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Wild Blueberry Nutrition Under Climate Change

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WILD BLUEBERRY NUTRITION UNDER CLIMATE CHANGE

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An Abstract of the Dissertation Presented
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The wild blueberry crop is one of the most important commercial crops in Manie, USA. Some new fertilizers claimed to enhance both conventional and organic wild blueberry production, but no scientific research has been done to test their effects. Besides, no scientific research has explored whether nutrition in wild blueberry plants is related to their physiological and morphological characteristics. Further, changed temperature and rainfall patterns related to global climate change have altered the growth pattern and nutrient economy of the wild blueberry system, bringing in new challenges to this traditional agricultural system. Therefore, the objectives of my dissertation were: 1) To characterize climate change patterns of wild blueberry production regions and determine their effects on crop health to manage the fields efficiently; (2) To test techniques using biochar-compost pellets and mulch to mitigate potential increased water deficits of wild blueberries, and 3) To quantify the effects of different commercial foliar fertilizer products on wild blueberry physiology and production,

To fulfill objective 1, trends in the temperature, precipitation, and potential evapotranspiration (PET) during the growing season (May–September) as well as their effects on the Maximum Enhanced Vegetation Index (EVI) were analyzed for 26 wild blueberry fields in Downeast Maine over the time period of 1980 to 2019 in Chapter 2. Annual and seasonal climate trends (temperature, rainfall, snow cover) from 1980 to 2020 for seven Maine counties with large wild blueberry areas were analyzed in Chapter 3. These analyses were conducted using Remote Sensing software and Geographic Information System (GIS) tools in Arc GIS Pro software. The air temperature of the studied wild blueberry fields in Downeast showed higher rates of increase than those of the entire region (blueberry fields and non-

blueberry production areas) during 1980-2019. An optimum temperature and PET for EVI of 22.4 °C and 145 mm/month suggest potential negative effects of further warming and increasing PET on crop health and productivity. Across all blueberry production fields, historical temperatures increased significantly in the fall and winter followed by summer, but not in the spring. Additionally, precipitation increased by 0.5–1.2 mm/year in the winter and fall, whereas no changes were found in the spring and summer. The rate of increasing temperature was comparatively slower in the warmer barrens located towards the south-west (Hancock to York). Moreover, the growing season lengthened towards the fall consistently in all locations, whereas lengthening towards the spring was inconsistent.

To fulfill objective 2, the effects of warming and soil amendments (mulch and biochar-compost mix) on the physiology, growth, and yield of six different genotypes of wild lowbush blueberry plants at two different locations (Jonesboro and Deblois) in Maine were tested in Chapter 4. Open-top chambers with active heating were used to simulate climate warming, and crop physiology and yiield were measured. Some physiological performance, growth, berry yield and size of wild blueberry plantsunder warming treatment. The studied biochar-compost mix retained more moisture in the studied wild blueberry field soil, which helped the plants grow better with better physiological performance whereas a 0.5” (1.3 cm) layer of mulch amendment was not sufficient. Moreover, the plants growing under the warmer environment along with the biochar-compost mix amendment had the highest fruit production. Therefore, it would be beneficial to use a soil amendment like biochar-compost mix for wild blueberry crops under predicted warmer and drier summers.

To fulfill objective 3, the impacts of seven foliar fertilizers and a standard granular fertilizer were tested on wild blueberries for one crop cycle (2019-2020) in a randomized complete block design with eight replicates in a conventional wild blueberry field in Maine, USA in Chapter 5. Soil-applied fertilizers containing N, P, K, as well as foliar fertilizers containingCa and/or plant hormones might benefit crop growth, but the impact on yield was limited. Moreover, wild blueberry physiology, morphology, and leaf nutrients in the vegetative year largely impact their yield in the following crop year. In Chapter 6, a follow up study was conducted on the use of nanocellulose (CNFs) with one of the foliar fertilizers to verify its promising result on wild blueberries found in Chapter 5. The effects of CNFs on the leaves of two wild lowbush blueberry species (*Vaccinium angustifolium* and *Vaccinium myrtilloides*) were investigated. Our study showed that the CNF addition significantly affected the surface wettability and water loss of the *V. myrtilloides* leaves but not the *V. angustifolium* leaves. The difference could be related to denser trichomes in *V. myrtilloides* leaves. Our study also revealed that the CNFs assisted the foliar fertilizer to disperse into smaller particles on the leaf surface of *V. angustifolium* species, which might have resulted in a higher average fruit yield, inviting further study.

DEDICATION

I dedicate my dissertation to my almighty Allah, my loving and super-supportive husband (SK Belal Hossen) and my baby girl (Inaya Hossen) in my womb who have continuously given me the strength, patience and motivation to keep going and complete this degree.

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TABLE OF CONTENTS

DEDICATION iii

ACKNOWLEDGEMENTS iv

LIST OF TABLES x

LIST OF FIGURES xii

CHAPTERS

1. INTRODUCTION 1

    1.1. Background and Importance of this Research 1

    1.2. Dissertation Structure 6

2. CLIMATE CHANGE PATTERNS OF WILD BLUEBERRY FIELDS IN DOWNEAST, MAINE OVER THE PAST 40 YEARS 7

    2.1. Abstract 7

    2.2. Introduction 8

    2.3. Materials and Methods 10

        2.3.1. Study Area 10

        2.3.2. Data Acquisition and Methodology 11

        2.3.3. Statistical Analysis 13

    2.4. Results 14

        2.4.1. Comparison of Historical Climate Change between Maine and the Wild Blueberry Fields of Downeast Maine 14

        2.4.2. Comparison of Historical Climate Change among the Wild Blueberry Fields in Downeast, Maine 20

        2.4.3. Relationship of Climate Variables with the Vegetation Index of Wild Blueberry Fields 23

        2.4.4. Wild Blueberry Fields Experienced Suboptimal Temperatures

During the Peak Season (July and August) 25
2.4.5. Potential Evapotranspiration (PET) Rate of the Wild Blueberry Fields 26
2.5. Discussion 29
3. SEASONAL CLIMATE TRENDS ACROSS THE WILD BLUEBERRY BARRENS OF MAINE, USA 33
3.1. Abstract 33
3.2. Introduction 33
3.3. Materials and Methods 38
3.3.1. Data Source and Acquisition 38
3.3.2. Data Analyses 39
3.4. Results 40
3.4.1. Annual Climate Changing Trends (1980–2020) in the Wild Blueberry Barrens 40
3.4.2. Seasonal Climate Changing Trends (1980–2020) in the Wild Blueberry Barrens 44
3.4.2.1 Summer (May–September) Climate in the Barrens 55
3.4.2.2 Winter (November–February) Climate in the Barrens 55
3.4.2.3 Fall (September–October) Climate in the Barrens 56
3.4.2.4 Spring (March–May) Climate in the Barrens 57
3.4.2.5 Growing Season in the Barrens 58
3.5. Discussion 58
3.5.1. Changes in Daytime and Nighttime Temperatures 59
3.5.2. Seasonal Variations in Climate Change and Implications 60
3.5.3. Spatial Variations in Climate Change and Implications 61
4. INFLUENCE OF SOIL AMENDMENTS ON SOIL WATER AVAILABILITY AND RESPONSES OF WILD LOWBUSH BLUEBERRIES TO WARMER CLIMATE 64
4.1. Abstract 64

4.2. Introduction 64
4.3. Methodology........ 67
4.3.1. Study area, Experimental Design, Application Materials and Methods........ 67
4.3.2. Data Collection and Measurement Methods........ 70
4.3.3. Data Analysis 72
4.4. Results........ 73
4.4.1. Air Temperature and Relative Humidity 73
4.4.2. Wild Blueberry Soil and Leaf Water Status 74
4.4.3. Wild Blueberry Physiology 77
4.4.4. Wild Blueberry Morphology 82
4.4.5. Wild Blueberry Water-Use and Nitrogen Source 83
4.4.6. Wild Blueberry Leaf and Fruit Nutrients........ 84
4.4.7. Wild Blueberry Quantity and Quality........ 88
4.4.8. Relationship among Wild Blueberry Plant Traits and Fruit Quantity and Quality 90
4.5. Discussion 92
5. ARE FOLIAR FERTILIZERS BENEFICIAL TO GROWTH AND YIELD OF WILD LOWBUSH BLUEBERRIES?........ 97
5.1. Abstract 97
5.2. Introduction 98
5.3. Materials and Methods........ 101
5.3.1. Study Area and Experimental Design........ 101
5.3.2. Application Materials and Methods........ 103
5.3.3. Measurement Methods 105
5.3.3.1 Physiological Traits........ 105
5.3.3.2 Structural Traits and Leaf Nutrient Concentrations 106
5.3.3.3 Crop Yield 107

5.3.4. Data Analysis 107

5.4. Results..... 108

5.4.1. Effects on Wild Blueberry Plant Physiology 108

5.4.2. Effects on Wild Blueberry Plant Morphology 112

5.4.3. Effects on Wild Blueberry Leaf Nutrients 114

5.4.4. Effects on Crop Yield 118

5.4.5. Relationships among All Wild Blueberry Plant Traits and Yield 118

5.5. Discussion 123

6. INTERACTIONS OF CELLULOSE NANOFIBRILS WITH A FOLIAR FERTILIZER AND WILD BLUEBERRY LEAVES: POTENTIAL TO ENHANCE FRUIT YIELD..... 127

6.1. Abstract 127

6.2. Introduction 127

6.3. Materials and Methodology..... 129

6.3.1. Materials..... 129

6.3.2. CNF Effects on Leaf Water Holding, Water Release, and the Interaction between Water and Wild Blueberry Leaves..... 130

6.3.3. Interaction among CNFs, Foliar Fertilizer, and Wild Blueberry Leaves 132

6.3.4. Foliar Fertilizer and CNF Effects on Wild Blueberry Leaves and Yield 133

6.3.5. Data Analysis 133

6.4. Results..... 134

6.4.1. CNF Effects on the Interaction between Water and Wild Blueberry Leaves..... 134

6.4.2. Interaction among CNFs, Foliar Fertilizer, and Wild Blueberry Leaves 136

6.4.3. CNF and Foliar Fertilizer Effects on Wild Blueberry Leaves and Yield 139

6.5. Discussion 140

7. CONCLUSIONS, LIMITATIONS, AND RECOMMENDATIONS 143

BIBLIOGRAPHY 146

APPENDICES...161

APPENDIX A: Supporting Information for Chapter 2 161

APPENDIX B: Supporting Information for Chapter 3 167

APPENDIX C: Preliminary Study for Chapter 4: The future of wild blueberries:

Testing warming impacts using open-top chambers 172

APPENDIX D: Supporting Information for Chapter 6 192

APPENDIX E: Supporting Information for Chapter 4 194

BIOGRAPHY OF THE AUTHOR 195

LIST OF TABLES

Table 2.1 Trend analysis of climate variables using Mann-Kendall test at the studied 26 wild blueberry fields of Downeast, Maine from 1980 to 2019. 20

Table 2.2 Correlation analysis of climate variables 21

Table 2.3 Generalized Linear Model (GLM) analysis between climate variables and geographic factors at the studied 26 wild blueberry fields of Downeast, Maine over 40 years from 1980 to 2019......22

Table 3.1 Historical trend analysis of annual climate variables using Mann–Kendall test. 43

Table 3.2 Correlation analysis of the increases in climate variables with the geographic factors for the studied wild blueberry barrens of Maine during different seasons 54

Table 3.3 Approximate last spring frost dates, first fall frost dates, and growing season period during the periods of 1980–1990, 1991–2000, 2001–2010, and 2011–2020 54

Table 4.1. Physical and chemical properties of wild blueberry soil for the studied six genotypes. 69

Table 4.2 Comparison in physical and chemical properties of wild blueberry soil among the studied treatments in September 2021 95

Table 4.3 Comparison in physical and chemical properties of wild blueberry soil among the studied treatments in August 2022 96

Table 5.1 Physical properties, chemical composition, application rates, and times of commercial fertilizer products used in the experimental design.......... 103

Table 5.2 Two-way Analysis of variance (ANOVA) results from the fertilizer effects on leaf chlorophyll concentration measured on different dates in 2019 and 2020 109

Table 5.3 Multiple linear regression analysis predicting the harvested wild blueberry yield in the crop year using measured physiological traits, morphological traits, leaf macro- and micro-nutrients... 121

Table 5.4 Bi-variate linear regression analysis predicting the harvested wild blueberry yield in the crop year using individual measured physiological traits, morphological traits, leaf macro- and micro-nutrients 122

Table 6.1 Chemical Properties, Application Rates, and Frequencies of the Applied Treatments of This study in the Conventional Wild Blueberry Field at the Blueberry Hill Farm, Jonesboro, Maine..... 132

Table A 1. Changes (increasing or decreasing) in climate variables over last 40 years from 1980 to 2019 at the studied 26 wild blueberry fields in Downeast, Maine...165

Table A.2. Regression analysis between climate variables and Maximum Enhanced Vegetation Index at the studied 26 wild blueberry fields in Downeast, Maine.. 166

Table B.1. Historical trend analysis of Seasonal climate variables using Mann–Kendall test, and comparison of a linear regression fitted slopes using slope t-test.. 169

Table D.1. Macro- and micro-nutrients in leaf tissues of wild blueberry plants treated with different treatments in Experiment 1...192

LIST OF FIGURES

Figure 2.1 Location and Temperature Map.. 11

Figure 2.2 Historical (1980 to 2019) changes in temperature..16

Figure 2.3 Historical (1980 to 2019) changes in precipitation.. 17

Figure 2.4. Sequential Mann-Kendall test statistics 19

Figure 2.5 Maximum Enhanced Vegetation Index (EVI_{max}) of the wild blueberry fields.......................24

Figure 2.6 The maximum temperatures from the fields 26

Figure 2.7 Historical comparison in average potential evapotranspiration (PET) 28

Figure 3.1 Locations of the studied wild blueberry barrens in different counties …................................ 36

Figure 3.2 Comparison of historical (1980 to 2020) annual climate parameters......................................41

Figure 3.3 Historical (1980 to 2020) changes with fitted linear regression trendlines for the annual climate parameters 42

Figure 3.4 Comparison of historical (1980 to 2020) summer climate parameters 45

Figure 3.5 Historical (1980 to 2020) changes with fitted linear regression trendlines for the summer climate parameters..46

Figure 3.6 Comparison of historical (1980 to 2020) winter climate parameters...................................... 47

Figure 3.7 Historical (1980 to 2020) changes with fitted linear regression trendlines for the winter climate parameters 48

Figure 3.8 Historical (1980 to 2020) changes with fitted linear regression trendlines for the temperature range..48

Figure 3.9 Comparison of historical (1980 to 2020) fall climate parameters.......................................49

Figure 3.10 Historical (1980 to 2020) changes with fitted linear regression trendlines for the fall climate parameters 50

Figure 3.11 Comparison of historical (1980 to 2020) spring climate parameters 51

Figure 3.12 Historical (1980 to 2020) changes with fitted linear regression trendlines for the spring climate parameters....52

Figure 3.13 Annual average temperature cycle for the periods of 1980–1990, 1991–2000, 2001–2010, and 2011–2020 53

Figure 4.1 The open-top chambers (OTC) and control plots used for this study in the wild blueberry field....68

Figure 4.2 Monthly changes in average (a) Atmospheric temperature (°C) and (b) Relative humidity (%) from June 2021 (vegetative year) to August 2022 (crop year).... 73

Figure 4.3 Diurnal changes in (a) Atmospheric temperature (°C) and (b) Relative humidity (%) during two typical sunny days (28 – 29 June 2021) 74

Figure 4.4 Seasonal changes in volumetric water content in the wild blueberry field soil across five different treatments on (a) Vegetative year 76

Figure 4.5 Comparison in midday leaf water potential of wild blueberry plants among five different treatments on a typical sunny day in vegetative year.... 77

Figure 4.6 Comparison in mean 79

Figure 4.7 Comparison in (a) Photosynthetic rate, (b) Stomatal conductance, (c) Transpiration rate, and (d) Water use efficiency of wild blueberry plants....80

Figure 4.8 Comparison in (a) Photosynthetic rate per stem and (b) Photosynthetic rate per land of wild blueberry plants 81

Figure 4.9 Comparison in (a) Number of leaves per stem, (b) Leaf size, and (c) Leaf mass per area of the wild blueberry plants 83

Figure 4.10 Comparison in (a) Water use efficiency (^{13}C isotope) and (b) Nitrogen (^{15}N isotope) of the wild blueberry plants 84

Figure 4.11 Comparison in (a-j) Nutrients: N, P, K, Ca, Mg, B, Cu, Fe, Mn, Zn of the wild blueberry leaves … 86

Figure 4.12 Comparison in (a-j) Nutrients: N, P, K, Ca, Mg, B, Cu, Fe, Mn, Zn of the wild blueberry fruits.. 87

Figure 4.13 Comparison in (a) Fruit yield, (b) Weight of blue fruits per stem, (c) Fresh weight of 100 berries, and (d) Berry sugar content of the wild blueberry plants 89

Figure 4.14 Principal Component Analysis (PCA) of physiological traits, morphological traits, major and minor leaf nutrient elements, and quantity and quality of wild blueberries.....................91

Figure 5.1 Experimental Design of this study in a conventional wild blueberry field at the Blueberry Hill Farm, Jonesboro, ME, USA…...................................... 102

Figure 5.2 Changes in leaf chlorophyll concentration of wild blueberry plants 110

Figure 5.3 Comparison in (a) Photosynthetic rate per leaf area and (b) Photosynthetic rate per leaf mass of wild blueberry plants in 2019 and 2020 across the studied nine different treatments 111

Figure 5.4 Comparison of final wild blueberry stem heights in 2020 (crop year) across the studied nine treatments. …..112

Figure 5.5 Comparison in (a) Total leaf area per stem, (b) Dry biomass of leaves per stem, and (c) Leaf mass per area (LMA) of wild blueberry plants in 2019 and 2020.......................113

Figure 5.6 Comparison in concentration of macro-nutrient elements per leaf mass of wild blueberry plants in 2019 and 2020 across the studied nine different treatments.....................115

Figure 5.7 Comparison in concentration of micro-nutrient elements per leaf mass of wild blueberry plants in 2019 and 2020 across the studied nine different treatments 117

Figure 5.8 Comparison in harvested yield of wild blueberries in crop year (2020) across the studied nine different treatments…...118

Figure 5.9 Principal Component Analysis (PCA) of physiological traits, morphological traits, major and minor leaf nutrient elements, and yield of wild blue-berries in (a) 2019 and (b) 2020 … 120

Figure 5.10 Wild blueberry yield in relation to stem length (a) and leaf area per stem (b) in the vegetative year (2019) and crop year (2020). 123

Figure 6.1 Comparisons in (a) leaf water loss over time, (b) time reaching 70% relative water content from saturation point, and (c) mass-based saturated water content of two wild blueberry species among applied treatments... 135

Figure 6.2 Comparisons in the contact angle using deionized a water droplet on (a) abaxial and (b) adaxial leaf surfaces of two wild blueberry species among the applied treatments.............136

Figure 6.3 Interactions of nanocellulose with the foliar fertilizer on wild blueberry leaves.................. 137

Figure 6.4 FTIR spectra of CNF, CNF-Poma washed, CNF-Poma unwashed, and Poma (inset)............. 138

Figure 6.5 Comparisons in (a) Midday leaf water potential (LWP), (b) Leaf size, (c) Leaf mass per area (LMA), and (d) Yield of wild lowbush blueberries 140

Figure A.1 Comparison of temperature… 163

Figure A.2 Comparison of temperature range..164

Figure B.1 A map showing different counties and climate regions of Maine...167

Figure B.2 The relationship between average temperature recorded by different weather stations at 2 ft and 6.5 ft from the ground surface in a wild blueberry field at the Blueberry Hill Farm, Jonesboro, Maine. 168

Figure B.3 Historical changes with fitted linear regression trendlines for the snow water equivalent throughout the studied wild blueberry barrens … 169

Figure C.1 Wild blueberry stems with roots growing from the rhizome, creating one individual wild blueberry plant referred to as one genet in the wild blueberry field… 173

Figure C.2 The open-top chambers (OTC) and control plot used for this study in the wild blueberry field........176

Figure C.3 Monthly averages under control, active heating and passive heating treatments 179

Figure C.4 (a) Soil Temperature, (b) Volumetric Water Content in soil, and (c) Leaf Water Potential of wild blueberry stems under three different treatments 180

Figure C.5 (a) Photosynthetic rate per leaf area, (b) Photosynthetic rate per mass, (c) Pre-dawn Fv/Fm (maximum potential quantum efficiency of photosystem II), (d) Stomatal conductance, (e) Leaf intercellular CO_2 concentration, and (f) Water use efficiency of wild blueberry stems growing........181

Figure C.6 Chlorophyll content per leaf mass........183

Figure C.7 Leaf and stem structural traits of the wild blueberry stems growing under different treatments........184

Figure C.8 Estimated CO_2 assimilation per individual stem of the wild blueberries growing under three different treatments 186

Figure D.1 Relationship between leaf mass per area (LMA) and yield of studied wild blueberries 192

Figure D.2 EDAX results of calcium percentage before and after filtration washing........193

Figure E.1 Seasonal changes in chlorophyll concentration per leaf area in the wild blueberry leaves …........ 194

CHAPTER 1

INTRODUCTION

1.1 Background and Importance of this Research

Wild blueberry crops have played an important role in Maine's agricultural economy for centuries where the crops are currently grown commercially on 44,000 acres of land. Two kinds of wild blueberry crops (Vaccinium species) exist in the field: (1) Common lowbush blueberry (*Vaccinium angustifolium*) (80-90% of the field) and (2) Velvet leaved lowbush blueberry (*Vaccinium myrtilloides*). The wild blueberry production system in Maine is a unique semi-natural agricultural system. Wild blueberry plants are initially established from seeds naturally (not planted) and then underground stems (rhizomes) develop. Roots then grow directly off of rhizomes creating a tightly woven mat across fields. Rhizomes grow within approximately 10 cm of the soil surface and produce upright stems above the soil surface. An individual wild blueberry plant, with its spreading rhizome system, is referred to as a genet (Bell et al. 2009). Each genet is genetically different from neighboring plants creating a complex mixture of genotypes in the wild blueberry field providing consumers with a rich diversity of flavors. This crop is managed on a two-year cycle: the plants grow vegetatively in the first year (prune year) after the previous year's harvest and pruning, and the plants flower and produce a fruit crop in the second year (crop year). After harvesting the fruits, growers prune the field either by mowing or burning.

Based on several past studies which identified soil-nutrition requirements for better wild blueberry production, N-P-K (Nitrogen-Phosphorous-Potassium) and diammonium phosphate (DAP) granular fertilizers have been proved to be the best (Percival et al. 2002; Percival & Sanderson 2004; Starast et al. 2007; Smagula 2011). Also, boron foliar fertilizer was found to be more efficient than boron granular fertilizer for wild blueberry crop as the leaves absorbed the foliar ones faster than the granular ones from soil because boron is a relatively immobile nutrient element (Perrin 2001; Eaton 1944). While the above-mentioned research on fertilizer efficacy has been conducted to explore growth and yield of wild blueberry crops, very limited studies explored only the photosynthetic performance of wild blueberry crops (Hicklenton et al. 2000; Percival et al. 2003, 2012; Yarborough 2004). There is still a lack of in-depth research on physiology and morphology of wild blueberry crop specially under climate variabilities as well as different fertilization management. For instance, leaf photosynthesis is directly related to plant productivity and crop yield (Zelitch 1982, Peng et al. 1991), and is therefore used as a good indicator of fertilizer efficacy. In order to deliver nutrients to the wild blueberry it is vital to understand where and when they absorb nutrients most efficiently. Using chlorophyll content and photosynthesis to identify the absorption of nutrients, researchers and growers would have a physiological explanation for why certain products are effective or not. Besides, new commercial foliar fertilizers consisting of different amounts of macro and micro-nutrients (N, P, K, Ca, Mg, Si) along with plant growth regulator hormones (cytokinin and gibberellin) have been claiming to be the most effective ones for wild blueberry crops without any scientific evidence. Therefore, robust investigations need to be conducted to test the efficacy of those different types of commercial foliar fertilizers on the wild blueberry crops' physiology and morphology. Since the photosynthetic rate in wild blueberry crops is low compared to other crops, wild blueberry yield could be greatly enhanced by fertilizer applications.

Besides fertilizers, biochar is currently recommended to use with N-rich fertilizer (Zhang et al. 2012; Wang et al. 2012) or compost (Hunt et al. 2010; Dias et al. 2010) for better crop production. Biochar is a carbon-rich byproduct resulting from pyrolyzing plant biomass under high-temperature and oxygen-deficient conditions for biofuel production (Lehmann 2007a; Laird et al. 2009). Many studies have demonstrated benefits of adding biochar to agricultural soils (Glaser et al. 2002; Marris 2006; Lehmann 2007b; Warnock et al. 2007) including better plant growth (Chan et al. 2008; Asai et al. 2009; Major et al. 2009; Graber et al. 2010; Hossain et al. 2010), improving soil water-holding capacity (Laird et al. 2010b), reducing nutrient leaching loss, which in turn can reduce fertilizer needs (Liang et al. 2006; Laird et al. 2010a). Since biochar is a byproduct of bioenergy production and can contribute to carbon sequestration, besides increasing crop yield and reducing fertilizer use, biochar can potentially provide a 'win-win-win' solution to meeting global environmental challenges (Laird 2008). Since Maine has vast resources of forest residuals, some fuel production companies in Maine have been producing biofuel from pyrolysis of wood pellets and they are throwing away the waste by-product produced as biochar. This biochar could be the cheapest potential available option to enhance wild blueberry productivity and reduce CO_2 emission. However, variation in plant and soil responses to biochar cannot be evaluated based on the literature because biomass material and pyrolysis conditions could significantly alter the structure, nutrient content, and pH (Novak et al. 2009). Interactions with climate, soil type (Tryon 1948; van Zwieten et al. 2010a), and fertilization status (van Zwieten et al. 2010b; Haefele et al. 2011) can also contribute to uncertainty in how biochar interacts with organisms. Therefore, investigations are needed for this specific biochar from wood pellets on wild blueberry field soil as well as crop physiology and production.

Generally, wild blueberry farmers are recommended to sample leaf tissues (after 90% tip die back of plants) from their crops to identify their nutrition requirements because lack of nutrients or excessive nutrients both would have adverse effects on the crop (Smagula 2011). Besides leaf nutrition analysis, monitoring crop physiology could be another, in fact much more efficient way to manage the wild blueberry crop with nutrition when and where needed. This is because leaf-nitrogen has been proven to have direct correlations with physiology (e.g., photosynthetic rate, chlorophyll-concentrations) and morphology (e.g., leaf mass per area) for different plant species (Wright et al. 2004; Zhang et al. 2015; Campbell et al. 1990; Fanizza et al. 1991; Monje & Bugbee 1992; Schaper & Chacko 1991; Takebe & Yoneyarna 1989; Wood et al. 1992a, 1992b). These researchers have been investigating direct correlations of leaf-nitrogen with chlorophyll-concentration measured by lightweight, easy to use chlorophyll meters for different fruit-trees and crops. The purpose of these investigations was to help the farmers so that they can easily identify the nutrition-levels of their crops by measuring chlorophyll-content of leaves rather than leaf nutrition analysis and can take immediate action. For instance, fertilizer application has been shown to improve yields in conventional fields when blueberry plants have less than the standard ranges of foliar nutrient levels (Santiago 2011), specifically less than 1.7% nitrogen or 0.13% phosphorus in leaves. If fertilizer is applied when and where it is not needed, it will stimulate weeds which may reduce blueberry yield (Drummond et al. 2009). In this case, if wild blueberry farmers can identify the nitrogen level by monitoring chlorophyll-concentration using a chlorophyll meter, it would save the time and cost of leaf nutrition analysis, minimize waste of fertilizer resources as well as result in optimal yield. Therefore, during the investigations on different foliar and organic fertilizers for better wild blueberry crop production it could be also explored if leaf nutrition of wild blueberry crops (from both conventional and organic wild blueberry fields) has any relationship with their physiology.

Dramatically changed temperature and precipitation patterns due to global climate change are threatening crops all over the world (Shrestha et al. 2017; Van Passel et al. 2017; Petersen 2019). Average annual global temperature has increased up to ~0.7°C within the last century (1906 to 2005) (Solomon et al. 2007). The average annual temperature in Maine, USA has increased ~1.8°C within the last 124 years (1895 to 2018) (Fernandez et al. 2020). While air temperatures have been increasing throughout Maine, the Coastal zone of Maine has experienced the highest rate of increase. Maine has three climate zones: Northern, Interior and Coastal, where long-term annual average temperatures from 1895 to 2018 are 3.4, 5.8 and 6.6°C respectively (Fernandez et al. 2020; NOAA 2019). Such variation in temperature across climate zones in Maine might not affect different plant and crop species in similar ways. This is because plant species often have different threshold values of atmospheric temperature or precipitation beyond which their physiological performance and growth patterns deteriorate. For instance, the temperature threshold for maximum photosynthesis of wheat (Triticum spp.) is 33°C (Badaruddin et al. 1999; Reynolds et al. 2000) whereas our recent preliminary study on wild blueberry (*Vaccinium angustifolium* Aiton) physiology showed that photosynthesis declined from the maximum when air temperature increased above 25°C (Tasnim et al. 2020). Atmospheric temperatures are further predicted to increase by 1.8 to 4ºC at the end of this century (IPCC 2014). Specifically, in Maine, USA, temperatures are expected to increase a further 2 to 6 ºC by the end of this century (The University of Maine Climate Institute 2015). Wild blueberry barrens are mostly distributed along the coastal zone of Maine where the annual average temperature has been increasing the most. While historical climate change information as well as climate change predictions are readily available for Maine, the local weather at different locations in Maine varies significantly as is evident from Maine’s three climate zones (Fernandez et al. 2020). Hence, wild blueberry fields at different locations in the coastal area of Maine might not be facing similar atmospheric temperature and precipitation changes and therefore might not have similar potential evapotranspiration rates. Under such circumstances, it would be ineffective to develop general management recommendations (e.g., irrigation, fertilizers) for all fields based solely on edaphic factors. Therefore, it is necessary to explore both historical climate change as well as potential evapotranspiration rates of specific wild blueberry fields to determine if these changes have significant physiological and growth performance effects.

As a consequence of increasing atmospheric temperatures, the physiology, growth, and yield of crops have been and will continue to be significantly influenced by frequent droughts and dry summers since crop production may be stimulated by 1 to 3ºC increases in temperate regions whereas crop production might be hindered by any degree of warming in tropical and subtropical regions (Easterling et al. 2007; Hatfield et al. 2011). Warmer temperatures due to climate change affect the development of temperate fruits and vegetables by impacting various plant processes i.e., photosynthesis, respiration, water uptake, and nutrient transport (Magan et al. 2011). Warming has been found to have negative effects on a variety of temperate crops (Lobell & Field 2007). For example, although temperate crops such as maize and wheat show increasing leaf photosynthetic rates due to warming (up to 33ºC), lower final biomass was observed (Reynolds et al. 2000; Badaruddin et al. 1999). Reynolds et al. (2000) also observed lower chlorophyll content and total canopy photosynthetic rate in temperate crops under warm environments. So far, the effects that future warming could have on small fruit crops, specifically wild blueberry (*Vaccinium angustifolium*), and other crops in Maine, USA have not been studied. Our preliminary investigation conducted in 2018 using open-top chambers with heating to manipulate warmer environment showed that the warming has changed the growth pattern of wild blueberries and has negative effects on their physiology due to decreased soil water availability and lower leaf nutrient concentrations (Tasnim et al. 2020) which could further affect wild blueberry production. Further robust investigation is needed on several different genets during both prune and crop year in the field to justify the outcomes from our preliminary investigation because it was conducted only on one genet in a single wild blueberry field during a crop year. Besides, to sustain wild blueberry production in a future with warmer and drier summers, management techniques need to be developed and tested to mitigate these negative effects. The negative effects of elevated temperatures can be mitigated with irrigation. However, irrigation systems are costly and result in low water use efficiency due to the low water-holding-capacity of the sandy soils on wild blueberry fields. Therefore, soil amendment techniques such as mulching and biochar-compost mix application need to be tested to enhance soil water and nutrient holding capacity. Mulching can decrease soil water loss while biochar-compost mix application potentially improves soil fertility and water-holding-capacity (Mukherjee and Zimmerman 2013, Liang et al. 2014).

1.2 Dissertation Structure

Five projects were carried out to address the above-mentioned challenges. under climate change. Thus, my dissertation including the following five chapters.

WILD BLUEBERRY NUTRITION UNDER CLIMATE CHANGE	
Climate Change	**Nutrient Management**
Chapter 2: Climate Change Patterns of Wild Blueberry Fields in Downeast, Maine over the Past 40 Years. **Chapter 3:** Seasonal Climate Trends across the Wild Blueberry Barrens of Maine, USA. **Chapter 4:** Influence of Soil Amendments on Soil Water Availability and Responses of Wild Lowbush Blueberries to a Warmer Climate.	**Chapter 5:** Are Foliar Fertilizers Beneficial to Growth and Yield of Wild Lowbush Blueberries? **Chapter 6:** Interactions of Cellulose Nanofibrils with a Foliar Fertilizer and Wild Blueberry Leaves: Potential to Enhance Fruit Yield.

