Influence of Biochar as a Soil Amendment on Soil Water Content and Wild Blueberry Physiology

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INFLUENCE OF BIOCHAR AS A SOIL AMENDMENT ON SOIL WATER CONTENT AND WILD BLUEBERRY PHYSIOLOGY

By
Abigayl Novak

A THESIS
Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science
(in Forest Resources)

The Graduate School
The University of Maine
May 2023

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INFLUENCE OF BIOCHAR AS A SOIL AMENDMENT ON SOIL WATER CONTENT AND WILD BLUEBERRY PHYSIOLOGY

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B.S. University of Maine, 2020
Thesis Advisors: Dr. Ling Li & Dr. Yongjiang Zhang

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Master of Science
(in Forest Resources)
January 2023

Maine wild (or lowbush) blueberries (*Vaccinium angustifolium* Ait.) continue to face challenges imposed by climate change. Reduced frequency of precipitation and increased drought conditions have negatively impacted this crop since it resides in sandy soils with limited retention of water and nutrients. The wild blueberry plants growing in water- and nutrient-poor sandy soils are likely to have poor resilience to drought, resulting in a decline in berry yield during drought years. Thus, there is an urgent need to find a drought management solution for wild blueberries. Compared with other drought management practices, such as irrigation systems, mulching, and adopting drought-resistant varieties, maximizing the water-holding
capacity using biochar is a promising solution due to its other benefits, like providing nutrients, immobilizing heavy metals, and increasing carbon storage. Numerous studies revealed that the effects of biochar additions on the improvement of the water retention capacity of sandy soils are significantly greater than other soil types, such as clay soils. Currently, in Maine, biochar can be produced as a byproduct of bioenergy in a medium-sized forest biomass Combine Heat and Power (CHP) plant with a production capacity of approximately 1,000 tons of biochar per year going to the landfill. Using the locally available biochar to amend the sandy soil in the wild blueberry fields might be a cost-effective approach. However, the physical properties of this biochar, and its influence on soil texture, soil pH, and wild blueberry physiology need to be addressed. This study aimed to characterize the biochar recycled from the local CHP plant, test biochar pH modification effectiveness for use in the wild blueberry fields, and investigate the role of biochar in improving the resistance of wild blueberries to drought in terms of physiological performance. First, biochar physical and chemical characterization was conducted in the laboratory. Other biochar materials made of similar feedstocks showed comparable qualities to our biochar. Then the biochar was modified using an acid treatment method (acetic acid and citric acid with 1% to 3% concentrations) to reduce pH from 11.4 to 6.0, aiming to maintain an acidic soil pH for wild blueberries when used in the fields. We also quantified the water holding capacity of sandy soils (S) amended with untreated biochar (B) at four ratios of 100S:0B (control), 50S:50B, 30S:70B, 10S:90B without fertilizer (Type I) and with fertilizer (Type II). Based on the analysis results and economic consideration, we determined the 50S:50B mixing ratio of sandy soils and biochar to be used in the controlled drought experiments in the greenhouse. Untreated biochar and pH-modified biochar were applied to wild blueberry soils in 2021 and 2022, respectively. The results showed that adding biochar to the sandy soils aided the
wild blueberry plants by delaying the onset of soil water deficits and leaf water stress in wild blueberries in the later summer drought in 2021. The midday leaf water potential ($\psi_{\text{leaf}}$), stomatal ($g_s$) conductance, and photosynthetic electron transport rate (ETR) of plants in biochar treated soils declined slower compared to those in soils without biochar. However, in the early summer drought accompanied by unexpected heatwaves in 2022, there was limited effect of biochar applications in plant drought response. Plants in biochar treated soils (BA soils) did not show a significant delay in the decline in $\psi_{\text{leaf}}$ compared with those in soils without biochar (NA soils), but BA soils aided in maintaining leaf chlorophyll concentrations after the heatwave. pH-adjusted biochar was applied to the soil in 2022 and was able to maintain a pH of 5.7, which was lower than the soil pH of 6.3 when untreated biochar was used in 2021. From this study, we concluded that 1) the biochar produced from the local CHP plant shows potential to be used as a soil amendment in wild blueberry fields; 2) as drought conditions continue to intensify and impact wild blueberries, amending proper biochar to sandy soils would be an effective method to mitigate the effect of rainfall shortages or climate drought.
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LIST OF ABBREVIATIONS

MPa ........................................................................................................ Megapascal
TLP ........................................................................................................... Turgor Loss Point
CHP ........................................................................................................ Combine Heat and Power
AA ................................................................................................................. acetic acid
CA ................................................................................................................ citric acid
RCBD .......................................................................................................... randomized complete block design
G1-G6 ........................................................................................................ genotype 1-6
\( \psi_{\text{leaf}} \) ................................................................................................. leaf water potential
\( g_s \) ........................................................................................................... stomatal conductance
ETR .............................................................................................................. electron transport rate
BA soils .................................................................................................. Biochar-amended soils
NA soils .................................................................................................. Non-amended soils
CHAPTER 1: CHARACTERIZATION AND MODIFICATION OF BIOCHAR FROM A COMBINED HEAT AND POWER (CHP) PLANT FOR AMENDING SANDY SOILS OF WILD BLUEBERRY FIELDS

ABSTRACT

Plant growth and yield in low water retention and nutrient-poor sandy soils are likely to be vulnerable to drought. Biochar is a charcoal-like material that, when used to amend soils, may improve water and nutrient retention. In this study, we investigated if biochar produced from a forest biomass fueled combined heat and power (CHP) plant waste-stream could be used as a soil amendment to help wild blueberry plants, an important economic crop in Maine, to combat drought. We first characterized the biochar’s properties, including bulk density, moisture content, porosity, surface area, functional groups, pH, and ash content, which agreed well with the properties of biochar derived from similar forest biomass and pyrolysis temperatures. Because the biochar that was sourced had a high pH of 11.40, we developed an acid treatment method to decrease the pH using acetic acid (1% and 2% concentration) and citric acid solutions (1.5% and 3% concentration) to make it suitable for wild blueberry applications. We found that all acid treatments significantly lowered the pH to a range of 5.0 to 6.5, close to the range of blueberry soil pH (4 to 5). Additionally, the ash content of biochar decreased from about 16.60% to 2.80%. We tested the water holding capacity of sandy soil (S) mixed with untreated biochar (B) (Type I) and the mixed samples with fertilizer added (Type II) at four ratios of 100S:0B (control), 50S:50B, 30S:70B, and 10S:90B. We found that the 50:50 mixing ratio for both Type I and Type II increased the water holding capacity by about 20% compared with control groups. Our results suggested that the biochar yielded as a byproduct in the CHP plant could be modified with simple low-concentration acid treatments to make it more applicable for application in sandy soils to increase
the water holding capacity and help mitigate negative impacts on wild blueberry plants caused by drought. Further, the sample preparation method (i.e., soaking and crystallization) developed in this study greatly increased the bulk density and fertility of the mixed samples, which might also aid in reducing the mass loss of fine biochar particles when applied in the fields.

**Keywords:** Biochar, Blueberry, Climate change, Porosity, Sandy soil, Water holding capacity

1. INTRODUCTION

The projected increase in climate variability imposes an enormous threat to agricultural systems (Chukalla et al., 2015; Clément et al., 2019; Fernández-Luqueño et al., 2010; Kundu et al., 2008; D. Yarborough, 2008). For agricultural systems with sandy soils, the impacts will be exacerbated due to the low water retention and capacity of sandy soils to buffer increasing variability in rainfall. Although certain types of crops, such as wild (or lowbush) blueberries (*Vaccinium angustifolium* Ait.), potatoes (*Solanum tuberosum*), and beans (*Phaseolus vulgaris*), can thrive and remain productive in sandy soils, these crops require considerable water and fertilizer because the coarse texture of sandy soils causes runoff of these substances (Chukalla et al., 2015; Clément et al., 2019; Fernández-Luqueño et al., 2010; Kundu et al., 2008; Yarborough, 2008). The severity, duration, and frequency of droughts are predicted to increase in many regions of the world, including the Northeastern United States (Fernandez et al., 2020; Field et al., 2012). Further, the increase in the annual average global temperature is expected to lengthen the warm season potentially requiring more irrigation to avoid crop water stress (Field et al., 2012). Wild blueberry crops are an important economic crop primarily grown in the Northeastern United States and Atlantic Canada, are experiencing climate change impacts. Approximately 70% of the wild blueberry farmlands in Maine are not equipped with irrigation systems (Yarborough, 2004).
Therefore, there is an urgent need for improving water- and fertilizer-use efficiencies, lowering the cost of water management practices, and stabilizing the growth and yield of the crops.

Biochar is a charcoal-like material, which is produced through pyrolysis of biomass such as forest logging, wood processing, and agricultural residues (Chen et al., 2018; Rehrah et al., 2014; Suliman et al., 2017). The application of biochar in soils to improve the organic carbon content and fertility can be traced back to 2,500 years ago when Amazonians made biochar and applied it in soils of the Amazon Basin of South America (Yuan et al., 2022). It has become a more prominent soil amendment due to its socio-economic and environmental benefits when used to amend soils (Chen et al., 2018; International Biochar Initiative, 2015; Kameyama et al., 2019; Lu & Zong, 2018; Xiao et al., 2018a; Zhao et al., 2017). Biochar has a highly porous structure, a relatively large surface area, various carbon structures (e.g., aromatic, and heterocyclic carbons), a wide range of pH, and abundant mineral elements (Chen et al., 2018; Rehrah et al., 2014; Suliman et al., 2017; Yuan et al., 2022). These characteristics can aid in its potential as a good soil amendment and be mixed with fertilizer or organic compost to help retain the water and nutrients. The effects of biochar additions on the improvement of water and nutrient retention capacity of sandy soils are significantly greater than other soil types, such as clay soil (Cheng et al., 2017; International Biochar Initiative, 2015; Ren et al., 2015; Wartelle & Marshall, 2000; Xiao et al., 2018a; Zhao et al., 2017). Using biochar to amend sandy soils should be able to increase the overall porosity and modify the pore size distribution of the soils, thereby helping hold large volumes of water even at elevated matric potentials (Pariyar et al., 2020; Yu et al., 2013; Zhao et al., 2013). Nutrient retention can be achieved by the trapping of nutrient-rich water (Chia et al., 2012). Thus, dissolved nutrients would be retained near the soil surface if the water is immobile or moves
slowly. Plants can access part of the nutrients in the retained soil solution as they transpire (Pyoungh Chung et al., 2014).

Biochar can be derived using a range of biomass feedstocks and pyrolysis conditions (e.g., temperature and time) through incomplete combustion in furnaces or using integrated pyrolysis or gasification reactors (Kameyama et al., 2019; Lu & Zong, 2018; Xiao et al., 2018b). When biochar is produced by the biomass-fueled combined heat and power (CHP) plants that generate heat and power as energy outputs and biochar as a waste product. This could provide low-cost biochar for agricultural applications. Moreover, local forest harvesting, and wood processing operations generate large amounts of forest biomass, including bark, leaves, branches, sawdust, wood shavings, and wood chips, which can provide easily accessible and sustainable biomass feedstocks for the CHP plants.

The characteristics of biochar depend greatly on the feedstocks’ characteristics, pyrolysis conditions, and pretreatment of feedstocks. Therefore, not all types of biochar may be ideal as a soil amendment for all soil types and plants. Maine wild blueberry crops are adapted to acidic environments with a soil pH of 4 to 5, where many other plants such as weeds cannot survive (Liu et al., 2016; Luo et al., 2020). When soil pH is above the optimal range, soil pH needs to be lowered by applying a number of remediation ways including adding fertilizers with sulfur or gypsum, pine needle litter, papermill sludge, and soilless substrates (Drummond et al., 2009; Imler et al., 2019; Rosen et al., 1990; Starast et al., 2007; Verheijen et al., 2019).
Figure 1.1 Relationship between pyrolysis temperature and pH of lignocellulosic biochar. The dash lines indicate the linear relationship of pH to pyrolysis temperature for each of these studies with each symbol representing the biochar tested during that study. (Note: Data in this graph was from (Guo et al., 2020).)

Literature review shows that the pH of biochar can vary between 5 and 12 depending on feedstock and the temperature of pyrolysis (Figure 1.1). Therefore, when adding biochar to soil as an amendment, it is critical to consider the pH of the biochar and potentially modify it to a suitable range based on the plant and soil types. For instance, alkaline biochar might need to be treated using acid treatments to decrease its pH and avoid greatly raising the soil pH after applying it in acidic soils for wild blueberry plants (Zhao et al., 2017).

