TF) under soil amendment (SA) and irrigation (IR) treatments in year-1 field at Uvalde

Source		SR mg/kg CO_2-C	AB µg/g	TB µg/g	AF µg/g	TF µg/g
SA	Control	34.93	26.47	782.38	10.89	140.50
	HS	36.41	25.87	802.19	8.30	136.11
IR	25%	27.51	27.53	715.75	6.64	114.91
	50%	40.08	25.86	851.88	11.79	138.65
	75%	27.98	25.30	793.88	11.26	165.41
	100%	47.10	25.99	807.63	8.69	134.25
P-value	SA	0.890	0.665	0.652	0.319	0.784
	IR	0.499	0.697	0.193	0.467	0.189
	SA × IR	0.677	0.307	0.143	0.969	0.635

c. Year-2 plant (2017)

The year 2 field experiment was aimed to test the long-term effects of HS on bell pepper growth and physiology performances. Due to similar results between severe and mild deficit (25% and 50% ET) irrigation from year-1 experiment, for year-2, we removed the 25% and kept the 50% irrigation as a low irrigation level. Similarity, since in year-1, there were no differences between moderate deficit and well-watered (75% and 100% ET) irrigation, we removed the 75% and kept the 100% irrigation as well-watered irrigation level.

Growth and physiology - The results (Table 14) showed that HS significantly increased plant height at the fruiting stage 2 months after irrigation treatment started (ITS), but decreased plant chlorophyll content (SPAD) and photosynthesis of the flowering period 1 month after ITS. The early decrease in transpiration observed in year-1 was not detected in year-2, which might indicate that the reduction in leaf gas exchange due to HS application are diminished in year-2. Deficit irrigation did not have effects on bell pepper growth and physiology performances.

Yield and quality - Since there were no significant interactions for yield between soil amendment and irrigation levels (Table 15), the effects of soil amendment and irrigation were analyzed separately. The results indicated that HS significantly increased bell pepper early yield of No.1 size fruits by 81.4 % compared to control, while early marketable and total yield increased numerically but not statistically by 5.5% and 6.4%, respectively. For total harvest, HS numerically increased marketable and total yield by 9.1% and 8.0%, respectively (Figure 8). In terms of irrigation, deficit (50% ET) treatment did not affect early harvest yield, but it decreased marketable and total yield from total harvest by 11.9% and 11.4% compared to well-watered (100% ET) treatment (Figure 9). Table 16 showed bell pepper quality from the Uvalde year-2 experiment. Overall, HS did not affect fruit quality, while low irrigation increased fruit sugar content (^{o} Brix) (*P*-value = 0.05) and decreased wall thickness (*P*-value = 0.08).

Root development - Root length, surface area and dry weight were numerically decreased by HS application in year-2, while HS also decreased root average diameter which was consistent with year-1. In terms of irrigation, all root parameters were decreased by deficit (50% ET) treatment, especially root dry biomass decreased by 32.6% compared to well-watered treatment (Table 17).

d. Year-2 soil (2017)

HS significantly increased soil electrical conductivity, nitrogen and organic carbon content by 28.8%, 40.7% and 20.4%, respectively (Table 18). Regarding soil microbial activity, HS significantly increased active bacteria population, and although not statistically significant, HS also increased soil respiration by two-fold, which might due to the promotion of total fungi population (115.9%). Low irrigation decreased soil total bacteria and fungi population, although not statistically significant (Table 19).

Table 14. Summary of morphological and physiological traits of bell pepper grown in year-2 experiment at Uvalde

Time	Source		PH cm	SD mm	SPAD	*Pn* μmol m^{-2} s^{-1}	g_s mol m^{-2} s^{-1}	*E* mmol m^{-2} s^{-1}
1	SA	Control	40.13	11.43	62.63 a *	18.29 a	0.70	13.20
		HS	42.04	11.84	58.34 b	16.81 b	0.60	12.32
	IR	50%	41.29	11.75	60.83	18.05	0.66	13.06
		100%	40.88	11.51	60.14	17.05	0.64	12.46
	P-value	SA	0.077	0.403	0.006	0.020	0.153	0.138
		IR	0.696	0.612	0.643	0.108	0.749	0.315
		SA × IR	0.639	0.885	0.144	0.164	0.143	0.438
2	SA	Control	52.96 b	13.76	49.99	17.67	0.68	8.88
		HS	56.13 a	14.47	49.61	17.17	0.75	9.02
	IR	50%	53.04	14.38	50.83	18.07	0.69	8.83
		100%	56.04	13.85	48.78	16.77	0.74	9.07
	P-value	SA	0.047	0.125	0.888	0.618	0.208	0.711
		IR	0.059	0.254	0.441	0.203	0.380	0.522
		SA × IR	0.312	0.165	0.325	0.896	0.915	0.938

Time is shown as monthly interval after deficit irrigation started for 2 months. PH: Plant height, SD: stem diameter, SPAD: chlorophyll content index, *Pn*: photosynthesis, g_s: stomatal conductance, *E*: transpiration. * Different letters within column from the same factor indicate significant differences at α = 0.05 according to LSD test.

Table 15. Source of variations and *P*-values from ANOVA on bell pepper year-2 yield in Uvalde

Source of variation	Early harvest					Total harvest				
	Fancy	No.1	MY	Cull	TY	Fancy	No.1	MY	Cull	TY
SA	0.427	0.003	0.374	0.670	0.448	0.469	0.161	0.527	0.457	0.480
IR	0.401	0.186	0.677	0.763	0.947	0.422	0.390	0.366	0.157	0.275
SA × IR	0.229	0.700	0.171	0.765	0.515	0.304	0.444	0.345	0.601	0.359

SA: soil amendment; IR: deficit irrigation; Early harvest: first 2 harvests; total harvest: 5 harvests; MY: marketable yield, sum of Fancy and No.1 yield; TY: total yield, sum of marketable and cull yield.

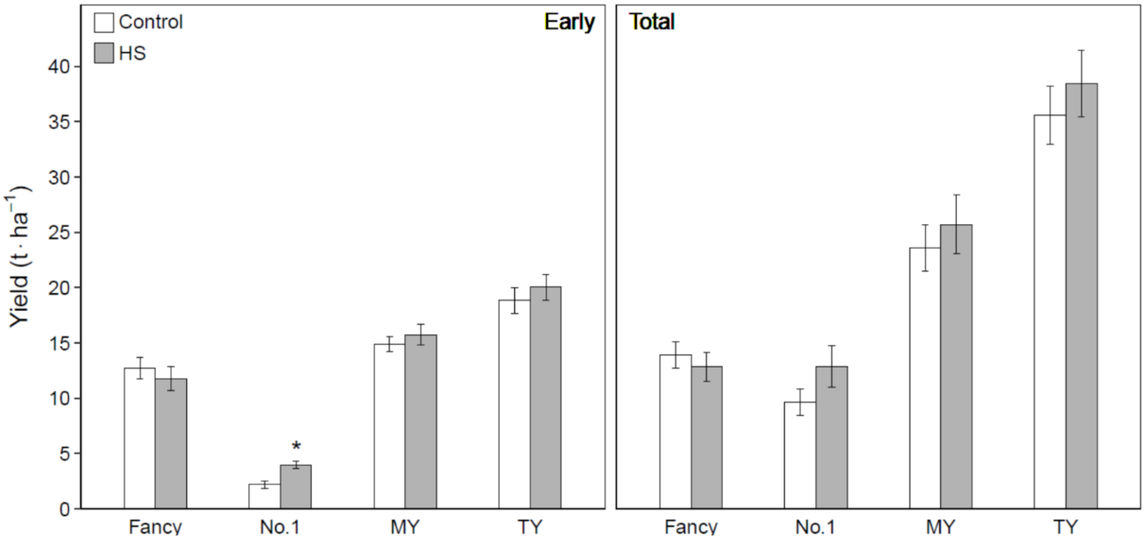

Figure 8. Bell pepper year-2 early and total harvest yield in response to soil amendment treatment in Uvalde.
MY: marketable yield, sum of Fancy and No.1 yield; TY: total yield, sum of marketable and cull yield. * indicated significantly different between HS and control at α = 0.05 based on LSD test.