CHAPTER 2

CLIMATE CHANGE PATTERNS OF WILD BLUEBERRY FIELDS IN DOWNEAST, MAINE OVER THE PAST 40 YEARS

2.1 Abstract

Maine, USA is the largest producer of wild blueberries (*Vaccinium angustifolium* Aiton), an important native North American fruit crop. Blueberry fields are mainly distributed in coastal glacial outwash plains which might not experience the same climate change patterns as the whole region. It is important to analyze the climate change patterns of wild blueberry fields and determine how they affect crop health so fields can be managed more efficiently under climate change. Trends in the maximum (T_{max}), minimum (T_{min}) and average (T_{avg}) temperatures, total precipitation (P_{total}), and potential evapotranspiration (PET) were evaluated for 26 wild blueberry fields in Downeast Maine during the growing season (May–September) over the past 40 years. The effects of these climate variables on the Maximum Enhanced Vegetation Index (EVI_{max}) were evaluated using Remote Sensing products and Geographic Information System (GIS) tools. We found differences in the increase in growing season T_{max}, T_{min}, T_{avg}, and P_{total} between those fields and the overall spatial average for the region (state of Maine), as well as among the blueberry fields. The maximum, minimum, and average temperatures of the studied 26 wild blueberry fields in Downeast, Maine showed higher rates of increase than those of the entire region during the last 40 years. Fields closer to the coast showed higher rates of warming compared with the fields more distant from the coast. Consequently, PET has been also increasing in wild blueberry fields, with those at higher elevations showing lower increasing rates. Optimum climatic conditions (threshold values) during the growing season were explored based on observed significant quadratic relationships between the climate variables (T_{max} and P_{total}), PET, and EVI_{max} for those fields. An optimum T_{max} and PET for EVI_{max} at 22.4 °C and 145 mm/month suggest potential negative effects of further warming and increasing PET on crop health and productivity. These climate change patterns and associated physiological relationships, as well as threshold values, could provide important information for the planning and development of optimal management techniques for wild blueberry fields experiencing climate change.

2.2 Introduction

Dramatically changing temperature and precipitation patterns due to global climate change are threatening crops all over the world (Shrestha et al. 2017, Van Passel et al. 2017, Petersen 2019). The average annual global temperature has increased up to ~0.7 °C within the last century (1906 to 2005) (Solomon et al. 2007). The average annual air temperature in Maine, USA has increased ~1.8 °C within the last 124 years (1895 to 2018) (Fernandez et al. 2020). While air temperatures have been increasing throughout Maine, the Coastal zone of Maine has experienced the highest rate of increase. Maine has three climate zones: Northern, Interior and Coastal, where long-term annual average temperatures from 1895 to 2018 are 3.4, 5.8 and 6.6 °C, respectively (Fernandez et al. 2020, NOAA-CAAG 2019). Such temperature variation across Maine might not affect different plant and crop species located at different climate zones in similar ways. This is because plant species often have different optimal and threshold values for atmospheric temperature or precipitation, beyond which their physiological performance and growth deteriorate. For instance, the threshold air temperature for maximum photosynthesis of wheat (Triticum spp.) is 33 °C (Badaruddin et al. 1999, Reynolds et al. 2000) whereas a recent study on wild blueberry (*Vaccinium angustifolium* Aiton) physiology showed that photosynthesis declined from the maximum when air temperature increased above 25 °C (Tasnim et al. 2020).

The wild blueberry crop is one of the most important fruit crops native to North America. It has a vital role in Maine's economy. This crop is commercially grown on almost 18,000 ha in Maine (Yarborough 2015). Maine has the highest production of wild blueberries in the world, with Washington County, Maine producing the most (Yarborough 2015). Recent research has shown that changing climate patterns (i.e., increasing temperature) could significantly alter its physiology, morphology, and growth patterns which would further affect the nutrient economy of wild blueberries (Tasnim et al. 2020). These changes will bring new challenges to this traditional agricultural system. To sustain wild blueberry production under the influence of a changing climate, management techniques (i.e., irrigation, fertilization) will need to be adjusted. In order to fine-tune management techniques for a specific field, vital information will be required such as air temperature, humidity or precipitation rates, and potential evapotranspiration rate (evaporation from soil and transpiration from plants), which affects the physiological and growth performance of the crop. In addition to the effects of a changing climate pattern, estimating and understanding the potential evapotranspiration has proven to be of major importance for developing new or improved agricultural management techniques (Bhatt and Hossain 2019). In fact, this would be especially important for wild blueberry fields due to the soils in which they are managed. These soils are characterized by low water-holding capacity and under increasing temperatures due to climate change, evapotranspiration stress is likely to occur. This is especially significant due to the fact that wild blueberry fields are not planted (Yarborough 2015). Existing plant populations in the landscape are nurtured and managed, thus growers are constrained in their responses to climate change.

Wild blueberry barrens are mostly distributed along the Coastal zone of Maine where the annual average temperature has been increasing the most. While historical climate change information and climate change predictions are readily available for Maine, the local weather at different locations in Maine varies significantly as is evident from Maine's three climate zones (Fernandez et al. 2020). Hence, wild blueberry fields at different locations in the Coastal area of Maine might not be experiencing the same atmospheric temperature and precipitation changes and might not have the same potential evapotranspiration rates. Under such circumstances, it would be ineffective to develop general management recommendations (e.g., irrigation, fertilizers) for all fields based solely on regional edaphic factors. Therefore, it is necessary to explore both historical climate change as well as potential evapotranspiration rates of specific wild blueberry fields to determine if these changes have significant physiological and growth performance effects. "Leaf-greenness", a proxy of crop health and productivity, representing chlorophyll and nitrogen concentrations in plants, could be a useful parameter to explore wild blueberry plant responses to climate change. One such measure, Enhanced Vegetation Index (EVI), has become a popular standard remote sensing tool adopted by scientists (Waring et al. 2006; Wu et al. 2011) because of its ability to eliminate canopy background and atmospheric noise, as well as its property of non-saturation, which are major issues of the commonly used measure, the Normalized Difference Vegetation Index (NDVI) (Huete et al. 2002). Therefore, we used EVI to indicate plant productivity for specific field sites in Downeast, Maine, and evaluated its relationship with changing climate using archived historical climate data over the past 40 years. We used Remote Sensing products and Geographic Information System (GIS) techniques and tools in Arc GIS Pro (Version 2.4.2) Software (Esri, Redlands, CA, USA) (ArcGIS Pro 2019) to quantify the climate change patterns of wild blueberry fields. The specific objectives of our study were:

1. To characterize the historical climate change patterns (maximum temperature, minimum temperature, average temperature, and precipitation) of different wild blueberry fields in Downeast, Maine over the last 40 years (1980 to 2019), and test whether wild blueberry fields show different climate change patterns compared to that of the region (state of Maine);
2. To quantify the historical changes in potential evapotranspiration (PET) of those wild blueberry fields by comparing between 1970–2000 and 2001–2014, as well as to determine the relationship between PET and temperatures for wild blueberry fields;
3. To establish relationships between climate variables (maximum temperature, minimum temperature, average temperature, and precipitation) during the growing season (May to September) and the Maximum Enhanced Vegetation Index (EVI_{max}) for the wild blueberry fields.

2.3 Materials and Methods

2.3.1. Study Area

The study area involved 26 wild blueberry fields in Downeast, Maine, USA. Two fields (field no. 3 and 10) are in Hancock County and 24 fields are in Washington County, Maine, USA (Fig. 2.1). Among all the wild blueberry fields in the Downeast region of Maine, 26 fields with 1 km^2 or larger area, were selected for the study. The land area threshold (1 km^2) was used because the remote sensing data products that we used had a spatial resolution of 1 km. The selected wild blueberry fields are marked with blue boundary and black mid-points as well as labeled with numbers 1 to 26 in Figures 2.1b - d.

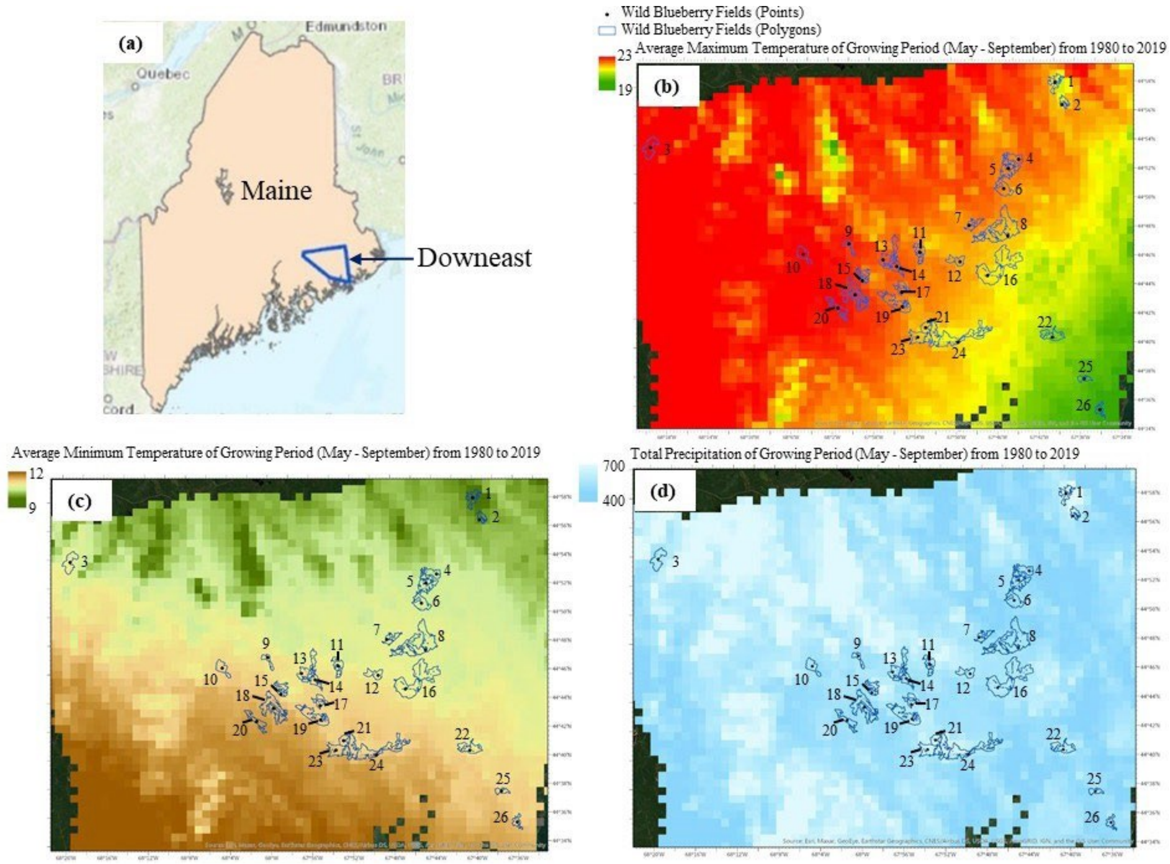

Figure 2.1 (a) A map of Maine showing the study location in Downeast, Maine by the dark blue boundary (a large part of the Washington Co. and a small part of the Hancock Co.), (b) Average maximum temperature (T_{max}, °C), (c) Average minimum temperature (T_{min}, °C), and (d) Total rainfall (P_{total}, mm) during the growing season (May to September) averaged over 40 years (1980 – 2019) in Downeast, Maine where twenty-six wild blueberry fields (area of each field ≥ 1 km^2) are shown as the 26 black points inside the field polygons (shown by light blue borders) and labeled with numbers 1 to 26.

2.3.2. Data Acquisition and Methodology

The polygons of all 26 wild blueberry fields in Maine (Fig. 2.1) were acquired from a Google Earth Pro KMZ file based on a field survey carried out by David Yarborough, Professor Emeritus of Horticulture and Wild Blueberry Specialist, University of Maine. The dataset of climate variables (maximum temperature, minimum temperature, average temperature, and total precipitation) during the growing season (May to September) over 40 years from 1980 to 2019 averaged across Maine, were acquired from the software, Climate Reanalyzer (https://ClimateReanalyzer.org, Climate Change Institute, University of

Maine, USA). The original data source for the climate variables were obtained from the website: NOAA Climate at a Glance (https://www.ncdc.noaa.gov/cag/statewide /time-series/).

The measures for monthly climate variables (maximum temperature, minimum temperature, and total precipitation) over 40 years from 1980 to 2019 were acquired for our study area from Daymet (https://daymet.ornl.gov/getdata) (Thornton et al. 1997; Thornton et al. 2018). These data were provided on a per-pixel basis at 1 km spatial resolution. Annual Maximum Enhanced Vegetation Index (EVI_{max}) data for 17 years (2001 to 2017) from the Downeast region of Maine were acquired from Google Earth. These data were originally obtained by the Moderate Resolution Imaging Spectroradiometer (MODIS) (https://lpdaac.usgs.gov/products/mod13a1v006/) (Didan 2015). The MODIS data (MOD13A1 Version 6) provides Maximum Enhanced Vegetation Index (EVI_{max}) values at a per-pixel basis at 500 m spatial resolution. The EVI corrects for some atmospheric conditions, minimizes canopy background noise, and maintains sensitivity over dense vegetation and high biomass conditions. The best available pixel values from all the acquisitions for the 16-day-period were selected based upon the criteria of low clouds, low view angle, and highest EVI_{max} value.

Remote sensing measures for Monthly Global Potential Evapotranspiration, averaged over the 31-year-period (1970 - 2000) were acquired from the Consortium for Spatial Information (CGIAR-CSI) GeoPortal (https://cgiarcsi.community) (Trabucco and Zomer 2019). These data were provided at a per-pixel basis at 1 km spatial resolution. The Remote sensing (MODIS data) measures for the Monthly Global Potential Evapotranspiration over the 15-year-period from 2000 to 2014 were acquired from the Numerical Terradynamic Simulation Group (NTSG) website from the University of Montana (http://www.ntsg.umt.edu/project/mod16#data-product). The spatial resolution of these MODIS data products (MOD16A2) was 1 km.

After acquiring the above-mentioned remote sensing measures, they were further analyzed using different tools in Arc GIS Pro 2.4.2 Software (ArcGIS Pro 2019). The detailed methodology adopted in Arc GIS Pro 2.4.2 Software is described in the Appendix A as supporting information. The measures of the climate variables, maximum enhanced vegetation index (EVI_{max}), and potential evapotranspiration (PET) were extracted for the studied 26 wild blueberry fields and then transferred from Arc GIS Pro to an Excel spreadsheet. Then, the comparisons and trendlines of historical climate change for the entire state of Maine and the 26 wild blueberry fields as well as relationships of the climate variables with the EVI_{max} were analyzed. Also, the comparisons of the PET among the two different time periods for the wild blueberry fields as well as the relationship between PET and EVI_{max} were tested.

2.3.3. Statistical Analysis

Statistical analyses were conducted using SPSS V23 (IBM Corp., Armonk, NY, USA) (IBM SPSS 2015), XRealStats (Addinsoft. XLSTAT 2020), and RStudio softwares. Changes (increasing or decreasing) in climate variables (T_{max}, T_{min}, $T_{avg,}$ $P_{total,}$) over the last 40 years from 1980 to 2019 at the studied 26 wild blueberry fields in Downeast, Maine as well as at the overall state of Maine were determined from linear regression trendlines (Table A.1). Also, trend analyses of these climate variables (T_{max}, T_{min}, $T_{avg,}$ $P_{total,}$) were conducted by the Mann-Kendall trend test, Sen's Slope estimator, and Sequential Mann-Kendall test. Mann–Kendall trend test results and the Sen's slope Q (Table 2.1) were computed using XRealStats (Addinsoft. XLSTAT 2020) where continuity correction was applied as well as the autocorrelation has been taken into account using the Hamed and Rao method (Hamed and Rao 1998). Also, the forward (UF) and backward (UB) curves of the Sequential Mann-Kendall test statistics were computed in RStudio software. A pearson correlation analysis was conducted between the climate variables (T_{max}, T_{min}, $T_{avg,}$ $P_{total,}$ PET, and increase in T_{max}, increase in T_{min}, increase in $T_{avg,}$ increase in P_{total}) and geographic factors (Latitude, Longitude, Elevation, Distance from the coast) (Table 2.2). Here, in order to adopt multiple analysis significance protection, the p-values were adjusted using Benjamini and Hochberg method at a false discovery rate (FDR) of 0.05 (Benjamini and Hochberg 1995). Generalized Linear Model (GLM) analysis using the Gaussian error distribution was also conducted between the climate variables (T_{max}, T_{min}, $T_{avg,}$ P_{total}) and geographic factors (Latitude, Longitude, Elevation, Distance from coast) considering the geographic locations as fixed factors (Table 2.3). Furthermore, univariate and multiple linear regression analyses were conducted to test the relationship between climate variables (T_{max} averaged over May to

September and P_{total} of May to September) and PET (average of May – September) for the 26 wild blueberry fields for 15 years (2000 – 2014). Univariate and multiple linear regression analyses in the form of a + bx (linear line), and a + bx + cx^2 (quadratic curve) models, respectively, were also conducted to explore the relationship between EVI_{max} and climate variables including T_{max} averaged over May to September, P_{total} of May to September, and PET averaged over May to September. We determined the model which best approximated the structure of the relationship using the coefficient of determination and its significance at $P < 0.001$. Similar regression analyses were also conducted for each of the studied 26 fields separately (Table A.2). The significant differences in PET between the 1970 - 2000 period and 2001 - 2014 period were tested by One-Way Analysis of Variance (ANOVA) for each month (January - December). Levene's test was conducted to meet the assumption of homogeneity of variances. Whenever the assumption was violated (when $P < 0.05$ in Levene's test) for any case, the significance was further tested using Brown-Forsythe test (Brown and Forsythe 1974) at significance levels of $P \leq 0.05$*, $P \leq 0.01$** and $P \leq 0.001$***. Furthermore, we conducted a two-way ANOVA test without replication (Randomized Complete Block design) considering the 12 months as a block variable, and the time periods of 1970 - 2000 and 2001 - 2014 as a categorical variable to determine if there was a significant difference between the two time periods during the overall 12 months.

2.4 Results

2.4.1. Comparison of Historical Climate Change between Maine and the Wild Blueberry Fields of Downeast Maine

Based upon the increasing linear trends in the maximum, minimum, and average temperatures (Fig. 2.2 and Table A.1) over the last 40 years (1980 - 2019), the temperature increment during the growing season was higher in the studied wild blueberry fields compared to the spatial average of the state of Maine. The maximum temperature during the growing season increased by 1.2±0.05°C over the last 40 years in the wild blueberry fields compared to 0.9±0.06°C in Maine (Fig. 2.2a). Interestingly, while there was a very slight difference in maximum temperatures (Fig. 2.2a) between the wild blueberry fields and Maine over the last 40 years, the difference in minimum temperatures between the wild blueberry fields and Maine was quite apparent (Fig. 2.2b). The minimum temperature increased by 1.6±0.03°C in the fields while it increased by 1.3±0.03°C in Maine (Fig. 2.2b). Consequently, the average temperature had a marked difference between the wild blueberry fields and Maine over the last 40 years. The average temperature increased by 1.3±0.04°C in wild blueberry fields while it increased by 1.1±0.05°C in Maine (Fig. 2.2c).

In contrast to the temperature changes, total precipitation during the growing season showed no overall changes over the last 40 years for both wild blueberry fields and Maine (Fig. 2.3a and Table A.1). However, for most of the years (36 out of 40 years) there were marked differences in total precipitation between the entire state of Maine and the wild blueberry fields (Fig. 2.3a). For instance, the total precipitation was higher in the wild blueberry fields compared to the state of Maine for 19 out of the last 40 years while for 17 years the total precipitation was lower in the fields compared to the state of Maine. Moreover, the total precipitation of the studied wild blueberry fields was higher than the long-term mean of 1980 to 2019 during 19 out of the 40 years while it was much lower during 21 out of 40 years (Fig. 2.3b).

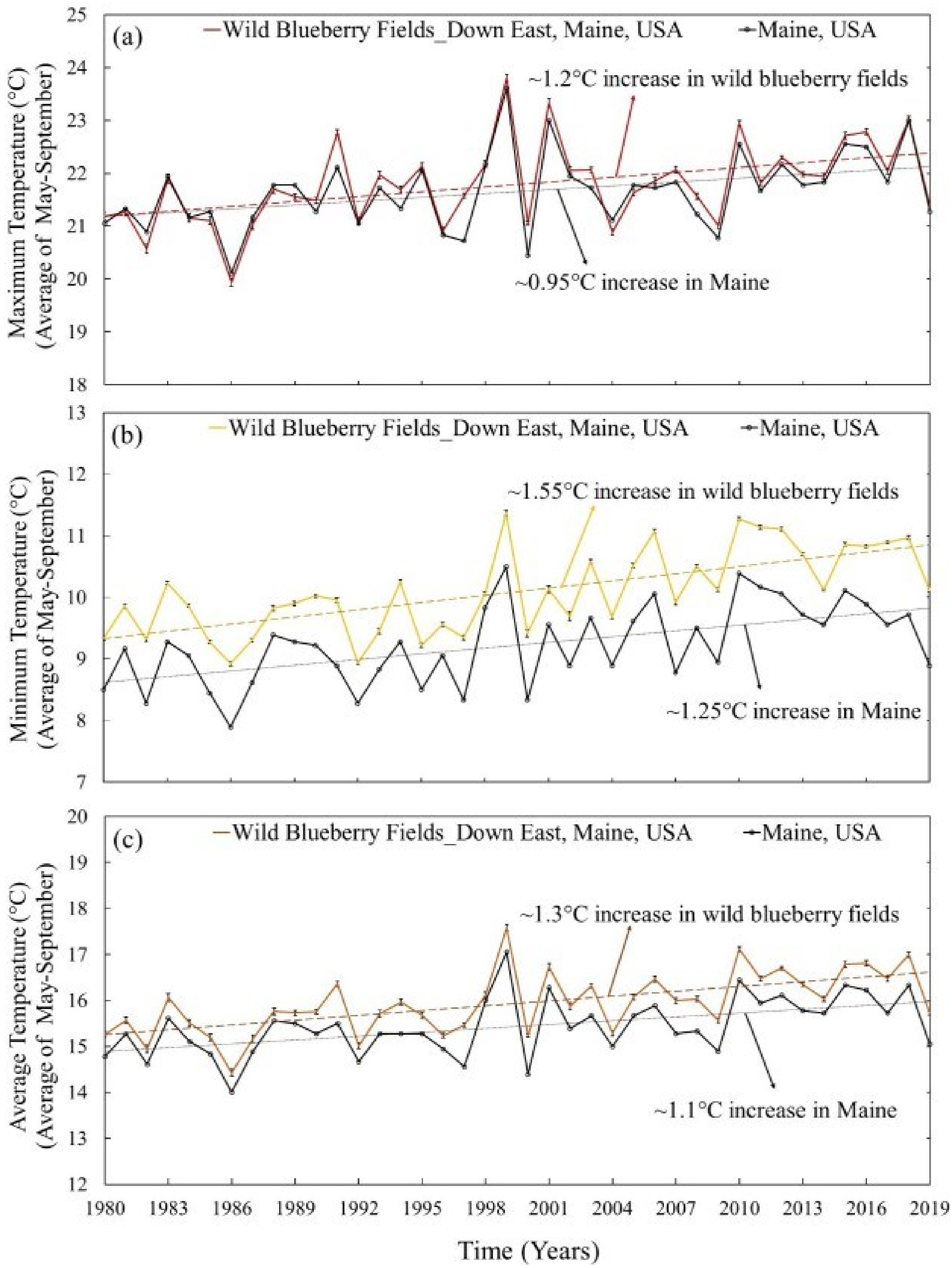

Figure 2.2 Historical (1980 to 2019) changes in (a) maximum temperature (average of May - September), (b) minimum temperature (average of May - September), and (c) average temperature (average of May - September) throughout the state of Maine (averaged spatially), as well as at the 26 wild blueberry fields (shown in Fig. 2.1) in Downeast, Maine. The climate variables from the fields are represented as the Mean ± Standard error (n = 26). The dotted lines are linear regression lines for the

state of Maine and the dashed lines are linear regression lines for the wild blueberry fields in Downeast, Maine over 40 years from 1980 to 2019.

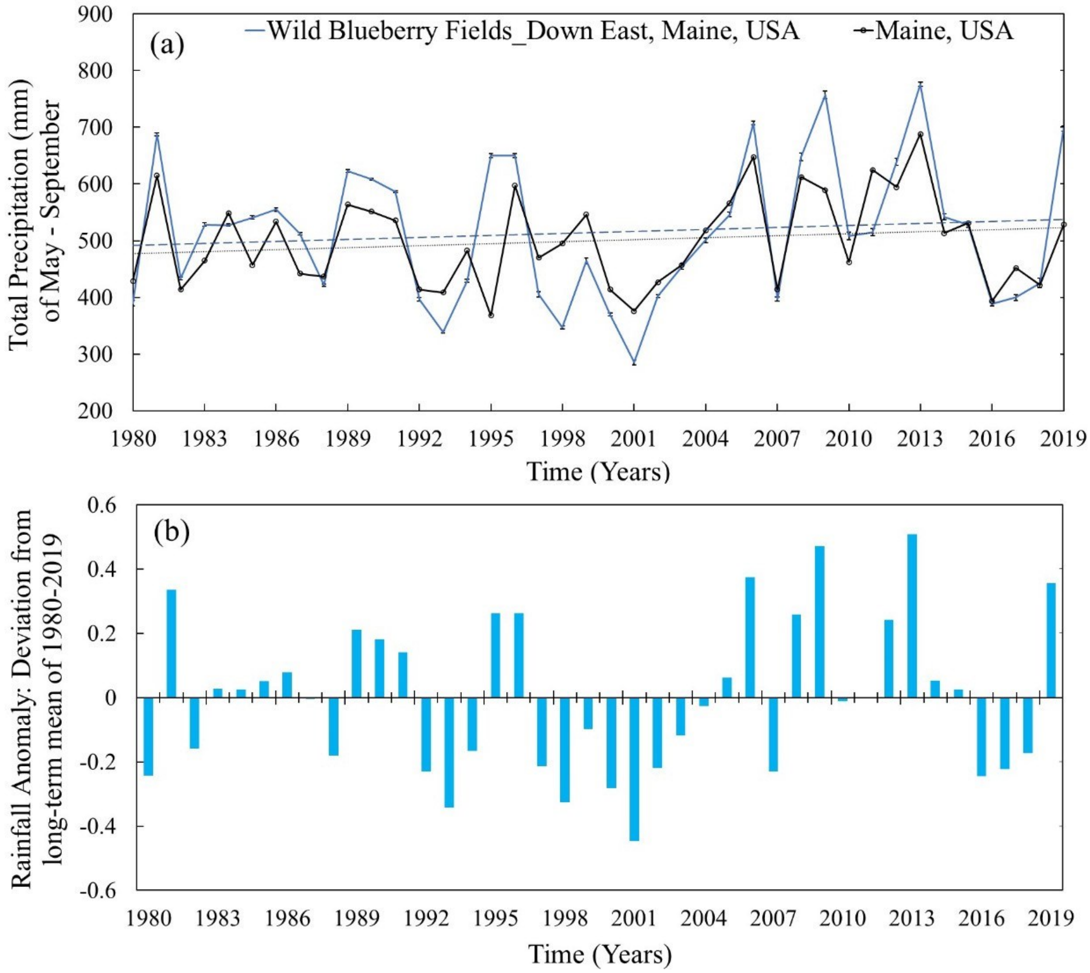

Figure 2.3 (a) Historical (1980 to 2019) changes in precipitation (total of May - September) throughout the state of Maine (averaged spatially), as well as at the 26 wild blueberry fields (shown in Fig. 2.1) in Downeast, Maine; The climate variables from the fields are represented as the Mean ± Standard error (n = 26). The dotted lines are linear regression lines for the state of Maine and the dashed lines are linear regression lines for the wild blueberry fields in Downeast, Maine over 40 years from 1980 to 2019. (b) Rainfall anomaly during 40 years from 1980 to 2019 (deviation in annual precipitation amounts from the long-term mean of 1980 to 2019) at the 26 wild blueberry fields (shown in Fig. 2.1) in Downeast, Maine.

Furthermore, significant increasing trends in historical temperature changes (maximum, minimum, and average temperatures, from 1980 to 2019) at the wild blueberry fields in Downeast, Maine were supported graphically by the upward UF curve (forward trend) mostly being > 0.0 and UB (backward trend) curve mostly being < 0.0 (Figs. 1.4a to 1.4c) and supported statistically by the Mann-Kendall test statistics (Table 2.1). In contrast, no significant trend was found in historical precipitation changes at those wild blueberry fields (Fig. 2.4d and Table 2.1).

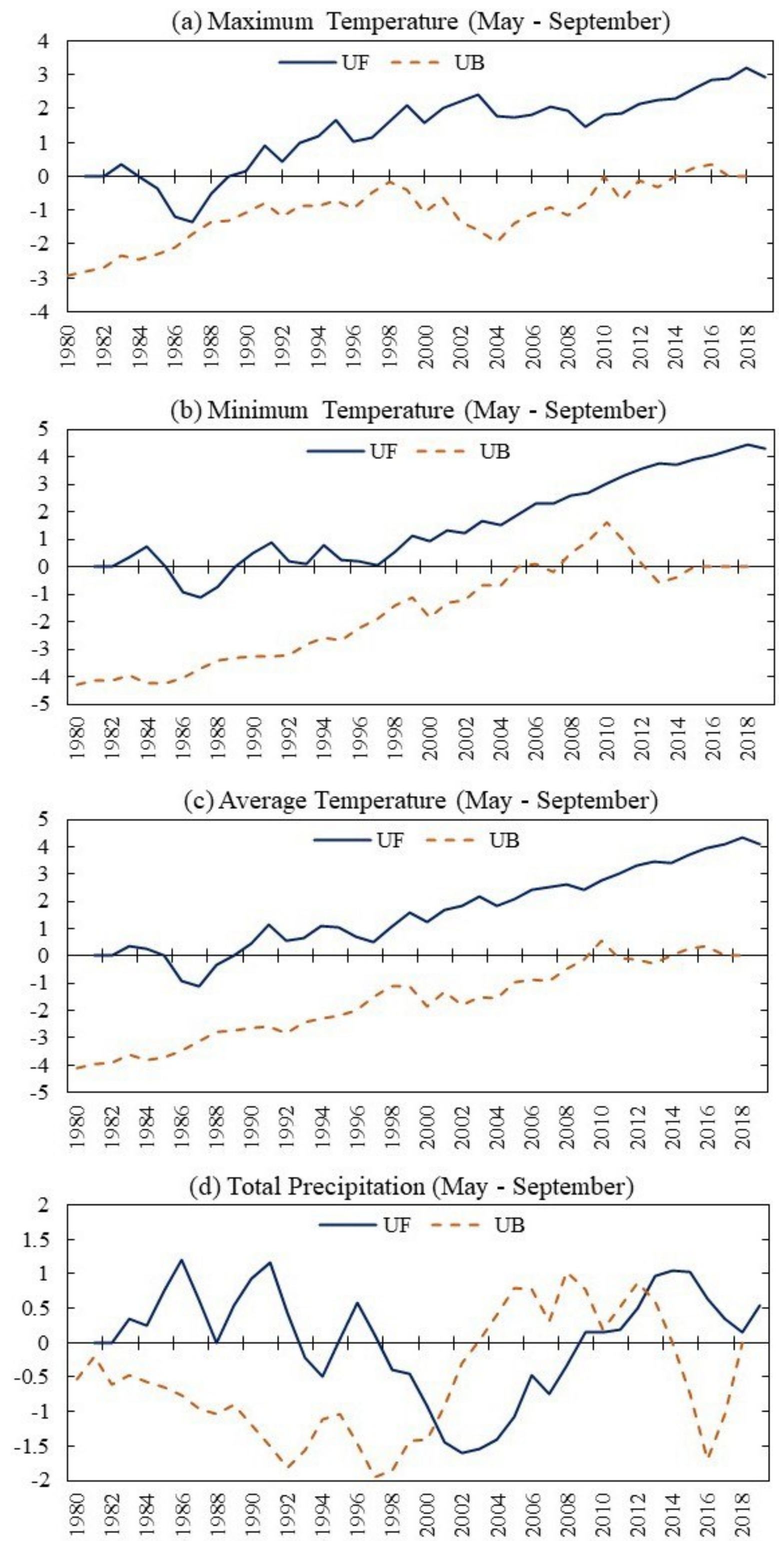

Figure 2.4 Sequential Mann-Kendall test statistics (UF and UB values) calculated from the (a) maximum temperature (average of May - September), (b) minimum temperature (average of May - September), (c) average temperature (average of May -

September), and (d) total precipitation (total of May – September) at the 26 wild blueberry fields (shown in Fig. 2.1) in Downeast, Maine.

Table 2.1 Trend analysis of climate variables (T_{max}, T_{min}, T_{avg}, P_{total}) using Mann-Kendall test at the studied 26 wild blueberry fields of Downeast, Maine from 1980 to 2019. T_{max}, T_{min}, and T_{avg} represent the maximum, minimum, and average temperatures, respectively averaged during growing period (May to September); P_{total} represents the total precipitation of the growing season (May to September).

Mann-Kendall Test	T_{max}	T_{min}	T_{avg}	P_{total}
Kendall's tau	0.323	0.474	0.454	0.06
Mann-Kendall Stat (S)	252	370	354	47
Var (S)	7366.67	7366.67	7366.67	7365.67
p-value (two-tailed)	0.003	< 0.0001	< 0.0001	0.592
alpha	0.05	0.05	0.05	0.05
Trend	Increasing	Increasing	Increasing	Not significant
Sen's slope Q	0.031	0.038	0.037	0.96

2.4.2. Comparison of Historical Climate Change among the Wild Blueberry Fields in Downeast, Maine

Marked differences in growing season maximum, minimum and average temperatures (Figs. 2.1b & 2.1c and Figs. A.1.1a, A.1.1b, A.1.1c & A.1.2) averaged over the last 40 years (1980 - 2019) were detected among the wild blueberry fields. In fact, these climate variables, water flux (PET) and their historical changes at the studied wild blueberry fields have significant relationships with their geographic factors (i.e., Latitude, Longitude, Elevation, and Distance from the coast) (Tables 2.2 & 2.3).