In this study, we sourced waste biochar from a local biomass CHP plant, which consumes about 100,000 tonnes of biomass feedstocks per year and produces approximately 1,000 tonnes of biochar currently disposed of with ash in landfills. The goal of this study was to investigate the
characteristics of this biochar type, how it can be modified to be suitable as a soil amendment for wild blueberries, and its influence on soil properties when used as an amendment in sandy soils. Our objectives were to 1) characterize the basic physical properties of locally sourced biochar to create a material information database, 2) determine an ideal mixing ratio of sandy soil and woody biochar without fertilizer and with fertilizer mixture to increase the water holding capacity, and 3) develop an effective and efficient post-treatment method to neutralize the pH of alkaline biochar for application to acidic soil.

2. MATERIALS & METHODS

2.1 MATERIALS

A biochar material collected from a local biomass CHP plant (Athens, ME, USA) was derived from low quality forest biomass (e.g., bark, branches, leaves, and wood chips) generated from forest operations and wood processing on deciduous and coniferous tree species (e.g., spruce, fir, maple, oak). The biochar was produced at a high temperature of approximately 800°C in the biomass furnace of the CHP unit. Incomplete combustion yielded biochar mixed with ash as a byproduct. The mixture of biochar and ash was transported in barrels to the BioEnergy Lab of the University of Maine. The biochar was separated from the mixture by using a laboratory sifting machine (Gilson Testing Screen, Model TM-3”, Gilson Company, Inc., Worthington, OH, USA) with the following screen sizes: 3.35mm, 1.40mm, 850um, and 425um (International Biochar Initiative, 2015). As the ash was removed from the biochar, fine biochar powders that were of a similar size to the ash were removed as well.

Acetic acid (liquid, pure) and citric acid (solids) were purchased from Fisher Scientific (Hampton, NH, USA), and Research Products International (Mt Prospect, IL, USA). And chosen to neutralize the biochar by considering their environmentally friendly nature, economic
feasibility, and the accessibility (Cheng et al., 2017; Ren et al., 2015; Wartelle & Marshall, 2000). Acetic acid (liquid, pure) was purchased from Fisher Scientific (Hampton, NH, USA), while citric acid (solids) was from Research Products International (Mt Prospect, IL, USA).

Prior to this study, 20 soil samples were collected from Wymans Farm (Milo, ME, USA) and were sent to the Maine Analytical Lab and Maine Soil Testing Service soil lab to analyze the soil composition and pH. The soil composition was measured based on soil type of sand, silt, or clay. The clay was measured by the hydrometer method. The sand was measured gravimetrically after wet sieving and the silt was calculated as the remainder of the sample. The pH was measured by using distilled water and adding the Mehlich buffer. The 20 soil samples were classified as sandy (11 samples), sandy loam (6 samples), loamy (2 samples), and loamy sand (1 sample). The pH of the soil samples ranged between 4.5 to 6.2. Ammonium sulfate [(NH₄)₂SO₄] fertilizer is commonly used in blueberry crop management and was purchased from Northeast Agriculture Sales Inc. (Detroit, ME, USA).

2.2 METHODS

2.2.1 CHARACTERIZATION OF BIOCHAR

The basic physical and chemical properties and morphology of untreated biochar samples were measured using different methods, these are described as follows. Biochar moisture content was measured by using a moisture balance (Ohaus MB23, Hogentogler & Co. Inc, Columbia, MD, USA). The samples had been stored in the lab for a few months before testing the moisture content. Three 5-gram samples were randomly taken from the storage container. Each sample was dried at 120°C for about 10 minutes to ensure the sample weight reached a constant value. Then the sample’s moisture content on a wet basis was reported from the reading of the moisture balance.
The porosity and medium pore diameter of biochar were measured by using a Mercury Intrusion Porosimetry (MIP) Autopore IV (Micromeritics Instrument Corporation, Norcross, GA, USA). This testing was conducted at a collaborative laboratory by an experienced operator. Two biochar samples were tested. The bulk density of biochar particles was measured following ASTM E873-82(2019) Standard Test Method for Bulk Density of Densified Particulate Biomass Fuels (ASTM, 2019). The weight of biochar particles that filled up a graduated cylinder (250 ml) was measured to calculate the bulk density following the equation 1.1. Three replicates were tested.

\[
\text{Bulk density, } g/cm^3 = \frac{\text{Mass}_{\text{dry}}, \ g}{\text{Volume of column packed by sample}, \ cm^3} \quad (\text{Eq.1.1})
\]

The total surface area of biochar was measured by using a Brunauer-Emmett-Teller (BET) surface analyzer (model: Micromeritics ASAP 2020, Micromeritics Instrument Corporation, Norcross, GA, USA). This testing was conducted at a collaborative laboratory by an experienced operator. Two randomly selected biochar samples were tested.

The functional groups of biochar samples were detected by conducting an ATR-FTIR analysis using a PerkinElmer Spectrum Two™ FTIR spectrophotometer (Shelton, CT, USA). Three biochar samples were scanned at a resolution of 4 cm\(^{-1}\) and 64 co-added scans in the wavenumber range of 4000–450 cm\(^{-1}\). Three replicate spectra were averaged for each sample. A normalization process was conducted after acquiring all the data using Origin Pro 2021b (Origin Pro, 2021). The spectra were normalized by dividing each signal of a spectrum by the total peak areas, which can minimize errors resulted from the sample size, shape, and mass.

The morphology of biochar was observed using a Scanning Electron Microscope (SEM) (AMRay 18201820; Amray scanning electron microscopes, Bedford, ME, USA). Biochar particles were mounted on stubs using carbon tape and then conductive silver paint was applied to the samples. The regions of interest of the samples were sputter coated with gold and palladium.
nm) using a Cressington 108 auto sputter coater (Ted Pella Inc., Redding, CA). The images were taken at an accelerating voltage of 3 kV.

### 2.2.2 MODIFICATION OF BIOCHAR pH

An acid treatment method was developed to modify the alkaline nature of the biochar acquired from the CHP plant, aiming to reduce the high pH to a neutral pH of 7 or lower. A total of five treatments were designed, including two diluted acetic acid (AA) solutions (1% and 2% concentrations by volume), two diluted citric acid (CA) solutions (1.5% and 3% concentrations), and deionized (DI) water (control). The DI water control group was used to examine the influence of a water washing process on biochar pH.

For measuring the pH of raw biochar (i.e., untreated biochar), ten (10) g of raw biochar were soaked into 200 ml of DI water, and the pH of the biochar slurry was measured by using a pH meter (Hanna Instruments, Smithfield, RI, USA). This test was repeated three (3) times using three (3) biochar samples randomly selected from the storage containers.

The biochar treatment procedure was described as follows. Approximately 200 mL of 1% of AA, 2% of AA, 1.5% of CA, 3% of CA solutions, and DI water were prepared in five beakers, respectively. Ten (10) g of biochar prewashed were added to each beaker and soaked for 5 minutes. Then, the pH of the biochar slurries was measured and recorded. After that, each beaker's solution was drained using a filtering funnel. About 200 ml of DI water was added into each beaker to wash the biochar, followed by a second pH measurement and recording. This process was repeated a few times (an average of five times) until the biochar pH reached a constant value, indicating water-soluble basic mineral compounds were removed from the biochar. After that, the five groups of biochar samples were oven-dried at 103°C until no water was in the biochar, which ensured all
the samples had the same initial moisture contents before doing the final measurement of biochar pH. Then, the oven-dried biochar samples were rewet by soaking into 200 ml of DI water. The final pH of the five samples was measured and recorded. This process was repeated three times and each time the raw biochar was randomly picked from the storage containers. Fifteen (15) treated biochar samples were made and tested.

2.2.3 ASH CONTENT ANALYSIS OF BIOCHAR

Thermogravimetric analysis (TGA) was conducted to determine if ash minerals were removed through the DI-water and acid solution treatments. All 15 treated biochar samples plus three (3) untreated biochar samples (control group) were placed in 18 crucibles in a TGA instrument (LECO 701, St. Joseph, MI, USA). Each crucible had approximately one gram of biochar sample. The samples were first dried at 103°C for 1 hour. Then the temperature of TGA was increased to 600°C at a rate of 15°C/minute and kept at 600°C for 1 hour to burn the samples completely. The remaining inorganic compounds (i.e., ash) were weighted and used to calculate the ash content following the ASTM E1755-01 (2020): Standard Test Method for Ash in Biomass (ASTM, 2020), and equation 1.2:

\[
\text{Ash content, \%} = \frac{\text{Mass of ash, } g}{\text{Initial mass of biochar sample, dry basis, } g} \times 100\% \\
\text{ (Eq. 1.2)}
\]

2.2.4 WATER HOLDING CAPACITY OF SANDY SOILS AMENDED WITH BIOCHAR

Two types of untreated biochar and sandy soil mixtures were prepared to test ratios of biochar and the effect of adding fertilizer on soil properties. Type I consisted of sandy soil (S) and biochar particles (B) mixed at four ratios of 100S:0B (control), 50S:50B, 30S:70B, and 10S:90B by volume. Type II was composed of sandy soil (S), biochar (B) and fertilizer (F). Unlike simply
blending soil, biochar, and fertilizer granules together, an exploratory approach was designed in this study, aiming to ensure a more homogeneous distribution of fertilizer in the biochar and sandy soil mixture and reduce the blowing off of lightweight biochar after applying in the fields. Ammonium sulfate fertilizer granules were firstly dissolved in water to form a saturated solution at room temperature (74.4 g per 100 g water at 20 °C). Then, the soil and biochar mixture samples with the four ratios used in Type I were soaked in the solution for 24 hours at room temperature. After that, the samples were dried at 50°C in a conventional laboratory oven to allow the fertilizer to be crystalized on the sandy soil and biochar particles (Figure 1.2a).

The water holding capacity of all of these biochar, soil, and fertilizer mixtures was determined using a modified column experiment based on the column test (Yu et al., 2013). We constructed a 150-mm in diameter (D) by 610-mm in height (H) acrylic column system comprised of three sections: upper column (200-mm H) to mimic a precipitation, middle column (254-mm H) for holding soil/biochar/fertilizer mixture, and bottom column (150-mm H) for collecting water (Figure 1.2b). The bottom of the upper and middle columns was drilled to form a perforated plate with 48 holes of 3-mm in diameter. A fine 25-mesh size screen was placed at the bottom of the middle column, allowing water to drain off only.
Figure 1.2 Water holding capacity test of biochar/soil mixed samples using a column experimental method. (a) the preparation process of Type II sand soil/biochar/fertilizer samples. The first step was to measure the biochar/soil ratio and create the saturated solution for the material. After that, the material was soaked for 24 hours, drained and oven-dried to obtain the water holding capacity. (b) The column system used for draining and the water holding capacity test. The sample was packed in the middle column; water was poured in the top column and a waterfall was formed by a perforated plate; and the excess water drained out from the sample was collected in the bottom column.

The sample was packed in the middle column of our custom column system with a thickness of 150 mm, leaving 100-mm of space above the sample. Next, 1000 to 4000 mL of water was poured through the upper column with more solution used for the fertilizer solution due to the dissolved solutes. The water in each sample drained by gravity until no water was dripping into the lower column. The drain time was recorded. Lastly, the sample was collected, weighted (Mass\(_{\text{wet}}\)), and then dried at 103°C in an oven until the mass reached a constant value (Mass\(_{\text{dry}}\)). There was a total of 24 samples run with three (3) replicates within each combination of 10S:90B, 30S:70B, and 50S:50B. We calculated water holding capacity using equation (3) (Yu et al., 2013). The bulk density of the soil and biochar mixture samples were calculated using equation 1.3.
\[
\text{Water holding Capacity,}\% = \frac{\text{Mass}_{\text{wet}} - \text{Mass}_{\text{dry}}}{\text{Mass}_{\text{dry}}} \times 100\%
\] (Eq. 1.3)

2.2.5 STATISTICAL ANALYSIS

The effects of acid treatments on biochar pH and ash content, and the effects of mixing ratios of soil and biochar on water holding capacity and bulk density were analyzed using an analysis of variances (ANOVA) and a paired comparison test using Origin Pro 2021b (Origin Pro, 2021). The significance level was 0.05 (p < 0.05).