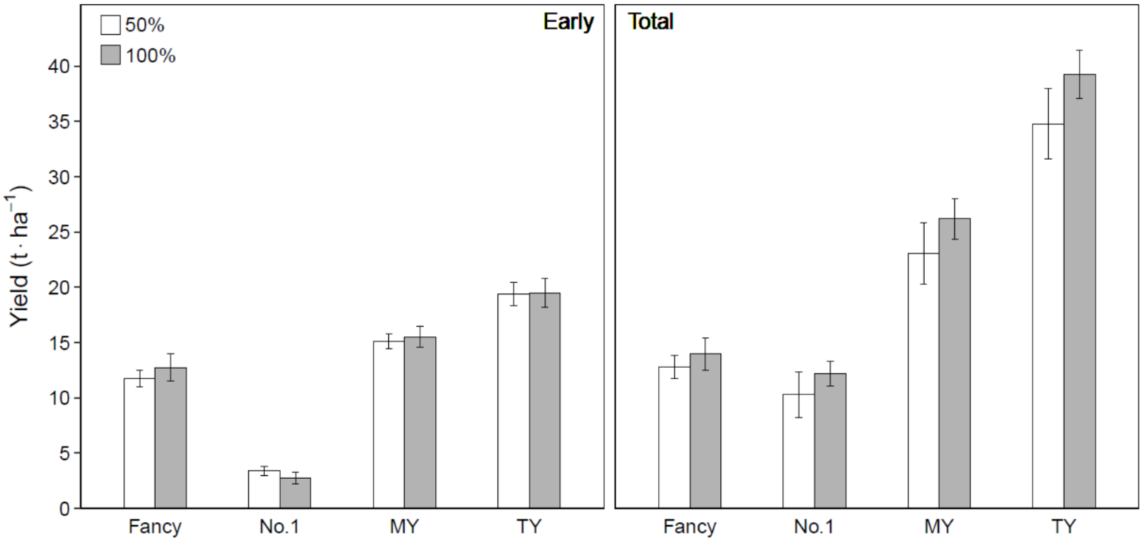

Figure 9. Bell pepper year-2 early and total harvest yield in response to different irrigation levels in Uvalde.
The percentage of irrigation levels is based on bell pepper evapotranspiration (ET) demands.
MY: marketable yield, sum of US Fancy and No.1; TY: total yield, sum of marketable and cull yield.

Table 16. Bell pepper year-2 quality in Uvalde

Source		° Brix	Firmness KgF	Wall thickness mm
SA	Control	3.64	1.51	6.44
	HS	3.64	1.52	6.19
IR	50%	3.71 a *	1.48	6.07
	100%	3.29 b	1.54	6.68
P-value	SA	0.979	0.834	0.248
	IR	0.050	0.590	0.082
	SA × IR	0.759	0.949	0.899

* Levels not connected by same letter are significantly different at α = 0.05 based on LSD test.

Table 17. Summary of plant root traits under soil amendment (SA) and irrigation (IR) treatments in year-2 field at Uvalde

Source		RL m	RSA cm^2	RAD mm	RDW g
SA	Control	14.07	173.55	0.39	0.42
	HS	11.63	138.69	0.37	0.35
IR	50%	11.73	136.57	0.37	0.31
	100%	13.97	175.67	0.40	0.46
P-value	SA	0.078	0.107	0.434	0.467
	IR	0.100	0.075	0.258	0.161
	SA × IR	0.111	0.416	0.303	0.712

RL: root length; RSA: root surface area; RAD: root average diameter; RDW: root dry weight.

Table 18. Summary of soil pH, electrical conductivity (EC), chemical component analysis and organic carbon (OC) under soil amendment (SA) and irrigation (IR) treatments in year-2 field at Uvalde

Source		pH	EC umhos/cm	NO_3-N mg/kg	P mg/kg	K mg/kg	Ca mg/kg	Mg mg/kg	S mg/kg	Na mg/kg	OC %
SA	Control	8.2 a *	403 b	5.9 b	64	868	12723	309	29	58	1.37 b
	HS	8.1 b	519 a	8.3 a	68	938	12299	313	31	54	1.65 a
IR	50%	8.2	409 b	7.4	67	900	12235	306	24 b	40 b	1.49
	100%	8.1	513 a	6.8	64	906	12787	317	35 a	72 a	1.53
P-value	SA	0.034	0.018	0.020	0.225	0.479	0.380	0.682	0.569	0.471	0.039
	IR	0.346	0.031	0.488	0.442	0.951	0.260	0.222	0.007	0.000	0.753
	SA × IR	0.748	0.957	0.900	0.381	0.619	0.997	0.950	0.798	0.818	0.790

* Levels not connected by same letter are significantly different at α = 0.05 based on LSD test.

Table 19. Summary of soil respiration (SR), active bacteria (AB), total bacteria (TB), active fungi (AF) and total fungi (TF) under soil amendment (SA) and irrigation (IR) treatments in year-2 field at Uvalde.

Source		SR mg/kg CO_2-C	AB µg/g	TB µg/g	AF µg/g	TF µg/g
SA	Control	26.19	11.56 b *	1259.06	1.55	74.84
	HS	54.80	17.94 a	1115.71	1.64	159.79
IR	50%	48.09	14.51	1120.13	1.49	84.37
	100%	32.90	14.99	1254.63	1.70	150.26
P-value	SA	0.116	0.032	0.404	0.932	0.304
	IR	0.380	0.853	0.432	0.831	0.419
	SA × IR	0.242	0.729	0.453	0.110	0.357

* Levels not connected by same letter are significantly different at α = 0.05 based on LSD test.

e. New year-1 plant (2017)

Growth and physiology - The new year-1 (2017) field experiment was aimed to repeat and validate the results from year-1 (2016). At the fruiting period 2 months after irrigation treatment started (ITS), HS significantly increased plant stem diameter with the well-watered treatment. At the flowering period 1 month after ITS, HS significantly decreased leaf transpiration while maintaining photosynthesis to the same level as control. In terms of irrigation, deficit stress significantly decreased plant photosynthesis rate by 15.6% and gas exchange performances (stomatal conductance) by 44.6% in late growth (fruiting stage) compared to well-watered treatment (Table 20).

Yield and quality - Table 21 shows the lack of significant interaction between soil amendment and irrigation levels for yield and fruit quality. Therefore, data was combined to investigate the effects of soil amendment and irrigation separately. Compared to control, plants grown in HS treated soil had significant decreases in marketable and total yield from early harvest: 22.2% and 19.5%, respectively, which was opposite to what we found in year-1 (2016). However, these significant reductions in yield were not observed from total harvest (Figure 10). Deficit irrigation significantly decreased early yield with a decrease in Fancy fruit size yield by 35.2% compared to well-watered irrigation. When combining all harvest, deficit stress decreased both Fancy and No.1 fruit yield by 43.0% and 21.4%, respectively (Figure 11). HS and irrigation did not affect bell pepper quality (Table 22), except that HS decreased the sugar content (3.98 °Brix) of bell pepper especially in well-watered irrigation compared to the control (4.45 °Brix).