Table 2.2 Correlation analysis of climate variables (T_{max}, T_{min}, T_{avg}, P_{total}, and PET) and the increases in climate variables (Increase in T_{max}, T_{min}, T_{avg}, and P_{total} from Table S1.1) and in PET with the geographic factors (Latitude, Longitude, Elevation, and Distance from the coast) from the studied 26 wild blueberry fields at the Downeast, Maine. T_{max}, T_{min}, and T_{avg} represent the maximum, minimum, and average temperatures, respectively averaged during the growing period (May - September) and P_{total} represents the total precipitation of the growing period (May - September) averaged over 1980 to 2019; PET represents the average potential evapotranspiration of growing period (May - September) averaged over 1970 to 2014. The increase in T_{max}, T_{min}, T_{avg}, and P_{total} represent the increments in those variables over 1980 to 2019 and increase in PET represents the increment in PET during 2001-2014 compared to 1970-2000 during growing season at the studied 26 wild blueberry fields in the Downeast, Maine. [Numbers are Pearson correlation coefficient (R) values. P < 0.001***; P < 0.01**; P < 0.05*, where Benjamini-Hochberg adjusted P values are used at a False Discovery Rate of 0.05].

Factors / Variables	Latitude	Longitude	Elevation	Distance from Coast
T_{max}	0.476*	-0.864**	0.542**	0.743**
T_{min}	-0.866**	-0.416*	-0.544**	-0.512*
T_{avg}	0.019	-0.95**	0.225	0.416
P_{total}	0.155	0.093	0.602**	0.122
PET	-0.04	-0.57**	0.493*	0.088
Increase in T_{max}	-0.15	0.945**	-0.338	-0.59**
Increase in T_{min}	0.056	0.313	0.444	-0.273
Increase in T_{avg}	0.003	0.893**	-0.082	-0.526*
Increase in P_{total}	-0.889**	0.253	-0.556**	-0.769**
Increase in PET	-0.005	0.365	-0.51*	-0.004

Table 2.3 Generalized Linear Model (GLM) analysis between climate variables (T_{max}, T_{min}, T_{avg}, P_{total}) and geographic factors (Latitude, Longitude, Elevation, Distance from the coast) at the studied 26 wild blueberry fields of Downeast, Maine over 40 years from 1980 to 2019. T_{max}, T_{min}, and T_{avg} represent the maximum, minimum, and average temperatures, respectively averaged during the growing season (May - September); P_{total} represents the total precipitation of the growing season (May - September).

Fixed Factors	T_{max}		T_{min}		T_{avg}		P_{total}	
	F[1]	P[2]	F	P	F	P	F	P
Latitude	33.64	< 0.001	44.82	< 0.001	0.025	0.87	0.04	0.84
Longitude	119.5	< 0.001	10.01	0.002	66.045	< 0.001	0.014	0.905
Elevation	43.94	< 0.001	17.26	< 0.001	3.49	0.062	0.607	0.436
Distance from coast	85.74	< 0.001	15.223	< 0.001	12.055	0.001	0.025	0.875

[1] F-statistics for fixed factors, degrees of freedom are x and y for the numerator and denominator degrees of freedom, respectively.
[2] Estimated P-values for generalized linear model fixed factors.

During the growing season, the maximum temperature difference among all fields was ~2°C (Fig. 2.1b and Fig. A.1.1a). The maximum temperature was the lowest (~20°C to 21°C) in fields near the coast (field #s 22, 25, and 26, Fig. 2.1b). The more distant fields from the coast (Fig. 2.1b), the higher the maximum temperatures were in those fields. Wild blueberry field #3 which is the farthest from the coast, located in Hancock Co., Maine (in the upper-left corner of the study area boundary in Fig. 2.1b) experienced the highest temperature (~22.5°C) among all fields. Maximum temperatures in the remaining fields (middle of the study area, Fig. 2.1b) ranged from ~21.5°C to ~22.5°C. The maximum temperature at those studied wild blueberry fields had a significant positive relationship with their latitude, elevation, and distance from the coast (Tables 2.2 & 2.3).

In contrast, the minimum temperature at those fields had a significant negative relationship with their latitude, longitude, elevation, and distance from the coast (Tables 2.2 and 2.3). Minimum temperatures were higher in fields near the coast (Fig. 2.1c). Also, fields farther from the coast (field #s 26 to 1 sequentially, 26 being the closest to coast and 1 to 3 being the farthest, Fig. 2.1c), had lower minimum temperatures. The overall difference in minimum temperatures during the growing season among all fields was ~1.0°C.

Interestingly, a contradictory trend in average temperatures was observed among fields compared to the maximum and minimum temperature differences (Fig. A.1.1c). For instance, three fields closest to the coast (field #s 22, 25, and 26, Fig. 2.1) and two fields farthest from the coast (field #s 1 and 2 in Fig. 2.1) had similar atmospheric average temperatures (~15.5°C to 15.8°C) during the growing season (Fig. A.1.1c and Table A.1.1). But field #3, although farthest from the coast, had the highest average temperature (~16.3°C) compared to all other fields because of its highest recorded maximum temperature (Fig. A.1.1c and Table A.1.1). The overall difference in average temperatures during the growing season among all those fields was 0.9°C.

In contrast to temperature changes, total precipitation during the growing season was similar among all fields (Fig. 2.1d and Fig. A.1.1d). No significant relationship was observed between the total precipitation and the geographic characteristics of those fields when analyzed with a generalized linear model (Table 2.3). However, a significant positive linear correlation was observed between total precipitation and elevation of the wild blueberry fields, but the increasing trends of total precipitation in the fields had a significant negative correlation with latitude, elevation, and distance from the coast (Table 2.2 and Table A.1.1). Therefore, based upon both correlation analysis and use of generalized linear models the relationship between geographic field characteristics and precipitation was not consistent.

2.4.3. Relationship of Climate Variables with the Vegetation Index of Wild Blueberry Fields

The Maximum Enhanced Vegetation Index (EVI_{max}) in wild blueberry fields during the growing season was significantly ($P < 0.001$) related to the maximum temperature (Fig. 2.5a) and total precipitation (Fig. 2.5b). A significant quadratic relationship ($P < 0.001$) was observed between EVI_{max} and maximum temperature (Fig. 2.5a), as well as, between the EVI_{max} and total precipitation (Fig. 2.5b).

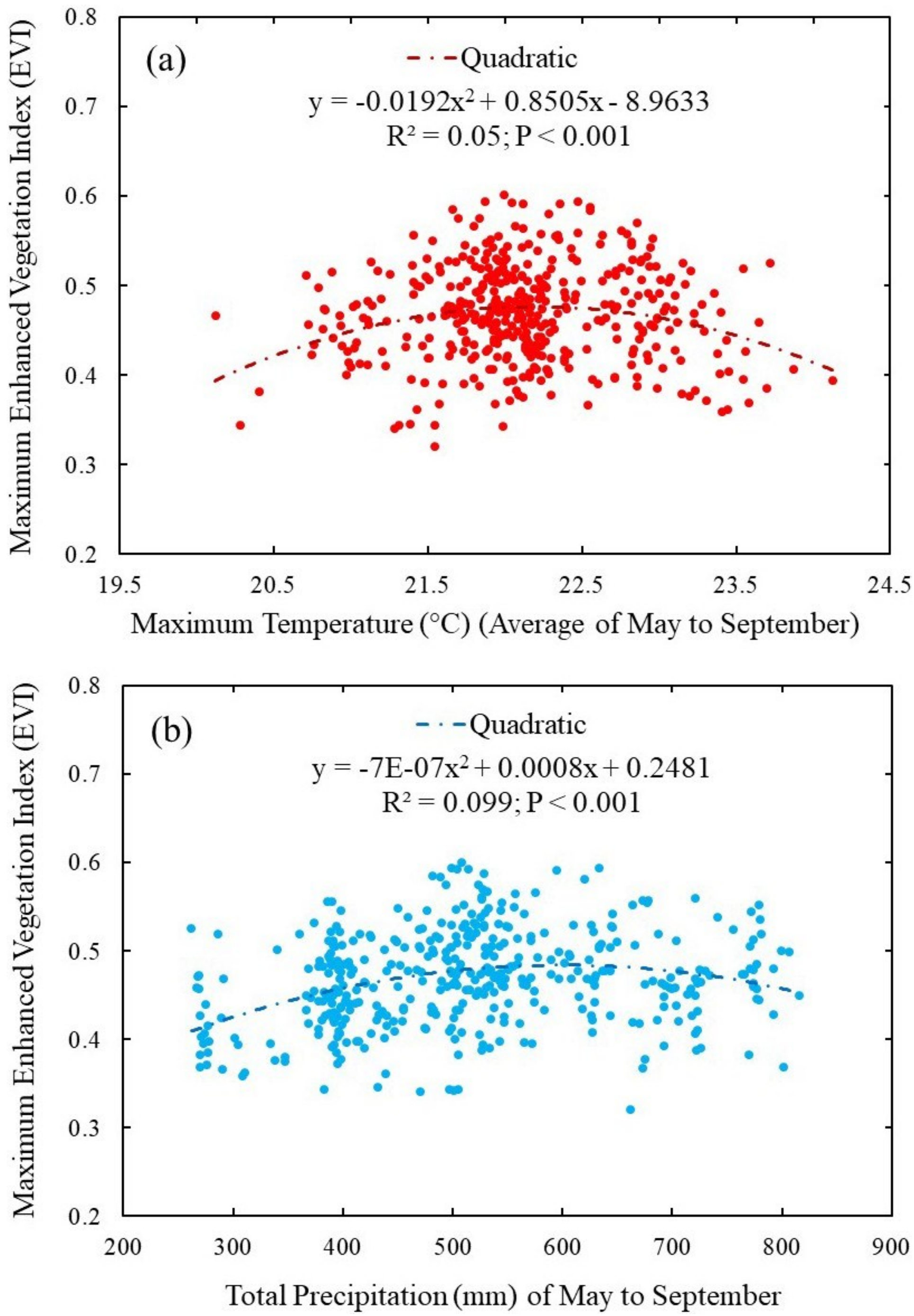

Figure 2.5 Maximum Enhanced Vegetation Index (EVI_{max}) of the wild blueberry fields in relation to (a) maximum temperature (average of May - September) and (b) precipitation (total of May - September), from 2001 to 2017. The observed data points (n = 26*17 = 442) are from the 26 wild blueberry fields for 17 years (2001 - 2017). The dashed lines are quadratic relationships fitted to the data with multiple linear regression (*P < 0.001*).

2.4.4. Wild Blueberry Fields Experienced Suboptimal Temperatures During the Peak Season (July and August)

More than half of the studied 26 wild blueberry fields have been experiencing an average maximum temperatures greater than 25°C during the peak season (July and August) for 15 - 20 years out of the last 40 years (1980 – 2019) (Fig. 2.6a). Here, 25°C was the potential threshold temperature for wild blueberries observed by Tasnim et al. (2020), above which wild blueberry photosynthesis started to decline. Furthermore, the average maximum temperature of the studied wild blueberry fields in July and August has increased ~1.7°C to 1.8°C over the last 40 years (Fig. 2.6b). Also, the average maximum temperature in July and August was observed to increase beyond the potential threshold temperatures of 22.4°C (Fig. 2.5a) and 25°C (based on a field study by Tasnim et al. (2020)) in the studied wild blueberry fields.

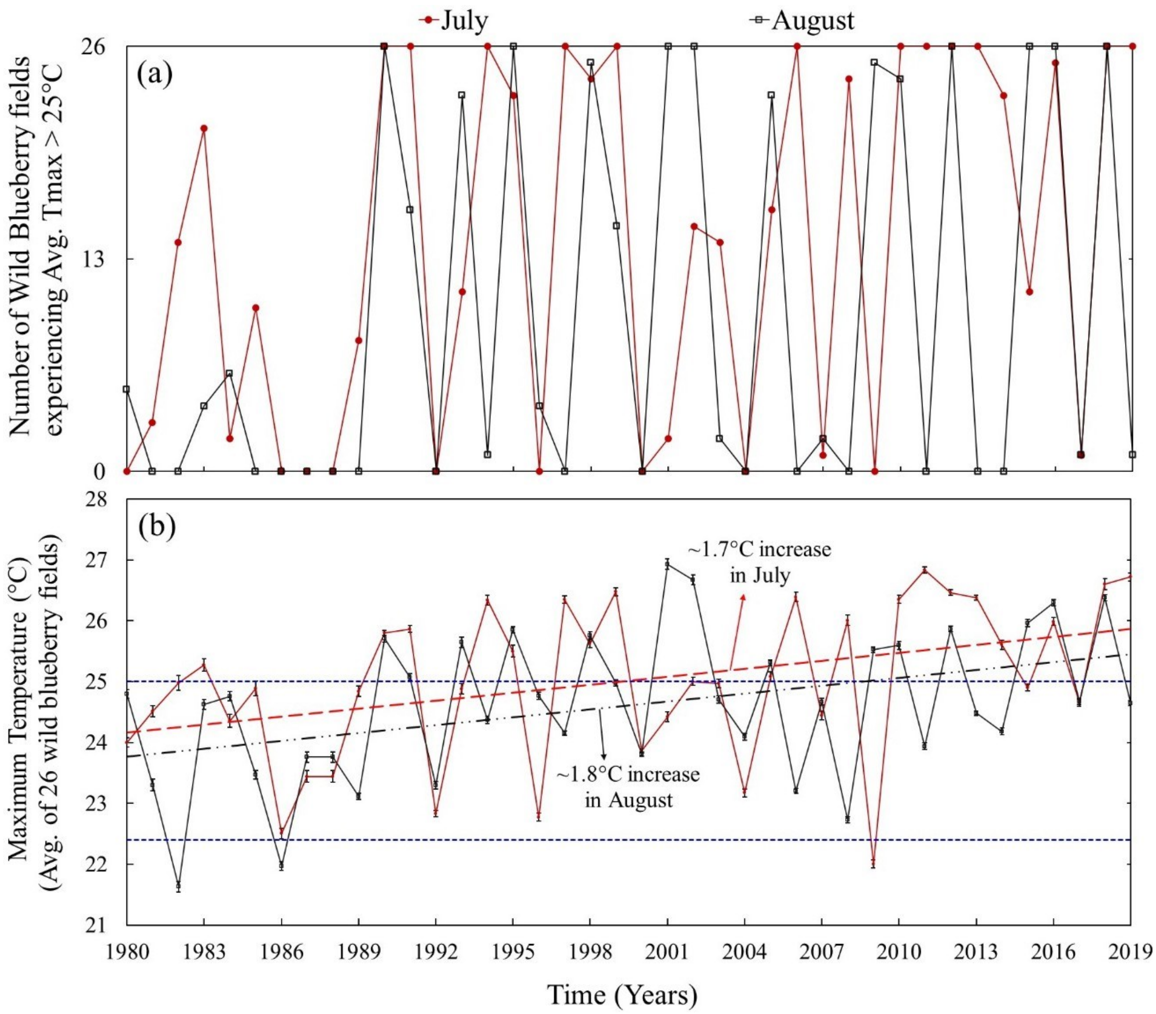

Figure 2.6 (a) Number of wild blueberry fields among the 26 fields (shown in Fig. 2.1) experiencing average maximum temperatures greater than 25°C during July and August over the last 40 years (1980 – 2019), (b) Historical (1980 to 2019) changes in maximum temperature during July and August of the 26 wild blueberry fields (shown in Fig. 2.1) in Downeast, Maine. The maximum temperatures from the fields are presented as Mean ± Standard error (n = 26). The red solid lines with circles and the black solid lines with squares represent July and August, respectively. The dashed red lines and black lines are linear regression lines fitted to the data observed in July and August, respectively. The blue dotted lines represent the threshold maximum temperatures of 22.4°C (observed from Fig. 2.5a) and 25°C (reported in the study by Tasnim et al. [2020]).

2.4.5. Potential Evapotranspiration (PET) Rate of the Wild Blueberry Fields

Average potential evapotranspiration (PET; mm/month) was significantly different ($F_{(1,2)} = 22.2$, $P < 0.001$) between the two time periods (1970 - 2000 and 2001 - 2014) during the 12 months. Also, the monthly total potential evapotranspiration (averaged over 1970 - 2000 and 2001 - 2014 period) was significantly different between the 1970 - 2000 and 2001 - 2014 periods for every month from January to December (Fig. 2.7a). Potential evapotranspiration (mm/month) was significantly higher during the 2001 - 2014 period compared to the 1970 - 2000 period in the months of April, June, July, and August to October (3.3 mm/month - 17.5 mm/month). In contrast, potential evapotranspiration (mm/month) was significantly higher during the 1970 - 2000 period compared to the 2001 - 2014 period in the months of January to March, May, and October to December (2.1 mm/month - 11.4 mm/month).

The average PET had a significant positive linear relationship ($R^2 = 0.42$; $P < 0.001$) with the average maximum temperature during the growing season in wild blueberry fields (Fig. 2.7b). A significant negative linear relationship ($R^2 = 0.55$; $P < 0.001$) was also observed between average PET and total precipitation (mm) during the growing season (relationship not shown). Furthermore, multiple regression showed that the average maximum temperature and total precipitation together explained 73% of the variance in average potential evapotranspiration ($R^2 = 0.73$; $P < 0.001$). Similar to the temporal dynamics of the climate variables, the average potential evapotranspiration (mm/month) during the growing season had a significant quadratic relationship ($P < 0.001$) with EVI_{max} in wild blueberry fields, although only 5% of the variance was explained in EVI_{max} (Fig. 2.7c). This suggests that while average evapotranspiration is a significant predictor of EVI_{max}, there are one or more other factors primarily responsible for driving EVI_{max}.

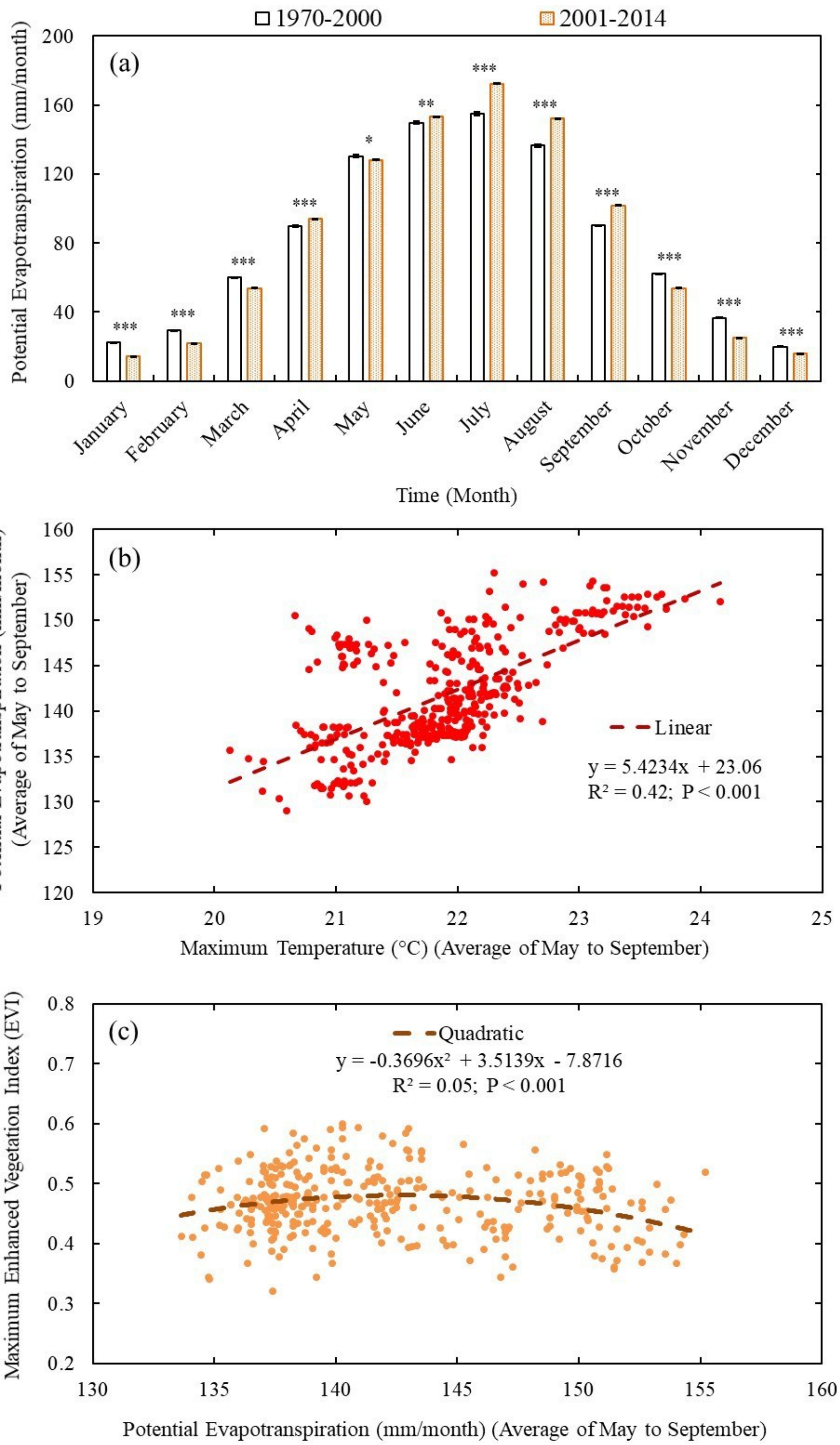

Figure 2.7 (a) Historical comparison in average potential evapotranspiration (PET) between 1970 - 2000 and 2001-2014 periods from January to December. The data are represented as the Means ± Standard errors (n = 26; where 26 represents the number of wild blueberry fields). Differences are significant at $P < 0.001$***; $P < 0.01$**; $P < 0.05$*. (b) The relationship between maximum temperature (average of May – September) and potential evapotranspiration (average of May - September) from 2000 to 2014.

The observed data points (n = 26*15 = 390) are from the 26 wild blueberry fields in Downeast Maine for 15 years (2000 - 2014). The dashed line in (b) is a linear relationship fitted to the data with multiple linear regression ($P < 0.001$). (b) The relationship between potential evapotranspiration (average of May - September) and Maximum Enhanced Vegetation Index (EVI_{max}) of the 26 wild blueberry fields in Downeast Maine from 2001 to 2014. The observed data points (n = 26*14 = 364) are from the 26 wild blueberry fields for 14 years (2001 - 2014). The dashed line in (c) is a quadratic relationship fitted to the data with multiple linear regression ($P < 0.001$).

2.5 Discussion

Our results indicate that climate change patterns in wild blueberry fields are different from the spatially averaged patterns of Maine. While temperatures have been increasing over the last 40 years everywhere in Maine (Fernandez et al. 2020), the temperature increment in wild blueberry fields during the growing season (May – September) is higher than that of the region (state of Maine). Our analysis (Mann-Kendall test) indicated significant increasing trends in historical temperature in those studied wild blueberry fields. Consequently, the potential evapotranspiration (PET) also increased in wild blueberry fields, which is strongly determined by maximum temperatures. In addition, the temporal climate change patterns we observed also varied spatially among wild blueberry fields depending on their geographic locations (i.e., latitude, longitude, elevation, and distance of the fields from the coast). Our study also revealed significant relationships among climate variables, PET, and the maximum enhanced vegetation index (EVI_{max}) for wild blueberry fields in Downeast, Maine which can provide guidelines for developing mitigation strategies against climate change.

The different climate patterns, especially higher temperatures, in wild blueberry fields compared to the state of Maine's overall average imply that we must not recommend management tactics for those fields based on Maine's aggregate climate patterns. This pattern is consistent with the designated three climate zones (Northern, Interior, and Coastal) in Maine which are characterized by different maximum, minimum, and average temperatures (Fernandez et al. 2020, NOAA-CAAG 2019). However, the observed patterns in average maximum temperatures across the wild blueberry fields during the growing season were different compared to the observed annual average maximum temperatures in those three climate zones. It was observed that the wild blueberry fields close to the coast experienced the lowest temperatures and fields farthest from the coast experienced the highest temperatures among the studied fields. This trend is contradictory to the fact that the Coastal climate zone of Maine has historically been the warmest followed by the Interior and Northern climate zones (Fernandez et al. 2020, NOAA-CAAG 2019). On the contrary, the observed minimum temperatures of those fields were in agreement with temperature patterns in the three Maine climate zones (Fernandez et al. 2020, NOAA-CAAG 2019). It was observed that fields near the coast experienced the highest minimum temperatures, and fields farther from the coast experienced the lowest minimum temperatures. But, the calculated average temperatures also followed the trend of maximum temperatures, as fields closest to the coast had slightly lower average temperatures than other fields. These contradictory responses could be because we studied temperature trends of wild blueberry fields during the growing season (May to September), whereas temperature trends in the three Maine climate zones are based on annual average temperatures which incorporates warming trends during the winter (Fernandez et al. 2020; NOAA-CAAG 2019).

Additionally, because many of the fields experienced warming at different rates, spatial variation should be considered when planning for future management strategies (i.e., irrigation, fertilization) under climate change. Since wild blueberry fields closer to the coast showed significantly higher increases in growing season temperatures (Table 2.2), growers who have fields closer to the coast need to pay more attention to the potential negative effects of warming on crop health and production. Meanwhile, fields closer to the coast also showed a higher increase in precipitation. This suggests a lower risk of water deficits in coastal fields. However, our analysis (sequential Mann-Kendall test) on climate change patterns in wild blueberry fields over the last 40 years demonstrated that air temperatures have been significantly increasing without significant change in precipitation. Hence, at similar precipitation rates, the higher temperature increases in more coastal fields, if they continue in the future would result in higher PET and hence higher soil and crop water loss. This dynamic needs to be considered in estimating the impacts of climate change on the water needs of wild blueberries on the sandy granitic soils in Maine.

Temperatures strongly determine water-flux (i.e., PET) in agricultural fields, which further affects crop water status, health, and productivity (Bhatt and Hossain 2019). Aprialdi et al. (1972) found a similar correlation between the atmospheric temperature and PET estimated using the Penman-Monteith equation (Allen et al. 1998, Walter et al. 2000), as shown in this study. Thus, the significant increase in atmospheric temperature during the growing season over the last 40 years explained the increase in PET in Maine wild blueberry fields. A predicted further increase in temperatures and thus PET will probably increase water deficits of the wild blueberry crops that grow naturally in sandy granitic soils with a low water-holding capacity. Remote sensing-based PET can be a useful tool for determining crop water deficits (Wambura and Dietrich 2020) and can be analyzed based on the PET-EVI relationship established in this study to infer the needs for irrigation.

The quadratic relationships between maximum EVI and the maximum temperature, and between maximum EVI and PET suggest that, after reaching a threshold maximum temperature (~22.4°C) and potential evapotranspiration (~145 mm/month), further increases in temperature and PET will cause declines in EVI. These threshold values suggest optimum conditions for wild blueberry health and productivity and can be used to infer effective management. Although these relationships had low coefficients ($R^2 < 0.1$) due to variations among fields, they imply future declines in Maximum EVI, and crop productivity with continued increases in air temperature and PET (Xiao et al. 2005, Wu et al. 2010). In support of this prediction, Tasnim et al. (2020) have shown that air temperature above 25°C results in a reduction of chlorophyll leaf content and photosynthetic performance in wild blueberry plants. This suboptimal temperature (≥ 25°C) appears to be a concern for at least half of our studied fields in the peak season (July and August). Moreover, the increasing maximum temperatures beyond the observed threshold temperatures of 22.4°C or 25°C in our studied wild blueberry fields in July and August over the past 40 years could be alarming. A temperature between 22.4°C and 25°C probably would not impact photosynthetic processes and related enzyme activities directly. However, a temperature increase beyond 22.4°C could increase soil and crop water loss, resulting in water deficits and consequently decreased photosynthesis. Wild blueberry crops growing on sandy soils can be sensitive to water loss. This is supported by the quadratic relationship between maximum EVI and PET in combating the potential negative effects of further warming. This potential negative effect of warming on wild blueberry production suggests the need for mitigation efforts, and irrigation or soil amendment techniques need to be considered in planning.

While use of temperature, precipitation, and potential evapotranspiration variables to predict changes in vegetation growth and productivity have been conducted for forest ecosystems (i.e., EVI) (Deng et al. 2007), our study is the first to explore climate change patterns in different fields of a fruit crop within a single production region. We have also established relationships between climate conditions, water flux, and the vegetation index for wild blueberry fields. These environmental variables, PET, and maximum EVI derived from remote sensing measurements can assist researchers in the development of optimal wild blueberry crop production models (Wambura and Dietrich 2020; Xiao et al. 2005; Wu et al. 2010; Deng et al. 2007; Jahan and Gan 2011). In turn, predictive crop production models should help wild blueberry growers to efficiently manage their crops during the current unprecedented era of climate change.

CHAPTER 3

SEASONAL CLIMATE TRENDS ACROSS THE WILD BLUEBERRY BARRENS OF MAINE, USA

3.1 Abstract

Wild blueberries in Maine, USA are facing threats from our changing climate. While summer climate variations have been affecting this important commercial crop directly, significant climate variations in other seasons also can be potentially detrimental to blueberry production. Therefore, we analyzed annual and seasonal climate trends (temperature, rainfall, snow cover) over the past 41 years (1980–2020) for seven Maine counties (Piscataquis, Washington, Hancock, Knox, Lincoln, Kennebec, York) with large wild blueberry areas. We found that, across all blueberry production fields (or "barrens"), historical temperatures increased significantly ($p < 0.05$) in the fall and winter followed by summer, but not in the spring. Additionally, precipitation increased slightly (0.5–1.2 mm/year) in the winter and fall, whereas no changes were found in the spring and summer. Furthermore, we found that historical temperatures were lower in Piscataquis (north-central) and Washington (north-east) counties, whereas in south-western counties (Hancock to York) experienced a relatively warmer climate. The rate of increasing temperature was comparatively slower in the warmer barrens located towards the south-west (Hancock to York). Moreover, the growing season lengthened towards the fall season consistently in all locations, whereas lengthening towards the spring was inconsistent. These findings inform the wild blueberry growers in different locations of Maine about the seasonal shifts occurring for their crop. This knowledge may assist with land management planning in order for the growers to prepare for future impacts.

3.2 Introduction

Wild lowbush blueberry has been one of the three most economically important commercial crops native to North America for hundreds of years. This crop was not planted; rather, it started to grow naturally on large fields, or "barrens", formed through glacial outwash plains 10,000 years ago (Hanes and Waring 2018). In fact, this naturally growing and evolving temperate crop is one of the largest crops produced in Maine, USA, managed by over 480 growers across the state, and covering 41,000 acres (Hanes and Waring 2018). These wild blueberry barrens are mainly distributed in the coastal climate region and a fraction of the interior climate region of Maine (Hanes and Waring 2018, Fernandez et al. 2020, Birkel and Mayewski 2018). These coastal regions followed by the interior region are experiencing faster annual atmospheric temperature increments and extremes, longer growing seasons, and more drastic rainfall events (Fernandez et al. 2020, Birkel and Mayewski 2018). Therefore, wild blueberries growing in those regions are also exposed and potentially vulnerable to such drastic climate changes.

Meantime, there may be benefits of a warmer climate. Based on analyzed relationships between historical climate parameters and blueberry yield in Maine, blueberry yield may increase under a warmer climate with higher precipitation (Birkel and Mayewski 2018). If increased precipitation came at appropriate times and amounts, it would improve soil moisture for the blueberries to counterbalance the water stress effects on this crop due to the warmer climate (increased water use under a warmer climate). On that note, wild blueberry barrens in the Downeast region of Maine are still vulnerable because they are experiencing a higher increase rate in temperature during their growing period compared to the rest of the state with no changes in rainfall (Tasnim et al. 2021). Additionally, coarse-grained sandy soils in the wild blueberry barrens cannot efficiently hold soil moisture for the plants. Under such circumstances with low soil moisture availability due to less rainfall and sandy soils, higher crop water loss further adversely affects the physiological, morphological, and yield performances of the wild blueberry plants (Tasnim et al. 2021, Tasnim et al. 2020, Barai et al. 2021). The Washington County is the largest (~70%) wild blueberry production region of Maine, USA (Maine Wild Blueberry Production Statistics 2019), and climate warming at a faster rate would directly affect the local economy by hurting the livelihoods of wild blueberry growers in this region.

In order to stay in business, growers need to be vigilant and take necessary management actions in their wild blueberry fields. Necessary actions include investing in irrigation due to frequent droughts (higher temperatures with less rainfall in summer) and adopting strategies to retain soil moisture due to the low moisture-holding capacity of wild blueberry soils (Tasnim et al. 2021, Tasnim et al. 2020, Barai et al. 2021). To adjust the management techniques accordingly, growers who manage fields in different towns and counties need to know the regions of most concern and the intensity of changing temperature and rainfall in their blueberry fields (Tasnim et al. 2021). Wild blueberry fields in the Washington County of Maine have already experienced considerable variations in temperature and precipitation. Such spatial variations significantly depend on the geospatial locations of the wild blueberry barrens and are related to the latitude, longitude, elevation, and distances from the coast (Tasnim et al. 2021).

Although a previous study reported the importance of considering spatial climate variations for managing these barrens, it focused on one major production county (Washington) (Tasnim et al. 2021). Wild blueberry barrens are also found in other counties (Hancock, Knox, Waldo, Lincoln, Kennebec, York) farther south and west from the Washington County along the coast (Fig. 3.1) and in small parts of northern Maine (Piscataquis and Penobscot Counties in the interior climate region). Therefore, it is important to learn and compare the extent of climate variations that barrens in all regions are experiencing. This would better inform the growers about the local climate variations in their fields, thus increasing their awareness and better enabling them to manage their fields accordingly. Moreover, comparing the climate variations across different counties in different latitudinal and longitudinal directions (Fig. 3.1) would indicate the temperatures to which this crop may migrate in order to find favorable growing conditions if the existing barrens cannot be managed properly.