3. RESULTS & DISCUSSION

3.1 CHARACTERISTICS OF BIOCHAR

3.1.1 PHYSICAL PROPERTIES OF RAW BIOCHAR

(a) A pile of biochar after sifting

(b) Particle sizes of biochar particles sampled

Figure 1.3 Untreated biochar particle size and shape. a) shows a pile of biochar at different sizes and b) shows the particle sizes of biochar particles sampled.
The size of the biochar particles ranged from 1 to 5 mm after sifting (Figure 1.3). The moisture content of biochar samples tested were between 29% to 38% when placed in the lab for a few months (Table 1.1). The porosity of two biochar samples were 52.66% and 30.29%. The median pore diameter of two samples were 14.50 µm and 17.48 µm. The surface area of two samples were about 302 m²/g to 402 m²/g (Table 1.1). The difference in these properties between biochar samples tested might be caused by the large variation in biomass feedstock, such as species and portions of trees (Pariyar et al., 2020; Zhao et al., 2013). Comparatively, the biochar’s porosity and surface area measured in this study agreed well with biochar derived from different forest biomass (e.g., pine sawdust, shaving, oak pellets, birch wood chips) through pyrolysis with temperatures ranging from 550 ºC to 650ºC (Ferraro et al., 2021; Guo et al., 2020; Lu & Zong, 2018). For instance, the porosity of coniferous forest biochar was about 57% and most of the pore size fell in the range of 6 to 25 µm (Lu & Zong, 2018). The surface area of biochar derived from black pine (Pinus nigra), poplar (Populus), and willow (Salix) was increased from about 350 m²/g to 500 m²/g (Ferraro et al., 2021).

Table 1.1 Summary of physical properties of untreated biochar received from the combine heat and power plant in Athens, ME, USA. Characterization of the biochar was done by using a moisture analyzer for moisture content, the median pore diameter and porosity of the biochar particles using the Mercury Intrusion Porosimetry (MIP) test, total surface area using Bruaner-Emmett-Teller (BET) test, and bulk density using ASTM E873.

<table>
<thead>
<tr>
<th>Property</th>
<th>Method</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Content (MC)</td>
<td>Moisture Analyzer</td>
<td>37.43%</td>
<td>29.73%</td>
<td>32.67%</td>
</tr>
<tr>
<td>Porosity of biochar particle</td>
<td>MIP</td>
<td>52.66%</td>
<td>30.29%</td>
<td></td>
</tr>
<tr>
<td>Median pore diameter</td>
<td></td>
<td>14.50 µm</td>
<td>17.48 µm</td>
<td></td>
</tr>
<tr>
<td>Bulk density, dry</td>
<td>ASTM E873</td>
<td>0.081 g/cm³</td>
<td>0.086 g/cm³</td>
<td>0.082 g/cm³</td>
</tr>
<tr>
<td>Total surface area</td>
<td>BET</td>
<td>402.21 m²/g</td>
<td>301.87 m²/g</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1.4 Pore diameter distribution (a) and cumulative pore volume (b) of biochar samples measured with biochar pore size decreasing from 0 µm to 350 µm.

The pore diameter distribution of untreated biochar ranged from 0 µm to 350 µm (Figure 1.4a). The two biochar samples (Figure 1.4a, b) showed a substantial increase in the pore volume
between 5 and 35 µm, which would be classified primarily as “micropores” (Kameyama et al., 2019). Micropores play an important role in holding water in place by capillarity forces (Chia et al., 2012). The advantage of woody biochar for holding excess water is that biochar keeps the original wood cell structure such as tracheids, vessels, and fibers that typically have diameters within this range (Figure 1.4a) (Held et al., 2021; Salvo et al., 2017; Tarmian et al., 2009). Moreover, about 60% of the total pores of the two biochar samples are micropores. Amending woody biochar into sandy soils therefore would likely increase the amount of micropores in the soils to achieve an increase in water holding capacity (Liu et al., 2017).

3.1.2 MORPHOLOGY OF RAW BIOCHAR

(a) Cross-section of biochar derived from xylem
(b) Biochar derived from bark

(c) Ash deposition on biochar surface
Figure 1.5 Scan Electron Microscopy (SEM) images of selected untreated biochar samples to show the morphological structure of the biochar (a. cross-section of biochar derived from xylem; b. biochar derived from bark; c. ash deposition on biochar surface; d. ash agglomeration in biochar)

Biochar scan electron microscopy (SEM) images showed the biochar derived from a mix of forest biomass: a xylem (Figure 1.5a) and bark sample (Figure 1.5b). Also, ash deposition and agglomeration were observed from the SEM images (Figure 1.5c and 1.5d). Ash in biochar may provide both benefits and negative impacts to the soils. Moreover, ash could be a supplementary source for some essential plant nutrients, such as K\(^+\), Na\(^+\), Zn\(^{2+}\) (Augusto et al., 2008; Buneviciene et al., 2021; Mittra et al., 2005). In addition, excess ash may block the ultramicropores (pore size in 0.1µm to 5 µm) and cryptopores (pore size <0.1 µm) of biochar to decrease the total surface area, which would reduce the adsorption capacity for organic and inorganic pollutants (Li et al., 2017; Klasson et al., 2014). Hence, proper ash removal approaches should be considered based on the end uses of the biochar.
3.1.3 FUNCTIONAL GROUPS OF RAW BIOCHAR

Figure 1.6 Fourier Transform Infrared (FT-IR) test of three oven-dried untreated woody biochar samples indicated by corresponding biochar sample 1 shown as a gray line, biochar sample 2 shown in red, and biochar sample 3 shown in blue. A relatively smooth spectrum shown in three biochar samples revealed that most functional groups, such as aliphatic C–H, olefinic C=C, aromatic CH₃, carboxylic acid functional groups -COOH, in biomass feedstocks were lost.

In line with other studies of biochar (Askeland et al., 2019), the fourier transform infrared (FT-IR) curves of our three oven-dried biochar samples revealed a relatively smooth nonlinear spectrum, and an increase of absorbance in the range of 2000 cm⁻¹ to 500 cm⁻¹ (Figure 1.6). The formation of amorphous structures likely caused the increase in absorbance of biochar samples at those wavelengths. The relatively smooth curves indicated that nearly no functional groups exist in our biochar samples (Chia et al., 2012). This is likely because at high pyrolysis temperatures (800 °C in this study) most functional groups in biomass feedstocks were lost. Functional groups that may drive spikes and absorbance and be present with lower pyrolysis temperatures include aliphatic C–H, olefinic C=C, aromatic CH₃, carboxylic acid functional groups -COOH (Abdel-
Fattah et al., 2015; Alburquerque et al., 2016; Askeland et al., 2019; Chen et al., 2017). Due to the absence of the carboxylic acid functional groups -COOH, biochar was prone to be neutral and alkaline.
3.2 ACID TREATMENT OF BIOCHAR FOR PH ADJUSTMENT

![Graph showing effects of ACID TREATMENT OF BIOCHAR FOR PH ADJUSTMENT](image)

**Figure 1.7** Effects of wash-treatment and acid-treatment on biochar pH through a paired comparison test (a) and ash content (b) fit with standard error bars (Note: The mean values of biochar pH and ash content were plotted. An error bar was added to each column showing the standard derivation (SD) of results. Different letters on bars indicate statistical significance in a paired comparison analysis. Letters shared in common between the mixing ratios indicate no significant difference.)
The mean ± SD values of untreated biochar pH was 11.40 ± 0.03 (Figure 1.7a). The biochar washed by water had a lower pH of 10.07 ± 0.05. Additionally, there was not a statistically significant difference between untreated biochar pH and water washed biochar pH. Both acetic acid and citric acid treatments further lowered the pH of the biochar washed, which ranged from 5 to 6.5. The weak acid treatments showed that they were able to treat the biochar to reduce the pH by 5 to 6.5 and there was not a statistically significant difference in pH between acetic acid treated biochar and citric acid treated biochar. Due to the similar concentrations of acetic acid and citric acid solutions, they acted similarly to reduce the pH.

As for ash content, we found that the ash content of untreated biochar (16.60 ± 1.09 %) was significantly reduced to 8.60 ± 0.33 % when the biochar was washed with water (Figure 1.7b). The ash contents of four types of acid-treated biochar samples were significantly lower than the untreated and water-washed biochar, which were 2.00 ± 0.27 % and 2.80 ± 0.27 %, respectively. But, similar to the result for pH, the acid treatments did not differ from each other (Figure 1.7b). Furthermore, the pH and ash content results reveal that because the ash contributes to causing a high biochar pH, removal of Calcium- and Magnesium- based salts in ash using acid treatments was an effective method to reduce the biochar pH.
3.3 WATER HOLDING CAPACITY OF BIOCHAR AND SOIL MIXTURES

Figure 1.8 Water holding capacity of soil/biochar samples (a) and soil/biochar/fertilizer samples (b) fit with standard error bars (Note: The mean values of water holding capacity of Type I and Type II samples were plotted. An error bar was added to each column showing the standard derivation (SD) of results. Different letters on bars indicate statistical significance in a paired comparison analysis. Letters shared in common between the mixing ratios indicate no significant difference.)
In Type I group of sand:biochar mixture (Figure 1.8a), the mean ± SD values of water holding capacity of sandy soil (100S:0B, control) was 30.4 ± 1.43 %. The averaged water holding capacity of the three soil/biochar mixture samples (50S:50B, 30S:70B, and 10S:90B) were all significantly higher than the control and reached as high as 72.2 ± 2.65 % for the 10S:90B group (Figure 1.8a). However, none of the mixtures containing biochar were significantly different from each other. Type II soil mixture showed a lower water holding capacity than that of Type I soil mixture by about 30% on average. This is likely because the fertilizer filled some of the pore spaces (interpores and intrapores) of the mixture samples. The mean ± SD values of water holding capacity of the Type II 100S:0B group was 12.24 ± 2.23 %, while the water holding capacity increased to about 30 % (50S:50B), 37 % (30S:70B), and 46 % (10S:90B). There was a statistically significant increase in water holding capacity with each increase in biochar (Figure 1.8b). The biochar samples showed that there was an increase in water holding capacity with increased biochar used, which are in line with other studies done on the water holding capacity of biochar (Basso et al., 2012; Fischer et al., 2019; Liu et al., 2016).
3.4 BULK DENSITY OF BIOCHAR AND SOIL MIXTURES

Figure 1.9 Bulk density of soil/biochar mixture samples fit with standard error bars (a) and soil/biochar/fertilizer mixture samples (b). Ratios of soil/biochar include 10S:90B, 30S:70B, 50S:50B, and 100S:0B. (Note: The mean values of bulk density of Type I and Type II samples were plotted. An error bar was added to each column showing the standard derivation (SD) of results. Different letters on bars indicate statistical significance in a paired comparison analysis. Letters shared in common between the mixing ratios indicate no significant difference.)
Amending untreated biochar into sandy soils significantly reduced the bulk density of the soils for Type I and Type II samples from about 1.15 ± 0.16 g/cm³ (100S:0B Type I, control) to 0.18 ± 0.03 g/cm³ (Figure 1.9a), and from 1.38 ± 0.10 g/cm³ (100S:0B Type II, control) to 0.34 ± 0.05 g/cm³ (Figure 1.9b). The density for Type II was slightly higher than that for Type I, likely because the ammonium sulfate fertilizer has a high density of 1.77 g/cm³. The decline in bulk density of the mixed samples became more significantly when more biochar and less sandy soils were mixed together as biochar has a very low bulk density (about 0.08 g/cm³ in Table 1.1) due to the highly porous structure (Luo et al., 2020; Verheijen et al., 2019). When incorporating biochar into the sandy soil, like wild blueberry fields, full consideration should be given to the fact that the bulk density will decrease with biochar. This could alter the soil amendment composition of the existing soils.