Table 20. Summary of morphological and physiological traits of bell pepper grown in new year-1 experiment at Uvalde

Time	Source		PH cm	SD mm	SPAD	*Pn* µmol m^{-2} s^{-1}	g_s mol m^{-2} s^{-1}	*E* mmol m^{-2} s^{-1}
1	SA	Control	41.13	11.67	62.24	19.66	0.59	10.43 a *
		HS	40.21	11.84	59.98	19.55	0.60	9.31 b
	IR	50%	41.21	11.94	61.03	19.24	0.57	9.65
		100%	40.13	11.57	61.20	19.97	0.62	10.09
	P-value	SA	0.385	0.704	0.145	0.816	0.753	0.005
		IR	0.305	0.415	0.911	0.135	0.360	0.241
		SA × IR	0.634	0.416	0.911	0.026	0.123	0.105
2	SA	Control	56.42	13.71 b	62.89	18.99	0.59	9.38
		HS	54.75	15.10 a	61.42	18.46	0.55	9.87
	IR	50%	55.00	13.99	63.40	17.14 b	0.41 b	8.07 b
		100%	56.17	14.81	60.91	20.31 a	0.74 a	11.18 a
	P-value	SA	0.297	0.004	0.429	0.413	0.596	0.363
		IR	0.464	0.080	0.184	< 0.001	< 0.001	< 0.001
		SA × IR	0.297	0.022	0.365	0.273	0.510	0.324

Time is shown as monthly interval after deficit irrigation started for 2 months. PH: Plant height, SD: stem diameter, SPAD: chlorophyll content index, *Pn*: photosynthesis, g_s: stomatal conductance, *E*: transpiration. * Levels not connected by same letter are significantly different at α = 0.05 based on LSD test.

Table 21. Source of variations and *P*-values from ANOVA on bell pepper new year-1 yield in Uvalde

Source of variation	Early harvest					Total harvest				
	Fancy	No.1	MY	Cull	TY	Fancy	No.1	MY	Cull	TY
SA	0.092	0.173	0.012	0.805	0.021	0.298	0.506	0.078	0.918	0.144
IR	0.011	0.816	0.026	0.943	0.040	0.032	0.003	< 0.001	0.079	< 0.001
SA × IR	0.958	0.523	0.432	0.274	0.333	0.968	0.679	0.735	0.704	0.873

SA: soil amendment; IR: deficit irrigation; Early harvest: first 2 harvests; total harvest: 5 harvests; MY: marketable yield, sum of Fancy and No.1 yield; TY: total yield, sum of marketable and cull yield.

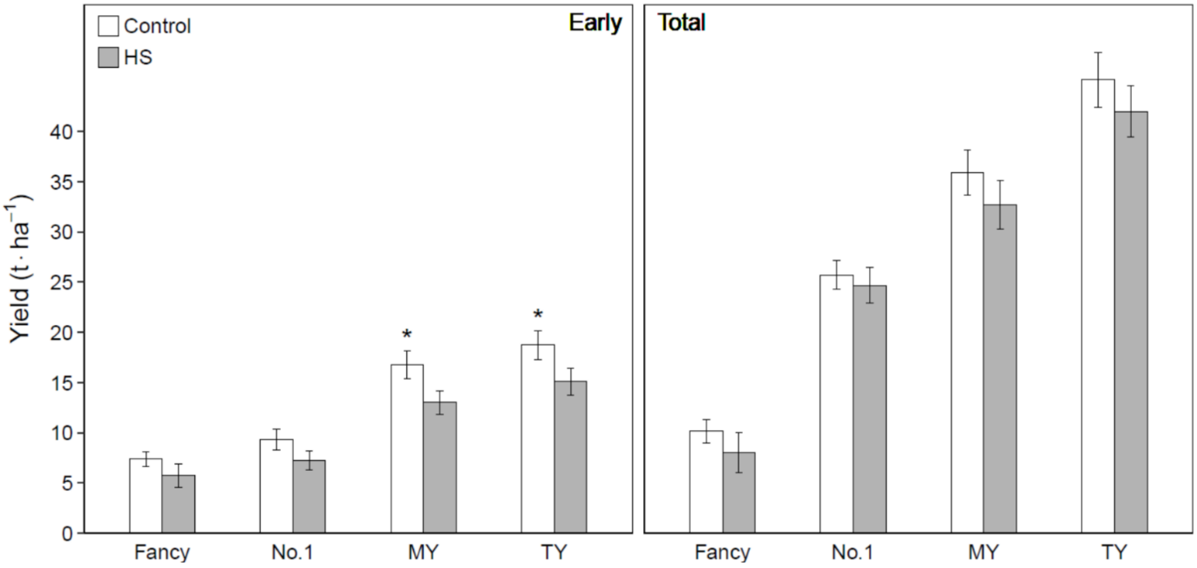

Figure 10. Bell pepper new year-1 early and total harvest yield in response to soil amendment treatment in Uvalde.
MY: marketable yield, sum of Fancy and No.1 yield; TY: total yield, sum of marketable and cull yield. * indicated significantly different between HS and control at α = 0.05 based on LSD test.

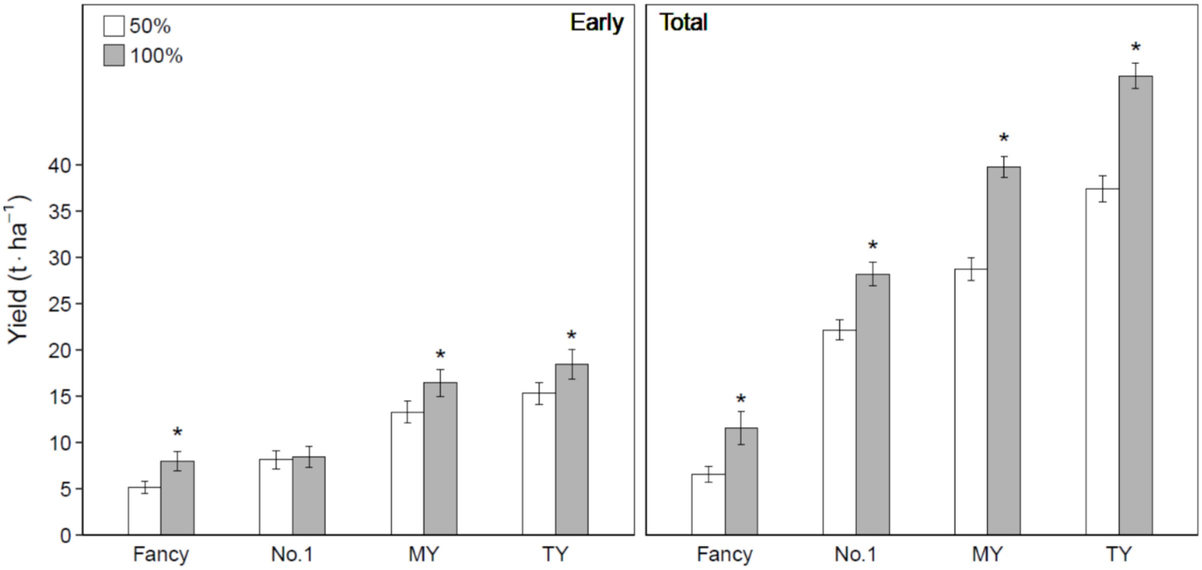

Figure 11. Bell pepper new year-1 early and total harvest yield in response to different irrigation levels in Uvalde.
The percentage of irrigation levels is based on bell pepper evapotranspiration (ET) demands. MY: marketable yield, sum of Fancy and No.1 yield; TY: total yield, sum of marketable and cull yield. * indicated significantly different between 50% and 100% irrigation at α = 0.05 based on LSD test.

Table 22. Bell pepper new year-1 quality in Uvalde

Source		° Brix	Firmness KgF	Wall thickness mm
SA	Control	4.31	2.07	5.63
	HS	4.14	2.03	5.28
IR	50%	4.24	1.90	5.35
	100%	4.21	2.20	5.56
P-value	SA	0.187	0.864	0.416
	IR	0.843	0.166	0.624
	SA × IR	0.037	0.162	0.629

Table 23. Summary of plant root traits under soil amendment (SA) and irrigation (IR) treatments in new year-1 field at Uvalde

Source		RL m	RSA cm^2	RAD mm	RDW g
SA	Control	11.07	115.98 b *	0.33	0.24
	HS	14.06	158.33 a	0.36	0.45
IR	50%	12.29	142.00	0.37 a	0.39
	100%	12.84	132.30	0.32 b	0.30
P-value	SA	0.064	0.047	0.150	0.089
	IR	0.709	0.612	0.025	0.422
	SA × IR	0.372	0.281	0.280	0.403

RL: root length; RSA: root surface area; RAD: root average diameter; RDW: root dry weight.
* Levels not connected by same letter are significantly different at $\alpha = 0.05$ based on LSD test.