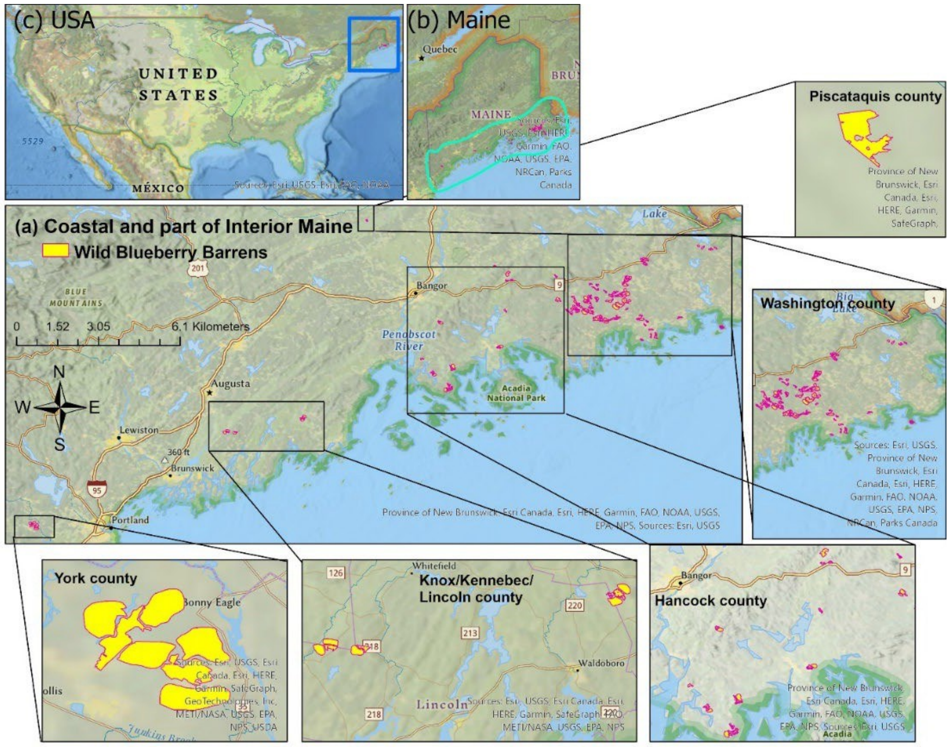

Figure 3.1 Locations of the studied wild blueberry barrens in different counties from north-central (Piscataquis) and north-east (Washington, Hancock) to south-west (Knox, Lincoln, Kennebec, York) at the (**a**) coastal and interior climate regions of (**b**) Maine at the north-east of (**c**) the United States of America. The yellow-colored polygons with pink-colored boundaries indicate the studied wild blueberry barrens (WBBs) (area of each barren ≥ 0.5 km^2). Different counties and climate regions of Maine are shown in Fig. B.1 in the supporting materials of this study.

In addition to the growing season (summer), other seasons (fall, winter, and spring) are also dramatically changing. For example, the growing season lengthened by 14 days over the last 20 years due to warmer spring and fall seasons, in addition to a shift in early fall frost and late spring frost dates (Fernandez et al. 2020, Birkel and Mayewski 2018, https://umaine.edu/climate-ag/farm-response-changing-weather/, Kukal and Irmak 2018, Drummond and Yarborough 2012). Such an extended growing season has been shown to be beneficial for some crops (Kukal and Irmak 2018), and cultivated crops can be managed with necessary actions in response to changing climate (https://umaine.edu/climate-ag/farm-response-changing-weather/). However, some crops such as the naturally growing wild blueberries cannot be managed like the cultivated crops. Some region-specific crops might face more negative impacts (pests, intense rainfall events, soil erosion, heat waves, seasonal droughts) than positive impacts (longer period for development through higher carbon assimilation for carbohydrate production) (Fernandez et al. 2020, Birkel and Mayewski 2018, Tasnim et al. 2020, Barai et al. 2021, https://umaine.edu/climate-ag/farm-response-changing-weather/, Kukal and Irmak 2018, Drummond and Yarborough 2012, Tasnim and Zhang 2021). Moreover, warmer winters were found to adversely affect temperate grasslands more than the warmer summer (Kreyling et al. 2019). In fact, abnormally higher temperatures in late winter and early spring can trigger the early development of plants and crops. In this scenario, they would be more susceptible to frost damage because unusually late last spring frost caused significant crop (apple, blueberry, peach) damage in some years (e.g., 2012, 2016, 2020) over the past decade (Fernandez et al. 2020, Birkel and Mayewski 2018, https://umaine.edu/climate-ag/farm-response-changing-weather/). Under such circumstances, seasonal climate variations for the wild blueberry barrens in temperate Maine need immediate assessment because we cannot rely on existing seasonal climate patterns for the whole state of Maine (Tasnim et al. 2021).

To this end, our study aims to: (1) quantify the historical (1980–2020) changes in maximum (T_{max} indicating daytime temperature), minimum (T_{min} indicating nighttime temperature) and average (T_{avg}) temperatures, total precipitation (P_{total}), and snow cover (snow water equivalent) annually and seasonally (summer, fall, winter, spring) for wild blueberry barrens in Maine; (2) characterize historical climate variations across different counties, from the north-central (Piscataquis) and north-east (Washington) regions, towards the south-west (Hancock, Knox, Lincoln, Kennebec, York) (Fig. 3.1); and (3) assess the growing season length and the timing of the last spring frost and first fall frost across different counties for the wild blueberry barrens located both closer and farther from the coast at different latitudinal and longitudinal directions in Maine, USA. Based on these analyses, our study also presents a detailed analysis of the potential positive and negative impacts of the studied climate parameters and trends on wild blueberries of Maine. This work intended to assist researchers and growers in developing proper strategies, and to reveal the spatial and temporal extent of climate change threats to this unique natural agricultural system.

3.3 Materials and Methods

3.3.1. Data Source and Acquisition

The dataset of both annual and monthly climate variables (maximum temperature, minimum temperature, average temperature, total precipitation, snow water equivalent) from 1980 to 2020 for North America was acquired as raster files from Daymet (**https://daymet.ornl.gov/getdata**, accessed on 17 May 2020) (Thornton et al. 2020a, 2020b). These raster files were provided on a per-pixel basis at 1 km spatial resolution. Daymet data source was chosen over other gridded climate data sources (i.e., PRISM) because of its higher spatial resolution, suitability for our study, and the fact that some studies did not find significant differences among the climate data acquired from different gridded data sources (Mehdipoor et al. 2018, Brust 2018). After acquiring the raster climate files from Daymet, we used different tools of Arc GIS Pro 2.4.2 Software (Esri Inc. 2019) to clip and extract the datasets for our studied wild blueberry barrens. These datasets were then transferred from Arc GIS Pro to an Excel spreadsheet (Microsoft, Redmond, WA, USA) for data arrangements and further analyses.

The polygons of the studied wild blueberry barrens in different counties of Maine (Fig. 3.1) were acquired from a Google Earth Pro (**https://www.google.com/earth/versions/#earth-pro**; accessed on 1 April 2020) KMZ file based on a field survey carried out by David Yarborough, Professor Emeritus of Horticulture and Wild Blueberry Specialist, University of Maine. Since the spatial resolution of the datasets for climate variables was 1 km, we separated the wild blueberry barrens into two categories based on their area: (1) 0.5–1 km^2 barrens and (2) >l km^2 barrens. Then we compared the climate variables for these barrens of two different sizes and found that they did not differ from each other (data not shown here). Therefore, we finally used the wild blueberry barrens of 0.5 km^2 and a larger area to analyze and compare the acquired climate variables for those barrens located in the studied different counties (Fig. 3.1 and Fig. B.1). Here, it is to be noted that we found more 0.5 km^2 and larger wild blueberry barrens in Washington and Hancock counties compared to the other studied counties (Knox, Lincoln, Kennebec, York, and Piscataquis) as shown in Fig. 3.1. Therefore, we used the climate variables averaged across all the barrens from the studied different counties as categorized in Fig. 3.1. Further, based on the annual average temperature cycle, we compared the approximate growing season length and the timing of the first fall frost and last spring frost during different periods (1980–1990, 1991–2000, 2001–2010, 2011–2020) for the wild blueberry barrens located in different counties of Maine. Growing season length was calculated based on the time duration when the average temperature was 55 °F (12.8 °C) and above. Last spring frost and first fall frost dates were determined when the average temperature was 32 °F (0 °C) on the last day of spring and the first day of fall, respectively.

The temperature data we used in this study were measured at 2 m (6.5′) height from the ground surface. The average wild blueberry plant height can be typically 20–30 cm (8”–12”). It is not unusual that the ground and shorter wild blueberry plants might experience significantly lower temperatures than the air temperatures measured at 2 m (6.5′). Therefore, we used the air temperature data measured at 2 m (6.5 ft) and also temperature measured with weather stations installed at ~60 cm (2′) close to the wild blueberry plants at the Blueberry Hill Farm, Jonesboro, Maine to fit a linear regression (Fig. B.2). For this purpose, we used available monthly average temperature data from 1980–1989 and 2010–2018. From the linear regression ($R^2 = 0.997$ in Fig. B.2), we found that the plants were experiencing similar temperatures recorded at 2 m (6.5′), which is the typical height that air temperatures are recorded with deployed weather stations.

3.3.2. Data Analyses

Statistical analyses of the acquired data were conducted using JMP Pro 16.2 (SAS Institute Inc., Cary, NC, USA) (JMP® 1989-2021) and XRealStats (Addinsoft, New York, NY, USA) (Addinsoft 2020). Changes (increases or decreases) in climate variables (Tmax, Tmin, Tavg, Ptotal, SWE) over the past 41 years from 1980 to 2020 in the studied wild blueberry barrens in different counties of Maine (Fig. 3.1 and Fig. B.1) were determined from linear regression trendlines. Trend analyses of the annual and seasonal climate variables were conducted using the Mann–Kendall (MK) trend test. Kendall’s tau with *p*-values and Mann–Kendall trend test results were computed using JMP Pro 16.2 and XRealStats

(JMP® 1989-2021, Addinsoft 2020) where continuity correction was applied, and the autocorrelation was taken into account using the Hamed and Rao method (Hamed and Rao 1998). This Hamed and Rao method is a modified version of the original MK test by Mann and Kendall. Further, the differences in slopes of the linear fitted lines among the barrens in different counties were analyzed using Student's *t*-test at the significance level of $p < 0.05$. Moreover, a Pearson correlation analysis was conducted between the changing rates of climate variables (increase in T_{max}, increase in T_{min}, increase in T_{avg}, increase in P_{total}, increase in SWE) and geographic factors (latitude, longitude, distance from the coast) for the studied wild blueberry barrens, as shown in Fig. 3.1. Here, in order to provide multiple analysis significance protection, the *p*-values were adjusted using the Benjamini and Hochberg method at a false discovery rate (FDR) of 0.05 (Benjamini and Hochberg 1995). Basically, FDR is a statistical approach used in multiple hypothesis testing to correct for multiple comparisons in order to correct for random events that falsely appear significant. This FDR can be controlled by the Benjamini–Hochberg method (Benjamini and Hochberg 1995), which uses sequential modified Bonferroni correction for analyzing significant differences in multiple comparisons.

3.4 Results

3.4.1. Annual Climate Changing Trends (1980–2020) in the Wild Blueberry Barrens

Based on the comparisons of temperature box plots (Fig. 3.2a–c), annual temperatures were higher in barrens of the studied coastal counties (Washington to York) compared to Piscataquis County towards the north. In fact, temperatures were observed to be gradually higher in barrens of the studied coastal counties farther south-west from Washington to York (Fig. 3.2a –c). In contrast, total annual precipitation was similar in barrens of all studied counties (Fig. 3.2d). In agreement with the trends observed for temperatures, snow water was found to be slightly higher in barrens of the Piscataquis County followed by Washington County, compared to other studied coastal counties farther south-west (Hancock to York) (Fig. 3.2e). Moreover, barrens in Piscataquis County in 2008 and Washington County in 2015 experienced unusually higher (outliers in Fig. 3.2e) snow cover.

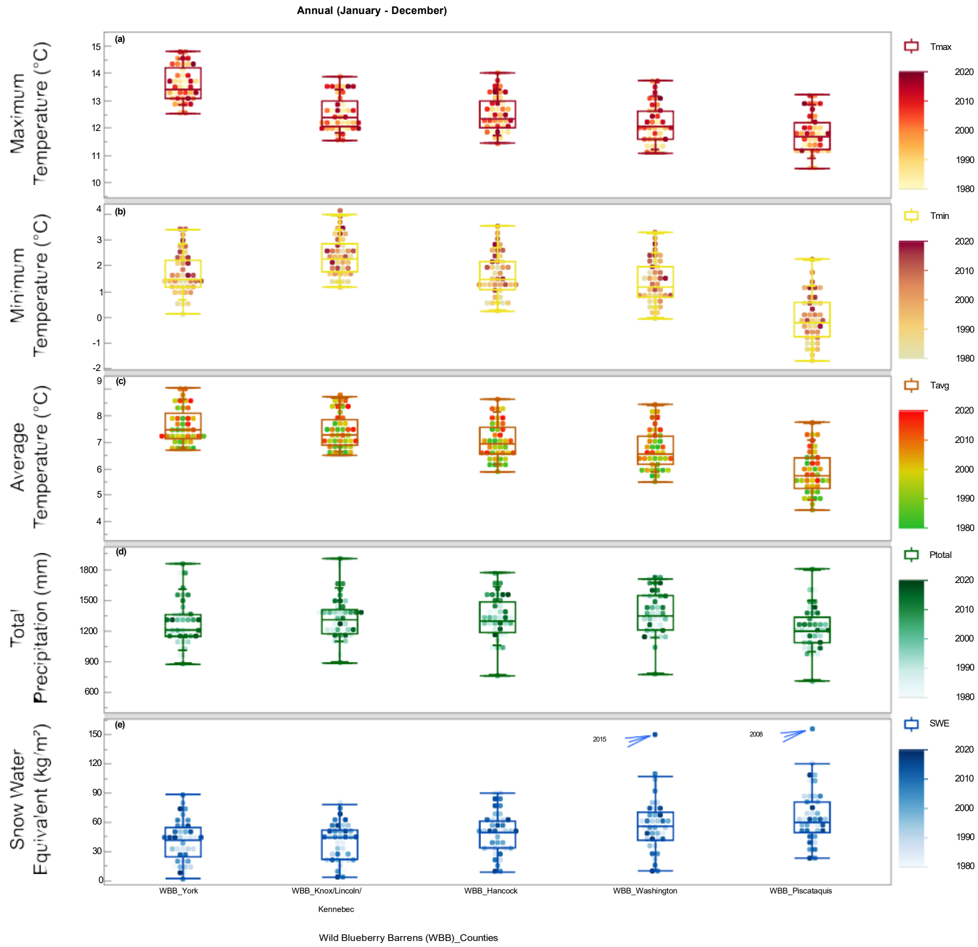

Figure 3.2 Comparison of historical (1980 to 2020) annual climate parameters: (**a**) maximum temperature, (**b**) minimum temperature, (**c**) average temperature, (**d**) total precipitation, and (**e**) snow water equivalent among the studied wild blueberry barrens (WBBs) in different counties from north-central (Piscataquis) and north-east (Washington, Hancock) to south-west (Knox, Lincoln, Kennebec, York) of Maine as shown in Fig. 3.1.

The annual maximum temperature increased significantly in the wild blueberry barrens in Piscataquis (1 °C) and Washington (1.3 °C) Counties, compared to other studied coastal counties farther south-west (Hancock to York), where temperature increment rates (0.2–0.6 °C) were not significant (Fig. 3.3a, Table 3.1). In contrast, the annual minimum temperature significantly increased (1.2–1.7 °C) in the barrens of all studied counties (Fig. 3.3b, Table 3.1). Consequently, the annual average temperature (Fig. 3.3c, Table 3.1) increased significantly (0.8–1.5 °C) in the barrens of all studied counties but the increase rate was higher in Piscataquis and Washington counties (1.3–1.5 °C) compared to other coastal counties

(0.8–1 °C) farther south-west. Moreover, the annual total precipitation increased in the barrens of all studied counties, but the increase was significant at a rate of 5.61 mm/yr (~0.22”/yr) only in Washington County (Fig. 3.3d, Table 3.1). The precipitation increase rate was significantly slower in Piscataquis County (2.93 mm/yr: 0.11”/yr) compared to the increase rate in other studied counties (4.4–5.6 mm: 0.17–0.22”/yr) (Table 3.1). There were no significant changes in historical snow cover trends (Fig. 3.3e and Fig. B.3, Table 3.1). The increasing trend in snow cover (15 kg/m^2) in the counties from Knox to York (Fig. 3.1 and Fig. B.1), and the decreasing trend in snow cover (15 kg/m^2) in Piscataquis County (Fig. 3.3e) from 1980–2020 were not significant.

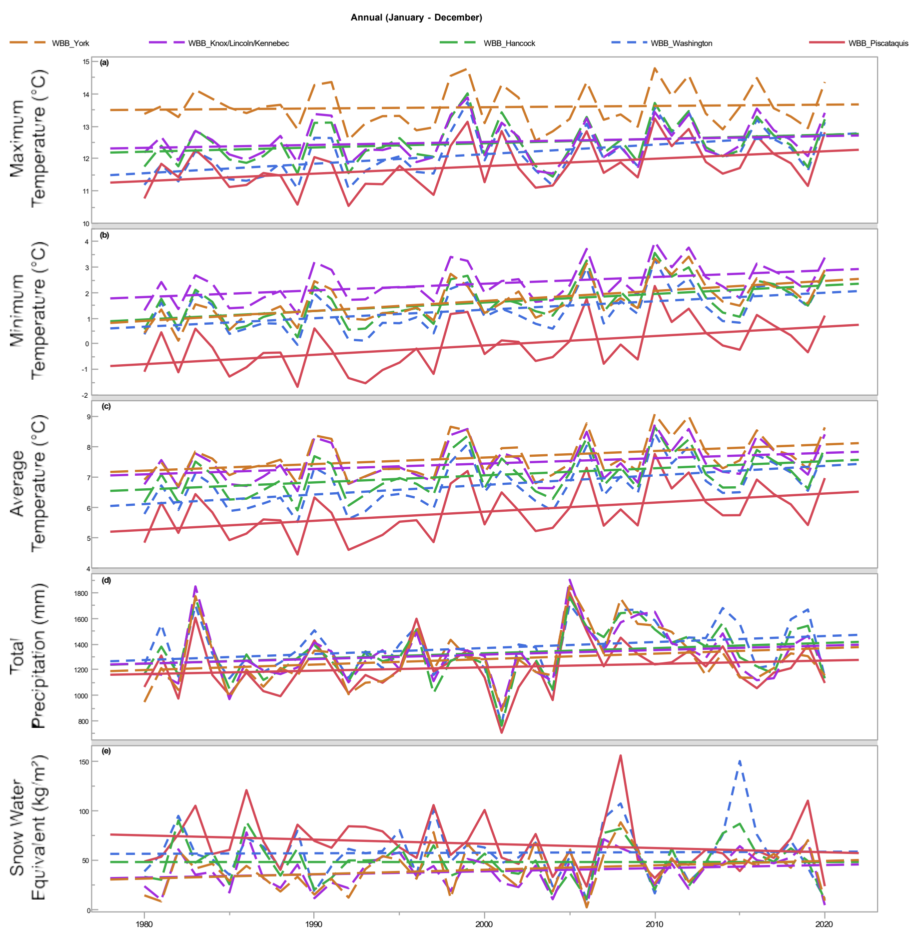

Figure 3.3 Historical (1980 to 2020) changes with fitted linear regression trendlines for the annual climate parameters: (**a**) maximum temperature, (**b**) minimum temperature, (**c**) average temperature, (**d**) total precipitation, and (**e**) snow water equivalent

throughout the studied wild blueberry barrens (WBBs) in different counties from north-central (Piscataquis) and north-east (Washington, Hancock) to south-west (Knox, Lincoln, Kennebec, York) of Maine as shown in Fig. 3.1.

Table 3.1 Historical trend analysis of annual climate variables using Mann–Kendall test, and comparison of linear regression fitted slopes using slope *t*-test among the studied wild blueberry barrens (WBBs) in different counties from north-central (Piscataquis) and north-east (Washington, Hancock) to south-west (Knox, Lincoln, Kennebec, York) of Maine (shown in Fig. 3.1) from 1980 to 2020. Red-colored parts indicate significant strength in historical climate trends. Different letters associated with the "Slope rate" indicate significant differences among the counties at a significance level of $p < 0.05$. Different letters "a–d" after the numbers indicate significant differences among the studied barrens in different counties.

Climate Variables	**Mann–Kendall and Slope *t*-Test**	**Wild Blueberry Barrens (WBB) Counties**				
		WBB_ Piscataquis	**WBB_ Washington**	**WBB_ Hancock**	**WBB_Knox/ Lincoln/Kennebec**	**WBB_ York**
T_{max} in Figure 3.3a	Kendall's tau	**0.25**	**0.36**	0.18	0.12	0.02
	p-value	**0.02**	**0.0009**	0.09	0.26	0.84
	Trend	**Increasing**			Increasing	
	Slope rate, °C	1a	1.3 a	0.6 b	0.4 bc	0.2 c
	°C/year	0.024a	0.032 a	0.014 c	0.01 c	0.005 d
T_{min} in Figure 3.3b	Kendall's tau	**0.31**	**0.36**	**0.35**	**0.31**	**0.44**
	p-value	**0.004**	**0.001**	**0.001**	**0.004**	**<0.0001**
	Trend			**Increasing**		
	Slope rate, °C	1.7 a	1.5 ab	1.5 ab	1.2 b	1.7 a
	°C/year	0.04 a	0.036 ab	0.036 ab	0.03 b	0.04 a
T_{avg} in Figure 3.3c	Kendall's tau	**0.30**	**0.36**	**0.28**	**0.23**	**0.26**
	p-value	**0.005**	**0.0008**	**0.009**	**0.03**	**0.01**
	Trend			**Increasing**		
	Slope rate, °C	1.3 ab	1.5 a	1 bc	0.8 c	1 bc
	°C/year	0.032 ab	0.036 a	0.024 bc	0.02 c	0.024 bc
P_{total} in Figure 3.3d	Kendall's tau	0.15	**0.23**	0.17	0.19	0.16
	p-value	0.18	**0.04**	0.12	0.08	0.13
	Trend	Increasing	**Increasing**		Increasing	
	Slope rate, mm	120 a	230 b	180 b	180 b	200 b
	mm/year	2.93 a	5.61 b	4.4 b	4.4 b	4.88 d
SWE in Figure 3.3e	Kendall's tau	−0.16	−0.0024	0.02	0.17	0.17
	p-value	0.13	0.98	0.82	0.12	0.12
	Trend	Decreasing	No change		Increasing	
	Slope rate, kg	15 a	0		15 b	15 b
	kg/year	0.36 a	0		0.36 b	0.36 b

3.4.2. Seasonal Climate Changing Trends (1980–2020) in the Wild Blueberry Barrens

Based on the comparisons among different seasons, historical maximum, minimum, and average temperatures increased significantly (Table 3.2 and Table B.1) in the blueberry barrens of Maine in the summer (Fig. 3.4 and Fig. 3.5), winter (Fig. 3.6, Fig 3.7 and Fig. 3.8), and fall (Fig. 3.9 and Fig. 3.10), but not in the spring (Fig. 3.11 and Fig. 3.12). Moreover, the overall historical temperature increase rates (Table B.1) were higher for the barrens in the fall (0.9–2.9 °C) and winter (0.4–2.1 °C) seasons than in the summer (0.2–1.9 °C). In agreement with these seasonal temperature variations, the growing season for the barrens has lengthened consistently towards the fall season (September–October) after summer (Table 3.3) because of the highest rate of increasing temperatures in the fall (Fig. 3.13). On the contrary, the lengthening of the growing season towards the spring season has been inconsistent (Table 3.3) because of the erratic fluctuations in spring temperatures over the past 41 years (Fig. 3.11, Fig. 3.12, and Fig. 3.13). In contrast to the temperature patterns, historical precipitation did not change significantly in the barrens during any season, where ~20–50 mm increments in precipitation over the past 41 years were observed only in the fall and winter seasons (Table B.1).

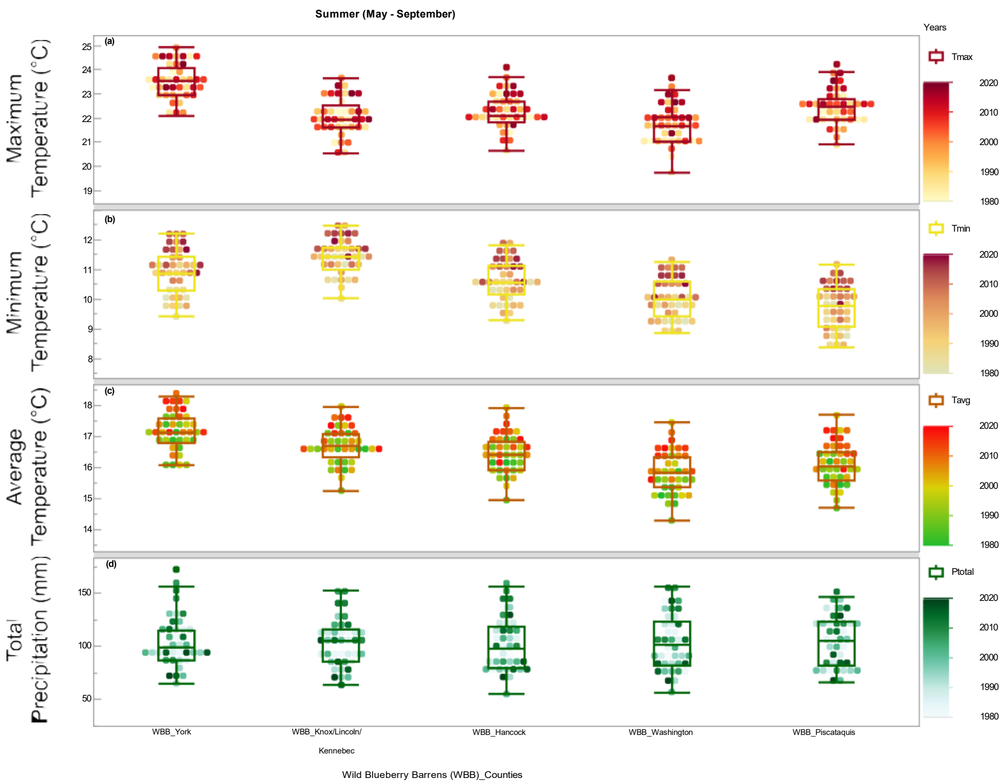

Figure 3.4 Comparison of historical (1980 to 2020) summer climate parameters: (**a**) maximum temperature, (**b**) minimum temperature, (**c**) average temperature, and (**d**) total precipitation among the studied wild blueberry barrens (WBBs) in different counties from north-central (Piscataquis) and north-east (Washington, Hancock) to south-west (Knox, Lincoln, Kennebec, York) of Maine as shown in Fig. 3.1.

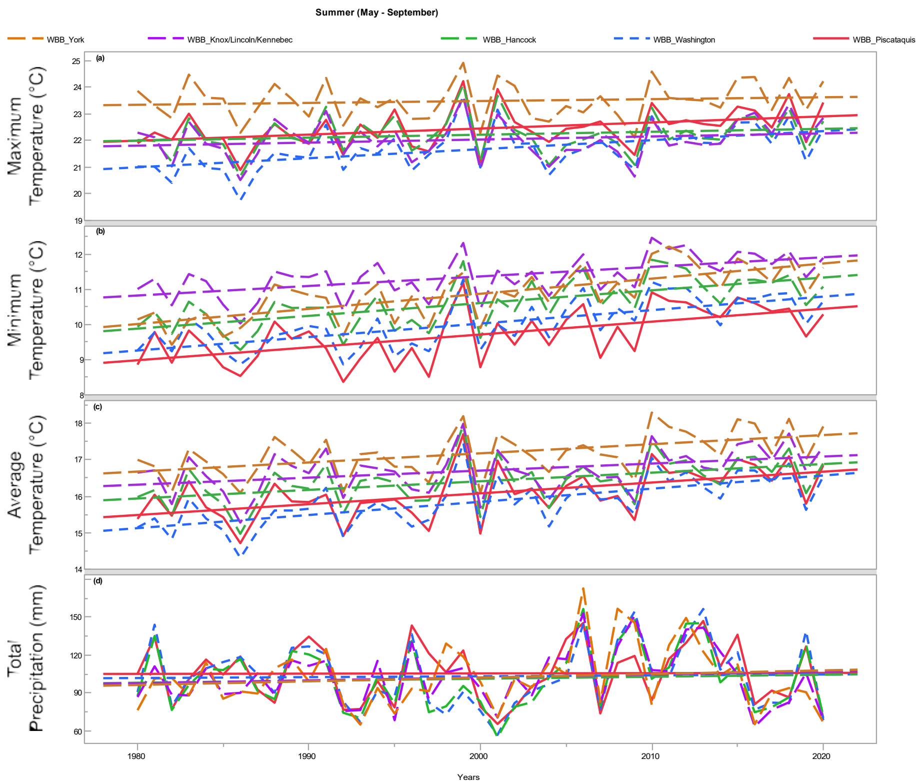

Figure 3.5 Historical (1980 to 2020) changes with fitted linear regression trendlines for the summer climate parameters: (**a**) maximum temperature, (**b**) minimum temperature, (**c**) average temperature, and (**d**) total precipitation, throughout the studied wild blueberry barrens (WBBs) in different counties from north-central (Piscataquis) and north-east (Washington, Hancock) to south-west (Knox, Lincoln, Kennebec, York) of Maine as shown in Fig. 3.1.

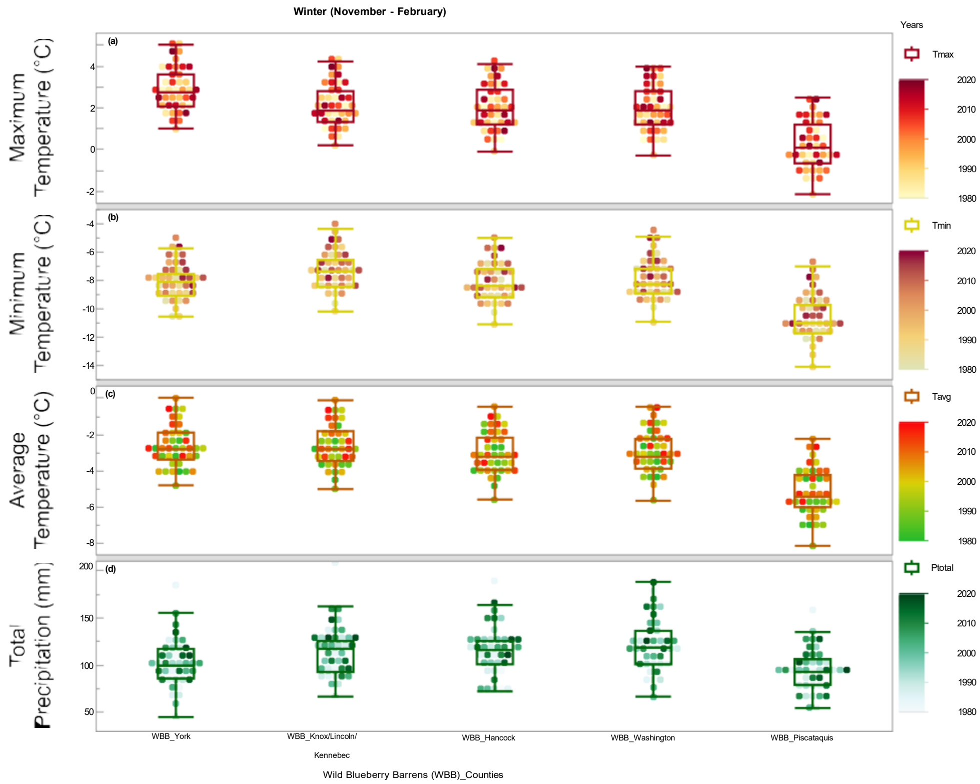

Figure 3.6 Comparison of historical (1980 to 2020) winter climate parameters: (**a**) maximum temperature, (**b**) minimum temperature, (**c**) average temperature, and (**d**) total precipitation among the studied wild blueberry barrens (WBBs) in different counties from north-central (Piscataquis) and north-east (Washington, Hancock) to south-west (Knox, Lincoln, Kennebec, York) of Maine as shown in Fig. 3.1.

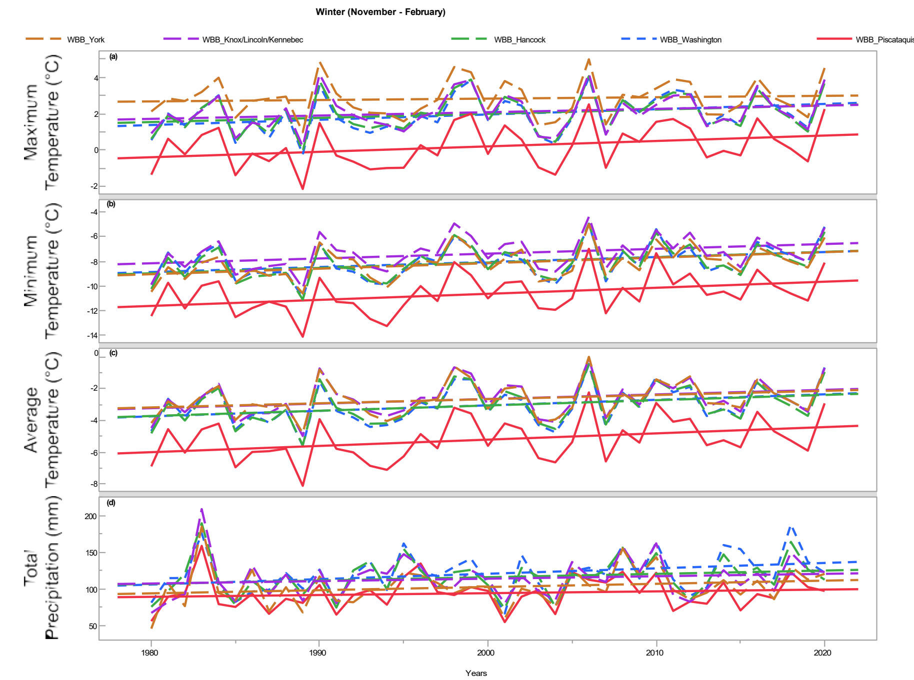

Figure 3.7 Historical (1980 to 2020) changes with fitted linear regression trendlines for the winter climate parameters: (**a**) maximum temperature, (**b**) minimum temperature, (**c**) average temperature, and (**d**) total precipitation, throughout the studied wild blueberry barrens (WBBs) in different counties from north-central (Piscataquis) and north-east (Washington, Hancock) to south-west (Knox, Lincoln, Kennebec, York) of Maine as shown in Fig. 3.1.