4. CONCLUSIONS

In this study, the characteristics of biochar sourced from a biomass combined heat and power (CHP) plant in Maine were studied to explore its feasibility to be used as a soil amendment for the sandy soils in wild blueberry fields. The biochar’s properties, including bulk density, moisture content, porosity, functional groups, pH, and ash content, showed its comparable qualities with other biochar products made of similar feedstocks. We found that an even mixing ratio (50S:50B) struck a balance that increased the beneficial properties of soils (increased water holding capacity, reduced bulk density) for future field studies. However, the beneficial effects of biochar on soil structural and water holding properties were partly offset by the addition of fertilizer when using the sample preparation method (i.e., soaking and crystallization) developed in this study. Due to the similar effects of weak acid treatments, a 2% of acetic acid solution could be used to decrease the pH of biochar to reduce the risk of increasing the alkalinity of the sandy
soils amended with biochar, creating a favorable environment for wild blueberry plants but a hostile environment for weeds and other plants to survive. We recommend doing a mid-term or long-term field study to investigate the influence of pH-modified biochar on the soil pH and weed growth in the wild blueberry fields. Overall, these results suggested that waste biochar recycled from the CHP plant may become an applicable soil amendment to the wild blueberry fields. After refining the operational feasibility of biochar applications in these settings, the use of pH-modified biochar in the sandy soils of wild blueberry fields could create a mutual sustainable solution to reduce the negative environmental impact of biochar waste in landfills and help wild blueberry plants to combat drought.
CHAPTER 2: BIOCHAR APPLICATION MITIGATED DROUGHT EFFECT ON WILD LOWBUSH BLUEBERRIES

ABSTRACT

Agricultural systems continue to face global climate changes including increasing drought conditions and heatwaves, which have negatively impacted crop growth and production. Crops residing in sandy soils, like the wild lowbush blueberries, are sensitive to drought because of the low water retention of sandy soils. Biochar material shows the potential to increase water-holding capacity due to its high porosity and surface area that could be incorporated into the existing sandy soil. By using biochar residual waste from a Combined Heat and Power (CHP) plant in Maine, we tested whether biochar application could mitigate the effect of rainfall shortage and climate drought on wild blueberry plants. Untreated biochar and pH-modified biochar were applied to wild blueberry soils in controlled drought experiments in 2021 and 2022. In year 1 (2021), the experiment was carried out in the late summer and early fall seasons, while in year 2 (2022), the experiment was done during the early summer. In 2021, midday leaf water potential ($\psi_{\text{leaf}}$), stomatal conductance ($g_s$), and photosynthetic electron transport rate (ETR) of plants in biochar-amended soils (BA soils) declined more slowly compared to the plants in soils without biochar (NA soils) during the drought treatment. Thus, biochar aided in mitigating late summer drought by delaying declines in $\psi_{\text{soil}}$, $\psi_{\text{leaf}}$, and $g_s$. In year 2, the plants experienced unexpected heatwaves during the early stage of drought treatment. Biochar application delayed declines in soil water potential but not $\psi_{\text{leaf}}$. Plants in BA soils maintained higher $g_s$ and ETR before and during the heatwave but showed a higher amount of leaf shedding compared to plants in NA soils. Biochar applications also aided in maintaining higher leaf chlorophyll concentrations after the heatwave. Wild blueberry plants showed high sensitivities in $g_s$ and leaf shedding to drought, while ETR was
less sensitive, and chlorophyll and anthocyanin concentrations were not sensitive. To conclude, biochar is effective in mitigating the effects of climate drought on wild blueberries, but its effect is limited for heatwaves.

**KEYWORDS:** Heatwave, Leaf Water Potential, Photosynthesis, Sandy Soils, Stomatal Conductance, *Vaccinium Angustifolium*, Water Holding Capacity

1. INTRODUCTION

Drought is a major limiting factor on agricultural production, and is predicted to intensify in many regions of the world (Dai, 2013; Fernandez et al., 2020; Pörtner et al., 2022). Drought effects may also be exacerbated by climate warming, as elevated temperatures increase plant and ecosystem water loss (Ciriaco da Silva et al., 2013; Tasnim et al., 2021; Zhang et al., 2016). As drought conditions continue to amplify, their impact on crops and livestock, and the cost of crops will influence the adaptability of the agricultural industry. It is seen that the economic impact of drought on agriculture is estimated to cost ~$30 billion per year and could continue to increase as the demand for agricultural production could double by 2050, while the freshwater supply is predicted to drop by 50% (Gupta et al., 2020). Moreover, extreme weather conditions such as rainfall anomalies, extreme droughts, and heatwaves can become more prevalent under climate change, causing high fluctuations in water availability in agricultural systems (Chavez et al., 2015; Cogato et al., 2019; Motha, 2011). The Northeastern United States is predicted to have less consistent rainfall events with increased intensity of rain, decreasing soil moisture content in the growing season, and creating an even more scarce water supply for crops (Bodner et al., 2015; Cogato et al., 2019; Fernandez et al., 2020; Wheaton & Kulshreshtha, 2017). In recent years, the Northeastern region has experienced an increase in drought conditions, which will continue and
impact crop productivity (Kang et al., 2009). Further, heatwaves that are described as hot days with temperatures above 32°C (90°F) for two or more consecutive days, (Masson-Delmotte et al., 2018; NOAA, 2022) are expected to increase in many regions. For instance, heatwaves in Maine are expected to increase by two- to four-fold by the 2050s (Fernandez et al., 2020). The most recent heatwave in Maine occurred in the summer of 2022 (WMTW, 2022). As a result of the challenges imposed by drought and warming on the agricultural systems, there is an urgent need for the development of solutions for drought mitigation.

Current drought management practices in agriculture involve irrigation systems, mulching, adopting drought resistance varieties, and maximizing the water holding capacity using soil amendment (Gumbrewicz & Calderwood, 2022; Hu et al., 2019; Nemali & van Iersel, 2006; Saha, Sekharan, & Manna, 2020; Saha, Sekharan, Manna, et al., 2020; Serena et al., 2020). Irrigation allows for regularly scheduled amounts of water to be given to the crops at a certain range that can be predetermined based on the crop and field type (Nemali & van Iersel, 2006; Ortiz et al., 2018). The appeal of irrigation systems is their ability to save time allowing plants to allocate their water resources to the plant stems and roots. The downside is the start-up cost being high as it ranges from $500 to $5,000 per acre (Sidibé et al., 2012). Additionally, with the predicted drop of 50% in freshwater (Serena et al., 2020), areas that have low water reservoirs could have a restricted threshold for the amount of water they can use (Moore & McEvoy, 2022). Mulching is another technique that growers use to reduce soil surface water loss, to adjust soil physical and chemical properties, and to improve crop productivity (Krogmann et al., 2008). With mulch being a topsoil application, it allows for the O horizon of the soil to hold moisture and prevent evaporation (Bot & Benites, 2005; Gumbrewicz, 2021). Mulching is able to reduce soil water loss and allows soils to hold more water and increase the rhizome growth of wild blueberries (Annis & Stubbs, 2008;
Gumbrewicz, 2021; Yarborough, 2012). Drought-resistant varieties have been developed, which allow crops to resist conditions with reduced access to water in drought conditions (Hu & Xiong, 2014; Luo et al., 2019). The use of soil amendments, such as biochar, can improve the water holding capacity of soils (Basso et al., 2012; Yu et al., 2013, 2017). However, the effect of biochar varies among studies (Blanco-Canqui, 2022).

Biochar has the potential to be a novel solution for drought management. Biochar is a charcoal-like material, which is produced using organic materials (i.e., biomass, such as forestry, logging, wood processing, and agricultural residues and wastes from farms and households) through pyrolysis at a wide temperature range from 300°C to 800°C (Braghiroli & Passarini, 2020; Rehrah et al., 2014; Suliman et al., 2017). Its light and airy porous structure allows for high water holding. As a soil amendment, biochar can be applied to manage drought conditions for drought-intolerant crops, such as soybean (Glycine max L.) (Latawiec et al., 2021; Wang et al., 2018; Zhu et al., 2019), potatoes (Solanum tuberosum) (Liu et al., 2017; Upadhyay et al., 2020), and wild blueberries (Vaccinium angustifolium) growing in sandy soils with low water holding capacity (Basso et al., 2012; De Melo Carvalho et al., 2014; Li et al., 2021). Short-term field and pot experiments have shown a positive effect of biochar on soil water holding capacity in some cases (Jeffery et al., 2011; Lorenz & Lal, 2014), but not in all studies (Blanco-Canqui, 2022). The physical and chemical properties of biochar depend on the biomass source, pyrolysis conditions, and chemical activation approaches (Kameyama et al., 2019; Lu & Zong, 2018), and different biochar feedstocks may show different effects in soil amendment for drought management. Additionally, alkaline biochar could increase soil pH by 0.5-1.0, which can be used to modify contaminated soils from heavy metals (Zhang et al., 2018), but might impact the performance of
crops that prefer acidic soils, like wild blueberry crops in this study. Therefore, detailed studies are needed for specific biochar and specific crops with certain soil types.

As a soil amendment, biochar could be an environmentally friendly solution to mitigate drought impact and secure crop production under climate change. Besides enhancing soil water holding capacity and mitigating drought effects, biochar has other beneficial effects on soil health, crop production, and climate change mitigation. Biochar adds to the overall organic carbon of the soil due to its ability to lock carbon (Biederman & Harpole, 2013; Jeffery et al., 2015). It also decreases nutrient leaching, increases soil nutrient holding such as P, Fe, Mg, and Zn (Biederman & Harpole, 2013; Gao et al., 2016; Sorrenti et al., 2016), filters out harmful heavy metals (Haider et al., 2021, 2022), increases the activity of microorganisms, and enhances microbial diversity (Biederman & Harpole, 2013; Hansen et al., 2017; Liu et al., 2022). When Jujube and cherry trees were amended with biochar in the soil, there was an increase in crop productivity (Gao et al., 2014; Li et al., 2019). Additionally, when different biochar feedstocks were used from wood, paper pulp, woodchips and poultry, uptake of water and nutrients improved with biochar-amended soils (Gao et al., 2014; Li et al., 2019).

The wild lowbush blueberry (Vaccinium angustifolium Aiton) industry is among one of the most prominent industries in New England, but is now under the threat of climate change and increasing drought (Barai et al., 2021; Tasnim et al., 2021). Maine is the largest producer of wild blueberries in the world (Calderwood et al., 2022), and the wild blueberry industry contributes to the economic revenue, social policies, and environmental conditions of Maine. Collectively, there are approximately 36,000 acres of commercial wild blueberry land in Maine, managed by nearly 485 farmers (Yechivi, 2020). Since the early 2000s, the number of harvested acres and yield outcomes have decreased. From 2015 to 2021, the harvested acres of wild blueberries declined by
1400 acres, as of 2015 there were 22,400 acres harvested, and 21,000 acres harvested in 2021 (Calderwood et al., 2022). Total yields also declined from around 100 million lbs. in regular years to 47 to 68 million lbs. in dry years such as 2017 and 2020 (Calderwood et al., 2022). The decrease in yield can be attributed to a variety of complex issues including extreme rainfall events and the predicted increase in climate variability (Fernandez et al., 2020). Over 70% of the wild blueberry farmlands in Maine do not have irrigation systems (Yarborough, 2004), which limits the yield in drought years (Barai et al., 2021; Schattman et al., 2021). Moreover, the primary soil type that wild blueberries grow in is acidic, sandy soil. Sandy soils can contain fine-grained metasandstone, granite, gneiss, and schist ranging from 0.25 mm - 2.00 mm (Ferwerda et al., 1997; Soil Texture Calculator, 2020). Sandy soils have a low water-holding capacity due to its pore size allowing more water to flow through. This results in excessive leaching of soluble soil nutrients, and increased runoff and erosion during heavy rain or manual watering (Li et al., 2019). Therefore, new techniques of drought management are urgently needed to enhance the capacity of the wild blueberry fields to buffer the impacts of drought.

Drought can lead to stomatal closure, declines in photosynthesis, limited plant growth, xylem embolism (hydraulic impairment), yield loss, tissue dehydration, and ultimately plant death (Gupta et al., 2020; Hoffmann et al., 2011; Mingeau et al., 2000; Pahadi, 2021). By improving water holding and delaying the onset of soil water deficit, biochar applications can improve plant physiological performances such as stomatal conductance ($g_s$) and photosynthesis, and delay damages such as chlorophyll degradation and leaf shedding when the water supply is limited. There has been little research on the drought response of wild blueberries (Glass et al., 2005; Pahadi, 2021) and how biochar applications can improve their performances under climate drought. Therefore, here, we aimed to characterize the physiological response of wild blueberries
to extreme drought, and test whether biochar applications to wild blueberry soils can help to delay the effects of climate drought (water withholding) on wild blueberry performance. By using controlled drought experiments, we investigated the physiological responses of wild blueberries (including stomatal conductance ($g_s$), photosynthesis indicated by ETR, electron transport rate of photosystem II, changes in chlorophyll and anthocyanin concentration, and leaf shedding) to extreme drought conditions, and determined the effects of the application of biochar residual sourced from a local biomass combined power and heat (CHP) plant in Maine on their responses. We hypothesized that 1) the biochar application will delay the onset of soil water deficit and decline in leaf water potential during drought treatment as biochar increases soil water holding (Li et al., 2021), and consequently 2) biochar applications will delay declines in $g_s$, ETR and chlorophyll concentration, and leaf shedding during the drought treatment. We also hypothesized that $g_s$, ETR, chlorophyll and anthocyanin concentrations, and leaf shedding of wild blueberries are sensitive to drought. Our study provides experimental evidence on the effect of biochar applications in mitigating the effect of climate drought. Because the plants experienced heatwaves during the drought experiment in year 2, the heatwave effects on plant performance and biochar effects in mitigating the effect of heatwaves were also discussed.