Root development - Roots from plants treated with HS had significantly higher surface area than control; in addition, HS also increased root length and dry weight although not statistically significant. Deficit irrigation increased root average diameter, but did not affect other root traits such as root length and root surface area (Table 23).

f. New year-1 soil (2017)

HS and deficit irrigation did not affect soil chemical changes except that HS increased organic carbon content by 27.4% although not significant. Interestingly, well-watered soil had slightly higher organic carbon (19.2%) than deficit irrigation (Table 24).

Table 24. Summary of soil pH, electrical conductivity (EC), chemical component analysis and organic carbon (OC) under soil amendment (SA) and irrigation (IR) treatments in new year-1 field at Uvalde

Source		pH	EC umhos/cm	NO_3-N mg/kg	P mg/kg	K mg/kg	Ca mg/kg	Mg mg/kg	S mg/kg	Na mg/kg	OC %
SA	Control	8.0	661	87	57	690	11957	210	28	31	1.82
	HS	8.0	664	62	42	666	11580	211	25	44	2.32
IR	50%	8.1	542	55	47	681	11804	211	26	36	1.85
	100%	8.0	783	94	52	675	11734	210	27	40	2.29
P-value	SA	0.707	0.993	0.691	0.230	0.633	0.409	0.911	0.217	0.105	0.079
	IR	0.265	0.381	0.546	0.678	0.891	0.875	0.914	0.611	0.560	0.113
	SA × IR	0.793	0.326	0.419	0.458	0.467	0.383	0.644	0.998	0.973	0.247

Results - Weslaco

a. Year-1 plant (2016)

Growth and physiology - Based on the results from Table 25, after irrigation treatment started (ITS), HS significantly decreased bell pepper leaf stomatal conductance and transpiration especially in severe and mild deficit treatments (25% and 50% ET) at the fruiting stage, while maintaining photosynthesis to the same level as control. Different irrigation levels did not affect plant growth and physiology performances.

Yield and quality - Table 26 shows the lack of significant interaction between soil amendment and irrigation levels for yield and quality. HS did not affect early yield, and only numerically increased total harvest yield by 7.4%. In terms of irrigation, there were no significant reduction in yield from deficit irrigation regardless of early or total harvest (Figure 12). Overall, HS and irrigation did not affect bell pepper quality, except that the severe deficit irrigation had a numerical but not significant increase in bell pepper ascorbic acid concentration (Table 27).

Root development - HS significantly increased root dry weight by 29.6% compared to control, while they numerically increased root length and root surface area; irrigation rates did not have a clear effect on root performance (Table 28).

Table 25. Summary of morphological and physiological traits of bell pepper grown in year-1 experiment at Weslaco

Time	Source		PH cm	SD mm	SPAD	*Pn* μmol m^{-2} s^{-1}	g_s mol m^{-2} s^{-1}	*E* mmol m^{-2} s^{-1}
1	SA	Control	52.94	16.28	68.19	20.71	0.30 a *	5.47 a
		HS	53.44	15.72	72.17	20.07	0.24 b	4.69 b
	IR	25%	54.56	16.56 ab	67.71	20.87	0.28	5.21
		50%	53.81	14.81 c	70.96	19.73	0.27	4.93
		75%	52.38	17.09 a	70.19	20.68	0.27	5.27
		100%	52.00	15.54 bc	71.85	20.27	0.26	4.90
	P-value	SA	0.813	0.199	0.065	0.317	0.007	0.048
		IR	0.803	0.004	0.533	0.596	0.838	0.855
		SA × IR	0.994	0.887	0.301	0.010	0.003	0.148
2	SA	Control	62.56	18.76	57.37	11.86	0.21	3.55
		HS	58.59	18.46	58.48	11.33	0.19	3.45
	IR	25%	63.94	19.44	57.99	11.08	0.17	3.13
		50%	54.75	18.35	57.54	11.86	0.21	3.68
		75%	63.25	18.17	57.04	11.58	0.20	3.51
		100%	60.38	18.48	59.13	11.87	0.22	3.66
	P-value	SA	0.204	0.703	0.604	0.604	0.706	0.840
		IR	0.158	0.675	0.910	0.939	0.745	0.835
		SA × IR	0.375	0.978	0.965	0.975	0.885	0.827

Time is shown as monthly interval after deficit irrigation started for 2 months. PH: Plant height, SD: stem diameter, SPAD: chlorophyll content index, *Pn*: photosynthesis, g_s: stomatal conductance, *E*: transpiration. * Levels not connected by same letter are significantly different at α = 0.05 based on LSD test.

Table 26. Source of variations and *P*-values from ANOVA on bell pepper year-1 yield in Weslaco

Source of variation	Early harvest TY	Total harvest TY
SA	0.454	0.076
IR	0.994	0.454
SA × IR	0.996	0.787

SA: soil amendment; IR: deficit irrigation; Early harvest: first 2 harvests; Total harvest: 4 harvests; TY: total yield, sum of marketable and cull yield.

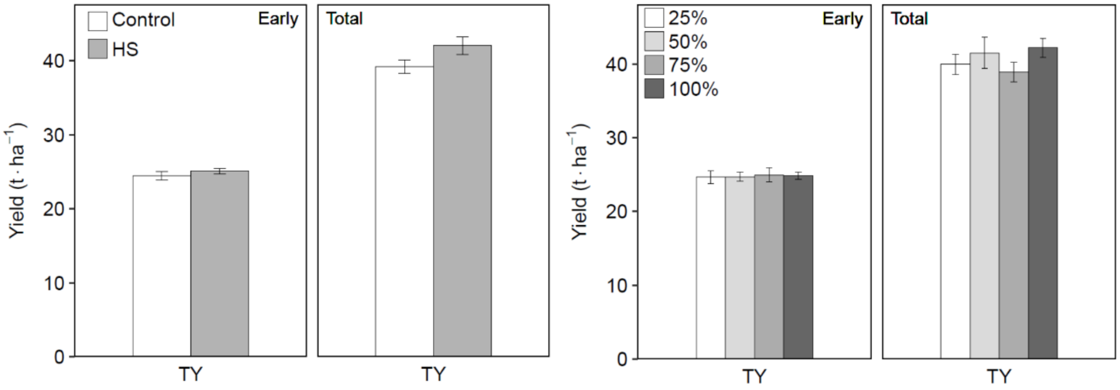

Figure 12. Bell pepper year-1 yield in response to different soil amendment and different irrigation levels in Weslaco.
The percentage of irrigation levels is based on bell pepper evapotranspiration (ET) demands; TY: total yield.