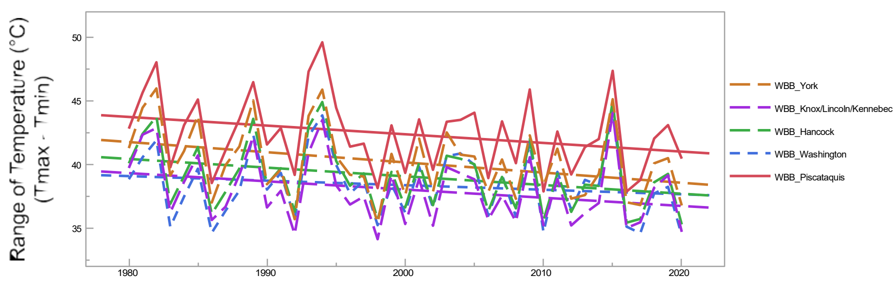

Figure 3.8 Historical (1980 to 2020) changes with fitted linear regression trendlines for the temperature range (difference between maximum temperature in summer and minimum temperature in winter throughout the studied wild blueberry barrens (WBBs) in different counties from north-central (Piscataquis) and north-east (Washington, Hancock) to south-west (Knox, Lincoln, Kennebec, York) of Maine as shown in Fig. 3.1.

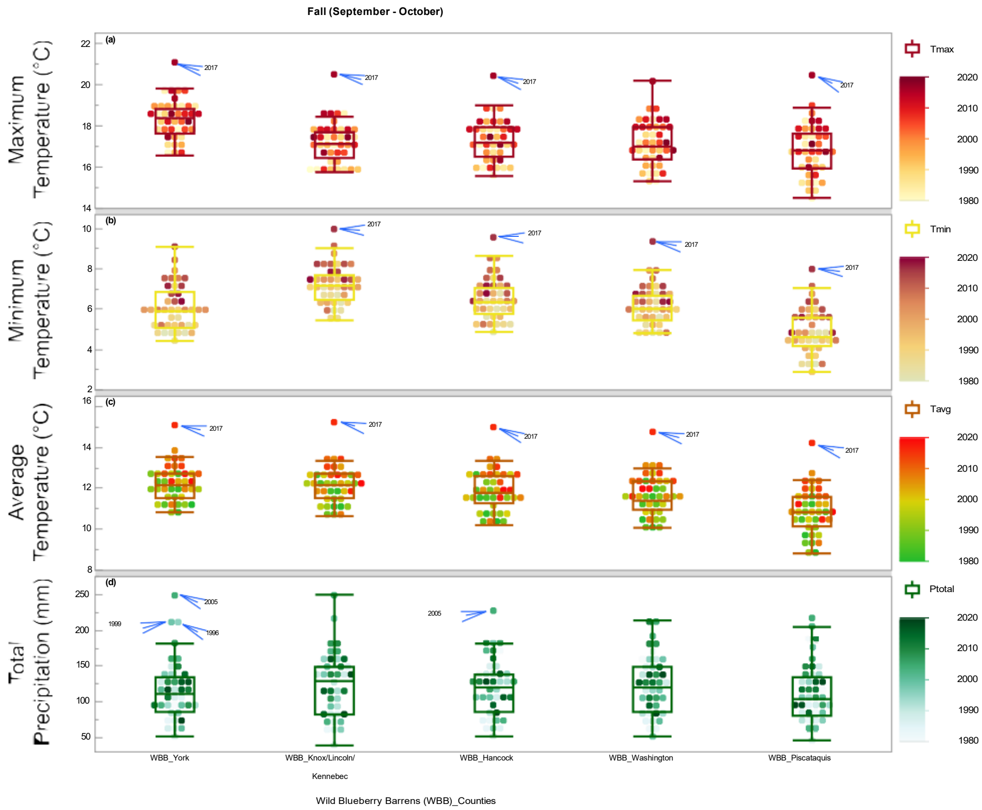

Figure 3.9 Comparison of historical (1980 to 2020) fall climate parameters: (**a**) maximum temperature, (**b**) minimum temperature, (**c**) average temperature, and (**d**) total precipitation among the studied wild blueberry barrens (WBBs) in different counties from north-central (Piscataquis) and north-east (Washington, Hancock) to south-west (Knox, Lincoln, Kennebec, York) of Maine as shown in Fig. 3.1.

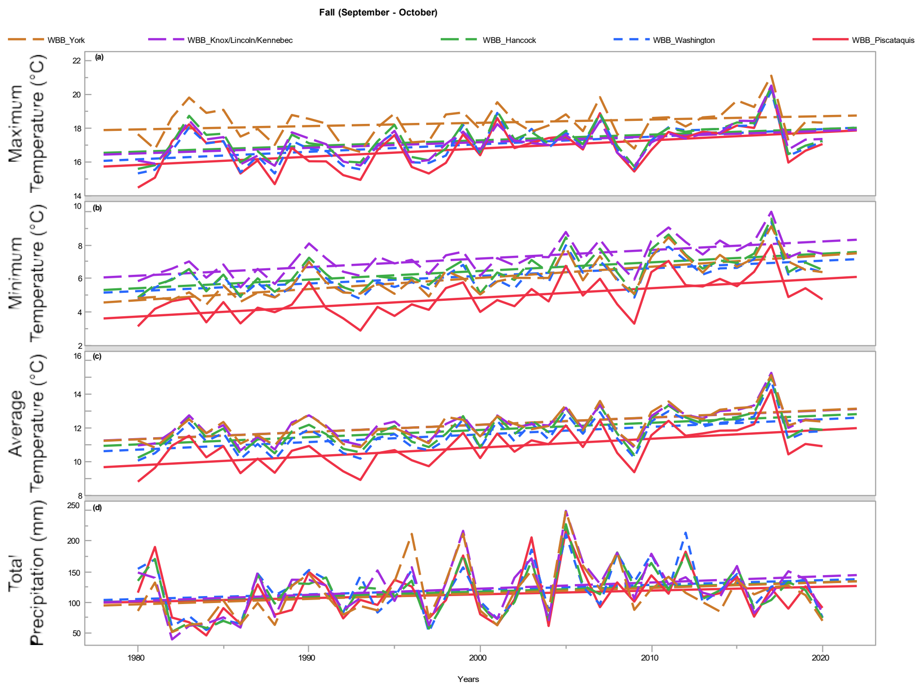

Figure 3.10 Historical (1980 to 2020) changes with fitted linear regression trendlines for the fall climate parameters: (**a**) maximum temperature, (**b**) minimum temperature, (**c**) average temperature, and (**d**) total precipitation, throughout the studied wild blueberry barrens (WBBs) in different counties from north-central (Piscataquis) and north-east (Washington, Hancock) to south-west (Knox, Lincoln, Kennebec, York) of Maine as shown in Fig. 3.1.

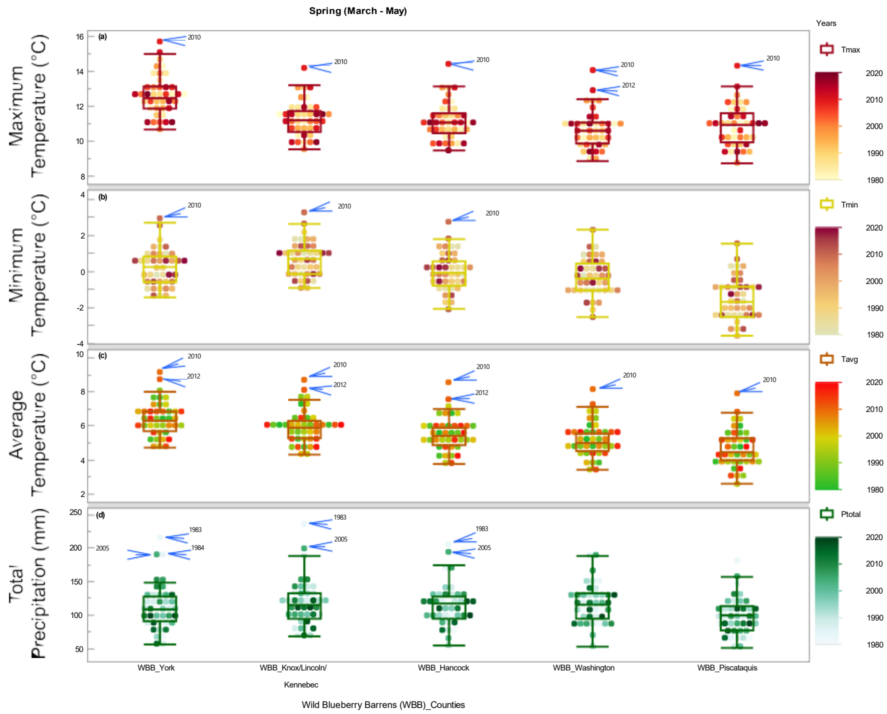

Figure 3.11 Comparison of historical (1980 to 2020) spring climate parameters: (**a**) maximum temperature, (**b**) minimum temperature, (**c**) average temperature, and (**d**) total precipitation among the studied wild blueberry barrens (WBBs) in different counties from north-central (Piscataquis) and north-east (Washington, Hancock) to south-west (Knox, Lincoln, Kennebec, York) of Maine as shown in Fig. 3.1.

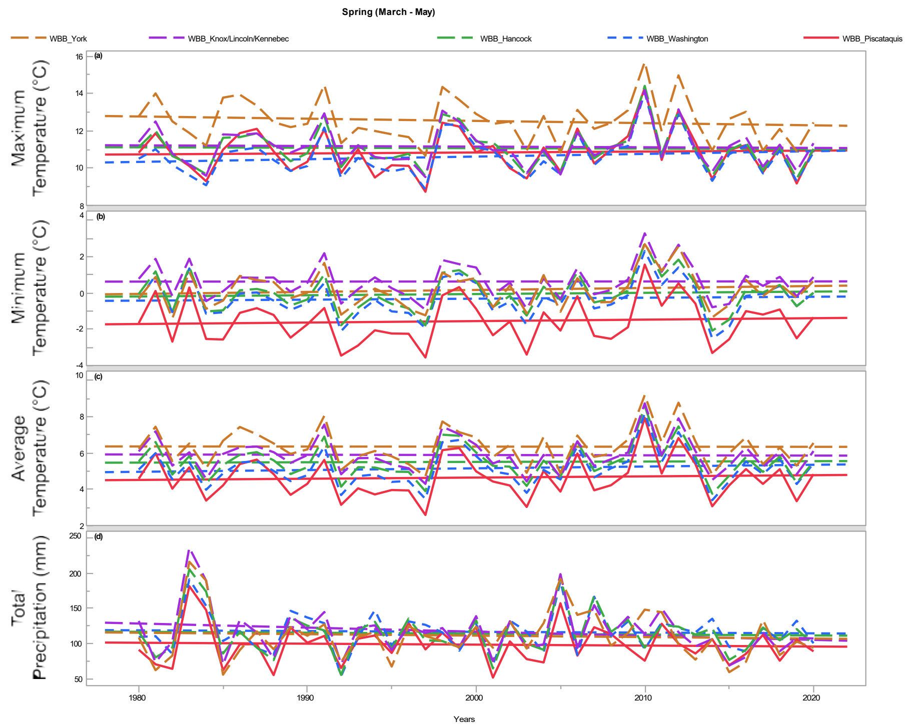

Figure 3.12 Historical (1980 to 2020) changes with fitted linear regression trendlines for the spring climate parameters: (**a**) maximum temperature, (**b**) minimum temperature, (**c**) average temperature, and (**d**) total precipitation, throughout the studied wild blueberry barrens (WBBs) in different counties from north-central (Piscataquis) and north-east (Washington, Hancock) to south-west (Knox, Lincoln, Kennebec, York) of Maine as shown in Fig. 3.1.

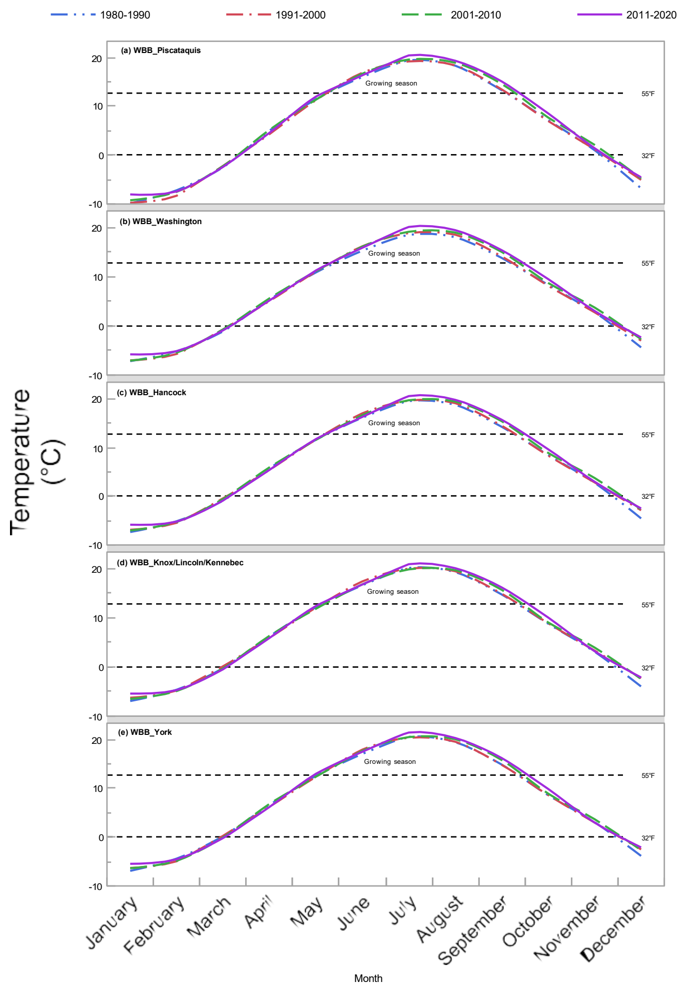

Figure 3.13 Annual average temperature cycle for the periods of 1980–1990, 1991–2000, 2001–2010, and 2011–2020 (represented by 4 lines of different colors and styles) in the studied wild blueberry barrens (WBBs) in (**a**) Piscataquis, (**b**) Washington, (**c**) Hancock, (**d**) Knox/Lincoln/Kennebec, (**e**) York counties of Maine as shown in Fig. 3.1. Dotted black lines indicate 32 °F (0 °C) and 55 °F (12.8 °C). Approximate last spring frost and first fall frost dates based on the 32 °F (0 °C) line, and growing season period based on the 55 °F (12.8 °C) line, are further detailed in Table 3.3.

Table 3.2 Correlation analysis of the increases in climate variables (increase in T_{max}, T_{min}, T_{avg}, P_{total}, and SWE) with the geographic factors (latitude, longitude, and distance from the coast) for the studied wild blueberry barrens of Maine (shown in Fig. 3.1) during different seasons at the significance level of $p < 0.05$ *, $p < 0.01$ **, and $p < 0.001$ ***.

Time Period/Seasons	Climate Variables	Latitude	Longitude	Distance from Coast
Annual (January–December)	Increase in T_{max}	0.15	**0.95 ***	**−0.63 **
	Increase in T_{min}	0.12	0.36	−0.31
	Increase in T_{avg}	0.11	**0.97***	**−0.59 **
	Increase in P_{total}	**−0.82 **	0.22	**−0.74 **
	Increase in SWE	−0.28	0.09	−0.19
Summer (May–September)	Increase in T_{max}	0.19	**0.93 **	**−0.58 *
	Increase in T_{min}	0.09	0.33	−0.23
	Increase in T_{avg}	0.14	**0.88 **	**−0.51 *
	Increase in P_{total}	**−0.82 **	0.32	**−0.73 **
Winter (November–February)	Increase in T_{max}	0.17	**0.91 **	**−0.54*
	Increase in T_{min}	0.12	**0.37*	−0.27
	Increase in T_{avg}	0.13	**0.90 **	**−0.53 *
	Increase in P_{total}	**−0.63 *	**0.54 *	**−0.64 *
Fall (September–October)	Increase in T_{max}	**0.34 *	**0.89 **	**−0.57 *
	Increase in T_{min}	0.17	**0.39 *	−0.26
	Increase in T_{avg}	**0.31 *	**0.86 **	**−0.55 *
	Increase in P_{total}	**−0.59 *	**0.57 *	**−0.62 *
Spring (March–May)	Increase in T_{max}	0.26	0.31	−0.28
	Increase in T_{min}	0.17	0.27	−0.24
	Increase in T_{avg}	0.22	0.33	−0.29
	Increase in P_{total}	−0.31	0.34	−0.33

Table 3.3 Approximate last spring frost dates, first fall frost dates, and growing season period during the periods of 1980–1990, 1991–2000, 2001–2010, and 2011–2020.

Wild Blueberry Barrens (WBB)_Counties	Period (Year)	Last Spring Frost	First Fall Frost	Growing Season Length and Period
WBB_Piscataquis in Figure 3.13a	1980–1990	25 March	17 November	116 days [23 May–17 September]
	1991–2000	26 March	19 November	116 days [23 May–17 September]
	2001–2010	23 March	23 November	124 days [23 May–25 September]
	2011–2020	26 March	21 November	131 days [19 May–28 September]
WBB_Washington in Figure 3.13b	1980–1990	20 March	24 November	114 days [28 May–20 September]
	1991–2000	18 March	29 November	119 days [25 May–22 September]
	2001–2010	17 March	04 December	124 days [25 May–27 September]
	2011–2020	19 March	04 December	126 days [25 May–29 September]
WBB_Hancock in Figure 3.13c	1980–1990	20 March	25 November	121 days [24 May–23 September]
	1991–2000	15 March	30 November	120 days [26 May–24 September]
	2001–2010	16 March	04 December	121 days [25 May–27 September]
	2011–2020	20 March	05 December	124 days [25 May–30 September]
WBB_Knox/Lincoln/ Kennebec in Figure 3.13d	1980–1990	16 March	27 November	127 days [18 May–23 September]
	1991–2000	14 March	04 December	128 days [18 May–24 September]
	2001–2010	16 March	04 December	129 days [20 May–27 September]
	2011–2020	19 March	04 December	136 days [16 May–02 October]
WBB_York in Figure 3.13e	1980–1990	14 March	28 November	131 days [15 May–24 September]
	1991–2000	14 March	03 December	130 days [16 May–24 September]
	2001–2010	15 March	04 December	134 days [16 May–28 September]
	2011–2020	17 March	04 December	141 days [13 May–02 October]

3.4.2.1. Summer (May–September) Climate in the Barrens

Based on the comparison of temperature box plots (Fig. 3.4a–c), summer maximum temperatures of the barrens were the highest in York County and slightly higher in Piscataquis County compared to other studied coastal counties (Fig. 3.4a). However, summer minimum temperatures and average temperatures were higher for the barrens in the studied coastal counties farther south-west from Washington to York (Fig. 3.4a–c). In contrast, summer precipitation (~ 50 to 180 mm) was similar for the barrens in all studied counties (Fig. 3.4d).

Historical summer temperature changing trends (Fig. 3.5a–c) followed the annual temperature changing trends (Fig. 3.3a–c) for the studied barrens. The summer maximum temperature of the wild blueberry barrens significantly increased in Piscataquis (1 °C) and Washington (1.5 °C) counties, compared to other studied coastal counties farther south-west (Hancock to York), where temperature increment rates (0.2–0.6 °C) were not significant (Fig. 3.5a, Table 3.2 and Table B.1). In contrast, the summer minimum temperature of the barrens significantly increased (1.4–1.9 °C) in all studied counties (Fig. 3.5b, Table 3.2 and Table B.1). Consequently, the summer average temperature (Fig. 3.5c, Table 3.2 and Table B.1) significantly increased (1–1.6 °C) in the barrens of all studied counties but the increase rate was higher in Piscataquis and Washington counties (1.3–1.6 °C) compared to other studied counties (1–1.1 °C). In contrast to the temperature changes, historical summer precipitation neither increased nor decreased in the barrens of the studied counties (Fig. 3.5d, Table 3.2 and Table B.1).

3.4.2.2. Winter (November–February) Climate in the Barrens

Based on the comparison of temperature box plots (Fig. 3.6a–c), winter temperatures were the lowest in the barrens in Piscataquis County compared to the similar higher temperatures observed in other studied coastal counties farther south-west (starting from Washington to York) (Fig. 3.6a–c). In contrast, the winter precipitation was slightly lower in the barrens in Piscataquis County (range: 50–130 mm) and slightly higher in Washington County (range: 70–190 mm) compared to other studied coastal counties (range: 50–170 mm) farther south-west (Hancock to York) (Fig. 3.6d).

Historical winter temperature changing trends (Fig. 3.7a–c) followed the summer temperature changing trends (Fig. 3.5a–c), whereas temperature increasing rates were higher in the winter than in the summer (Table B.1). Winter maximum temperatures increased in the barrens with a marginal significance (p = 0.05) in Piscataquis (1.3 °C) and Washington (1 °C) counties compared to other coastal counties (Hancock to York), where temperature increment rates (0.4–0.9 °C) were not significant (Fig. 3.7a, Table B.1). In contrast, winter minimum temperatures of the barrens significantly increased (1.9–2.1 °C) in all counties (Fig. 3.7b, Table B.1). Winter average temperatures (Fig. 3.7c, Table B.1) of the barrens significantly increased (1.5–1.7 °C) in Piscataquis, Washington, and Hancock counties compared to other coastal counties farther south-west (1.3 °C). Similar to the increasing temperature trends, the historical winter precipitation increased (20–55 mm total per winter) in the barrens of all studied counties, where the increasing trend was only significant for Washington County (Fig. 3.7d, Table B.1).

In agreement with the warmer summer and warmest winter trends, the difference between the summer maximum and winter minimum temperatures significantly decreased in the barrens of the studied coastal counties farther south-west (Hancock to York) compared to Washington and Piscataquis Counties (Fig. 3.8, Table 3.2). The reduction rate in temperature range was the highest in York County (3.6 °C) and lowest in Washington County (1.5 °C), compared to other studied counties (2.9 – 3 °C) (Fig. 3.8, Table 3.2).

3.4.2.3. Fall (September–October) Climate in the Barrens

Based on the comparison of temperature box plots (Fig. 3.9a–c), fall temperatures were slightly lower in the barrens in Piscataquis County compared to other studied coastal counties (Washington to York) with almost similar temperatures. However, higher maximum, minimum, and average fall temperatures as outliers in the year 2017 were found in the barrens of all studied counties, which represent an abnormally warmer fall outside of the historical temperature range (Fig. 3.9a–c). In contrast, fall precipitation was similar in the barrens of all studied counties, ranging from ~50 to 250 mm (Fig. 3.9d), which was slightly higher than the summer (~50 to 180 mm) and winter (~50 to 190 mm) precipitation ranges. Interestingly, higher precipitation than the historical precipitation range was observed in the barrens towards the south-west counties (Hancock in 2005 and York in 1995, 1999, 2005) during a few random years (outliers in Fig. 3.9d).

Historical fall temperatures of the wild blueberry barrens (Fig. 3.10a–c) significantly increased in all studied counties, where temperature increase rates were higher than the rates of summer (Fig. 3.5a–c) and winter (Fig. 3.7a–c, Table 3.2 and Table B.1). Fall maximum temperature increase rates were significantly higher in the barrens in Piscataquis (2.2 °C) and Washington (2.1 °C) counties compared to the rates (0.9–1.5 °C) in other studied coastal counties farther south-west (Hancock to York). Fall minimum temperature increase rates were significantly higher in the barrens of all studied counties and even higher (2–2.9 °C) than the fall maximum temperature increase rates (Fig. 3.10b, Table 3.2 and Table B.1). Consequently, the fall average temperature significantly increased (1.9–2.35 °C) in the barrens of all studied counties (Fig. 3.10b, Table 3.2 and Table B.1). In contrast, historical fall precipitation increased (20–55 mm total per fall) in the barrens of all studied counties, but the increasing trend was not significant and there was no significant difference among counties (Fig. 3.10b, Table 3.2 and Table B.1).

3.4.2.4. Spring (March–May) Climate in the Barrens

Based on the comparison of temperature box plots (Fig. 3.11a–c), spring maximum temperatures were the highest in the barrens in York County compared to other studied counties with similar maximum temperatures (Fig. 3.11a). Spring minimum and average temperatures were slightly lower in the barrens in Piscataquis County, whereas temperatures were higher in other studied coastal counties farther south-west from Washington to York (Fig. 3.11b,c). Additionally, outliers of higher maximum, minimum, and average fall temperatures in the years 2010 and 2012 were found in the barrens of all studied counties, which represent abnormally warmer springs outside of the historical temperature range (Fig. 3.11a–c). In contrast, spring precipitation was similar (ranging from ~50 to 200 mm) for the barrens in all studied counties (Fig. 3.11d). However, higher precipitation than the historical precipitation range was observed in the barrens towards the south-west counties (Hancock to York in 1983 and 2005) during a few random years (outliers in Fig. 3.11d). In contrast to the historical temperature changes during other seasons, historical spring temperature and precipitation trends did not show any changes in the wild blueberry barrens from any of the studied counties (Fig. 3.12, Table 3.2 and Table B.1).

3.4.2.5. Growing Season in the Barrens

The growing season extended consistently towards the fall as the final dates of the season were delayed from the third week of September to early October after summer (Fig. 3.13, Table 3.3). In contrast, the growing season extension towards the spring was inconsistent, as the starting dates of the growing season erratically fluctuated from mid-May to late May over the past 41 years (Fig. 3.13, Table 3.3). In agreement with these trends, first fall frost dates shifted gradually from the third week of November to early December, whereas the last spring frost dates were random from mid-March to late-March over the past 41 years. Moreover, in agreement with the observed higher temperatures in the barrens towards the south-west (Hancock to York), they were also found to have longer growing seasons than in the barrens towards the north-east (Piscataquis and Washington) (Fig. 3.1 and Fig. B.1, Table 3.3).

3.5 Discussion

Our study found that different seasons, day and night times, and different regions showed different climate change patterns over the past 41 years, which has important implications for wild blueberry management. Nighttime is warming faster than daytime in the wild blueberry barrens of Maine, both annually and seasonally. We also found that historical temperatures significantly increased annually driven by the highest rates of warming in the fall and winter seasons followed by summer in the blueberry barrens of Maine. Consequently, our study found that the growing season has been extending towards the fall season, but the extension has been inconsistent towards the spring season. In contrast, precipitation was found to have only increased significantly in Washington County barrens annually, which was driven by the significant precipitation increase in the winter season. In agreement with such rising temperatures and precipitation in the winter season, there have been no significant changes in snow cover on the wild blueberry barrens of Maine. Although temperatures have not increased significantly in the spring, outlier temperatures in some years during spring (2010, 2012) found in all counties indicate that sudden and abnormally warmer springs can happen. Moreover, the range of temperatures (difference between maximum temperature in summer and minimum temperature in winter) significantly decreased, implying that the temperature variations among different seasons declined over time. In terms of variations among the counties, barrens in Piscataquis (north-central) and Washington (north-east) counties experienced lower temperatures than other studied coastal counties farther south-west (Hancock to York). On the contrary, the rates of maximum temperature increase in the barrens of those counties (Hancock to York) with higher temperatures were significantly lower in the summer and winter. Our study is the first to access and report diurnal, seasonal, annual, and spatial climate patterns for the wild lowbush blueberry barrens of Maine, USA. These findings, along with their potential effects discussed below, will better inform the wild blueberry researchers and growers of Maine to be prepared to manage this crop accordingly after it has faced drastic climate change over recent decades.

3.5.1. Changes in Daytime and Nighttime Temperatures

During the diel cycle, minimum nighttime temperatures increased faster than maximum daytime temperatures in the wild blueberry barrens of Maine in summer, fall, and winter, which is a worldwide pattern (Davy et al. 2017, Cox et al. 2020). This phenomenon may disturb the balance in physiological functions and development of plants, adversely affecting their carbon assimilation, storage, and respiration (Cox et al. 2020, Peng et al. 2013, Peraudeau et al. 2015, Zhang et al. 2016). This is because photosynthesis (carbon assimilation) in plants happens during the daytime only, which is affected by the maximum daytime temperature, but respiration occurs throughout the day and night, which is affected by both daytime maximum and nighttime minimum temperatures (Peraudeau et al. 2015, Zhang et al. 2016, Atkin et al. 2013). Moreover, respiration is more sensitive to temperature compared to photosynthesis (Peraudeau et al. 2015, Zhang et al. 2016). Therefore, the increase in respirational loss of carbon may be higher than the increase in photosynthesis under warming. In fact, the respiration of forest and crop systems has been proven to be enhanced by both warmer days and nights (Peng et al. 2013, Peraudeau et al. 2015, Zhang et al. 2016, Atkin et al. 2013). As a result, warmer days and nights may adversely affect the carbon cycle, diminishing the net carbon assimilation of the wild blueberry crop system. In order to verify this, both respiration rates and photosynthetic rates need to be measured and studied under rising temperatures. This adverse effect related to climate change may be crucial for the yield of this important commercial crop in Maine.

3.5.2. Seasonal Variations in Climate Change and Implications

In terms of rising trends in seasonal temperatures, temperatures were found to increase faster in the fall and winter seasons than in the summer for the wild blueberry barrens of Maine. This pattern agrees with the warming and ice melting in the arctic. A significant amount of ice melting occurred, as a difference of 50% was observed in the sea ice covering the ocean between 1980 and 2016 at the end of the summer (Birkel and Mayewski 2018, Francis 2015). This greater warming in the fall season has also led to the extension of the growing season for the wild blueberries towards the fall. Unusually warmer fall seasons have shown delayed leaf senescence (Stuble et al. 2021), early flower initiation, and fall bloom in plants including temperate species (Khanduri et al. 2008, Rai et al. 2015, Sherry et al. 2007, Simonson et al. 2022) such as wild blueberries. First fall frost in the coastal and interior climate regions of Maine was previously shown to be around October–November (Kukal and Irmak 2018), which has been delayed up to early December since 1980 in the wild blueberry barrens. Such a lengthy growing season has increased the risk of frost damage for the wild blueberry leaves and buds. Warmer winter and spring seasons have been shown to advance the development and maturity of plant stages such as bud break, flowering, and leafing out before the last and late spring frost. This has caused considerable damage to the plants, directly affecting their yield (https://umaine.edu/climate-ag/farm-response-changing-weather/, Kukal and Irmak 2018, Drummond and Yarborough 2012, Stuble et al. 2021, Khanduri et al. 2008).

In contrast to the climate observations of coastal Maine and the overall state of Maine (Fernandez et al. 2020, Birkel and Mayewski 2018), our study did not show significant increases in historical spring temperatures. However, abnormally higher temperatures observed from the barrens of all studied counties in the last decade (e.g., 2010 and 2012 springs) are rather concerning. This is because such sudden warmer temperatures in the spring can trigger the plants to recover from the winter dormancy and start development (bud breaks and flowering) (Drummond and Yarborough 2012). Consequently, they may experience damages from late spring frost events, which have been observed to be more frequent within the last decade (2010–2020) and could potentially continue in Maine (Fernandez et al. 2020, Birkel and Mayewski 2018, https://umaine.edu/climate-ag/farm-response-changing-weather/, Kukal and Irmak 2018, Drummond and Yarborough 2012, Stuble et al. 2021, Khanduri et al. 2008). Furthermore, temperate species, such as wild blueberries, experiencing sudden warm and wet winters with no increase in snow cover may suffer from winter damage during their hardening and de-hardening processes with insufficient protection from snow-pack (Fernandez et al. 2020, Birkel and Mayewski 2018, https://umaine.edu/climate-ag/farm-response-changing-weather/, Rai et al. 2015, Pearson 2019, Vitasse et al. 2014, Wildung and Sargent 1988). In agreement with all seasons becoming warmer, the temperature difference between the summer maximum temperature and winter minimum temperature has shortened significantly and quickly. Such considerable change places wild blueberries that rely on a two-year cycle and a specific climate at risk due to an imbalance in certain seasonal temperature variations, which is required for a balanced plant life cycle (Kukal and Irmak 2018, Stuble et al. 2021, Khanduri et al. 2008, Rai et al. 2015, Pearson 2019, Vitasse et al. 2014, Wildung and Sargent 1988).

3.5.3. Spatial Variations in Climate Change and Implications

In terms of spatial variations, barrens in the warmer counties towards the south-west (Hancock to York) experienced a slower rate in temperature increase compared to the counties towards the north-east (Washington and Piscataquis). This phenomenon, in which barrens at higher latitudes and longitudes are warming faster (Table 3.2), agrees with previous studies undertaken both globally (Screen 2014) and locally (Tasnim et al. 2021). The reason for this, as explained by Screen (Screen 2014), is that the wind from the north, having negative temperature anomalies (colder days), is warming up more rapidly than the wind from the south, having positive temperature anomalies (warmer days). This has been a global and historical occurrence as the air temperature is significantly affected by the corresponding wind direction (Screen 2014).