2. MATERIALS & METHODS

2.1 PLANT AND BIOCHAR MATERIALS

The wild blueberry (*Vaccinium angustifolium*) plants, transplanted from Wyman’s farm in Deblois, ME, USA, were used for the greenhouse experiments conducted in 2021 (year 1) and 2022 (year 2). During the transplant, roots and rhizomes were intact and attached to sandy soils to minimize damage and transplant shock. Plants from five (year 1) or six (year 2) different genotypes were collected. The genotypes were determined by the morphological traits of the plants in the
field (Barai et al., 2022; Beers et al., 2019). A single wild blueberry plant with rhizome and root system was planted in 9L buckets with surrounding 9 1-inch drainage holes. The plants were then transported to a greenhouse (4.87 m x 13.7 m) located on the Rogers Farm of the University of Maine. Shade cloth was used to avoid overheating. During both years, new plants were used, where year 1 plants were transplanted in July, and year 2 plants were transplanted in May.

Wild blueberries are typically managed on a two-year crop cycle, where the first-year acts as the vegetative growth year and the following as the fruit-bearing (harvesting or crop) year. After each harvest year, farmers often mow the aboveground plant parts or prune their fields by burning the field using straw or oil burners (Gumbrewicz, 2021). Here, we used plants in their vegetative growth year.

The biochar material was obtained from a local combined heat and power plant (CHP). The CHP plant generates electricity and heat through burning low-quality forestry biomass, such as bark, branches, leaves, and wood chips, and biochar and ash as waste. The physical and chemical properties of the biochar derived from forest products were tested before the experiment. Raw biochar was used in 2021 and pH-modified biochar was used in 2022. For 2022, the biochar pH was modified using 2% acetic acid to decrease the pH from 11.40 to around 7.00. Ammonium sulfate (Nitrogen-based) fertilizer and Sulfate of Potash (Potassium-based) fertilizer were purchased from Northeast Agriculture Sales Inc. (Detroit, ME, USA) and used as the fertilizer mixture.

2.2 EXPERIMENTAL DESIGN

A randomized block design of two factors (biochar treatment and drought treatment; two by two) and five genotypes (year 1) or six genotypes (year 2) was implemented. The pots for growing individual wild blueberry plants in the greenhouse were also randomly assigned into five
(year 1) or six blocks (year 2). Genotype was used as a random factor. In year 1, genotype 4 died before the drought experiment due to transplant shock, so it was removed from the experiment. In each block for each year, there was one replicate of each unique treatment combination of drought (2 levels), biochar (2 levels), and genotype (4-6 levels). Therefore, there were 16 (year 1) and 24 (year 2) pots of wild blueberries for a total of 80 pots (year 1) and 144 pots (year 2) (Figure 2.1, 2.2). After this, wild blueberries were transported to the greenhouse for an establishment period of 21 days during both years to acclimate to the greenhouse environment. They were then put on a regularly watered schedule at 8:00 and 16:00 for 10 minutes at a rate of 1.3L per hour.
Figure 2.1 Experimental layout of drought experiment in 2021 in the greenhouse (4.87 meters by 13.7 meters). 80 wild blueberry plants were arranged in 5 blocks in a randomized complete block design (RCBD) with each treatment combination appearing once per block, for a total of 16 replicates per treatment combination in 5 experimental blocks. Experimental blocks are denoted as dark blue squares. Treatments are as follows: biochar with drought treatment (blue closed squares), no biochar with drought treatment (green closed squares), biochar control (no drought treatment; blue open squares), no biochar control (green open circles). Five different genotypes of wild lowbush blueberries were used in the experiment (G1 to G5).
Figure 2.2 Experimental layout of drought experiment in 2022 in the greenhouse (4.87 meters by 13.7 meter). 144 wild blueberry plants were arranged in 6 blocks in a randomized complete block design (RCBD) with each treatment combination appearing once per block, for a total of 24 replicates per treatment combination. Experimental blocks are denoted as dark blue squares. Treatments are as follows: biochar with drought treatment (blue closed squares), no biochar with drought treatment (green closed squares), biochar control (no drought treatment; blue open squares), no biochar control (green open circles). Six different genotypes of wild lowbush blueberries were used in the experiment (G1 to G6).

The same treatments were implemented each year with a slight modification of biochar application. In year 1, raw biochar was used and in year 2 pH-modified biochar was used. The four treatments include: biochar with drought treatment, no biochar with drought treatment, biochar regularly irrigated, and no biochar regularly irrigated. For the biochar amended soils (BA soils), the soils were mixed with 50% sandy soil from Wyman's farm and 50% biochar with an addition
of ammonium sulfate and sulfate of potash at a 1:1 ratio by volume. The fertilizer mixture was mixed in with the sandy soil and biochar homogeneously. For the drought treatment, watering stopped when the drought treatment began and the plants were allowed to dry down naturally. The drought treatment started on August 10 of 2021, and July 4 of 2022. When the mean value of the wild blueberry plants reached a midday leaf water potential ($\psi_{\text{leaf}}$) of $-7$MPa the drought was terminated. The control crops were regularly irrigated twice a day using irrigation lines, in the morning and early evening. Each block contains five or six different genotypes, drought and control conditions, and biochar or no biochar to account for the differences of each added factor.

The relative humidity and the temperature changes inside and outside were recorded every five minutes using a ZL6 weather station (Metergroup Inc., Pullman, WA, USA) for year 1 (2021) and an Onset HOBO weather station for year 2 (2022) (Onset, Cape Cod, Massachusetts, USA). During year 1 (2021) and year 2 (2022), the relative humidity ranged from 30% to 97% inside the greenhouse. The soil water potentials of the top 5 cm (1.96 inches) layer ($n = 3$ for biochar in drought and no biochar in drought treatments) were measured using TEROS 21 soil water potential sensors connected to ZL6 data loggers (Metergroup Inc., Pullman, WA, USA). Soil temperatures were measured using a Fieldscout TDR 150 Soil Moisture Meter (Spectrum Technologies Inc., Aurora, IL, USA). Temperatures inside the greenhouse were typically 1 to 3°C higher than the daily max ambient temperatures outside of the greenhouse, which ranged from 2.8 to 24°C (Figure S1).

2.3 PRESSURE VOLUME RELATIONSHIPS

In order to determine the turgor loss point ($\Psi_{\text{tlp}}$) and other water relation characteristics, pressure volume analysis (Pahadi, 2021; Tyree and Hammel, 1972; Zhang and Zheng, 2012) was
carried out on two samples per genotype using samples collected from the control plants of the six genotypes studied. Here we aimed to find the overall value and variations of all genotypes rather than that for each genotype. An approximate 2.5-cm-long section of the terminal branch including leaves was enclosed with an aluminum foil-wrapped ziplock bag the evening before the measurement day. Following this, the enclosed samples were cut in the early morning and the samples were taken within 30 minutes and transported to the laboratory within 10 to 15 minutes for measurement. The water potential of a shoot with more than two leaves was measured using a pressure chamber (Model 1505D; PMS Instrument Company, Corvallis, OR USA) and the weight of fresh leaf was measured using a high precision analytical balance (RADWAG X2 PLUS, NE, USA). This process was repeated over time at a 10 minute to an hour time interval until at least eight points were obtained and reached -4.00 MPa. The leaf area of the sample was measured by a leaf area meter (LI-3100; Li-Cor Biosciences, Lincoln, NE, USA) and samples were oven-dried at 65 °C for 72 h. The $\Psi_{\text{tlp}}$ from the pressure volume curves were visualized by plotting leaf relative water content (%), calculated by leaf water (g) divided by the saturated water content (g), against $\Psi$ (Fig. S2). Using the information from the pressure–volume curve and the leaf water potential values, we estimated the turgor loss point ($\Psi_{\text{tlp}}$), the relative water content at each measurement (%), capacitance, and modulus of elasticity ($\varepsilon$) according to methods described by Bartlett et al., 2012 (Bartlett et al., 2012; Pahadi, 2021).

### 2.4 LEAF WATER POTENTIAL AND STOMATAL CONDUCTANCE

To estimate the maximum water stress of the crops during the drought treatment, midday leaf water potential ($\psi_{\text{leaf}}$) was measured using a leaf pressure chamber (Model 1505D; PMS Instrument Company, Corvallis, OR USA). At least 12 samples were taken on a three-day interval or longer depending on the previous leaf water potential ($\psi_{\text{leaf}}$) measurement and how fast the
plants were drying. After collection between 11:00 and 14:00 (all samples collected within 30 minutes), they were enclosed in a sealed bag, placed in a dark cooler, and transported to the University of Maine Plant Physiology Laboratory within 10 to 15 minutes of collection. In coordination with midday leaf water potential ($\psi_{\text{leaf}}$), midday stomatal conductance ($g_s$) was also measured using a LI-600 portable porometer (LI-600; Li-Cor Biosciences, Lincoln, NE, USA). To estimate the photosynthesis of the wild blueberries, a Y(II) Meter (Opti-Sciences Inc., Hudson, NH, USA) was used to measure the electron transport rate (ETR) for two leaves per plant (Stratoulias et al., 2015). The ETR was measured under ambient conditions in the greenhouse.

2.5 MONITORING SOIL pH

To show the changes in soil pH amended with raw biochar and pH-modified biochar in the wild blueberries, the soil pH was measured by making a soil slurry and tested by using the Fisherbrand pH strips on each plant. Raw biochar with an averaged pH value of 11.40 ± 0.05 was directly used in the sandy soils in year 1. For year 2, Biochar with pH modified using a 2% acetic acid solution reduced the biochar pH to 6-7. This was done once per week in year 1 (2021) and before, during, and after the drought in year 2 (2022) by using a spatula to make a 2-5 mm hole in the soil to gather a representative sample (Fisher Scientific, Pittsburgh, PA, USA). These soil pH samples were taken with sandy soils or a combination of sandy soils and biochar.

2.6 LEAF CHLOROPHYLL AND ANTHOCYANIN CONCENTRATIONS

Leaf chlorophyll concentration was measured using a chlorophyll meter (SPAD 502; Minolta Corp., Osaka, Japan), and the anthocyanin concentration was measured by an ACM-200 anthocyanin meter (Opti-Sciences Inc., Hudson, NH, USA). These were taken once per week on all plants from the establishment through the drought period on a representative leaf of each plant.
2.7 PLANT GROWTH, LEAF BROWNING AND LEAF DROPPING

For the growth of plants, stem basal width (two cm above the soil surface) was taken with a caliper (General Tools & Instruments 3-in Digital Caliper) and plant height was measured on all plants with a measuring tape (Komelon 30-ft Measuring Tape) once per week for year 1 and at three times (before the drought, mid-drought, and at the end of drought) for year 2. Leaf browning and leaf dropping were estimated on two days in 2021 and four days in 2022. Each plant from each treatment combination was measured from six genotypes and in six blocks, totaling 36 plants. In total, four measurements were conducted on June 13, 2022 (day -21, before drought), July 20, 2022 (day 16, drought), August 11, 2022 (day 42, drought), and August 19, 2022 (day 49, drought). Each plant was visually inspected by observing the color change of leaves and estimating the percentage of leaves on the plant turning brown and the percentage of brown leaves dropped from the plant. The leaves that dropped into the pots were untouched, and the next measurement counted the old leaves in the new calculation. Plants that died during the establishment period (i.e., day -21, before drought) were excluded from the measurements. If plants had all leaves turning brown (100% leaf browning) or dropped into the pot (100% leaf dropping), they were deemed to have experienced canopy dieback. The measured value of 100% was carried forward to the next measurement. The results of leaf browning and leaf dropping in a percentage scale were converted to scores for complete accuracy and a clear protocol following the criteria: 0 (0%), 1 (1% to 19%), 2 (20% to 39%), 3 (40% to 59%), 4 (60% to 79%), 5 (80% to 99%), and 6 (100%) (Blackman et al., 2019). In each measurement, the number of plants with the same score was counted and reported in percentages. The plants that died during the establishment period were excluded from the measurements.
2.8 STATISTICAL ANALYSIS