Table 27. Bell pepper year-1 quality in Weslaco

Source		° Brix	Firmness KgF	Wall thickness mm	Ascorbic acid µg/g FW
SA	Control	3.64	1.82	4.81	779.72
	HS	3.75	1.98	4.99	869.19
IR	25%	3.59	1.80	4.89	1092.75
	50%	3.68	2.23	5.30	786.27
	75%	3.54	1.77	4.62	526.43
	100%	3.99	1.81	4.79	892.38
P-value	SA	0.713	0.380	0.422	0.651
	IR	0.686	0.219	0.214	0.251
	SA × IR	0.908	0.924	0.674	0.833

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Table 28. Summary of plant root traits under soil amendment (SA) and irrigation (IR) treatments in year-1 field at Weslaco

Source		RL m	RSA cm^2	RAD mm	RDW g
SA	Control	9.67	103.90	0.34	0.19 b *
	HS	11.39	126.47	0.35	0.27 a
IR	25%	11.28	121.78	0.34	0.21
	50%	9.37	98.88	0.34	0.18
	75%	11.08	121.33	0.35	0.26
	100%	10.38	118.74	0.37	0.26
P-value	SA	0.108	0.052	0.312	0.023
	IR	0.562	0.416	0.308	0.207
	SA × IR	0.327	0.178	0.641	0.414

RL: root length; RSA: root surface area; RAD: root average diameter; RDW: root dry weight.
* Levels not connected by same letter are significantly different at $\alpha = 0.05$ based on LSD test.

b. Year-1 soil (2016)

At the end of the experiment, HS increased soil nitrate-nitrogen content by 166% compared to control (*P*-value = 0.067). HS also significantly decreased active fungi population by 37.8%, which might cause the reduction in soil respiration. Irrigation did not significantly affect soil chemical and microbial properties. However, an interesting pattern of soil respiration was found under different irrigation levels: from severe to well-watered treatments, soil respiration was increased until moderate deficit (75% ET), then decreased in well-watered (100% ET) treatment (Tables 29, 30).

Table 29. Summary of soil pH, electrical conductivity (EC), chemical component analysis under soil amendment (SA) and irrigation (IR) treatments in year-1 field at Weslaco

Source		pH	EC umhos/cm	NO_3-N mg/kg	P mg/kg	K mg/kg	Ca mg/kg	Mg mg/kg	S mg/kg	Na mg/kg
SA	Control	7.9	707	5.9	40	416	4481	324	85	139
	HS	7.9	710	15.7	38	385	4255	316	85	143
IR	25%	7.9	714	6.8	42	399	4359	323	80	141
	50%	7.9	709	9.8	38	404	4473	324	92	147
	75%	8.0	680	17.2	39	406	4313	318	80	124
	100%	7.9	731	9.5	38	392	4326	316	88	151
P-value	SA	0.180	0.950	0.067	0.751	0.095	0.124	0.476	0.996	0.711
	IR	0.269	0.941	0.524	0.853	0.938	0.851	0.923	0.826	0.265
	SA × IR	0.811	0.905	0.911	0.746	0.879	0.976	0.939	0.775	0.507

Table 30. Summary of soil respiration (SR), active bacteria (AB), total bacteria (TB), active fungi (AF) and total fungi (TF) under soil amendment (SA) and irrigation (IR) treatments in year-1 field at Weslaco

Source		SR mg/kg CO_2-C	AB µg/g	TB µg/g	AF µg/g	TF µg/g
SA	Control	7.95	28.90	551.69	8.18 a *	132.57
	HS	5.31	29.38	560.75	5.09 b	142.41
IR	25%	4.69	30.16	592.75	7.56	167.38
	50%	6.94	27.68	525.75	5.98	139.25
	75%	8.94	28.76	545.38	6.65	129.33
	100%	5.95	29.95	561.00	6.36	114.00
P-value	SA	0.102	0.778	0.786	0.018	0.454
	IR	0.287	0.699	0.539	0.816	0.050
	SA × IR	0.369	0.585	0.755	0.862	0.779

* Levels not connected by same letter are significantly different at $\alpha = 0.05$ based on LSD test.

c. Year-2 plant (2017)

Growth and physiology - Based on the results from Table 31, HS significantly decreased bell pepper leaf gas exchange as well as the rate of photosynthesis at the fruiting stage 2 months after irrigation treatment started (ITS). HS also decreased plant height at the same period (*P*-value = 0.061). In terms of irrigation, deficit irrigation decreased plant chlorophyll content (SPAD) at the flowering stage 1 month after ITS, while it did not affect leaf gas exchange regardless of plant growth periods.

Yield and quality - There were no significant interactions between soil amendment and irrigation levels for total harvest yield (Table 32). HS treated field had a 13.7% lower marketable yield than control, which mainly because of the significantly reduction (43.8%) in No.1 size fruit yield. However, HS increased Fancy size fruit yield by 34.1% compared to control. Deficit irrigation (50% ET) reduced marketable yield by 12.5% compare to well-watered (100% ET) treatment, but the negative effect was not significant (Figure 13). In terms of quality, HS significantly increased the wall thickness of bell pepper in deficit irrigation (4.89 mm) compared to control (4.25 mm), while had no effects in well-watered treatment (Table 33).

Root development - HS significantly increased root dry weight by 54.2%, which was consistent with the results from year-1, and the increase in root biomass especially happened in well-watered treatment (0.45 g) compared to control (0.13 g). HS also increased root length and surface area although the promotion was not statistically significant. Deficit irrigation did not affect root performance (Table 34).

Table 31. Summary of morphological and physiological traits of bell pepper grown in year-2 experiment at Weslaco

Time	Source		PH cm	SD mm	SPAD	*Pn* μmol m^{-2} s^{-1}	g_s mol m^{-2} s^{-1}	*E* mmol m^{-2} s^{-1}
1	SA	Control	43.44	13.92	63.06	11.56	0.23	6.78
		HS	44.31	14.08	63.39	12.11	0.22	6.64
	IR	50%	43.94	13.30	60.44 b *	12.05	0.23	6.79
		100%	43.81	14.71	66.19 a	11.62	0.22	6.64
	P-value	SA	0.698	0.855	0.891	0.572	0.871	0.787
		IR	0.956	0.124	0.028	0.660	0.769	0.764
		SA × IR	0.657	0.769	0.372	0.743	0.390	0.410
2	SA	Control	62.38	18.43	48.31	8.94 a	0.22 a	6.50 a
		HS	56.38	16.76	51.64	6.05 b	0.11 b	3.83 b
	IR	50%	62.13	17.95	48.09	7.83	0.16	4.94
		100%	56.63	17.24	51.85	7.16	0.17	5.40
	P-value	SA	0.061	0.134	0.208	0.003	0.011	0.003
		IR	0.084	0.517	0.157	0.449	0.643	0.572
		SA × IR	0.808	0.913	0.987	0.916	0.971	0.976

Time is shown as monthly interval after deficit irrigation started for 2 months. PH: Plant height, SD: stem diameter, SPAD: chlorophyll content index, *Pn*: photosynthesis, g_s: stomatal conductance, *E*: transpiration. * Levels not connected by same letter are significantly different at α = 0.05 based on LSD test.

Table 32. Source of variations and *P*-values from ANOVA on bell pepper year-2 yield in Weslaco

Source of variation	Total harvest		
	Fancy	No.1	MY
SA	0.202	0.004	0.072
IR	0.330	0.718	0.098
SA × IR	0.540	0.529	0.816

SA: soil amendment; IR: deficit irrigation; Total harvest: a 1-time harvest. MY: marketable yield, sum of Fancy and No.1 yield.

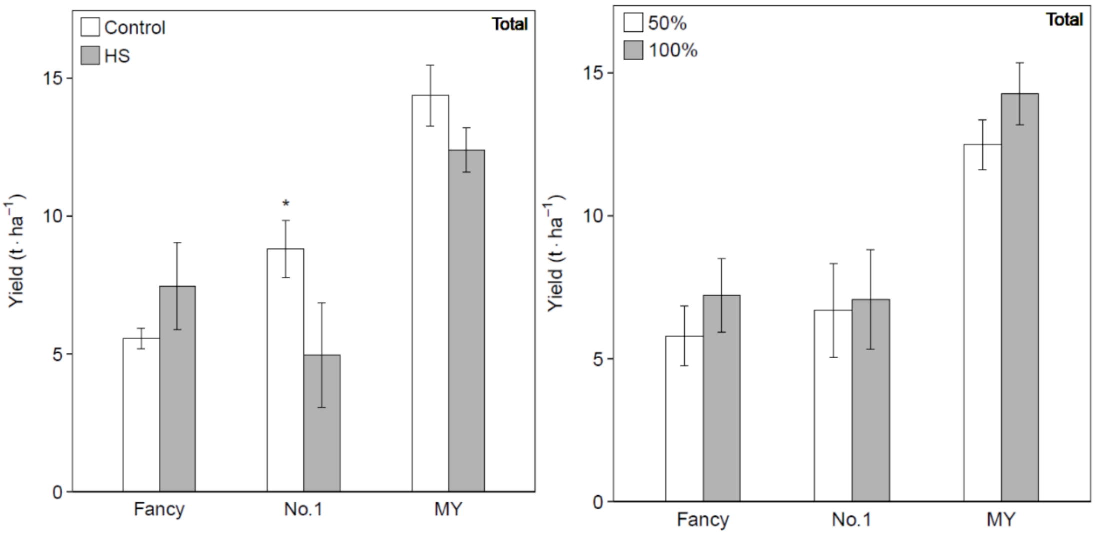

Figure 13. Bell pepper year-2 yield in response to different soil amendment and irrigation levels in Weslaco.