Overall, wild blueberry barrens everywhere in Maine continue to experience a warmer climate over time with no additional rainfall in summer. Temperate crops such as wild blueberries can potentially thrive in warmer summers but, at the same time, more soil moisture either from natural rainfall or irrigation would be required, which is crucial and necessary for survival (Birkel and Mayewski 2018, Barai et al. 2021). However, despite the lack of a clear summer precipitation trend over 1980–2020, we noted the occurrence of a particularly wet decade of 2005–2014, following dryness in the early 2000s, and preceding three dry years of 2016–2018 (Birkel and Mayewski 2018, Simonson et al. 2022). These phenomena, along with the historically increasing heavy precipitation events in Maine (MCC STS 2020), indicate an intensification of the hydrologic cycle. This would potentially result in more total rainfall from heavy precipitation events, interspersed with a period of dryness during the growing season. Such climate trends are particularly unhelpful for the wild blueberry system as the coarse-grained soil would quickly drain the water from heavy rainfall and wild blueberries would not get enough soil moisture to grow during the dry periods. Hence, it is typically recommended to irrigate wild blueberries with a low volume of water more frequently (https://extension.umaine.edu/blueberries/factsheets/irrigation/guide-to-efficient-irrigation-of-the-wild-blueberry/). Irrigation and soil management are particularly crucial because the wild blueberry barrens in Maine have experienced frequent drought events during the growing season (Barai et al. 2021, MCC STS 2020).

Furthermore, warmer winters may potentially hurt wild blueberry production more than warmer summers in a temperate region such as Maine, as a previous study showed adverse effects of winter warming on temperate grasslands rather than summer warming (Kreyling et al. 2019). For instance, soil respiration was higher under winter warming than in summer. Moreover, plant roots and microorganisms suffered from greater frost damage during a few days with a sudden extreme drop in temperature due to less snow cover and thermal insulation under warming (Kreyling et al. 2019). Such winter warming has been shown to be more intense at higher latitudes (Kreyling et al. 2019, Screen 2014). Therefore, the barrens located in the regions towards the north-east may be more vulnerable to the winter climate changes compared to summer changes. However, our study showed that warming rates are more dependent on the longitudinal directions, and on the distances of the barrens from the coast, than on the latitudinal directions during all seasons (Table 3.2).

Such spatial climate variations along with their variations and extremes during different seasons imply that the wild blueberry barrens also need attention during seasons in addition to summer. In fact, to avoid adverse seasonal climate effects and adapt to the drastic seasonal variations, management strategies and actions are already being considered for different cultivated crops in Maine (https://umaine.edu/climate-ag/farm-response-changing-weather/). However, it is rather complicated and difficult to apply those precautionary management strategies (i.e., double cropping, crop cover and rotation, etc., detailed in (https://umaine.edu/climate-ag/farm-response-changing-weather/)) for the naturally growing wild blueberries, as those strategies are more relevant and suitable for cultivated crops. Therefore, unique strategies need to be developed and tested for this crop, prioritizing seasonal variations and their immediate irreversible effects. For instance, actions need to be planned to protect this crop from irreversible stress due to summer moisture deficits during droughts, erosion from heavy precipitation events, warmer winters, and spring frost damage, which have become more frequent.

CHAPTER 4

INFLUENCE OF SOIL AMENDMENTS ON SOIL WATER AVAILABILITY AND THE RESPONSE OF WILD BLUEBERRIES TO WARMER CLIMATE

4.1 Abstract

Wild blueberry, a crucial agricultural industry of northeastern North America, has been experiencing an unprecedented threat of warming due to climate change. Under these conditions, a preliminary study has shown that warming due to climate change increased crop and soil water loss and thus decreased soil water content and nutrient availability in wild blueberry fields. Research is needed to investigate potential mitigation techniques for sustaining the production of wild blueberries in drier soil under a warmer climate. Therefore, in 2021 and 2022, we investigated the effects of warming and soil amendments (softwood bark mulch and biochar-compost mix) on the physiology, growth, and yield of six different genotypes of wild lowbush blueberry plants at two different locations in Maine. Our results indicate that physiological performance and growth of wild blueberry plants followed by berry yield and weight were higher under a warmer climate. In addition, the studied biochar-compost mix retained more moisture in the soil, which resulted in better physiological plant performance whereas the 0.5" layer of mulch amendment was not sufficient. Moreover, the plants growing under the warmer environment along with the biochar-compost mix amendment had the highest fruit production. Therefore, it might be beneficial to use a soil amendment like biochar-compost mix to avoid crop water stress under the predicted hotter and drier summers. It is to be noted that, prior to providing this recommendation to the growers, more crop cycles need to be investigated at different wild blueberry production regions.

4.2 Introduction

Global climate change has already imposed threats to crop production worldwide while warming, increased climate variability and extremes have also been intensifying (IPCC 2021). Wild lowbush blueberry (*Vaccinium angustifolium*) is an economically important crop in North America, with an annual production of over 100 million pounds (Brazelton 2013). The cropped areas of wild blueberry in Eastern

Canada and the Northeastern United States are expected to expand to meet the growing demand for this fruit. In fact, the wild blueberry crop is also an important crop economically and culturally in North America, especially in Maine, where the crop is grown commercially on 44,000 acres of land. However, wild blueberry production in this region has been threatened by climate change in recent years including warming (Tasnim et al. 2021) and frequent drought (Barai et al. 2021). Previous investigation by Tasnim et al. (2020) using open-top heated chambers to create a warmer environment showed that warming has changed the growth pattern of wild blueberries. Moreover, the warmer environment had adverse effects on wild blueberry physiology due to increased crop water loss and decreased soil water and nutrient availability (Tasnim et al. 2020), which could potentially affect wild blueberry production. Wild blueberry plants were thought to be drought-resistant (Percival et al. 2003, Glass et al. 2005), but their fruit production could be highly dependent on the occurrence of long-term drought (Barai et al. 2021). The importance of water availability on wild blueberry production is supported by the fact that the yield of this perennial crop can be significantly influenced by the soil water availability of the previous four years (Barai et al. 2021). Therefore, to sustain wild blueberry production in the future with warmer and drier summers, management techniques need to be developed and tested to mitigate potential adverse effects of dry soils on the blueberry yield under warmer climates.

Theoretically, any adverse effects of warmer temperatures and dry soils can be mitigated with irrigation. However, irrigation systems are costly and wild blueberry crops typically grow in acidic and sandy loam soils with low water water-holding capacity that require frequent nutrient inputs and pH management to maintain optimal yield. Because of the low water use efficiency in this crop system, wild blueberry fields are typically irrigated with low amount of water in higher frequencies rather than with a higher amount of water at a time (https://extension.umaine.edu/blueberries/factsheets/irrigation/guide-to-efficient-irrigation-of-the-wild-blueberry/). In addition, the frequency of intense rainfall events is increasing despite the more frequent drier summers (MCC STS 2020, Tasnim et al. 2022). Such intense weather patterns will further disrupt the wild blueberry agricultural system in Maine. Under such changing climate, the use of soil amendments enhancing the water holding capacity of wild blueberry soil could be a useful technique as an alternative to costly irrigation systems.

The use of soil amendments, such as mulch and biochar-compost mix could be a sustainable approach for improving soil water and nutrient holding capacity and plant performance in wild blueberry fields. Mulching can maintain moderate soil temperatures and retain soil water and nutrients while biochar-compost mix can improve soil water holding and nutrient availability (Hunt et al. 2010, Mukherjee and Zimmerman 2013, Liang et al. 2014, Li et al. 2021, Tasnim et al. 2024). Since Maine has vast resources of forest (wood) residuals, some companies are producing biofuel from wood pellets through pyrolysis. Biochar is produced as a waste-byproduct of this process and can be potentially available to enhance wild blueberry productivity in Maine. However, the effects of those soil amendments (mulch, biochar-compost) on wild blueberry crop performance might vary compared to the previous studies conducted on other plant species because plant responses to biochar amendments depend on soil type and quality, the forms of biochar, rates, and plant physiological status. Therefore, a comprehensive investigation is needed to reveal the interactive effects of warming and soil amendments on the physiology, growth, and yield of wild lowbush blueberry plants is crucial for the sustainability of this unique agricultural system.

The main objective of this study was to evaluate the effects of warming and soil amendments (mulch and biochar-compost mix) on the physiology, growth, and yield of wild lowbush blueberry plants. In order to fulfill this broad objective, we conducted a study to answer the following questions: (1) Will warming enhance or diminish the physiological performance, growth and yield of wild lowbush blueberries? (2) How will the soil amendments (mulch and biochar-compost mix) interact with wild blueberry soils under a warmer environment? and (3) How will wild blueberry plants respond to the separate and combined effects of warming and biochar-compost mix application? This study will provide valuable information on the management of wild lowbush blueberry crops under warmer and drier climatic and soil conditions and contribute to its sustainability and resilience.

4.3 Methodology

4.3.1. Study area, Experimental Design, Application Materials and Methods

We selected three different wild lowbush blueberry (*Vaccinium angustifolium*) genets in each of two locations in Maine: Blueberry Hill Farm in Jonesboro, ME, and Wyman's wild blueberry field in Deblois, ME, USA. Within each of six genotypes, we marked out five plots (Fig. 3.1a) in early June in the vegetative year (2021). Notably, the two selected fields are under different management regimes. Wyman's blueberry field has been frequently irrigated and fertilized, while Blueberry hill farm field is neither irrigated nor fertilized. However, for this experiment, we did not use irrigation in the Wyman's field to minimize soil water differences between the fields at Deblois and Jonesboro throughout this experiment. We collected soil samples from each of the six genets separately and sent them for a comprehensive soil test (Table 3.1) to the Analytical Soil Testing Laboratory, University of Maine, Orono, ME. The soil sample was collected up to a 6" depth from the soil surface. The soil pH and organic matter of the Deblois field soil were 3.7 - 4.2 and 9.3 - 20.5%, respectively, whereas the soil pH and organic matter of the Blueberry hill farm field soil were 4.6 - 5.2 and 4.5 - 9.5%, respectively.

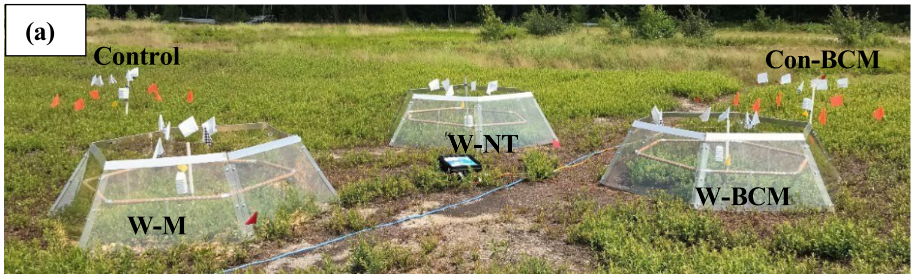

Figure 4.1 The open-top chambers (OTC) and control plots used for this study in the wild blueberry field: (a) Two control flagged plots with no warming chamber (one plot with no treatment presented as "Control"; one plot with biochar-compost mix on the soil surface presented as "Con-BCM") and three OTCs (warming chamber with no treatment, warming chamber with mulch, and warming chamber with biochar-compost mix on the soil surface presented as "W-NT", "W-M" and "W-BCM", respectively; (b) Example of control flagged plot with no OTC; (c) Schematic diagram of OTC with heating (to supply additional heat) [Hexagon dimensions: 100 cm (ground); 55 x 70 cm (top); 100 cm (radius)] (Tasnim et al. 2020).

Table 4.1. Physical and chemical properties of wild blueberry soils for the studied six genotypes, UMaine Compost and recommended optimum ranges for wild blueberry soil on May 2021 (prior to application of warming and soil amendments) from the studied wild blueberry fields in Deblois and Jonesboro, ME.

Physical & Chemical Properties	Optimum range	UMaine Compost	BHF genets			Deblois genets		
			C1	C2	C3	C4	C5	C6
pH	4.0-4.5	8	4.6	5.2	4.9	4.1	4.2	3.7
Organic matter (%)	5-8	79.7	9.9	4.5	5.6	16.3	9.3	20.5
CEC (me/100 g)	>5	68.7	6.2	2.7	4.1	6.3	4.9	7.8
Nitrate-N (ppm)	20-30	849	1	1	< 0.5	< 0.5	< 0.5	< 0.5
Ammonium-N (ppm)	<10	15	3	5	3	6	8	22
Phosphorous (lb/A)	10-40	6335	12.9	8.4	7.6	25.3	17.8	41.1
Potassium (% saturation)	2.1 - 3.0	57.2	1.7	3.2	1.9	5.4	3.3	4.2
Calcium (% saturation)	20 - 30	19.7	28.8	26.9	32	12.5	6.3	17
Magnesium (% saturation)	5-10	23.1	9.2	9.3	8.7	6.2	4.3	5.6
Sulfur (ppm)	>50	518	24	44	53	131	126	113
Copper (ppm)	0.25 - 0.6	0.7	0.28	0.1	0.11	0.16	0.31	0.23
Iron (ppm)	6-10	23.5	51.4	35.8	49.1	31.9	30.6	25.3
Manganese (ppm)	4-8	97.4	12.4	6	8.2	17.1	7.6	18.8
Zinc (ppm)	1-2	3.3	3.2	1.2	2.2	2.6	1.1	3
Boron (ppm)	0.5 - 1.2	8.7	0.3	0.3	0.3	0.3	0.4	0.4
Sodium (ppm)	< 200	4725	N/A	N/A	N/A	N/A	N/A	N/A

Within each genotype, we marked two open plots with flags (Fig. 4.1b) that had no warming chamber: one plot was not treated (referred to as "Control"), and the other open plot was treated with biochar-compost mix (referred to as "Con-BCM"). Three other plots in each genotype had open-top chambers (OTC) with a heating system inside (Figs. 4.1a & 4.1c). Based on a preliminary investigation of wild blueberry crops by Tasnim et al. (2020), this warming chamber would increase the ambient temperature by 3-5 °C. Out of these three chambers in Fig. 4.1a, the soil surface inside one chamber was not treated (referred to as "W-NT") and the soil surface inside two other chambers was treated with softwood bark mulch (referred to as "W-M") and biochar-compost mix (referred to as "W-BCM"), respectively. Here it is to be noted that, we did not have a treatment of mulch in an open plot because of lack of space in each genotype. We applied 0.5” (1.27 cm) deep softwood bark mulch on the soil surface and applied the biochar-compost mix (ratio of 1:1) at a rate of 7.5 yd^3/A ($1.4x10^{-3}$ m^3/m^2). Biochar was provided by the Maine wood pellets co., and also contained ash. After receiving the biochar at the University of Maine, we removed the ash from the biochar by mechanical sieving. Compost was provided by the University of Maine composting facility. At the time of collecting and using that compost, we also sent a sample of compost for comprehensive testing to the Analytical Soil Testing Laboratory, University of Maine, Orono, ME.

4.3.2. Data Collection and Measurement Methods

In mid-June 2021, we installed weather stations in the middle of each plot (Fig. 4.1) for real-time monitoring of the atmospheric temperature and relative humidity using Watchdog 1000 series micro stations (Spectrum Technologies, Inc, Aurora, IL 60504) and HOBO weather stations (ONSET Computer Corporation, Bourne, MA). We marked six random stems in each plot to monitor stem length, leaf number, and chlorophyll concentrations every two weeks from mid-June to November 2021. During this time, we also measured soil moisture in each of the plots using a Fieldscout TDR 150 soil moisture meter (Fieldscout TDR 150, Spectrum Technologies Inc.) at 6 random locations throughout each plot. Chlorophyll concentration was measured by a CCM-200 plus chlorophyll content meter (Opti-Sciences Inc., Hudson, NH, USA). We also conducted gas-exchange measurements on three random plants from each plot using a portable photosynthetic measurement system (li-6800; Li-Cor Biosciences, Lincoln, NE, USA) on a sunny day in mid-July between 10:00 and 15:00 h solar time at a photosynthetic photon flux density of 1500 $\mu mol.m^{-2}.s^{-1}$. To quantify crop water status, we collected one wild blueberry stem from each plot at midday 12:00-12:30 h solar time and measured midday leaf water potential by a leaf pressure chamber (PMS Inc., Albany, OR, USA). We conducted this measurement twice: once in July and once in August 2021. Once on 18 July and once on 19 August 2021, we collected six random stems from each plot to quantify leaf number, leaf area, leaf dry biomass, and leaf nutrition. We measured leaf area using an LI-3000A area meter (Li-Cor, Lincoln, NE, USA), then the leaves were oven-dried at 70ºC to constant mass and weighed. Leaf mass per area (LMA) was determined as leaf dry mass divided by leaf area ($g.m^{-2}$). Then we ground those dried samples and sent them to the University of Maine Soil and Plant Tissue Testing Laboratory in Orono, Maine for leaf tissue nutrient testing. We also sent dried, ground, and homogenized samples to UC Davis Stable Isotope Facility (Davis, CA, USA) for natural abundance carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) measurement to determine water use efficiency, and nitrogen uptake, respectively. We also collected soil samples from each plot on October 2 and sent them to the Analytical Soil Testing Laboratory, University of Maine, Orono, ME for a full soil health test.

We started this experiment in June 2021 (start of the vegetative year) and continued until August 2022 (end of crop year). We continued to monitor soil moisture and leaf chlorophyll concentrations every two weeks during May-August 2022. We also conducted gas-exchange measurements on three random stems from each plot using the portable photosynthetic measurement system (li-6800; Li-Cor Biosciences, Lincoln, NE, USA) on a sunny day in mid-June between 10:00 and 15:00 h solar time at a photosynthetic photon flux density of 1500 $\mu mol.m^{-2}.s^{-1}$. To quantify crop water status, we collected one wild blueberry stem from each plot at midday 12:00-12:30 h solar time on 15 June 2022 and measured midday leaf water potential with a leaf pressure chamber (PMS Inc., Albany, OR, USA). We harvested and measured yield from the warming chambers on the third week of July and from the control plots on the first week of August when fruit maturation reached 90-95%. We used a 4 sqft quadrat at the center of each plot and collected all the stems from that area to quantify the stem density and measured the fresh weight (actual yield) of blue matured fruits after hand-picking them from those stems. We also separated 100 random berries from each of those samples to measure their fresh weight and then we oven-dried the berries at 70ºC to constant mass and weighed. Then we ground those dried berry samples and sent them to the University of Maine Soil and Plant Tissue Testing Laboratory in Orono, Maine for the nutrient testing. We also subsampled 80 gm of berries and smashed them to make purée and three subsamples from each sample were deposited on a handheld PAL-BRIX/ACID F5 refractometer (Atago, Saitama, Japan) to measure berry sugar content as Brix (%). When we harvested the stems, we also separated eight random stems from each plot to quantify leaf number, leaf area, leaf dry biomass, and leaf nutrition. We measured leaf area, then the leaves were oven-dried at 70ºC to constant mass and weighed. Leaf mass per area (LMA) was determined as leaf dry mass divided by leaf area ($g.m^{-2}$). Then, we ground those dried samples and sent them to the University of Maine Soil and Plant Tissue Testing Laboratory in Orono, Maine for leaf tissue nutrient testing. We also sent dried, ground, and homogenized samples to UC Davis Stable Isotope Facility (Davis, CA, USA) for natural abundance carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) measurement to determine water use efficiency, and nitrogen, respectively. We also collected soil samples from each plot in August after the harvesting and sent them to the Analytical Soil Testing Laboratory, University of Maine, Orono, ME for a comprehensive soil test along with testing the total carbon and total nitrogen in those samples.

4.3.3. Data Analysis

Statistical analyses were carried out using JMP Pro 16.2 (SAS Institute Inc., Cary, NC, USA). Analysis of Variance (ANOVA) based upon a randomized complete block design (RCB) as well as a series of General linear models were used to compare the soil and leaf water status, physiological, morphological as well as fruit yield and quality measurements. These analyses were followed by a LSD (least significant difference) post-hoc test ($\alpha = 0.05$). For all these analyses, treatments were considered as a fixed factor, locations and genotypes were considered as random factors, and Bonferroni confidence interval adjustment was applied. Here, it is to be noted that no overall significant differences were found in measurements while comparing between the two studied sites in Jonesboro, Maine and Deblois, Maine (not shown here). A Principal Component Analysis (PCA) was applied to all the physiological, morphological, leaf nutrients, fruit quality and quantity measurements using the multivariate platform in JMP Pro 16.2. The two highest PCs (PC 1 and PC 2 and their explained percentage of total variance) were used to plot the PCA scores and PCA loadings for the data taken in 2021 and 2022 separately. For all the above-mentioned analyses, data were transformed by the square root prior to analysis if and where necessary.

4.4 Results

4.4.1. Air Temperature and Relative Humidity

During the experimental period of this study from June 2021 to August 2022, the monthly average temperature in the warming chambers was approximately 1 to 3°C higher than the ambient temperature in the control plots (Fig. 4.2a). Also, the monthly average relative humidity in the warming chambers was ~5 to10% lower than the ambient relative humidity in the control plots (Fig. 4.2b).

Over a 24-hour period, the air temperature in the warming chambers was ~3 to 5°C higher during the day and ~1 to 2°C higher during the night than in the control plots (Fig. 4.3a). The relative humidity in the warming chambers was ~6 to 10% lower than in the control plots (Fig. 4.3b).

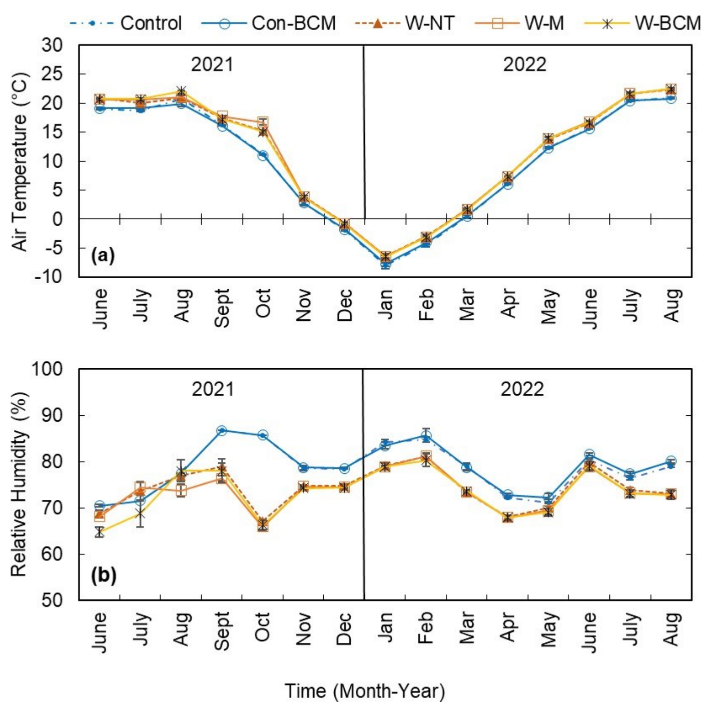

Figure 4.2 Monthly changes in average (a) Atmospheric temperature (°C) and (b) Relative humidity (%) from June 2021 (vegetative year) to August 2022 (crop year). "Control" represents the plot with no warming chamber and no treatment; "Con-

BCM" represents the plot with no warming chamber and treated with biochar-compost mix; "W-NT" represents the plot with warming chamber and no treatment; "W-M" represents the plot with warming chamber and treated with softwood bark mulch; "W-BCM" represents the plot with warming chamber and treated with biochar-compost mix.

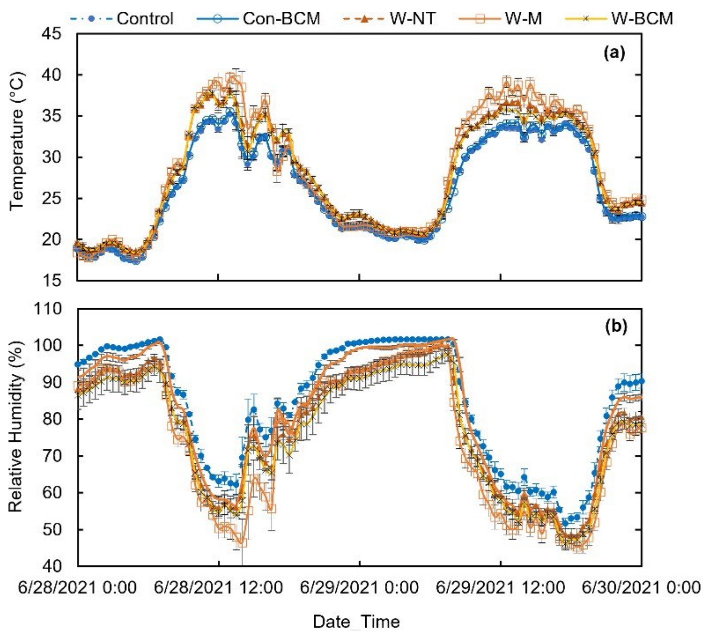

Figure 4.3 Diurnal changes in (a) Atmospheric temperature (°C) and (b) Relative humidity (%) during two typical sunny days (28 – 29 June 2021). "Control" represents the plot with no warming chamber and no treatment; "Con-BCM" represents the plot with no warming chamber and treated with biochar-compost mix; "W-NT" represents the plot with warming chamber and no treatment; "W-M" represents the plot with warming chamber and treated with softwood bark mulch; "W-BCM" represents the plot with warming chamber and treated with biochar-compost mix.

4.4.2. Wild Blueberry Soil and Leaf Water Status

In the vegetative year (2021), soil moisture content was slightly lower (almost negligible) in warming chambers with no treatment and mulch (W-NT & W-M) than in the control plot from mid-June to mid-August (Fig. 4.4a). However, from mid-August until mid-October, soil moisture content was distinctively (~5-8%) lower in the W-NT and W-M plots than in the control plot. In contrast, during the whole season from mid-June to mid-October, plots treated with biochar-compost mix (Con-BCM & W-BCM) had consistently higher soil moisture than the control plot. Especially, the Con-BCM plot had the highest soil moisture content followed by the W-BCM plot and control plot.

Similarly in the crop year (2022), soil moisture content was significantly lower in warming chambers with no treatment and mulch (W-NT & W-M) than in the control plots during the growing season (May to July) (Fig. 4.4b). In contrast, soil water content was significantly higher in the warming chamber with biochar-compost treatment (W-BCM) compared to in other warming chambers (W-NT and W-M). In fact, the soil moisture content in the W-BCM plot was similar to the control plots (Control and Con-BCM) (Fig. 4.4b).

In 2021, the average soil moisture from the whole season (Fig. 4.4c), in the Con-BCM was significantly higher than other treatments, followed by W-BCM and Control, with W-BCM plot showing the highest soil moisture among the warming chambers (Fig. 4.4c). Significantly lower soil moisture compared to the control plot was observed in W-NT and W-M. This trend was consistent during the growing season in crop year (2022) (Fig. 4.4c). However, the soil moisture content was not significantly different among the Control, Con-BCM and W-BCM plots.

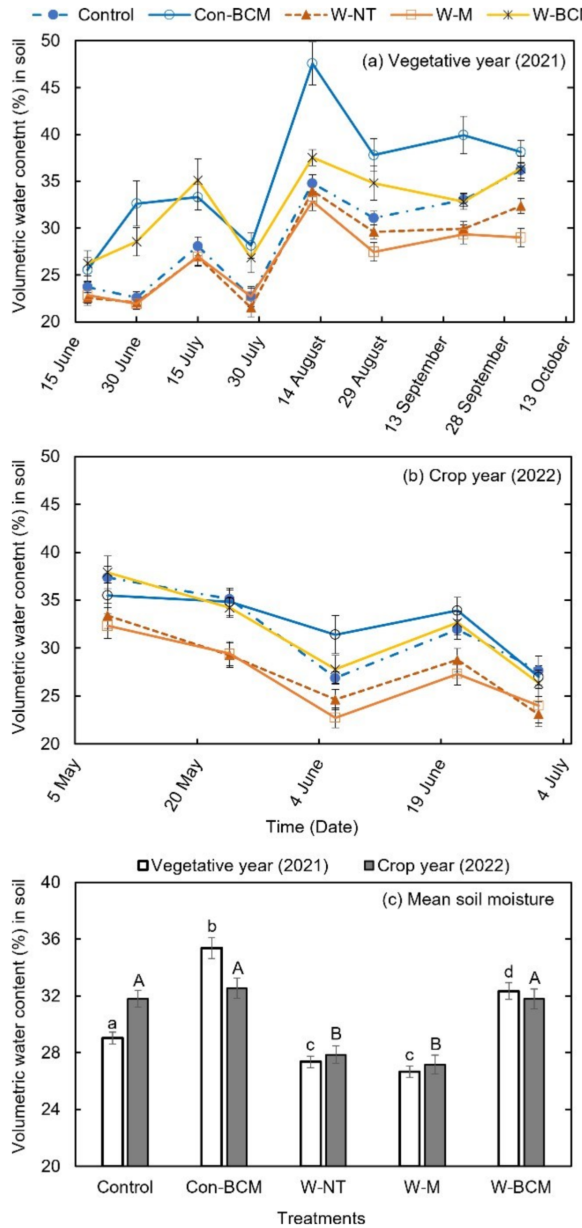

Figure 4.4 Seasonal changes in volumetric water content of the wild blueberry field soils across five different treatments on (a) Vegetative year (June to early October 2021) and (b) Crop year (May to August 2022); (b) Comparison in mean soil moisture content by treatment type over all field season data collection dates on vegetative year (June to early October 2021) and crop year (May-August 2022). Error bars indicate the standard error of the mean. Different letters indicate significant differences at the significance level of $p < 0.05$. "Control" represents the plot with no warming chamber and no treatment; "Con-BCM" represents the plot with no warming chamber and treated with biochar-compost mix; "W-NT" represents the plot with warming chamber and no treatment; "W-M" represents the plot with warming chamber and treated with softwood bark mulch; "W-BCM" represents the plot with warming chamber and treated with biochar-compost mix.

Despite the significant differences in soil moisture content, there were no significant differences in leaf water potential of wild blueberry plants growing under different treatments (Fig. 4.5) indicating that those plants did not differ in water deficits during both vegetative and crop years. On average, in the vegetative year (July 2021), plant midday leaf water potentials ranged from ~-1.2 MPa to -1.3 MPa, and ranged from ~-0.9 MPa to -1 MPa during the crop year (June 2022).

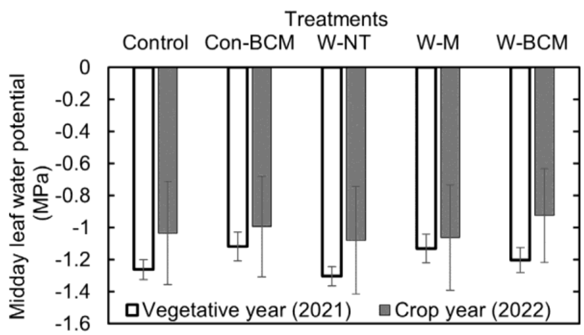

Figure 4.5 Comparison in midday leaf water potential of wild blueberry plants among five different treatments on a typical sunny day in the vegetative year (27 July 2021). and crop year (15 June 2022). Error bars indicate the standard error of the mean. Here, no significant differences were observed among the treatments at the significance level of $p < 0.05$. “Control” represents the plot with no warming chamber and no treatment; “Con-BCM” represents the plot with no warming chamber and treated with biochar-compost mix; “W-NT” represents the plot with warming chamber and no treatment; “W-M” represents the plot with warming chamber and treated with softwood bark mulch; “W-BCM” represents the plot with warming chamber and treated with biochar-compost mix.

4.4.3. Wild Blueberry Physiology

Based on the seasonal average, no significant differences were found in chlorophyll concentration per leaf area (Fig. 4.6a) and leaf mass (Fig. 4.6b) among the studied treatments during the vegetative year (2021). In crop year (2022), significant differences were found in both chlorophyll concentration per leaf area (Fig. 4.6a) and leaf mass (Fig. 4.6b) while comparing controls with warming treatments. Wild blueberry plants growing in warming chamber with biochar-compost mix had the highest and significantly higher chlorophyll concentration per leaf area and leaf mass compared to the other treatments.

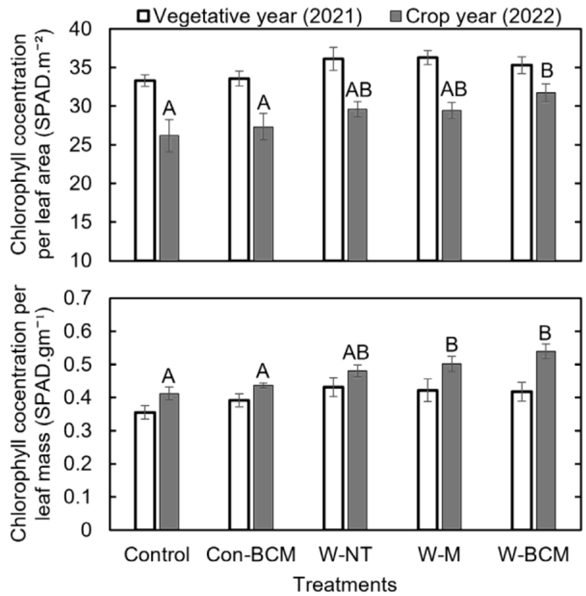

Figure 4.6 Comparison in mean (a) Chlorophyll concentration per leaf area and (b) Chlorophyll concentration per leaf mass by treatment type over all field season data collection dates in vegetative year (mid-June to mid-October 2021) and crop year (May to August 2022). Error bars indicate the standard error of the mean. Different letters indicate significant differences whereas no letters indicate no significant differences among the treatments at the significance level of $p < 0.05$. "Control" represents the plot with no warming chamber and no treatment; "Con-BCM" represents the plot with no warming chamber and treated with biochar-compost mix; "W-NT" represents the plot with warming chamber and no treatment; "W-M" represents the plot with warming chamber and treated with softwood bark mulch; "W-BCM" represents the plot with warming chamber and treated with biochar-compost mix.