To visually show how different treatments impacted leaf water potential, stomatal conductance, chlorophyll concentration, anthocyanin concentration, height, diameter, and soil pH during the drought experiment, time series plots of averages across genotypes and blocks were made using RStudio (RStudio Team, 2020). To determine whether there were significant effects of different treatments, day, and block, a linear model, lm, was used to conduct an analysis of variance (ANOVA) to test the effects of biochar treatment (biochar or no biochar), drought treatment (yes or no), block, and day. The day factor was analyzed as the change over time effect, and the first model was used to determine if the day factor had an interaction with other factors. If the day showed a significant interaction, another model was run on each day to see if there was any significant effect of biochar treatment, drought treatment, and interaction between biochar and drought while still accounting for the block effect. From the second (per day) model, the main effects were reported on the graphs. The significant effect of biochar treatment was denoted by the asterisk (*) symbol, and the effect of drought treatment by hashtag (#) symbols. In order to determine if there was a significant effect of biochar treatment 2021 for day 64 of 2021, a model was run on only this factor due to the absence of data of the non-drought treatments. For each model, if interactions between the factors were not significant, then * was changed to + to properly report the values. Genotype was not included as a factor due to limited replication of measurements from the same genotype under any combination of block, treatment, drought, or day. Inaccurate measurement values (obvious equipment and data entry errors, such as negative gs values, extremely high gs and ETR not possible for wild blueberries, close to zero leaf water potential values confounded by leaf tissue death under severe drought conditions) were assessed carefully and not included in the analyses.
The relationships between midday leaf water potential ($\psi_{\text{leaf}}$) and physiological variables (midday $g_s$, ETR, and chlorophyll concentration) were analyzed and plotted using RStudio (RStudio Team, 2020). Linear or exponential relationships were fitted to the data based on the shape of the relationship.
3. RESULTS

3.1 CHANGES IN SOIL WATER POTENTIAL AND LEAF WATER POTENTIAL DURING THE DROUGHT TREATMENT

Figure 2.3 The midday ($\psi_{\text{leaf}}$) of year one (a) and year two (b) over time. For day 64 of Year 1 (2021) and day 43 of year 2 (2022), values of some treatments are missing due to limited resource or sample availability. Treatments are as follows: biochar in drought (green closed squares); no biochar in drought (green closed circles); biochar irrigated (blue open squares); no biochar irrigated (blue open circles). Horizontal solid lines indicate the turgor loss point ($\Psi_{\text{tlp}}$) at -2.45 ± 0.08 MPa of all genotypes, and the horizontal dotted line at -4.00 MPa represents the period of extreme drought for wild blueberry plants (Pahadi, 2021). Values are mean midday ($\psi_{\text{leaf}}$) ± SE values with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment color. Drought significance indicated by # and biochar treatment differences indicated by *. ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$ (applies to * and # significance).

In year 2 (2022), the daily minimum soil water potentials of biochar-amended soils (BA soils) and non-amended soils (NA soils) were close to zero before the drought treatment and started to decrease on day 13 of drought treatment (Figure S2). BA soils showed a slower rate of decline
in water potential and the average reached -5.1 MPa on day 24, when that of NA soils reached -8.0 MPa (Figure S2). At the end of the drought treatment (day 64), the average daily minimum water potential of BA soils reached -11.2 MPa, while that of NA soils reached -16.3 MPa (Figure S2). No differences were found in soil temperatures among different treatments in both 2021 and 2022 (Figure S3).

There was no decline in midday $\psi_{leaf}$ during the early stage of the drought treatment, from day 1 to day 20 in year 1 (2021; Figure 2.3a) and from day 1 to day 19 in 2022 (Figure 2.3b). Midday $\psi_{leaf}$ of drought-treated plants started to decline on day 21 in 2021 and day 20 in 2022. Irrigated plants grown in BA soils showed no difference in midday $\psi_{leaf}$ compared to irrigated plants in NA soils in both 2021 and 2022 (Figure 2.3).

There was a significant effect of biochar treatment on midday $\psi_{leaf}$ during the drought treatment ($p < 0.001$; Appendix B). Plants grown in NA soils reached the turgor loss point ($\Psi_{tlp}$; -2.45 ± 0.08 MPa) on day 20 in 2021, while plants grown in BA soils reached $\Psi_{tlp}$ on day 33 in 2021 based on the relationship between Midday $\psi_{leaf}$ and day (Figure S4). Therefore, there was a difference of approximately 13 days in reaching $\Psi_{tlp}$ during year 1 (2021) between these two treatments (Figure S4). Turgor loss point ($\Psi_{tlp}$) was estimated from the pressure-volume (PV) curve analysis of all the studied genotypes (Table 2; Figure S5). Further, the plants in BA soils reached -4.00 MPa (extreme drought with PLC close to 90%) (Pahadi, 2021) 16 days later in 2021 (Figure S4). At the end of the drought treatment (day 64 in 2021), the plants in NA soils had a mean value of -6.81 ± 0.33 MPa, and those in BA soils in drought at a mean value of -4.50 ± 0.49 MPa, showing a significant difference of 2.31 MPa ($p < 0.001$, Figure 2.3a).

During year 2 (2022), plants grown in BA soils and NA soils in drought had similar midday $\psi_{leaf}$ until day 18 and 19 (Figure 2.3b). There was a sharp decline in midday $\psi_{leaf}$ of plants under
drought treatment (both BA and NA soils) from day 18 to day 20 that may be driven by a heatwave (Figure S1e). Midday $\psi_{\text{leaf}}$ reached a mean value of $-3.91 \pm 0.93$ MPa on day 19. Therefore, there is no difference in year 2 (2022) in time reaching the $\Psi_{\text{tlp}}$ (Figure S4). The heatwaves resulted in massive leaf shedding, and both plants in BA and NA soils experienced extreme canopy dieback. This made it difficult to capture further midday $\psi_{\text{leaf}}$ measurements and hindered the ability to capture midday $\psi_{\text{leaf}}$ toward the extreme drought point of $-4$MPa due to the limited existing number of leaves. On day 48 of year 2 (2022) (at the end of the drought treatment), the plants grown in BA soils in drought had a mean midday $\psi_{\text{leaf}}$ value of $-7.30 \pm 0.54$ MPa. During year 2 (2022), we found significant interactions between biochar treatment and days ($p < 0.001$), and between drought and days ($p < 0.001$) (see Appendix B).
Table 2.1 Water relations characteristics calculated from pressure-volume curves. $\psi_{\text{TLP}}$ (MPa): the water potential at turgor loss; $\text{RWC}_{\text{TLP}}$ (%): relative water content at the turgor loss; $e$ (MPa): Elasticity, $C_{\text{FT}*}$ (mol m$^{-2}$ MPa$^{-1}$): leaf hydraulic capacitance before turgor loss; $C_{\text{TLP}*}$ (mol m$^{-2}$ MPa$^{-2}$): leaf hydraulic capacitance after turgor loss. Values are means ± standard errors ($n = 12$).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean ± SE</th>
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<tbody>
<tr>
<td>$\psi_{\text{TLP}}$ (MPa)</td>
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</tr>
<tr>
<td>$\text{RWC}_{\text{TLP}}$ (%)</td>
<td>93.96 ± 1.13</td>
</tr>
<tr>
<td>$e$ (MPa)</td>
<td>42.58 ± 7.21</td>
</tr>
<tr>
<td>$C_{\text{FT}*}$ (mol m$^{-2}$ MPa$^{-1}$)</td>
<td>0.89 ± 0.22</td>
</tr>
<tr>
<td>$C_{\text{TLP}*}$ (mol m$^{-2}$ MPa$^{-2}$)</td>
<td>2.34 ± 0.66</td>
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3.2 CHANGES IN LEAF STOMATAL CONDUCTANCE AND ETR DURING THE DROUGHT

![Graphs](https://via.placeholder.com/150)

**Figure 2.4** The midday $g_s$ of year one (a) and year two (b) over time. For day 64 of Year 1 (2021), values of irrigated treatments are missing due to limited resource availability. Treatments are as follows: biochar in drought (green closed squares); no biochar in drought (green closed circles); biochar irrigated (blue open squares); no biochar irrigated (blue open circles). Values are mean stomatal conductance ($g_s$) ± SE values with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment color. Drought significance indicated by # and biochar treatment differences indicated by *. ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$ applies to * and # significance).

In 2021, $g_s$ of irrigated plants in both biochar-amended soils (BA soils) and non-amended soils (NA soils) ranged from 0.04 to 0.25 mol m$^{-2}$s$^{-1}$ (Figure 2.4a). Low $g_s$ values during early drought treatment in 2021 could be due to environmental factors such as high temperatures. In 2022, $g_s$ of irrigated plants ranged from 0.06 to 0.24 mol m$^{-2}$s$^{-1}$ (Figure 2.4b). Overall, drought treatment resulted in significant declines in stomatal conductance ($g_s$) in both years (Figure 2.4). The declines in $g_s$ of plants in NA soils started on day 19 in 2021 and day 14 in 2022. In 2021,
plants in NA soils reached minimum level \( g_s \) (\( =< 0.03 \) mol m\(^2\)s\(^{-1}\)) on day 23, while plants in BA soils on day 36 (Figure 2.4a). In 2022, plants in NA soils reached minimum \( g_s \) on day 33, while plants in BA soils on day 23 (Figure 2.4b).

![Figure 2.5](image)

**Figure 2.5** Photosynthetic electron transport rate (ETR) over time during the drought treatment of year one (a) and year two (b). Treatments are as follows: biochar in drought (green closed squares); no biochar in drought (green closed circles); biochar irrigated (blue open squares); no biochar irrigated (blue open circles). Values are mean ETR ± SE values with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment color. Drought significance indicated by # and biochar treatment differences indicated by *.* ***\( p < 0.001 \), **\( p < 0.01 \), and *\( p < 0.05 \) (applies to * and # significance).

In 2021, no significant differences in ETR were found between irrigated plants and plants under drought treatment (Figure 2.5a). The slight declining trend over time could be because of leaf senescence. Drought-treated plants in BA soils and NA soils maintained mean values of ETR of 71.76 ± 5.98 to 40.74 ± 16.98 μmol m\(^{-2}\)s\(^{-1}\) and 59.24 ± 4.41 to 11.20 ± 2.24 μmol m\(^{-2}\)s\(^{-1}\), respectively (Figure 2.5a). In 2022, declines in ETR of drought-treated plants in NA soils started
on day 14; drought-treated plants in NA soils showed significantly lower ETR compared to irrigated plants and drought-treated plants in BA soils on day 14 (Figure 2.5b). Declines in ETR of drought-treated plants in BA soils started on day 17. On day 23 and days after, no differences in ETR were found between drought-treated plants in BA and NA soils, while both showed significantly lower values compared to the irrigated controls (Figure 2.5b). There was a significant interaction between biochar treatment and drought ($p < 0.05$).

3.3 CHANGES IN LEAF CHLOROPHYLL AND ANTHOCYANIN CONCENTRATIONS DURING THE DROUGHT TREATMENT

Figure 2.6 The anthocyanin (ACM) concentration and chlorophyll (SPAD) concentration during year one (a,c) and year two (b,d) of the drought experiment. Treatments are as follows: biochar in drought (green closed squares); no biochar in drought (green closed circles); biochar irrigated (blue open squares); no biochar irrigated (blue open circles). Values are mean anthocyanin ± SE and chlorophyll ± SE with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment color. Drought significance indicated by # and biochar treatment differences indicated by *. ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$ applies to * and # significance).
Overall, drought treatment showed no significant impact on anthocyanin concentration in both 2021 and 2022 (Figure 2.6a, c). In 2021, anthocyanin concentration of drought-treated plants in both biochar amended soils (BA soils) and non-amended soils (NA soils) on day 9, while that of irrigated plants in BA and NA soils started to increase on day 14 (Fig. 2.6a). In 2022, biochar treatment showed a significant impact on anthocyanin concentration before the drought treatment and during the early stage of drought treatment (days 2 and 9). In 2022, anthocyanin concentration (Figure 2.6c) remained relatively low throughout the drought experiment, compared to a distinct increase in 2021.