The percentage of irrigation levels is based on bell pepper evapotranspiration (ET) demands; MY: marketable yield, sum of Fancy and No.1 yield. * Levels not connected by same letter are significantly different at α = 0.05 based on LSD test.

Table 33. Bell pepper year-2 quality in Weslaco

Source		° Brix	Firmness KgF	Wall thickness mm
SA	Control	5.66	1.40	4.71
	HS	5.53	1.34	4.96
IR	50%	5.60	1.38	4.57 b *
	100%	5.59	1.36	5.10 a
P-value	SA	0.607	0.629	0.129
	IR	0.962	0.868	0.006
	SA × IR	0.124	0.473	0.029

* Levels not connected by same letter are significantly different at $\alpha = 0.05$ based on LSD test.

Table 34. Summary of plant root traits in year-2 field at Weslaco

Source		RL m	RSA cm^2	RAD mm	RDW g
SA	Control	8.24	92.91	0.35	0.24 b *
	HS	8.97	106.14	0.38	0.37 a
IR	50%	9.40	108.99	0.37	0.32
	100%	7.81	90.06	0.36	0.29
P-value	SA	0.611	0.404	0.167	0.035
	IR	0.281	0.242	0.781	0.613
	SA × IR	0.251	0.084	0.175	0.006

RL: root length; RSA: root surface area; RAD: root average diameter; RDW: root dry weight. * Levels not connected by same letter are significantly different at α = 0.05 based on LSD test.

Table 35. Summary of soil pH, electrical conductivity (EC), chemical component analysis and organic carbon (OC) under soil amendment (SA) and irrigation (IR) treatments in year-2 field at Weslaco

Source		pH	EC umhos/cm	NO_3-N mg/kg	P mg/kg	K mg/kg	Ca mg/kg	Mg mg/kg	S mg/kg	Na mg/kg	OC %
SA	Control	8.1	267	6.0	56	534 a *	5640	381	61	87	0.43 b
	HS	8.2	261	7.1	48	474 b	5394	365	49	94	0.64 a
IR	50%	8.2	268	6.7	51	497	5553	371	49	88	0.58
	100%	8.2	260	6.4	52	510	5481	375	61	94	0.49
P-value	SA	0.220	0.827	0.242	0.103	0.019	0.074	0.108	0.509	0.597	0.030
	IR	0.740	0.779	0.787	0.819	0.548	0.568	0.609	0.514	0.650	0.294
	SA × IR	0.604	0.150	0.971	0.887	0.027	0.467	0.903	0.479	0.197	0.265

* Levels not connected by same letter are significantly different at α = 0.05 based on LSD test.

Table 36. Summary of soil respiration (SR), active bacteria (AB), total bacteria (TB), active fungi (AF) and total fungi (TF) under soil amendment (SA) and irrigation (IR) treatments in year-2 field at Weslaco

Source		SR mg/kg CO_2-C	AB µg/g	TB µg/g	AF µg/g	TF µg/g
SA	Control	19.94	16.08	513.18	2.64	16.01
	HS	40.19	16.79	624.97	3.27	21.37
IR	50%	24.80	15.98	567.57	4.22	16.84
	100%	35.33	16.89	570.59	1.69	20.54
P-value	SA	0.120	0.763	0.107	0.723	0.181
	IR	0.396	0.699	0.963	0.179	0.343
	SA × IR	0.593	0.057	0.067	0.350	0.565

d. Year-2 soil (2017)

HS significantly increased soil organic carbon by 48.8%, but decreased potassium content especially in well-watered condition (453 mg/kg) compared to control (568 mg/kg). Although not statistically significant, HS also increased soil respiration by 2-fold, total bacteria population by 21.8% and total fungi population by 33.6%. Deficit irrigation did not affect soil chemical properties, but it decreased soil respiration by 29.8% although not statistically significant (Tables 35, 36).

e. New year-1 plant (2017)

Growth and physiology - Based on the results from Table 37, HS slightly decreased leaf gas exchange at the fruiting stage 2 months after ITS. Although not statistically significant compared to control, HS also increased plant height at the same period (*P*-value = 0.057). Deficit irrigation did not affect plant morphological and physiological traits, except that deficit stress decreased plant height at the fruiting stage (*P*-value = 0.07).

Yield and quality - There was a significant interaction between soil amendment and irrigation levels on Fancy size fruit yield from total harvest (Table 38). HS significantly increased Fancy size fruit yield by 71.9% compared to control especially under well-watered (100% ET) treatment. Deficit irrigation did not affect marketable yield. In addition, HS and deficit irrigation did not have significant impacts on bell pepper quality (Tables 39).

Root development - Although not statistically significant, roots from HS treated plants had lower root length and surface area, but higher root dry weight compared to control. Deficit irrigation had numerically lower root length, surface area and dry weight compared to well-watered treatment (Table 40).

f. New year-1 soil (2017)

HS slightly increased soil organic carbon (33.3%) compared to control, while had no effects on other soil chemical properties. In terms of deficit irrigation, it significantly increased soil pH but decreased magnesium content in soil (Table 41).

Table 37. Summary of morphological and physiological traits of bell pepper grown in new year-1 experiment at Weslaco

Time	Source		PH cm	SD mm	SPAD	*Pn* μmol m^{-2} s^{-1}	g_s mol m^{-2} s^{-1}	*E* mmol m^{-2} s^{-1}
1	SA	Control	47.13	14.37	70.13	12.29	0.23	7.40
		HS	45.44	13.88	70.24	11.30	0.21	6.90
	IR	50%	47.06	14.27	70.09	12.40	0.24	7.51
		100%	45.50	13.97	70.28	11.19	0.21	6.78
	P-value	SA	0.410	0.433	0.945	0.275	0.320	0.285
		IR	0.445	0.638	0.916	0.187	0.087	0.125
		SA × IR	0.194	0.908	0.390	0.164	0.093	0.067
2	SA	Control	57.31	17.36	61.95	7.22	0.14	5.57
		HS	62.25	18.09	62.52	5.85	0.11	4.60
	IR	50%	57.44	17.85	63.56	6.68	0.13	5.23
		100%	62.13	17.61	60.91	6.40	0.12	4.95
	P-value	SA	0.057	0.371	0.762	0.148	0.135	0.125
		IR	0.070	0.770	0.165	0.762	0.640	0.657
		SA × IR	0.940	0.977	0.923	0.588	0.224	0.262

Time is shown as monthly interval after deficit irrigation started for 2 months. PH: Plant height, SD: stem diameter, SPAD: chlorophyll content index, *Pn*: photosynthesis, g_s: stomatal conductance, *E*: transpiration.

Table 38. Source of variations and *P*-values from ANOVA on bell pepper new year-1 yield in Weslaco

Source of variation	Total harvest		
	Fancy	No.1	MY
SA	0.050	0.842	0.415
IR	0.497	0.392	0.639
SA × IR	0.042	0.966	0.507

SA: soil amendment; IR: deficit irrigation; Total harvest: a 1-time harvest. MY: marketable yield, sum of Fancy and No.1 yield.