In the vegetative year (2021), plants in the warming treatments had higher photosynthetic rates (Fig. 4.7a), stomatal conductance (Fig. 4.7b), and transpiration rates (Fig. 4.7c) compared to those in the control plots. Especially, plants growing in the biochar-compost treated soil inside warming chambers had significantly higher photosynthetic rates, stomatal conductance, and transpiration rates compared to the other treatments. Consequently, plants growing in the warming chambers (W-NT, W-M and W-BCM) showed significantly lower water use efficiency than the plants in control plots where plants in Con-BCM plot showed the highest water use efficiency (Fig. 4.7d).

Similarly, in crop year (2022), plants in the warming treatments had higher photosynthetic rates (Fig. 4.7a), stomatal conductance (Fig. 4.7b), and transpiration rates (Fig. 4.7c) compared to those in the control plots. However, the addition of biochar-compost and mulch treatments did not affect the photosynthetic rates, stomatal conductance, and transpiration rates of the plants growing in the warming chambers. Although the plants in the control plot with the biochar-compost mix had slightly higher average photosynthetic rates, stomatal conductance, and transpiration rates compared to the control plot with no soil amendment, the difference was not significant (Fig. 4.7a-c). On the other hand, the water use efficiency of the wild blueberry plants growing showed no significant differences among different treatments in the crop year (Fig. 4.7d).

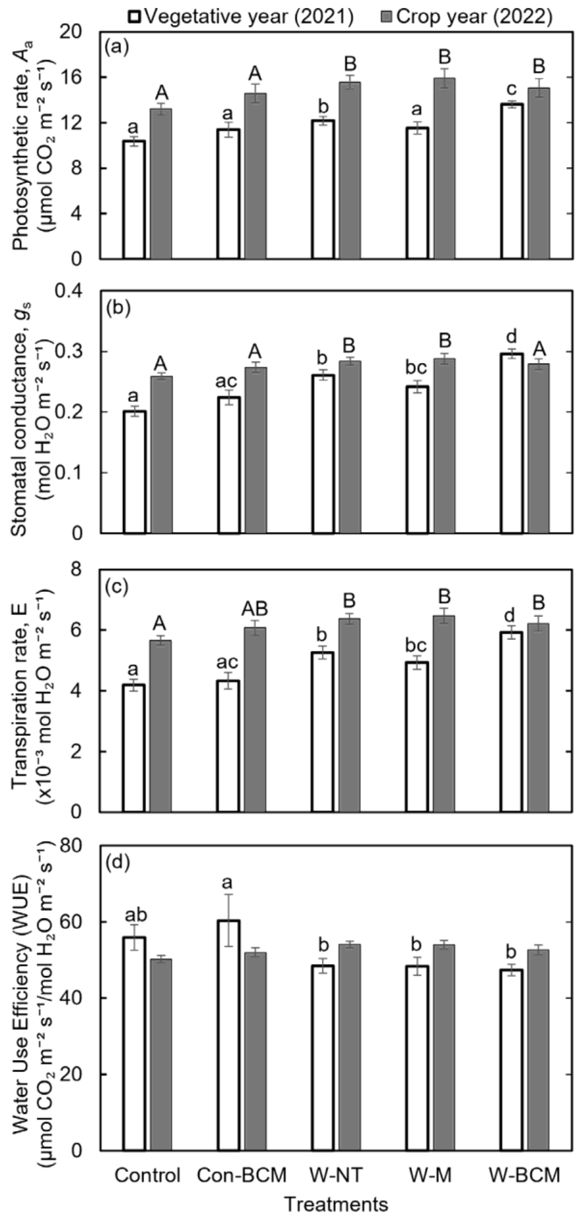

Figure 4.7 Comparison in (a) Photosynthetic rate, (b) Stomatal conductance, (c) Transpiration rate, and (d) Water use efficiency of wild blueberry plants among five different treatments on typical sunny days in vegetative year (11 and 12 July 2021) and crop year (15 June 2022). Error bars indicate the standard error of the mean. Different letters indicate significant differences whereas no letters indicate no significant differences among the treatments at the significance level of $p < 0.05$. "Control" represents the plot with no warming chamber and no treatment; "Con-BCM" represents the plot with no warming chamber and treated with biochar-compost mix; "W-NT" represents the plot with warming chamber and no treatment; "W-M" represents the plot with warming chamber and treated with softwood bark mulch; "W-BCM" represents the plot with warming chamber and treated with biochar-compost mix.

In the vegetative year (2021), both the photosynthetic rate per stem (Fig. 4.8a) and per land area (Fig. 4.8b) were the lowest in control plots (Control and Con-BCM) and significantly the highest in W-BCM plot compared to the other treatments. On the contrary, in the crop year (2022), both the photosynthetic rate per stem (Fig. 4.8a) and per land area (Fig. 4.8b) were the highest in W-NT plots among all treatments which were significantly higher compared to the control plots (Control and Con-BCM) only.

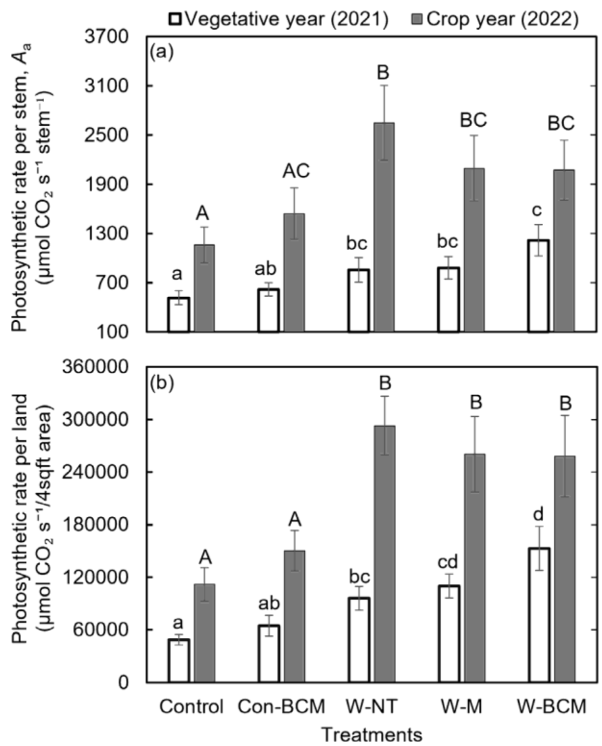

Figure 4.8 Comparison in (a) Photosynthetic rate per stem and (b) Photosynthetic rate per land of wild blueberry plants among five different treatments on typical sunny days in vegetative year (11 and 12 July 2021) and crop year (15 June 2022). Error bars indicate the standard error of the mean. Different letters indicate significant differences among the treatments at the significance level of $p < 0.05$. “Control” represents the plot with no warming chamber and no treatment; “Con-BCM” represents the plot with no warming chamber and treated with biochar-compost mix; “W-NT” represents the plot with warming chamber and no treatment; “W-M” represents the plot with warming chamber and treated with softwood bark mulch; “W-BCM” represents the plot with warming chamber and treated with biochar-compost mix.

4.4.4. Wild Blueberry Morphology

At the end of the growing season in the vegetative year (August, 2021), plants in the warming chambers (W-NT, W-M, W-BCM) had a higher number of leaves per stem than that of the control plots (Control, Con-BCM) especially the W-BCM plot had significantly the highest number of leaves among all the treatments (Fig. 4.9a). In contrast, at the end of the growing season in crop year (August, 2022), plants in the W-NT and W-BCM plots had a significantly higher number of leaves per stem than the Control, Con-BCM and W-M plots (Fig. 4.9a). On the other hand, no significant differences were found in leaf size (Fig. 4.9b) among different treatments in both the vegetative (2021) and crop (2022) years.

Plants in the control plots (Control and Con-BCM) had higher leaf mass per area (LMA) than the warming treatments (W-NT, W-M, W-BCM) in the vegetative year (2021) (Fig. 4.9c). LMA was especially significantly higher in the Control plot compared to that of the warming treatments. In crop year (2022), no difference was found in LMA among different treatments (Fig. 4.9c).

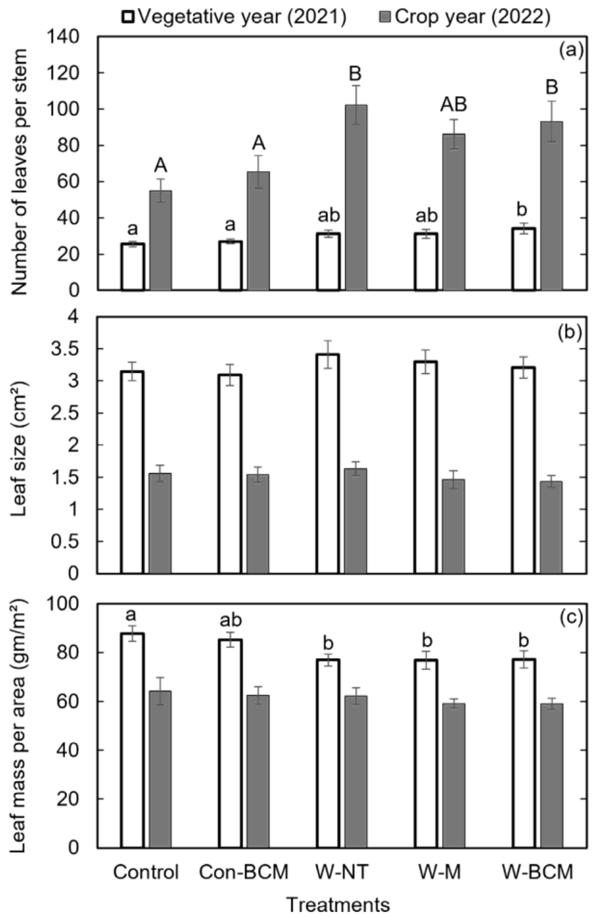

Figure 4.9 Comparison in (a) Number of leaves per stem, (b) Leaf size, and (c) Leaf mass per area of the wild blueberry plants among five different treatments in vegetative year (19 August 2021) and crop year (4 August 2022). Error bars indicate the standard error of the mean. Bars with no letters above indicate no significant differences among the treatments whereas different letters indicate significant differences at the significance level of $p < 0.05$. "Control" represents the plot with no warming chamber and no treatment; "Con-BCM" represents the plot with no warming chamber and treated with biochar-compost mix; "W-NT" represents the plot with warming chamber and no treatment; "W-M" represents the plot with warming chamber and treated with softwood bark mulch; "W-BCM" represents the plot with warming chamber and treated with biochar-compost mix.

4.4.5. Wild Blueberry Water-Use and Nitrogen Source

At the end of the growing season in both vegetative (2021) and crop years (2022), no significant differences were found in water use efficiency and nitrogen source of the growing wild blueberry plants among different treatments (Fig. 4.10).

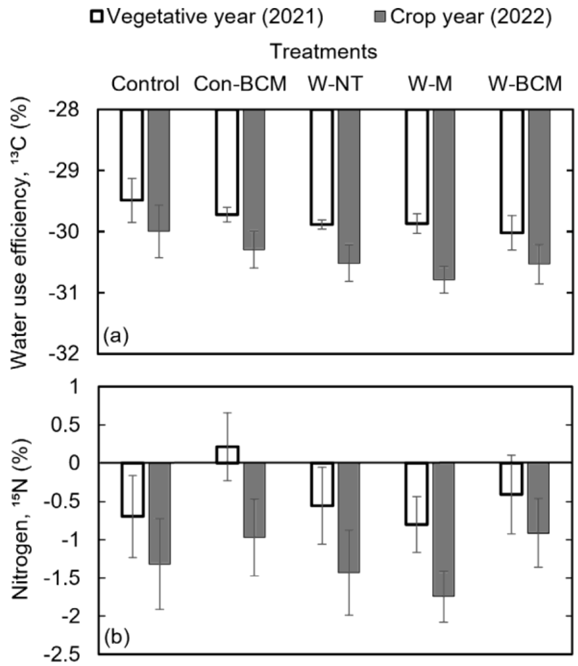

Figure 4.10 Comparison in (a) Water use efficiency (^{13}C isotope) and (b) Nitrogen (^{15}N isotope) of the wild blueberry plants among five different treatments in vegetative year (19 August 2021) and crop year (4 August 2022). Error bars indicate the standard error of the mean. Bars with no letters above indicate no significant differences among the treatments at the significance level of $p < 0.05$. "Control" represents the plot with no warming chamber and no treatment; "Con-BCM" represents the plot with no warming chamber and treated with biochar-compost mix; "W-NT" represents the plot with warming chamber and no treatment; "W-M" represents the plot with warming chamber and treated with softwood bark mulch; "W-BCM" represents the plot with warming chamber and treated with biochar-compost mix.

4.4.6. Wild Blueberry Leaf and Fruit Nutrients

Among the macro-nutrients (N, P, K, Ca, Mg in Figs. 4.11a-e), leaf nitrogen (N) and phosphorus (P) levels (Figs. 4.11a,b) were lower than the required nutrient level whereas leaf potassium (K), calcium (Ca) and magnesium (Mg) levels (Figs. 4.11c-e) were similar or higher than the required nutrient level under all studied treatments in both vegetative (2021) and crop (2022) years. While comparing the treatments, differences in the macro-nutrients were not significant in the studied years except for potassium (Fig. 4.11c) in the crop year (2022) where it was significantly higher in Con-BCM followed by W-BCM than other treatments.

In contrast to the macro-nutrients, all micro-nutrients (B, Cu, Fe, Mn, Zn in Figs. 4.11f-j) were similar or higher than the required nutrient level under all studied treatments in both vegetative (2021) and crop (2022) years. While comparing the treatments, differences in the micro-nutrients were not significant in the studied years except for manganese (Fig. 4.11i) in the crop year (2022) where it was significantly higher in W-M followed by W-NT than other treatments.

Regarding the macro-nutrients (Figs. 4.12a-e) of wild blueberries in crop year, phosphorus (Fig. 4.12b) and potassium (Fig. 4.12c) levels were significantly higher in the controls than the warming treatments whereas no significant differences were found in nitrogen (Fig. 4.12a), calcium (Fig. 4.12d), and magnesium (Fig. 4.12e) levels among the studied treatments. Also, no significant differences were found in any of the micro-nutrients (B, Cu, Fe, Mn, Zn in Figs. 4.12 f-j) of wild blueberries among the studied treatments.

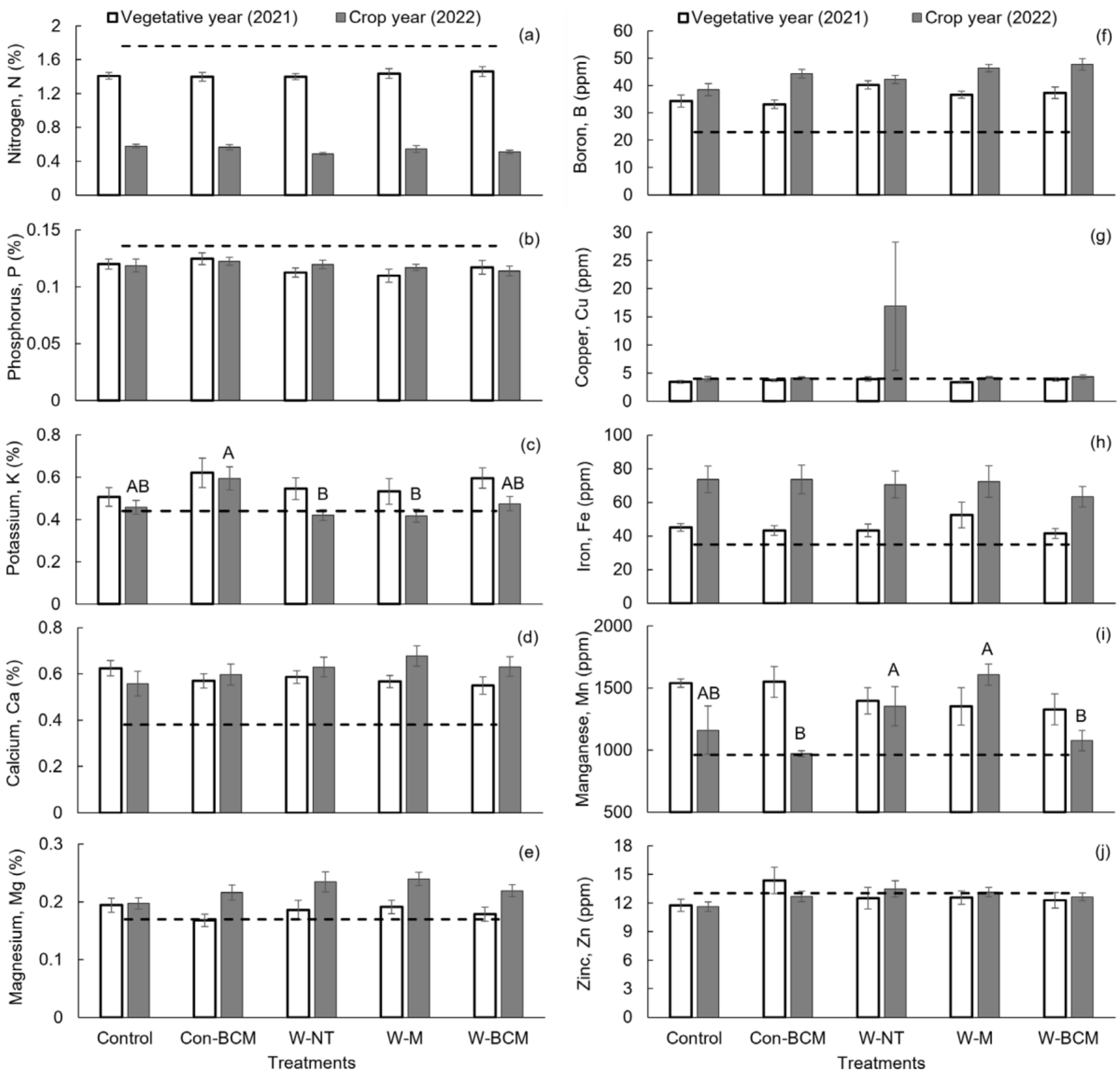

Figure 4.11 Comparison in (a-j) Nutrients: N, P, K, Ca, Mg, B, Cu, Fe, Mn, Zn of the wild blueberry leaves among five different treatments in vegetative year (19 August 2021) and crop year (4 August 2022). Error bars indicate the standard error of the mean. Bars with no letters above indicate no significant differences whereas different letters indicate significant differences among the treatments at the significance level of $p < 0.05$. Dashed lines represent the optimum nutrient levels required in wild blueberry leaves. "Control" represents the plot with no warming chamber and no treatment; "Con-BCM" represents the plot with no warming chamber and treated with biochar-compost mix; "W-NT" represents the plot with warming chamber and no treatment; "W-M" represents the plot with warming chamber and treated with softwood bark mulch; "W-BCM" represents the plot with warming chamber and treated with biochar-compost mix.

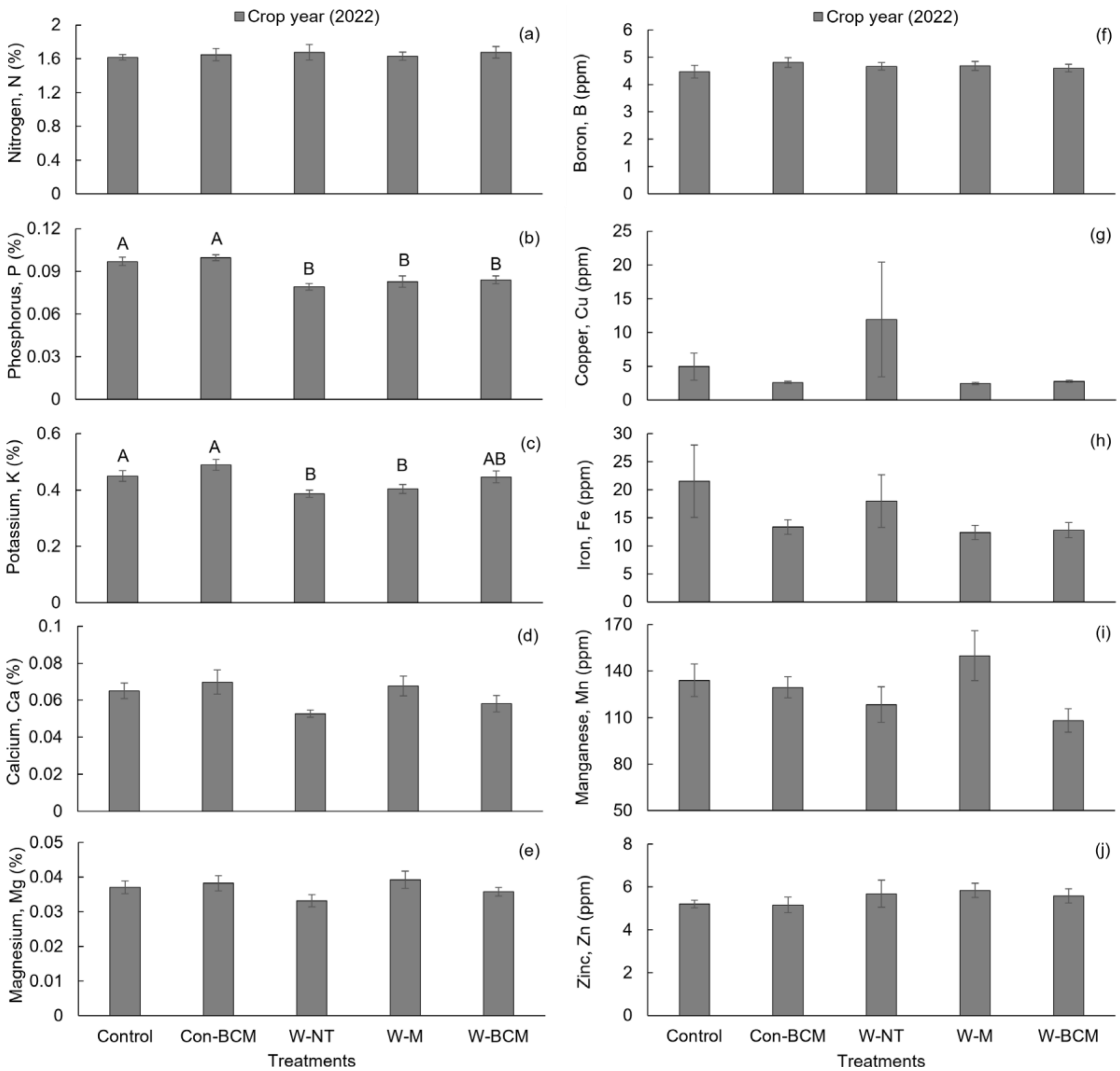

Figure 4.12 Comparison in (a-j) Nutrients: N, P, K, Ca, Mg, B, Cu, Fe, Mn, Zn of the wild blueberry fruits among five different treatments in crop year (4 August 2022). Error bars indicate the standard error of the mean. Bars with no letters above indicate no significant differences whereas different letters indicate significant differences among the treatments at the significance level of $p < 0.05$. "Control" represents the plot with no warming chamber and no treatment; "Con-BCM" represents the plot with no warming chamber and treated with biochar-compost mix; "W-NT" represents the plot with warming chamber and no treatment; "W-M" represents the plot with warming chamber and treated with softwood bark mulch; "W-BCM" represents the plot with warming chamber and treated with biochar-compost mix.

4.4.7. Wild Blueberry Quantity and Quality

In crop year (2022), fruit yield (Fig. 4.13a), the weight of matured blue fruits per stem (Fig. 4.13b) and the fresh weight of 100 berries (Fig. 4.13c) were significantly higher in the warming treatments (W-NT, W-M, W-BCM) than the control plots (Control, Con-BCM). Control and Con-BCM plots had similar fruit production (Fig. 4.11a-c) whereas W-BCM had the highest average yield (Fig. 4.11a-b) among the warming treatments, but the difference was not significant compared to the W-NT and W-M plots. In contrast, wild blueberry quality did not differ among the studied treatments based on berry sugar content (% of Brix in Fig. 4.13d).

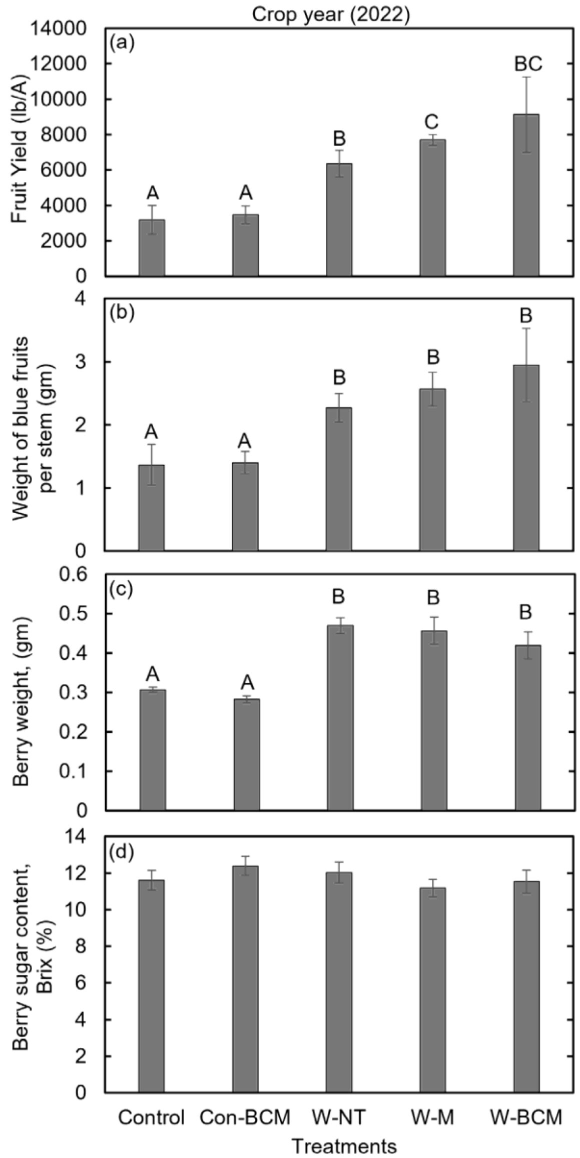

Figure 4.13 Comparison in (a) Fruit yield, (b) Weight of blue fruits per stem, (c) Fresh weight of 100 berries, and (d) Berry sugar content of the wild blueberry plants among five different treatments in crop year 2022. Error bars indicate the standard error of the mean. Bars with no letters above indicate no significant differences among the treatments whereas different letters indicate significant differences at the significance level of $p < 0.05$. "Control" represents the plot with no warming chamber and no treatment; "Con-BCM" represents the plot with no warming chamber and treated with biochar-compost mix; "W-NT" represents the plot with warming chamber and no treatment; "W-M" represents the plot with warming chamber and treated with softwood bark mulch; "W-BCM" represents the plot with warming chamber and treated with biochar-compost mix.

4.4.8. Relationship among wild blueberry plant traits and fruit quantity and quality

The PCA1 of vegetative year traits explained 26.8%, while PCA2 explained 20.2% of the total variance. For the crop year traits, PCA1 explained 22.1%, while PCA2 explained 21.7%. Based on the principal component analysis of all measured traits where vegetative (plant traits of 2021 and fruit quantity and quality of 2022 in Fig. 4.14a) and crop (both plant traits, and fruit quantity and quality of 2022 in Fig. 4.14b) years are analyzed separately, more closely related relationships were found from the vegetative year compared to the crop year. In fact, a higher percentage of the total variance was explained by the first two principal components in the vegetative year (26.8% by PC1 and 20.2% by PC2 in Fig. 4.14a) than in the crop year (22.1% by PC1 and 21.7% by PC2). Moreover, plant physiological traits such as leaf photosynthetic rate (A_a) and leaf chlorophyll concentration (SPAD) in the vegetative year (Fig. 4.14a) and crop year (Fig. 4.14b) were highly related to the berry quantity .

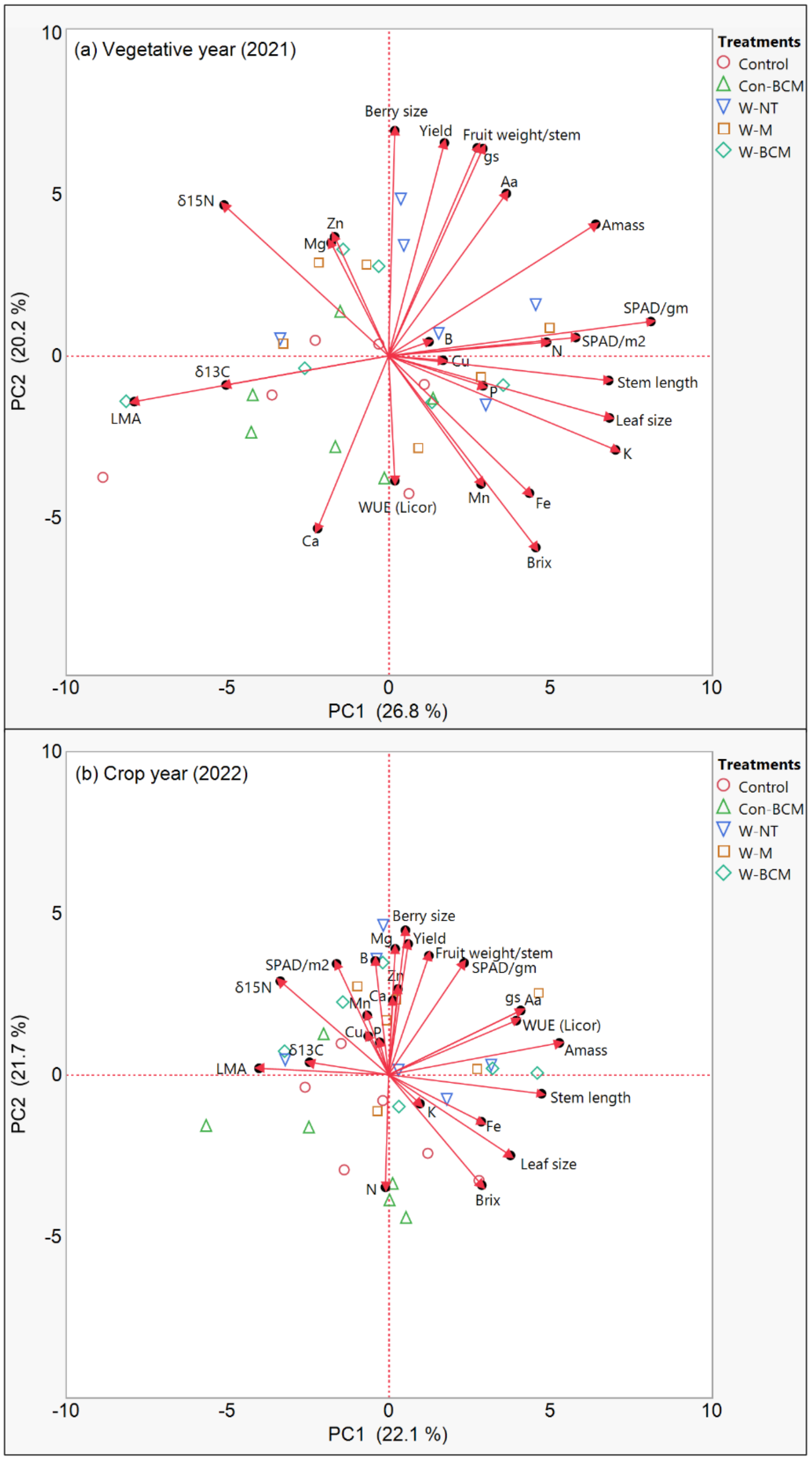

Figure 4.14 Principal Component Analysis (PCA) of physiological traits (SPAD/m², SPAD/gm, A_a, A_{mass}), morphological traits (stem length, leaf size, leaf mass per area), major and minor leaf nutrient elements (N, P, K, C, Ca, Mg, B, Al, Cu, Zn, Mn, Fe), and quantity and quality of wild blueberries (yield, fruit weight per stem, berry size, brix) in (a) Vegetative year (2021) and (b) Crop year (2022). Red arrows indicate the PCA loadings of different traits. Different colored shapes in the background indicate

the PCA scores for different studied treatments. “Control” represents the plot with no warming chamber and no treatment; “Con-BCM” represents the plot with no warming chamber and treated with biochar-compost mix; “W-NT” represents the plot with warming chamber and no treatment; “W-M” represents the plot with warming chamber and treated with softwood bark mulch; “W-BCM” represents the plot with warming chamber and treated with biochar-compost mix.

4.5 Discussion

Our results revealed that warming of 3 to 5°C significantly reduced the moisture of wild blueberry soils, while biochar-compost mix amendment used in this study can mitigate this negative effect, possibly by enhancing soil water retention (Li et al. 2021, Novak et al. 2024). In contrast, 0.5” (1.3 cm) layer of mulch is not effective. Our study also revealed that the wild lowbush blueberry plants were performing better under a warmer environment indicated by their higher physiological performance, growth, and production. Furthermore, the plants growing under the warmer environment along with the biochar-compost mix amendment were overall performing the best. While physiological performance, growth and fruit quantity of wild blueberry plants were distinctively affected by the warming and soil amendment treatments, the leaf nutrients and fruit quality (sugar content and nutrients) did not show any distinctive response towards those treatments. Lastly, our study established that physiological performance, especially the photosynthetic rate of wild blueberry plants in the vegetative year had a higher impact on the final production of wild blueberries compared to that of the crop year.