In 2021, when drought treatment occurred during late-summer, drought treatment showed no significant impact on chlorophyll concentration, while biochar showed a significant impact on three days (days 0, 9, and 23; Figure 2.6c). Chlorophyll concentrations of plants in NA soils started to decline on day 9, while that of plants in BA soils (both irrigated and under drought treatment) on day 14 (Figure 2.6c). This is supported by the significantly higher chlorophyll concentration of plants in BA soils compared to plants in NA soils on day 9. In 2022, when drought treatment occurred during early summer, no declines in chlorophyll concentrations were found in irrigated plants and drought-treated plants in BA soils (Figure 2.6d). In contrast, drought-treated plants in NA soils showed distinct declines on day 22, as suggested by significantly lower values compared to irrigated plants and drought-treated plants in BA soils (Figure 2.6d). There was also a significant interaction between the biochar treatment and days ($p < 0.01$, Appendix B) for chlorophyll concentration. The increase in anthocyanin concentration and decrease in chlorophyll concentration of irrigated plants in 2021 were likely associated with fall senescence.
3.4 LEAF BROWNING, LEAF DROPPING, AND GROWTH DURING THE DROUGHT TREATMENT

![Figure 2.7](image)

**Figure 2.7.** The leaf browning (a) and leaf dropping (b) of plants with four treatments during the drought experiment in year 2 (2022). Only the data related to plants with a score of 6 (i.e., 100% leaf browning or 100% leaf dropping) were reported in Figure 7. The full set of analyses on leaf browning and leaf dropping for all plants (scores from 0 to 6) was conducted, and the data were plotted in Figure S5. Treatment name is at the top and the dates of measurements are at the bottom of each graph. Treatments are as follows: biochar in drought (green closed squares); no biochar in drought (green closed circles square); biochar irrigated (blue open squares) no biochar irrigated (blue open circles).

In 2021, plants in biochar-amended soils (BA soils) showed less leaf browning on day 36 and 43 during the drought treatment compared to plants in non-amended soils (NA soils) (Figure S6). In 2022, no leaf browning and leaf dropping was observed on day -21 (Figure 2.7a,b), suggesting plants under all treatment groups were well-established and acclimated to the greenhouse environment. During the drought treatment, there were 21.8 %, 65.6 %, and 75 % of total plants in BA soils showing 100 % leaf browning on day 16, day 42, and day 49, respectively,
all of which were higher than those in NA soils by about 11 % to 30 %. In contrast, the percentages of plants with 100 % leaf browning of irrigated and non-irrigated plants in NA soils did not exceed 25 % on day 49. For leaf dropping, the drought treated plants in BA soils also had the highest percentage of total plants with 100 % leaf dropping: 22.0 % on day 16, 37.5 % on day 42, and 37.5 % on day 49. For the drought treated plants in NA soils, about 20.6 % of the plants lost all leaves on day 49. Comparatively, fewer plants with 100 % leaf dropping were observed in irrigated plants in BA soils and non-irrigated plants in NA soils. On day 49, about 16.7 % of total plants in the irrigated plants in BA soils showed all leaves dropped, whereas about 8.3 % in the non-irrigated plants of NA soils. Overall, wild blueberry plants in BA soils turned brown quicker and dropped more leaves than those in NA soils after the heatwave on days 18 to 20 in 2022 (Figure S6).

The plant height and diameter did not show significant increases throughout the experiment in both years (Figure S7a-d). In 2021, the mean height was 16.63 ± 0.44 cm in plants grown in BA soils in drought, and 18.25 ± 0.43 cm in irrigated plants grown in BA soils. The diameters were 1.19 ± 0.06 mm and 1.37 ± 0.06 mm for the former and later, respectively. In year 2, the mean height was 19.81 ± 0.63 cm in plants grown in BA soils in drought and 18.83 ± 0.65 cm in irrigated plants in BA soils. The diameter was 1.75 ± 0.06 mm and 1.87 ± 0.08 mm for the former and later, respectively.
3.5 LEAF PHYSIOLOGY (STOMATAL CONDUCTANCE, ETR, AND CHLOROPHYLL CONCENTRATION) IN RELATION TO LEAF WATER POTENTIALS

Figure 2.8 The relationship between midday stomatal conductance ($g_s$) and midday leaf water potential ($\psi_{leaf}$) for year one (a) and year two (b). The lines are an exponential model ($y = y_0 + a \times \exp(b \times x)$) fitted to the mean values. Treatments are as follows: biochar in drought (green closed squares); no biochar in drought (green closed circles); biochar irrigated (blue open squares); no biochar irrigated (blue open circles). Values are mean $g_s \pm$ SEs and midday $\psi_{leaf} \pm$ SEs with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment color.

The relationship between midday $g_s$ and $\psi_{leaf}$ was well-described by an exponential decline model for both 2021 and 2022 data (Figure 2.8). The midday $\psi_{leaf}$ for wild blueberries to reach 90% decline in midday $g_s$ were -2.10 MPa in 2021 and -6.11 MPa in 2022. The estimated water potential value for 90% decline in 2022 was not realistic due to a lack of data from -2 to -3 MPa (Fig. 2.8b). The relationship between ETR and midday $\psi_{leaf}$ was well-described by linear lines (Figure 2.9). When the midday $\psi_{leaf}$ was between 0.00 MPa to -2.00 MPa, the average ETR values
ranged from 30 to 70 umol m\(^{-2}\)s\(^{-1}\) in 2021 and between 60 to 100 umol m\(^{-2}\)s\(^{-1}\) in 2022. According to the relationship, the midday \(\psi_{\text{leaf}}\) for wild blueberries to reach a 90% decline in ETR were -7.97 MPa in 2021 and -8.72 MPa in 2022. When the midday \(\psi_{\text{leaf}}\) was -4 MPa, the ETR values were 41 and 42 umol m\(^{-2}\)s\(^{-1}\) in 2021 and 2022, respectively. No relationship between chlorophyll concentration and \(\psi_{\text{leaf}}\) was found in both years (Figure 2.10).

![Figure 2.9](image)

**Figure 2.9** The relationship between midday leaf water potential (\(\psi_{\text{leaf}}\)) and photosynthetic electron transport rate (ETR) in year one (a) and year two (b). The data were fitted with a linear regression \(y = mx+b\). Treatments are as follows: biochar in drought (green closed squares); no biochar in drought (green closed circles); biochar irrigated (blue open squares); no biochar irrigated (blue open circles). Values are mean ETR ± SE and mean midday leaf water potential (\(\psi_{\text{leaf}}\)) ± SE values with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment color. Two values in (b) as indicated by arrows were not included in the regression.
Figure 2.10 The relationship between midday leaf water potential ($\psi_{\text{leaf}}$) and chlorophyll concentration (SPAD) in year one (a) and year two (b). Treatments are as follows: biochar in drought (green closed squares); no biochar in drought (green closed circles); biochar irrigated (blue open squares); no biochar irrigated (blue open circles). Values are mean SPAD ± SEs and mean midday leaf water potential ($\psi_{\text{leaf}}$) ± SEs with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment color.
3.6 SOIL pH

![Figure 2.11](image)

**Figure 2.11** The soil pH of raw biochar in year one (a) and pH-modified biochar in year two (b) during the drought experiment. Treatments are as follows: biochar in drought (green closed squares); no biochar in drought (green closed circles); biochar irrigated (blue open squares); no biochar irrigated (blue open circles). The line at approximately 5.33 (2021) and 5.70 (2022) shows the pH in the control with no biochar indicating the optimal soil pH for wild blueberries. Values are mean soil pH ± SE values with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment color. ***** p < 0.001, ** p < 0.01, * p < 0.05 applies to * and # significance).

There was no significant change in soil pH over time during the experiment period in both 2021 and 2022 (Figure 2.11). In the first year (Figure 2.11a), the soils amended with biochar (BA soils) (pH not modified) showed significantly higher (p < 0.001) pH (6.30 ± 0.07), compared to non-amended soils (NA soils) (pH 5.33 ± 0.06). In 2022, when pH-modified biochar was used, there was no significant difference in pH among all treatments (NA and BA soils; Figure 2.11b).
4. DISCUSSION

Our study reveals the positive effects of biochar application in delaying the onset of soil water deficit, and consequently, biochar is effective in delaying the influence of rainfall shortage (climate drought) on plant physiological performances of wild blueberries. At least during the early stage of drought treatment, plants in biochar-amended (BA soils) were able to maintain higher values of $g_s$ and ETR compared to those in non-amended (NA soils). The delayed decline of soil water potential ($\psi_{soil}$) and midday leaf water potential ($\psi_{leaf}$) could be mainly due to the effect of biochar in enhancing the water holding of sandy soils (Li et al., 2021). Interestingly, when plants underwent an unexpected heatwave (2022; days 18 to 20), a delay in the decline of midday leaf water potential ($\psi_{leaf}$) was not found despite higher soil water potentials in BA soils. This could have been because of higher $\psi_{soil}$, plants grown in BA soils were able to maintain higher $g_s$ and ETR compared to that in NA soils even during the heatwave. Despite high leaf browning and shedding during the heatwave, plants grown in BA soils maintained higher leaf chlorophyll concentrations after the heatwaves compared to plants in NA soils.

4.1 BIOCHAR DELAYED THE DECLINE OF LEAF WATER POTENTIAL DURING THE DROUGHT TREATMENT

As hypothesized, we found that biochar applications delayed the declines in midday $\psi_{leaf}$ when the water supply stopped. Plants in biochar-amended soils (BA soils) reached leaf turgor loss point 13 days later, and reached the water potential of hydraulic impairment (-4 MPa; Pahadi, 2021) 16 days later, compared to plants in non-amended (NA soils) in 2021 when there was no heatwave effect. From the perspective of management, this two-week delay of drought impact will allow growers to finish harvesting or take mitigation actions such as temporary irrigation. The physical mechanism underlying this shows there is increased soil water holding by biochar, which
allows soils to conserve more water during rainy days or irrigation. This could be used by plants during the early stage of climate drought (rainfall shortages). Biochar retains water due to its porous structure (Amoakwah et al., 2017; Zhou et al., 2019), and enhances the soil water-holding capacity (Basso et al., 2012; DeLuca & Gundale, 2009; Lehmann & Joseph, 2009; Wang et al., 2018). Our findings are consistent with previous reports (Gao et al., 2016, Haider et al., 2021; Sorrenti et al., 2016; Upadhyay et al., 2020; Wang et al., 2018; Zhu et al., 2019), and suggest that biochar is a potential candidate for future use as a mitigation technique to combat drought.

However during an unexpected heatwave (days 18 to 20 of 2022), biochar applications did not result in delayed declines in $\psi_{\text{leaf}}$, and plants growing in BA soils were also impacted heavily by the heatwaves. Indeed, plants growing in BA soils showed higher canopy dieback compared to those in NA soils. Under heatwaves, our plants experienced high water loss due to high evaporative demands (high vapor pressure deficits, VPD). Even though BA soils have a higher amount of available water compared to NA soils, the plants could reached the same low water potentials as those in NA soils. This agrees with other studies showing that heatwaves can result in low water potentials, xylem embolism, and plant mortality even when soils have enough water (McDowell et al., 2008; Pivovaroff et al., 2016; Schönbeck et al., 2022). The impact of heatwaves on wild blueberry fields has not been studied, but recent studies suggest that warming will negatively impact wild blueberries (Tasnim et al., 2020). The potential effect of biochar applications in plant response to heatwave asks for further studies. Interestingly, biochar can help rice in flooded areas during heatwaves (Huang et al., 2021).
4.2 BIOCHAR HELPED MAINTAIN $g_s$, ETR AND CHLOROPHYLL DURING THE DROUGHT

Our results show that wild blueberry plants growing in biochar amended soils (BA soils) under drought conditions maintained higher stomatal conductance ($g_s$) values compared to plants in non-amended soils (NA soils) (Figure 2.4a). This could be attributed to higher soil moisture content facilitated by enhanced soil water holding in BA soils. Biochar is especially effective in increasing soil water holding capacity of sandy soils (Li et al., 2021). However, under the heatwaves (Figure 2.4b, S1e), biochar applications only showed an effect on $g_s$ before and during the heatwave (days 18 to 20), and then $g_s$ declined to the observed minimum level after the heatwave. Higher $g_s$ of plants grown in BA soils during the heatwave could be because of higher soil water potentials ($\psi_{soil}$) compared to NA soils, which did not result in ABA (abscisic acid) synthesis in roots to simulate stomatal closure (Ali et al., 2017; Mahmoud et al., 2022). These same plants treated with biochar also showed a higher leaf browning and leaf dropping compared to plants in NA soils (Figure 2.7). This finding suggests that when crops are experiencing rainfall shortages, biochar amendment in soils can delay the plants from experiencing soil water deficits and drought effects. However, when they are experiencing heatwaves, high soil water content and high $g_s$ of plants in BA soils could result in large transient plant water loss and leaf dieback due to high transpirational demand (high VPD) during the heatwave (Figure S8).