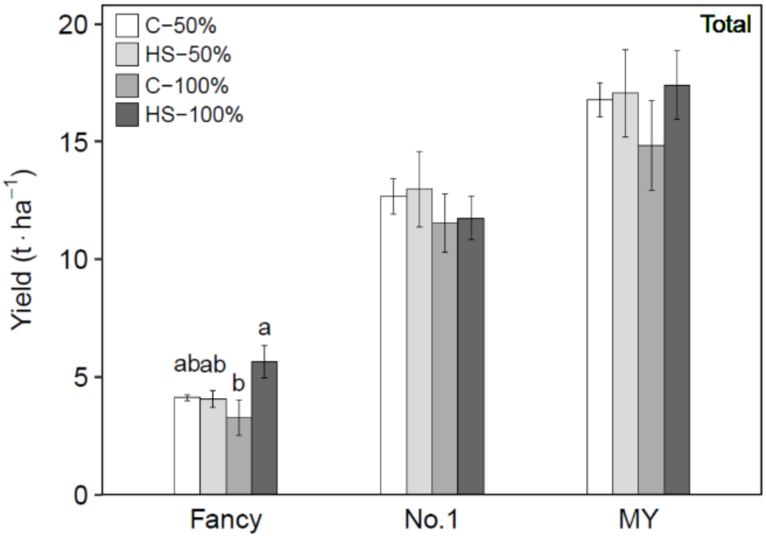

Figure 14. Bell pepper new year-1 yield in response to the interaction of soil amendment and irrigation treatments in Weslaco.
The percentage of irrigation levels is based on bell pepper evapotranspiration (ET) demands; MY: marketable yield, sum of Fancy and No.1 yield. Different letters among treatments indicate significantly different at α = 0.05 based on LSD test.

Table 39. Bell pepper new year-1 quality in Weslaco

Source		° Brix	Firmness KgF	Wall thickness mm
SA	Control	5.26	1.55	4.85
	HS	5.51	1.75	4.78
IR	50%	5.23	1.78	4.82
	100%	5.55	1.53	4.81
P-value	SA	0.220	0.167	0.787
	IR	0.120	0.082	0.964
	SA × IR	0.220	0.527	0.534

Table 40. Summary of plant root traits in new year-1 field at Weslaco

Source		RL m	RSA cm^2	RAD mm	RDW g
SA	Control	8.19	84.34	0.32	0.30
	HS	6.93	76.25	0.34	0.32
IR	50%	7.12	73.51	0.32	0.27
	100%	7.99	87.08	0.34	0.34
P-value	SA	0.239	0.551	0.481	0.908
	IR	0.408	0.326	0.506	0.573
	SA × IR	0.320	0.271	0.391	0.285

RL: root length; RSA: root surface area; RAD: root average diameter; RDW: root dry weight.

Table 41. Summary of soil pH, conductivity, chemical component analysis and organic carbon (OC) under soil amendment (SA) and irrigation (IR) treatments in new year-1 field at Weslaco

Source		pH	Cond	NO_3-N	P	K	Ca	Mg	S	Na	OC
			umhos/cm	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%
SA	Control	8.1	215	11.9	48	543	5788	381	28	54	0.30
	HS	8.2	231	10.8	48	582	5777	385	35	68	0.40
IR	50%	8.2 a *	222	11.0	47	533	5759	378 b	28	59	0.35
	100%	8.1 b	224	11.6	49	592	5806	388 a	35	62	0.35
P-value	SA	0.170	0.314	0.555	0.794	0.330	0.891	0.241	0.277	0.137	0.126
	IR	0.026	0.856	0.740	0.165	0.158	0.559	0.025	0.240	0.754	0.921
	SA × IR	0.917	0.987	0.460	0.072	0.584	0.527	0.202	0.522	0.865	0.448

* Levels not connected by same letter are significantly different at $\alpha = 0.05$ based on LSD test.

Discussion

The results from HS application on bell pepper growth and soil environmental changes showed both similarities but also differences between field and controlled conditions. In general, field weather conditions are more variable, which led to inconsistent impacts of HS application. The positive plant responses caused by HS in the controlled environments needed to be explored in the field for the potential future applications. The following discussion will mainly focus on the effects of HS on biota growth, crop yield, and the mitigation of water stress.

a. Biota growth and yield

Currently, information about the impacts of HS on vegetable crop yield is limited. Most of the studies were conducted under greenhouse or other controlled environments, and very few in field conditions. *Ciarkowska* et al. (2017) found that the high application rate (around 0.33 kg/m^2 in 11 L pot with diameter 22 cm and height 30 cm) of lignite-derived liquid HS (6% humic acids) increased celery and leek yield grown in greenhouse by more than 2-fold, compared to the low application rate (around 0.16 kg/m^2). *Hartz and Bottoms* (2010) tested 4 liquid commercial HS (6% to 11% humic acids), and found that HS did not appear to efficiently increase dry biomass of romaine lettuce (*Lactuca sativae* L.) grown in greenhouse (within 1 L pot), and yield of tomato (*Lycopersicon esculentum* Mill.) grown in field conditions at the application rate of 0.02 to 0.04 kg/m^2. In addition, *Azcona* et al. (2011) tested and compared the liquid HS derived from composted sewage sludge (HSS) and leonardite (HSL, an oxidation product of lignite) in a greenhouse environment, and they found that a higher application rate of HSS (around 0.07 kg/m^2 within 2 L pot) significantly increased pepper (*Capsicum annuum* L. cv. Piquillo) growth such as plant height, leaf area and net photosynthesis at the vegetative stage, but the final yield was similar to the control.

Two-year experiments in Uvalde - In our bell pepper study, a similar early vegetative growth promotion was detected from the results in Uvalde with clay soil, which led to the promotion of bigger fruit yield from early harvest (Fancy size increased by 33.8%), as well as increasing the marketable and total yield from early harvest by 28.9% and 32.8%, respectively. The early yield improvement is important for farmers to compete in high value markets. However, the early boost effect was not translated into improvement in the final marketable and total yield. Interestingly, the effects of HS on plant early growth and production promotion continued in the second year, although the effects diminished and only improved the No.1 size fruit yield. At the end of the experiment, HS treated field did not differentiate nutrient retention based on the soil chemical analysis as compared to control field. Within the same fertilization input, the total nutrient uptake in plants should be similar between control and HS treatment. Therefore, the early yield promotion effects of HS seemed to be caused by different nutrient uptake rate across the different plant growth stages – a faster uptake rate during early growth, following by a slower uptake in late growth. However, *Azcona* et al. (2011) concluded that the positive effect of HS on pepper growth was not related to the improved nutrient concentrations in plant tissues; instead, it might be associated with other growth-promoting factors such as auxin-like activity from HS or hormone generated by microbes that are entrapped in HS. But they only conducted the analysis of nutrient concentration at the end of plant growth, and they used soilless growth medium. Therefore, we suggest future work to address the mechanisms by which HS promote early plant growth especially in different soil types. This will require testing nutrient concentration from plant tissues and soil during different growth periods.

HS did not affect bell pepper quality in the consecutive two-year field experiments, which might indicate the subtle effects of HS on fruit quality composition.

Unlike the results from year-1 in 2016, the soil microbial activity from the second year in 2017 was accelerated due to HS application, showing a long-term soil microbial improvement from HS. This might suggest positive reactions between HS and heterotrophic or autotrophic bacteria, which can benefit from the consumption of certain compounds in HS such as organic acids or amino acids (*Valdrighi* et al., 1996). The additional organic compounds can be reflected by an increase in organic carbon content due to HS application. At the same time, microbes can also take the advantage of HS as electron acceptors for their respiration to provide energy (*Lovley* et al., 1996). However, research on specific type of microbes and their influences to soil environment is still lacking and needs further investigation.