The electron transport rate (ETR) values (Figure 2.5) agree with the $g_s$ that plants in BA soils were able to maintain higher photosynthesis under drought due to the delay of soil water deficits. This was also seen in year 1, where there are more consistent ETR values throughout the whole drought experiment, and in year 2 before the heatwaves. Further, although the effects of heatwaves in year 2 decreased both $g_s$ and ETR to the minimum, and resulted in a relatively high
degree of leaf browning and dropping, the remaining leaves of plants in BA soils showed that they were able to maintain high chlorophyll concentrations through the end of the drought treatment. This is because even with the heatwaves and high leaf dieback, the water content of BA soils still remained higher compared to NA soils. The effect of biochar in mitigating climate drought was also reported in other crops such as wheat, cabbage seeds, rapeseeds, quinoa, and potatoes (Akhtar et al., 2015; Haider et al., 2020; Khan et al., 2021; Yang et al., 2020; Yildirim et al., 2021). Additionally, higher chlorophyll concentration in plants grown in BA soils compared to that in NA soils was also seen in other studies (Hafeez et al., 2017; Zhu et al., 2019; Zulfiqar et al., 2022). Studies that show this trend indicated that the chlorophyll concentration (chlorophyll a, b) was higher in wheat crop (*Triticum aestivum* L.) and soybean (*Glycine max* (L.) Merr.) grown in BA soils (Hafeez et al., 2017; Zulfiqar et al., 2022). The mechanism is unclear but this could be due to the ability of biochar in keeping more water in the soil even if the water supply is limited, which allows plants to uptake more mineral nutrients through transpiration (Qi et al., 2022; Yuan et al., 2022; Zhang et al., 2018).

There were declines in chlorophyll concentration (Figure 2.6c) in plants under all treatments toward the end of the drought treatment in year 1, but no declines of chlorophyll concentration were found in irrigated and drought-treated plants in BA soils in year 2. Thus, the year 1 decline in chlorophyll concentration was driven by leaf senescence in the late summer and fall in year 1. However, drought-induced declines in chlorophyll concentrations were also found in wild blueberries in year 2 (NA soils under drought) and in other studies (Percival et al., 2012). The increase in anthocyanin concentration (Figure 2.6c) in year 1 is also probably related to leaf senescence (Zhou, 2015), as no increase in year 2 (early summer) was found. However, drought treatment did accelerate anthocyanin synthesis and leaf senescence in year 1. Overall, we can see
through this study that plants in BA soils delay the onset of soil water deficits, suggesting that biochar increases accessible water, which translates to higher levels of ETR, $g_s$, and chlorophyll concentration.

4.3 THE SENSITIVITIES OF DIFFERENT PHYSIOLOGICAL PROCESSES TO DROUGHT

We found that both $g_s$ and photosynthesis indicated by ETR (Davies & Johnson, 1982; Pahadi, 2021; Tasnim & Zhang, 2021) of wild blueberries are sensitive to changes in $\psi_{\text{leaf}}$, as suggested by the exponential and linear decline relationships (Figure 2.8, 2.9). Midday $g_s$ declined to the observed minimum level (90% decline) at -2.10 MPa (before the turgor loss point). In contrast, ETR was still high even at -4 MPa, suggesting that the photosynthetic electron transport of wild blueberries was less sensitive than $g_s$, and water use efficiency increased during the drought. Stomatal closure is a common strategy to conserve water when plants are under water deficits (Davies & Johnson, 1982). A high stomatal sensitivity to drought could help them to save water, but at a cost of declined photosynthetic carbon gain (Zhang et al., 2009). An increase in water use efficiency during drought could partly compensate for this negative effect and was also found in other angiosperm plants (Yang et al., 2021). Meanwhile, leaf chlorophyll concentration was not sensitive to drought as chlorophyll concentration did not differ among all treatments (Figure 2.6c) and no relationships were found between chlorophyll concentration and water potential (Figure 2.10). Wild blueberry crops have typical water potentials of -1.37 to -2.5 MPa during the day (Barai et al., 2022; Glass et al., 2005), suggesting that stomatal closure could happen during the day. Notably, no diameter or height growth was found in drought-treated or irrigated wild blueberry plants during the experiment. This could be because the growth of wild blueberries had finished before the drought treatment (spring).
4.4 BIOCHAR EFFECTS ON SOIL pH

Biochar did change soil pH in our study. During year 1, the pH of biochar amended soils (BA soils) ranged from 6 to 7, significantly higher than the average pH of 5.4 in non-amended soils (NA soils) (Figure 2.11). This introduced a problem because wild blueberries naturally live in soils with a pH < than 5 (Drummond et al., 2009). Although no effects of changed pH on plant performance were found in this study, long-term concerns should be considered. An increase in pH in the wild blueberry fields could alter the existing environment and weed pressure, which might require mitigation management such as soil application of sulfur. For year 2, we addressed this concern by treating the biochar with 2% acetic acid, and the soil pH was lowered from 6 to 7 in year 1 (Figure 2.11a) to an average pH of approximately 5.7 in year 2 (Figure 2.11b). Thus, the weak acid treatment was an effective approach to decrease the pH of biochar. Considering the low concentration (2%) of acetic acid solution used, it would be accessible and affordable for growers when they need to adjust the pH of biochar materials. It could be adopted by biochar manufacturers and farmers to treat alkaline biochar before applying it to wild blueberry fields. Nevertheless, long-term studies will need to be conducted to assess the long-term effects of the biochar treatment on soil pH and weed growth and control.

5. CONCLUSIONS

To conclude, our study found that adding biochar as a soil amendment aids the wild blueberry plants by delaying the onset of soil water deficits and leaf water stress in wild blueberries during rainfall shortages. During heatwaves, biochar application and improved soil water conditions could help maintain high stomatal conductance and photosynthesis of wild blueberries, but this can result in high water loss and leaf shedding. However, improved soil water conditions can help plants to maintain better water status and physiological performance after the heatwave.
As drought conditions continue to intensify and impact the world (Kang et al., 2009; Lobell & Gourdji, 2012), mitigation strategies are urgently needed to secure crop production. When water resources remain a crucial constraint for some crops, biochar application can be a promising technique to mitigate the effect of rainfall shortages or climate drought. Additionally, we found that weak acetic acid is effective in modifying pH of biochar. Further research using field experiments can be done to confirm the findings of our greenhouse study.
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### APPENDIX A: Characteristics of biochar derived from different biomass feedstocks and pyrolysis processes (Guo et al., 2020)

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Pyrolysis conditions</th>
<th>pH</th>
<th>Ash (%)</th>
<th>Organic Carbon (%)</th>
<th>Total N (g/kg)</th>
<th>Total P (g/kg)</th>
<th>Total K (g/kg)</th>
<th>CEC (cmolc/kg)</th>
<th>Surface area (m²/g)</th>
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## APPENDIX B. ANOVA results

### Year 1 (2021)

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<th>Stomatal Conductance</th>
<th>ETR</th>
<th>Soil pH</th>
<th>Height</th>
<th>Diameter</th>
<th>Anthocyanin Content</th>
<th>Chlorophyll Content</th>
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<td><strong>Biochar</strong></td>
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<td>0.02555 *</td>
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<td><strong>Drought</strong></td>
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<td>0.669843</td>
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<td><strong>Days</strong></td>
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<td>1.003e-07 ***</td>
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<td>&lt; 2.2e-16 ***</td>
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### Year 2 (2022)

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<th>ETR</th>
<th>Soil pH</th>
<th>Height</th>
<th>Diameter</th>
<th>Anthocyanin Content</th>
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<td>1.075e-05 ***</td>
<td>4.902e-10 ***</td>
<td>0.394903</td>
<td>0.024282 *</td>
<td>0.0001186 ***</td>
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<td><strong>Days</strong></td>
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<td>&lt; 2.2e-16 ***</td>
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**APPENDIX C.** One-way ANOVA of biochar pH (a) and biochar ash content (b)

(a) One-way ANOVA of biochar pH

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(b) One-way ANOVA of biochar ash content

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**APPENDIX D.** One-way ANOVA of bulk density of soil/biochar mixture (a) and soil/biochar/fertilizer mixture (b).

(a) One-way ANOVA of soil/biochar mixture

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(b) One-way ANOVA of soil/biochar/fertilizer mixture

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APPENDIX E. Supplemental materials

a) Outside ambient relative humidity and temperature of the first year greenhouse experiment. Some of the data was lost during the experiment due to miss connection.

b) Inside greenhouse relative humidity and temperature during year 1 greenhouse experiment.
c) Outside ambient relative humidity and temperature of year 2 greenhouse experiment.

d) Inside greenhouse relative humidity and temperature during year 2 greenhouse experiment
e) Comparison of temperature inside of greenhouse (2021 vs. 2022). The green dotted boxes show the differences in temperature between the different years when the heatwaves were expected to occur.

**Figure S1.** Temperature and relative humidity using the Z logger and HOBO weather station for year one (a,b) and year two (c,d) (Onset, Cape Cod, Massachusetts, USA) during the drought experiment. Comparison of temperatures inside of the greenhouse on the same time and same date from July 15 to August 5 in 2021 and 2022 (e), revealing heatwaves frequently occurring in 2022.
Figure S2. Daily minimum soil water potential in year two (2022) during the drought experiment. The data are means ± SEs (n = 3). Blue squares indicate biochar-amended soils in drought, and green closed circles are non-amended soils in drought.
Figure S3. Mean soil temperatures during the drought treatment in year one (a) and year two (b). Temperatures were measured using the soil moisture meter (TDR). No differences were detected among different treatments. Values in the data are means ± SE (n = 80 (2021), n= 144 (2022)).
Figure S4. The midday ($\psi_{\text{leaf}}$) of the first (a) and year two (b) over time plotted with a linear regression to predict the exact day that each treatment intersects with -2 MPa. Red fitted line indicates the Biochar in Drought and the black being No Biochar in Drought.
Figure S5. Pressure volume relationships of the wild blueberries. The relationship between the leaf relative water content (%) and leaf water potential (Ψ, MPa). Points indicate measurements from all samples of six genotypes. The vertical break line denotes the turgor loss point. The solid green lines are linear regressions fit to the data. Break green lines and gray lines are 95% confidence bands and 95% prediction bands, respectively.
(a) Leaf browning of biochar in drought


(b) Leaf browning of no biochar in drought


(c) Leaf browning of biochar irrigated


(d) Leaf browning of no biochar irrigated

Figure S6. The leaf browning (a-2d) and leaf dropping (3a-3d) of plants with four treatments during the drought experiments in year 1 (2021) and year 2 (2022). The treatment type is at the top and the dates of measurements are at the bottom of each graph. Legends of score and the corresponding percentage range are at the right of each graph. For the leaf browning, 0 means that the leaf browning percentage is 0% and the leaves on the plant are all green, while 6 means that leaf browning percentage is 100% and the leaves on the plant are all brown. For the leaf dropping, 0 means that the leaf dropping percentage is 0%, while 6 means that leaf dropping percentage is 100%. n is the number of total plants measured in each graph. The plants that died during the establishment period were excluded from the measurements.
Figure S7. The plant basal diameter (a,c) and height (b,d) of year one (top; a, b) and two (bottom; c,d) drought experiment showing the treatments of biochar in drought (green closed squares), no biochar in drought (green closed circles), biochar control (blue open squares), no biochar control (blue open circles). Values are mean diameter ± SE and leaf height ± SE with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment color.
Figure S8. Vapor pressure deficit (VPD) calculated using temperature and relative humidity from the ZL6 logger of year two (2022) (Onset, Cape Cod, Massachusetts, USA) during the drought experiment.
APPENDIX F: Peer-Reviewed Publications

   [https://doi.org/10.3390/su142214937](https://doi.org/10.3390/su142214937)

   [https://doi.org/10.3390/w13040407](https://doi.org/10.3390/w13040407)
BIOGRAPHY OF THE AUTHOR

Abigail (Abby) Novak grew up in New York and Maine. She graduated from Hampden Academy in Hampden, Maine in 2016. She attended the University of Maine and graduated in 2020 with a Bachelor’s of Science degree in Ecology and Environmental Sciences. She decided to remain at the University of Maine and entered the Forest Resources graduate program in the fall of 2020. After receiving her degree, Abby will be pursuing her Ph.D. in plant physiology with a focus on plant hormones. Abby is a candidate for the Master of Science degree in Forest Resources from the University of Maine in May 2023.