Considering plant roots, HS significantly decreased average root diameter while maintaining root length and surface area to the same level as the control in year-1. This revealed that the application of HS could induce finer roots for water and nutrient absorption, but this effect was diminished in the second year. Based on a germination study of HS application (2 mM C L^{-1} based on humic acid content) in maize (*Jindo* et al., 2012), due to the auxin (IAA) compound contained in HS, the proton pump activity in root plasma membrane was promoted, as well as the principal and lateral root growth, and these effects were more obvious when the source materials of HS contained more carboxylic and hydrophobic groups. In our study, the solid product we used was derived from lignite using ammonoxidation procedure (the reaction of a given substrate with oxygen in aqueous ammonia), in which method the carboxylic and hydrophobic contents will be decreased as mentioned by (*Karapanagioti* et al., 2010). Therefore, the significant effects of HS on plant root development in the field might diminish due to the specific type of HS we used.

Two-year experiments in Weslaco - In the Weslaco location with sandy soil, a similar plant early growth promotion effect from HS was observed in the year-1 experiment (2016). Plant root biomass accumulation also increased due to HS application in both years, but there was not an increase in finer roots as observed in Uvalde with clay soil. In terms of yield, HS slightly increased bell pepper total harvest yield in year-1. In the second year (2017) from one time over harvest, HS increased the bigger size (Fancy) yield but decreased the smaller size (No.1) yield, leading to a decrease in marketable yield. This indicated a similar promotion of fruit size when compared to Uvalde, although this yield promotion in bigger fruit could not offset the decrease in smaller fruit yield. It is expected that HS might be more effective to enhance the existing poor fertility of sandy soil; however, similar to Uvalde, the impacts of HS on bell pepper yield seemed not to be related to the soil nutrient changes. In another sandy soil study with potato, *Selim* et al. (2009) found an increase in crop yield in response to liquid HS application at a rate 120kg/ha (or 0.12 kg/m^2), and the change in yield was indeed related to soil nutrient concentrations change. Different observations once again explained that HS had inconsistent effects on soil environmental changes.

In year-1, HS decreased soil active fungi with a slight increase in nitrogen content, which was in agreement with *Allison* et al. (2007). In year-2, HS slightly increased soil microbial population and activity, especially soil respiration. It has been reported that soil respiration reflects the soil CO_2 efflux generated by soil biota activity, which is mainly influenced by temperature and water content of soil (*Davidson* et al., 1998). In this study, the soil samples collected to perform the soil respiration test was from the plant rhizosphere, a zone where respiration is mainly generated by the decomposition of the rhizosphere carbon (*Epstein*, 1996). Our study showed that HS application increased soil organic carbon, providing more carbon resources for soil microbes to consume. Moreover, the decomposition rate is sensitive to soil temperature changes. Therefore, in further investigation, a soil temperature sensor is also highly recommended to install in order to observe the dynamic changes of soil temperature.

New year-1 experiments in both locations (Uvalde and Weslaco) - Overall in both locations, similar plant growth promotions by HS were observed in new year-1 (2017) experiments. In Uvalde, HS significantly increased plant stem diameter and root surface area, but early yield was significantly decreased by HS application, which was a conflicting result as compared to year-1 experiment (2016). Since the increase in early yield occurred only in year-1, HS did not seem to have a consistent promotive effects on crop yield in the clay soil conditions, which might limit the scope of HS application as mentioned by *Hartz and Bottoms* (2010). However, root growth was greatly promoted as a result of HS application, which might indicate that the beneficial effects from HS were more translated into root development instead of shoot growth (as well as yield). In Weslaco, compared to the results from year-1 (2016), HS did not have obvious effects on plant morphology and physiology changes, but significantly increased bell pepper fancy size yield under well-watered condition. This was similar to the results from year-2 (2017), except that the beneficial effects of HS on fruit development occurred under more appropriate environments.

b. Mitigation of water stress

Changing in plant physiology - In the previous study conducted under controlled environments, we found HS had the potential to mitigate short-term severe or mild water stress by reducing plant transpiration and moisture loss while keeping the photosynthesis rate. In the field experiment, the reductions in leaf stomatal conductance and transpiration caused by HS application were observed in both locations regardless of the growing season (year-1, year-2, new year-1), and these effects tended to occur rapidly after irrigation treatment started (1 month after ITS). But sometimes, such effects occurred along with a reduced photosynthetic rate. With limited information, we can only speculate that HS might have functions to catalyze some physiological reactions such as the change of ROS, ATP synthase, ATP and RuBP contents, or ABA concentration that can control leaf photosynthesis rate and stomatal conductance, which will ultimately affect plant photosynthesis and gas exchange performance.

Root - Plant shoot growth is highly associated with root development. In the initiate hypothesis, we speculated that HS could ameliorate plant shoot performance in deficit stress by significantly increasing root growth. The results of this study confirmed that hypothesis - HS had profound effects on plant root development regardless of irrigation levels. However, root samples were only obtained from the plant final growth stage. There are still lack of observations about the plant early root development responses, especially under low irrigations (sever or mild deficit stress), when leaf gas exchange rapidly decreased due to the HS application. Therefore, the dynamic change of root development across growing stages might also be worthwhile to investigate.

Regarding to lignite-derived HS, this study provided new information on phenotypic changes of bell pepper grown in deficit stress. As mentioned earlier, there are only few studies addressing the relationship between HS and plant gas exchange (*Azcona* et al., 2011; *Dunstone* et al., 1988). A detailed study relating enzyme activity, ABA and gene expression levels for root traits would be valuable to further explore and elucidate specific HS effects and mechanisms on plant root and shoot physiological responses.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Summary

By testing and evaluating the effects of HS application on bell pepper growth and soil quality changes under controlled environment, we found HS-based soil amendment differentially affected bell pepper plant performance depending on soil moisture and soil types. HS improved plant growth under water limiting conditions due to reduced moisture loss and root growth stimulation. These two aspects were critical for plant water and nutrient absorption, and provided increased plant tolerance under water limited conditions. HS had short-term potential to mitigate severe water stress and promote plant growth under moderate stress conditions. The beneficial response of HS was greater in poor soil conditions (sand) than in rich soil (clay). The enhancement of microbial growth from HS was a key potential effect improving soil health, as well as an essential factor to improve plant-microbial interaction. Overall, HS application led to some short-term positive soil environmental changes under both controlled environments.

The field experiments in two diverse locations (Uvalde, Weslaco) and two seasons revealed the mid- to long-term effects of HS on bell pepper growth, yield and quality, as well as soil chemical and microbial properties under different irrigation levels and soil types. HS increased bell pepper early growth and early yield in clay soil (Uvalde), especially due to an early yield promotion of the more valuable Fancy size fruit. But in sandy soil (Weslaco), the effects on plant early growth and yield promotion was inconsistent among different seasons. HS had little or no effects on bell pepper quality regardless of soil types. HS decreased plant gas exchange performance during the vegetative growth period after irrigation treatment started; meanwhile, HS increased plant root development in both locations. In the field study, HS did not greatly change the soil chemical environment over two years, but increased soil organic carbon content, soil respiration and microbial activity, while they decreased the activity of fungi due to the increase in nitrogen content.

As mentioned by *Brown* et al. (2014), information about chemical structure, molecular nature and mineral concentration of HS is still in need for further investigation in order to be considered for specific agricultural uses. Moreover, a detailed study relating enzyme activity, ABA concentration changes and root dynamics is required for a better understanding of the specific mechanisms of mitigation and promotion effects of HS.

Conclusions

In conclusion, the future potential use of lignite-derived HS as soil amendment could be focused on three functions: a) improve seedling vigor, especially root development; b) promote biota growth, improve crop yield and soil nutrient retention; and c) relief and mitigate plants grown under abiotic stress conditions.

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