The warmer environment significantly lowered the soil moisture during the growing season, as previously found in the wild blueberry crop system (Tasnim et al., 2020). However, soil moisture in the warming chamber with no soil amendment (W-NT) was barely lower than the control before mid-August possibly because of a higher amount of organic matter (9-20% in Table 4.1, which is 2-3 times higher than the optimum level) on the studied field soils (Valette et al. 1994). It is possible that the soil organic matter was decomposing and mineralizing during the growing period (June to August 2021) and as a result, the organic matter content reduced to 8-11% at the end of seasons in both vegetative and crop years (Tables 4.2, 4.3). Therefore, the soil moisture was lower in the warming chamber than in the control from mid-August to October in the vegetative year (2021) as well as in the crop year (May – July 2022). Further, a 0.5” layer of mulch was not effective in retaining soil water. Previous researchers also reported that 0.5” layer of mulch is not deep enough and recommended applying at least a 2-3” layer of wood mulch in wild blueberry fields to conserve soil moisture (Hunt et al. 2010, Gumbrewicz and Calderwood 2022). On the other hand, the use of biochar-compost mix resulted in significantly higher soil moisture under both ambient and warmer environments throughout the whole season. It could be because biochar helps sandy soil, like the wild blueberry soils, hold more water (Mukherjee and Zimmerman 2013, Liang et al. 2014, Li et al. 2021, Tasnim et al. 2024). Therefore, applying biochar materials to sandy soils could reduce irrigation costs by increasing soil water retention, thereby saving water (Kroeger et al. 2021). In contrast, plants under a warming environment did not show higher water deficits, unlike reported by a previous study by Tasnim et al. (2020). This is possibly because the days when we measured the water potential were not dry (and low vapor pressure deficits).

Overall better physiological performance, growth as well as higher berry yield of wild blueberry plants growing on the biochar-compost treated soils could be at least partly due to higher soil moisture availability (Li et al. 2021, Agegnehu et al. 2017, Ariz et al. 2015). Interestingly, higher stomatal conductance, transpiration rate, and photosynthetic rate under the warmer environment suggest that they were consuming more water and more productive in carbon assimilation than the plants growing in the ambient environment. Higher rates of photosynthesis than all other treatments of plants in biochar-compost treated soil under warmer environments agree with their higher number of leaves and chlorophyll concentration under a warmer environment. These could at least partly explain the higher final fruit yield and fresh weight of berries under the warmer environment, especially with the biochar-compost treatment. These observations agree with previous studies on many temperate crops, which benefited from 1 to 3ºC warming compared to ambient temperature (Easterling et al. 2007, Hatfield et al. 2011). Wild blueberry crops grow in a temperate climate and therefore is expected to benefit from warmer climates. On the contrary, this study contradicts a previous similar study on wild blueberries under warmer environments where plants were water-stressed under warmer climates and drier soil followed by hindered physiological performance (Tasnim et al. 2020). This could be because wild blueberry plants in this study were not water-stressed with more than sufficient organic matter and plant available water in the soil under a warmer environment. This is also justified by a recent study on wild blueberries by Barai et al. (2021) that wild blueberry plants should be performing well even under warmer climates with sufficient available soil moisture. However, plants growing in the ambient environment had higher water use efficiency, especially in plants in the biochar-compost treated soil compared to the plants growing in the warmer environment.

Interestingly, our study further suggests that the physiological performance, especially the photosynthetic rate of wild blueberry plants in the vegetative year is a better indicator of fruit quantity compared to those in the crop year, which agrees with a previous study conducted on wild blueberries in Maine (Tasnim et al. 2022). Moreover, it has been well established for many crops that photosynthesis is potentially a major determinant of crop production (Tasnim et al. 2022, Zelitch 1982, Peng et al. 1991). The positive relationship between fruit yield and photosynthetic rate per stem and per land found in this study supported this statement.

Table 4.2 Comparison in physical and chemical properties of wild blueberry soil among the studied treatments in September 2021 (at the end of growing season) and recommended optimum ranges for wild blueberry soil. Soil properties for different treatments are represented as mean of six replicated soil samples ± standard error. "Control" represents the plot with no warming chamber and no treatment; "Con-BCM" represents the plot with no warming chamber and treated with biochar-compost mix; "W-NT" represents the plot with warming chamber and no treatment; "W-M" represents the plot with warming chamber and treated with softwood bark mulch; "W-BCM" represents the plot with warming chamber and treated with biochar-compost mix.

Physical & Chemical Properties	Optimum range	Treatments				
		Control	Con-BCM	W-NT	W-M	W-BCM
Texture	-	Sandy loam				
Sand (%)	-	66±1.9	69.8±1	69.9±1.5	69.3±0.8	69.7±1.62
Silt (%)		24.4±1.5	20.5±0.6	20.9±1.1	21.7±0.7	1.5±1.3
Clay (%)		9.6±0.5	9.7±0.4	9.2±0.4	9±0.2	8.8±0.4
pH	4.0-4.5	4.4±0.1	4.8±0.1	4.4±0.1	4.5±0.1	4.7±0.2
Organic matter (%)	5-8	9.8±0.7	9.4±1	10±1	9.7±1	9.8±1.2
Particulate organic matter (%)	1.5-3.0	2.8±0.3	2.7±0.5	2.9±0.4	2.6±0.4	2.8±0.6
Respirable, CO_2-C (mg/Kg)	140-300	128±15	146±22	150±25	173±14	166±17
Potentially mineralizable nitrogen (mg/Kg)	50-100	36.8±5.5	40.3±4.7	42.8±3.7	36.3±3	46.5±3.3
Plant available water (%)	>9	13.5±0.7	12.6±1.1	13.2±0.9	12.9±0.9	13.4±0.9
CEC (me/100 g)	>5	6.5±0.8	6.5±0.7	6.3±0.5	6±0.6	6.5±0.4
Nitrate-N (ppm)	20-30	1.3±0.2	1±0	1.7±0.4	1.5±0.2	1±0
Ammonium-N (ppm)	<10	3.3±0.4	2.8±0.4	4.3±0.6	3.7±0.5	3±0.3
Phosphorous (lb/A)	10-40	10.9±1.1	16.2±3.2	12.4±1.5	14.3±1.9	17.7±1.9
Potassium (% saturation)	2.1 - 3.0	2.4±0.3	6±0.9	2.5±0.2	3.2±0.6	5.3±0.3
Calcium (% saturation)	20 - 30	20.3±6.1	35.6±2	21.1±5.3	23.6±4.9	31.5±3.9
Magnesium (% saturation)	5-10	7.1±1.5	8.6±0.6	7.2±1.7	7.3±1	7.6±0.8
Sulfur (ppm)	>50	62.2±17.9	46.2±8.9	57±13.4	47.2±15.4	42.2±10.9
Copper (ppm)	0.25 - 0.6	0.2	0.2	0.1	0.2±0.1	0.2
Iron (ppm)	6-10	29±3.2	36.2±6.9	26.2±2.2	34.3±5	33.2±3.4
Manganese (ppm)	4-8	13.2±4.1	14.3±2.1	12.5±2.6	12.3±1.8	13.9±0.8
Zinc (ppm)	1-2	3±0.7	3.6±0.3	3±0.4	2.9±0.5	3.2±0.2
Boron (ppm)	0.5 - 1.2	0.3	0.4±0.1	0.3	0.3	0.4

Table 4.3 Comparison in physical and chemical properties of wild blueberry soil among the studied treatments in August 2022 (at the end of growing season) and recommended optimum ranges for wild blueberry soil. Soil properties for different treatments are represented as mean of six replicated soil samples ± standard error. "Control" represents the plot with no warming chamber and no treatment; "Con-BCM" represents the plot with no warming chamber and treated with biochar-compost mix; "W-NT" represents the plot with warming chamber and no treatment; "W-M" represents the plot with warming chamber and treated with softwood bark mulch; "W-BCM" represents the plot with warming chamber and treated with biochar-compost mix.

Physical & Chemical Properties	Optimum range	Treatments				
		Control	Con-BCM	W-NT	W-M	W-BCM
pH	4.0-4.5	4.6±0.1	4.8±0.1	4.6±0.2	4.6±0.2	5±0.1
Organic matter (%)	5-8	10.7±0.9	9.9±0.7	8.4±0.9	10.1±1.8	8.2±1.1
CEC (me/100 g)	>5	5.9±0.5	6.8±0.3	5.1±0.2	5.5±0.6	5.2±0.9
Total Carbon (%)	-	6.1±0.6	6.1±0.5	4.9±0.3	5.7±0.9	4.9±0.7
Total Nitrogen	-	0.3	0.3	0.2	0.2	0.2
Nitrate-N (ppm)	20-30	0.8±0.4	0.8±0.3	0.8±0.2	0.8±0.3	1
Ammonium-N (ppm)	<10	2.8±0.3	2.5±0.2	4.2±1.3	3.8±0.6	2.7±0.3
Phosphorous (lb/A)	10-40	15.2±2	17.3±1.6	11.4±2.6	13±1.7	14.4±2.9
Potassium (% saturation)	2.1 - 3.0	2.5±0.3	6.6±1	2.6±0.2	3±0.4	6.9±0.8
Calcium (% saturation)	20 - 30	18.5±5.7	29.4±3.9	19.6±6.2	19.8±4.6	28±4.3
Magnesium (% saturation)	5-10	6.6±1.5	8.6±0.5	6.1±1.3	7.2±1.2	7.8±0.9
Sulfur (ppm)	>50	47.2±10.5	32.3±5	71±11.1	57±15	42.3±5.8
Copper (ppm)	0.25 - 0.6	0.2	0.2	0.2	0.2	0.2
Iron (ppm)	6-10	54.6±10.7	45.7±6.9	41.7±5.6	55±13	39.8±6.3
Manganese (ppm)	4-8	7.7±1.9	7.3±1	8.5±2.3	8.7±1.5	8±2.2
Zinc (ppm)	1-2	2.7±0.5	3.5±0.5	3.9±1.5	2.7±0.4	2.5±0.6
Boron (ppm)	0.5 - 1.2	0.4	0.4	0.3	0.4	0.4±0.1

CHAPTER 5

ARE FOLIAR FERTILIZERS BENEFICIAL TO GROWTH AND YIELD OF WILD LOWBUSH BLUEBERRIES?

5.1 Abstract

Wild lowbush blueberry is an economically and culturally important crop in North America. Different fertilizer companies have been advertising their foliar fertilizer products to the wild blueberry growers, claiming better growth and production of this crop with no scientific proof. Although foliar fertilization has shown to be efficient for delivering micronutrients in deficit for different crops by reducing soil activation and environmental contamination, limited research has been done in wild blueberries. It is still unknown how foliar fertilizers affect the physiology, growth, and yield of this crop. Therefore, we tested the impacts of seven foliar treatments containing macro- and micro-nutrients and plant hormones (Seacrop16, Salvador, Agro-Phos applied in 2019 and Kali-T, Nano-Gro, Poma, Poma + Nanocellulose applied in 2020) on this crop for one crop cycle from vegetative year (2019) to crop year (2020). We tested these products against the standard soil-applied granular fertilizer called Diammonium phosphate (DAP) and control (no fertilizer) in a randomized complete block design with eight replicates in a conventional wild blueberry field in Maine, USA. In 2019, no significant differences across the applied treatments were observed in crop physiology and growth except in leaf chlorophyll concentration. In 2020, there was significantly higher leaf chlorophyll concentration in SeaCrop16 and Poma+Nanocellulose plots, but significantly lower photosynthetic rates in DAP and SeaCrop16 treated plots compared to the control. Meanwhile, no significant differences in plant height, leaf characteristics, or blueberry yield were found among the treatments. Overall, mobile nutrients (N, P, K) from soil applied fertilizers and foliar fertilizers containing other immobile nutrients (Ca) and/or plant hormones might benefit crop growth, but the impact on yield is limited. We also reveal that the wild blueberry physiological and morphological traits and leaf nutrients in the vegetative year are more related to the crop yield than those traits in the crop year. This implies that a combination of wild blueberry physiology, morphology, and leaf nutrients in the vegetative year largely impact their yield in the following crop year.

5.2 Introduction

The wild lowbush blueberry production system in North America is a unique seminatural agricultural system where two wild blueberry species exist in a field: (1) common low-sweet lowbush blueberry (*Vaccinium angustifolium* Aiton) (80–90% of the field) and (2) velvet leaved sour-top lowbush blueberry (*Vaccinium myrtilloides* Michx.). Wild blueberry fields are initially established from seeds naturally occurring (not planted), and then underground stems (rhizomes) develop. Roots then grow directly off of rhizomes creating a tightly woven mat across fields. Rhizomes grow within ~10 cm of the soil surface, and upright stems (~10 to 60 cm) above the soil surface are produced that ultimately bear fruit. An individual wild blueberry plant, with its spreading rhizome system, is referred to as a genet (Bell et al. 2009). Each genet is visually, genetically, and physiologically different (Bell et al. 2009, Tasnim and Zhang 2021) from neighboring plants creating a complex mixture of genets in the wild blueberry fields, which provides consumers with a rich diversity of flavors. This crop is managed in a two-year cycle: the plants grow vegetatively in the first year (vegetative year) after the harvest and pruning of the previous year, and the plants flower and produce fruits in the second year (crop year). After harvesting the fruits, the plants become dormant, and wild blueberry growers prune the field by either mowing or burning to encourage growth in the following crop cycle.

Maine, USA, is the largest production region of wild blueberry in the U.S., where approximately 485 growers manage this unique crop commercially on 41,000 acres of land (USDA NASS 2021). Wild blueberries are the second largest crop in Maine (USDA NASS 2021), with cultural and economic importance. Fertilizer companies advertise and sell foliar fertilizers to the growers without scientific evidence specific to wild blueberry. They claim that foliar fertilizer products consisting of different amounts of macro- and micro-nutrients, along with plant growth regulators (PGR), can improve nutrient uptake by the plants, further improving the growth and yield of wild blueberries. Soil-applied granular fertilizers such as monoammonium phosphate (MAP), diammonium phosphate (DAP), and ammonium sulfate have been the most common industry standard products for wild blueberry production. One of the major issues is that weed management is closely tied to nutrient management in wild blueberries. Because of the low soil pH, rhizomatous nature of the crop, and field-grown aspects of this system, any granular fertilizer applied is available for weeds to take up in addition to the crop (Yarborough and Bhowmik 1993). Often fertilizers provide nutrients to the weeds allowing them to out-compete slow-growing blueberry plants (Yarborough and Bhowmik 1993). Wild blueberry plants require acidic soil (soil pH of 4 to 4.5), making it harder for the plants to take up the required nutrients (Yarborough and Bhowmik 1993, Taiz et al. 2015). To overcome these issues, foliar fertilizers could be a solution because nutrients would be applied to the leaves where they can be quickly and readily absorbed with less potential for soil activation or environmental contamination (Karlsons and Osvalde 2019). The challenge behind applying foliar products is the waxy leaf cuticle on the blueberry leaves, which physiologically makes foliar fertilizer sprays ineffective when nutrients are applied or environmental conditions are unfavorable (Hart et al. 2006, Wach and Błazewicz-Wo´zniak 2012). In this case, spraying foliar fertilizers containing adjuvants or spraying additional adjuvants like cellulose nanofibers (Zhang et al. 2022) with the foliar fertilizers might aid in getting the applied nutrients or plant growth regulators (PGR) through the waxy leaf.

The traditional wild blueberry fertilizers are nitrogen and phosphorus based granular fertilizers, including MAP and DAP (Yarborough and Smagula 2013). Based on several past studies identifying nutrition requirements for improved wild blueberry yield, N-P-K (Nitrogen-Phosphorous-Potassium), MAP, and DAP granular fertilizers have been shown to work best, providing them the most important nutrients (N, P, K) (Yarborough and Smagula 2013, Collins and Drummond 2018, Starast et al. 2007, Percival et al. 2002, Percival and Sanderson 2004). While nitrogen and phosphorus are the most important nutrients for plants, they also require other macro- and micro-nutrient elements such as Calcium and Boron (Smagula 1993, Chen et al. 1998). Foliar-applied liquid boron was found to be more efficient for faster nutrient uptake by wild blueberry plants when compared to soil-applied granular boron due to the relative immobility of the boron element (Perrin 2001, Eaton 1944, Eaton 2007). To date, there have been very few studies that have investigated the effects of micronutrients through foliar sprays on wild blueberry, where mixed results were reported (Smagula 1993, Chen et al. 1998, Perrin 2001, Eaton 1944, Eaton 2007). In contrast, multiple studies on highbush blueberry plants reported foliar fertilizers to be effective in cases of leaf micronutrient deficiencies, which therefore improved yield in highbush blueberries (*Vaccinium corymbosum* L.) (Karlsons and Osvalde 2019, Hart et al. 2006, Wach and Błazewicz-Wo´zniak 2012). Due to the lack of sufficient investigation of foliar fertilizers containing macro- and micro-nutrients, plant growth regulators, and adjuvants, it is vital to evaluate such commercial foliar products before recommending them to wild blueberry growers.

Additionally, the physiology of wild blueberries is relatively understudied. Few studies, to our knowledge, have explored the photosynthetic performance of this crop (Tasnim and Zhang 2021, Hicklenton et al. 2000, Percival et al. 2003, 2012, Yarborough 2004, Tasnim et al. 2020). Also, no study so far has related the wild blueberry photosynthesis and other physiological and morphological traits to fruit yield. This information is needed to understand better the effects of different fertilizers on the physiology and yield of this crop. Photosynthesis is directly related to plant productivity and crop yield (Zelitch 1982, Peng et al. 1991), and is therefore used as a good indicator of fertilizer efficacy, though the relationship has not yet been established for wild blueberries. By using physiological traits like leaf chlorophyll concentration and photosynthesis to identify the absorption of nutrients, researchers and growers would have a physiological explanation for why certain products are effective or not. Therefore, robust investigations that test the efficacy of different commercial foliar products on the physiology, morphology, and production of wild blueberries are long overdue. To this end, the objectives of this study were to:

1. Assess the impacts of common commercial foliar fertilizer products containing different nutrient elements and plant growth regulators on wild blueberry physiology, morphology, growth, and yield during one crop cycle from 2019 (vegetative year) to 2020 (crop year).
2. Explore relationships among physiological traits, morphological traits, leaf nutrient elements, and the wild blueberry yield under different foliar fertilizer treatments.

5.3 Materials and Methods

5.3.1. Study Area and Experimental Design

Six different foliar fertilizer products were tested against one standard granular fertilizer, Diammonium phosphate (DAP) (Table 5.1) and control (no fertilizer) in a randomized complete block design with eight replicates (Figure 5.1) in a conventional wild blueberry field at the Blueberry Hill Farm, Jonesboro, ME, USA. Six soil samples were collected at a depth of 6” (15 cm) with a soil sampling probe from the entire study location across the field when the experimental blocks were laid out (May 2019). Those soil samples were mixed for homogeneity and sent as one sample to the University of Maine Soil Testing Lab, Orono, ME, USA, for a comprehensive soil test. The soil pH and organic matter (OM) were 4.8 and 12.1%, respectively, slightly higher than the recommended optimum levels (4 to 4.5 pH, and 5 to 8% OM) for wild blueberries. Each experimental plot was 6’ x 30’ (1.83 m x 9.14 m) represented in blue in Figure 5.1, and there was a 3’ (0.91 m) buffer zone between the experimental plots (Figure 5.1). In each experimental plot, at least two different wild blueberry genets were identified and flagged for measurements. This study was conducted for one crop cycle, starting in the vegetative year of 2019 and finishing in the crop year of 2020.

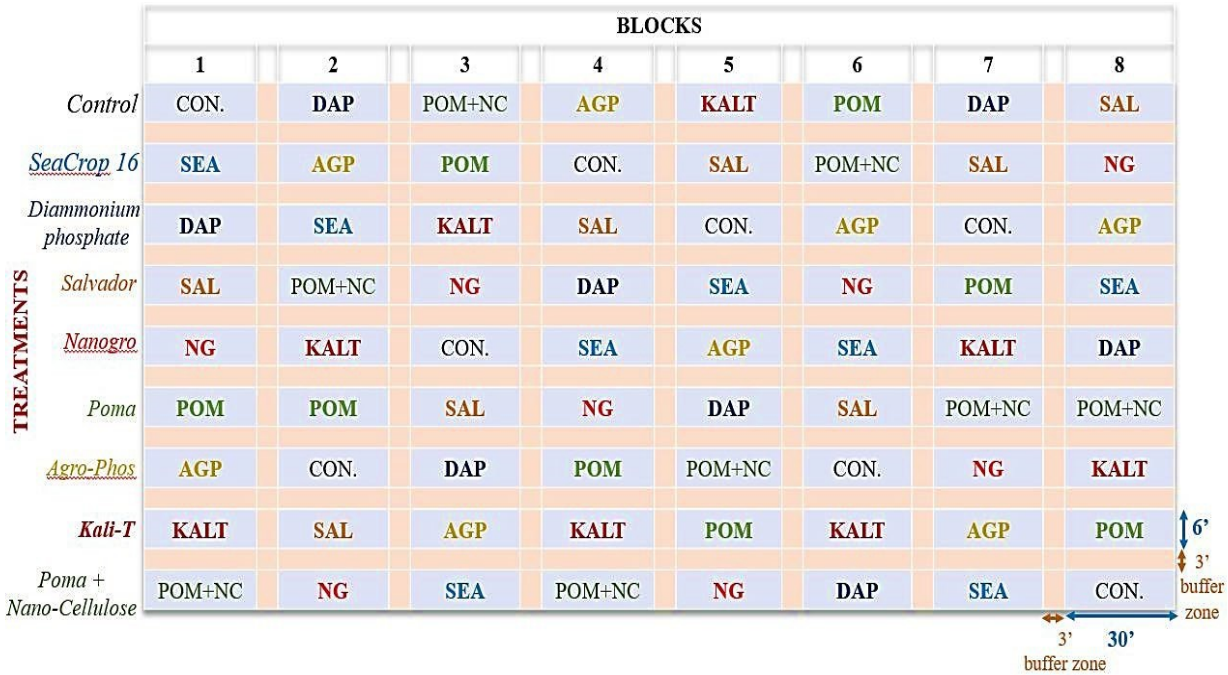

Figure 5.1 Experimental Design of this study in a conventional wild blueberry field at the Blueberry Hill Farm, Jonesboro, ME, USA.

Table 5.1 Physical properties, chemical composition, application rates, and times of commercial fertilizer products used in the experimental design (Figure 5.1) of this study in a conventional wild blueberry field at the Blueberry Hill Farm, Jonesboro, ME, USA during the crop cycle from 2019 (vegetative year) to 2020 (crop year).

Fertilizer Treatments	Type	Content	Application		
			Year	Rate	Frequency
DAP	Granular (broadcast)	Diammonium Phosphate	Vegetative (2019)	440 lbs./A (80 lbs. N/A)	One time
SeaCrop16	Liquid (backpack sprayer)	Cytokinin (Plant Growth regulator)		[2]41 oz/A	4 times: June to September
Salvador		N-P-K (14-4-6) + Micronutrients		[1]0.5 gal/A	
Agro-Phos		N-P-K (0-29-5) + 4% Mg			
Nano-Gro		N-P-K (7-10-1) + Gibberellic acid	Crop (2020)	[3]4 oz/A	3 times: June to July
Kali-T		N-P-K (2-0-24) + Si		[1]0.5 gal/A	
Poma		N-P-K (0-0-0) + 6% Ca + adjuvant			
Poma + Nano-cellulose (NC) slurry		Poma + Cellulose Nanofibrils (3% solid content)		[1](0.5 gal Poma+27.6 kg NC slurry)/A	

Note: Mixing rate with water: [1]242 gal of water/A; [2]154.88 gal of water/A; [3]15.13 gal of water/A

5.3.2. Application Materials and Methods

Fertilizer products for this study were chosen based on interest from a few fertilizer companies to sell their products to the wild blueberry growers in ME, USA. Fertilizer products were applied at the recommended rate according to the label or company representative in their recommended year (Table 5.1). Four products in this study are foliar fertilizers (Salvador, Kali-T, Agro-Phos, and Poma in Table 5.1) from the Agro-100 Global Inc., QC, Canada, which contain different amounts of macro- and micro-nutrient elements. Salvador is rich in nitrogen, phosphorus, and potassium, and complemented with magnesium, sulfur, and micronutrients (boron, iron, manganese, molybdenum, zinc) that are indispensable for plant growth. Kali-T is concentrated in potassium and enriched with nitrogen. About 70% of the potassium is in the form of potassium hydroxide and potassium carbonate, which are more easily absorbed by the leaves and the least phytotoxic. Agro-Phos is highly concentrated in phosphorus, includes soluble potassium and magnesium, and is assimilable by the foliar route. Poma is a calcium acetate-based liquid foliar nutrient which is also a chlorine- and nitrogen-free solution containing a multifunctional adjuvant (adhesive, penetrating, damping, anti-foaming, and tension-active agents) that allows calcium to remain in solution longer on the leaf without being washed out and helps to penetrate the leaf more easily. Calcium in Poma works as an osmo-regulator to help the stabilization of plant cell membranes to prevent drought and/or late frost damage (Taiz et al. 2015) and also plays a role in berry skin development. Out of the other two foliar products (Table 5.1), Seacrop16 produced by North American Kelp, Waldoboro, ME, USA, and NanoGro produced by Aqua-Yield, Sandy, UT, USA, are fertilizers with plant growth regulator (PGR) active ingredients. The active ingredient in Seacrop16 is kelp extract which naturally contains cytokinin, a growth hormone associated with enhanced plant growth and bud development (cell division), which may serve as an alternative to traditional fertilizers (Peltonen-Sainio 1997, Zodape et al. 2008). NanoGro is N-P-K (7-10-1) mixed with gibberellic acid, another plant growth hormone known to promote and elongate cells (Taiz et al. 2015). Aqua-Yield claims that the NanoGro product can increase fruit set when applied during bloom. We also tested a nanocellulose product called Cellulose Nanofibril (CNF). We tested this CNF material as an additive for foliar products, which has been recently shown to be effective for foliar pesticide application and retention on leaves (Zhang et al. 2022). CNF is made from wood-derived fiber (pulp) that has been micro-refined to the nano level of several hundredths of a micron and smaller. This cellulose nanofibril is the world's most advanced biomass material. We tested this material since CNF is derived from plant fibers, and thus the environmental impact from production and disposal is low. The University of Maine Process Development Center (PDC), Orono, ME, USA, supplies this cellulose nanofibril product (CNF) to academic, public, and private research groups interested in evaluating and developing applications for this material. The Process Development Center (PDC) is the only facility in the United States that can manufacture cellulose nanofibril (CNF) at a rate of one ton per day by mechanical fibrillation. The CNF material was hypothesized to help the foliar fertilizer as an adjuvant, sticking to the wild blueberry leaves and allowing the leaves to absorb the nutrients through the waxy cuticle. We chose the Poma product applied with the CNF to test our hypothesis. Poma was the only one containing an immobile macronutrient (Ca) with adjuvant out of all products, although there was no scientific proof regarding the efficiency of the adjuvant. Hence, testing with another potential adjuvant (CNF) (Zhang et al. 2022) would reveal if the adjuvant in Poma was sufficient or needed an additional adjuvant to efficiently work through the wild blueberry waxy cuticle. DAP was included in this study as a traditional standard soil-applied granular fertilizer and applied at the recommended rate by the University of Maine Soil Testing Lab, Orono, ME, USA, based on the foliar test results conducted in 2018. Products recommended for vegetative and bud development were applied in 2019 as vegetative-year products, and products associated with flower and fruit development were applied in 2020 as crop-year products. In 2019, vegetative-year foliar products were mixed with water and applied using a back sprayer on 12 June, 9 July, 21 August, and 10 September. The DAP fertilizer treatment was applied one time as a broadcast application by hand on 12 June 2019. In 2020, crop-year products were mixed with water and applied using a back sprayer on 17 June, 9 July, and 29 July.

5.3.3. Measurement Methods

5.3.3.1. Physiological Traits

Six wild blueberry stems from each treatment plot were randomly selected to monitor chlorophyll and anthocyanin concentration from June to October in the vegetative year (2019). Again, in the crop year (2020), eight stems from each treatment plot were randomly selected to monitor chlorophyll and anthocyanin concentrations from June to September. Chlorophyll concentration (SPAD) was measured using an MC-100 chlorophyll concentration meter (Apogee Instruments, Inc., Logan, UT, USA), and Anthocyanin concentration (ACM) was measured using an ACM-200 (Opti-sciences, Hudson, NY, USA). Leaf photosynthetic rates were measured in leaves from two stems in each treatment plot with a portable photosynthetic measurement system through gas-exchange measurements (li-6800; Li-Cor Biosciences, Lincoln, NE, USA) on a sunny day (July 15th) in the vegetative year (2019) between 10:00 and 15:30 h solar time at a photosynthetic photon flux density of 1500 $\mu mol.m^{-2}.s^{-1}$. The measurements were completed under an ambient CO_2 concentration of around 350 to 370 μmol.mol^{-1}, with temperatures ranging from 23.5 to 27.1 °C, and relative humidity ranging from 57% to 83%. Gas exchange measurements were taken from one stem in each treatment plot on a sunny day (July 16th) in the crop year (2020) between 10:00 and 15:00 h solar time. These measurements were completed under an ambient CO_2 concentration between 360 to 380 μmol.mol^{-1}, with temperatures ranging from 24 to 28 °C, and relative humidity ranging from 50% to 75%.

5.3.3.2. Structural Traits and Leaf Nutrient Concentrations

Six wild blueberry stems from each treatment plot were randomly selected to monitor stem heights from June to July (until 100% tip-die back of plants when their height increments leveled off) in the vegetative year (2019). In the crop year (2020), eight stems from each treatment plot were randomly selected for final stem height measurements. In the vegetative year (2019), twelve stems from each genet in each treatment plot (24 stems at two samples in each plot) were collected in July 2019 (after 100% tip-die back) to measure total leaf area per stem, leaf dry biomass per stem, and leaf mass per area. In the crop year (2020), during the harvesting period in early August, eight stems from two genets in each treatment plot (4 stems from each genet) were collected to measure leaf number, leaf dry biomass per stem, and leaf nutrients. Twelve random leaves from each of the 8 stems in each treatment plot were collected to measure leaf area per stem, leaf dry biomass, and leaf mass per area. Leaf area was determined using an LI-3000A area meter (Li-Cor, Lincoln, NE, USA), and the leaves were oven-dried at 70 °C to constant mass and weighed using a precision balance (0.0001 g). The dried leaf samples were ground and sent to the University of Maine Soil and Plant Tissue Testing Laboratory in Orono, ME, USA, for leaf nutrition analysis. Total carbon (C) and total nitrogen (N) in leaf tissue samples were quantified using a Leco TruMac CN analyzer (Midland, ON, Canada). The rest of the macro-nutrients (P, K, Ca, Mg) and micro-nutrients (Al, B, Cu, Fe, Mn, Zn) in leaf tissue samples were quantified using a Thermo-Fisher model iCAP 6300 radial view ICP-OES (Waltham, MA, USA).

5.3.3.3. Crop Yield

Wild blueberries from all the treatment plots were harvested on 13 August 2020, using a hand rake and a walk-behind harvester. The two harvesting modes provided a more exact yield via the hand rake and a more realistic yield via the harvester for each treatment plot. In two locations at the center third of each treatment plot, a 4sqft (0.37 m^2) quadrat was hand raked and weighed to obtain a quadrat yield. Following the collection of the quadrat yield, a 3 ft (0.91 m) wide walk-behind harvester was used to harvest a 90 sq ft (8.36 m^2) strip down the center of each treatment plot to obtain a representative 'whole plot' yield. Prior to harvesting, each plot was visually ranked on a scale of 0–3, with 0 indicating unusually low berry coverage and 3 indicating optimum berry coverage to account for bare patches or lower fruiting clones. The ranks were converted to a corresponding percent cover and multiplied by the whole plot yield and quadrat yield to obtain an estimated yield for each. The estimated yield represents an estimated potential yield under different fertility treatments. The estimated whole plot and quadrat yields were averaged by treatment plot to report the final 'yield' in this study.

5.3.4. Data Analysis

Statistical analyses were carried out using SPSS V21 (IBM Corp., Armonk, NY, USA) and JMP Pro 16.2 (SAS Institute Inc., Cary, NC, USA). Frequently monitored measurements during the growing season, including stem heights, chlorophyll concentration, and anthocyanin concentration, were analyzed by a two-way analysis of variance (ANOVA) using a General linear model univariate procedure testing the effects of time (measurement dates), treatments, and any interaction between time (dates) and treatments. Analysis of Variance (ANOVA) based upon a randomized complete block design (RCB) as well as a series of General linear models were used to compare the single date measurements (leaf photosynthetic rates, leaf area, leaf biomass, leaf mass per area, leaf nutrients, and harvest yield). These analyses were followed by a Tukey's pairwise comparison and LSD (least significant difference) post-hoc test (α = 0.05). For all these analyses, treatments were considered as a fixed factor, experimental blocks were considered as a random factor, and Bonferroni confidence interval adjustment was applied. A Principal Component Analysis (PCA) was applied to all the physiological, morphological, leaf nutrients, and yield measurements using the multivariate platform in JMP Pro 16.2. The two highest PCs (PC 1 and PC 2 and their explained percentage of total variance) were used to plot the PCA scores and PCA loadings for the data taken in 2019 and 2020 separately. Further, multiple linear regression analysis and bivariate linear regression analyses were conducted in predicting the crop yield (dependent variable) using all the physiological, morphological, and leaf nutrient measurements (independent variables) in JMP Pro 16.2. For all the above-mentioned analyses, data were transformed by the square root prior to analysis if and where necessary.

5.4 Results

5.4.1. Effects on Wild Blueberry Plant Physiology

Overall, applied fertilizer treatments significantly affected the measured wild blueberry leaf chlorophyll concentrations on different dates throughout the growing season (Table 5.2 and Figure 5.2) in both vegetative and crop years. In the vegetative year (2019), plants treated with DAP fertilizer consistently had the highest leaf chlorophyll concentration throughout the summer among all treatments (Figure 5.2a). Both DAP and Salvador treated plants had significantly higher leaf chlorophyll concentrations at the end of July 2019 (Figure 5.2c) when plants reached their seasonal peak chlorophyll concentration, compared to plants under other treatments (Figure 5.2a). In the crop year (2020), overall leaf chlorophyll concentration levels were lower than the observed levels in the vegetative year. Interestingly, the effects from DAP fertilizer diminished in the crop year (2020), whereas a significantly higher leaf chlorophyll concentration was observed in the SeaCrop16 and Poma+Nanocellulose treated plants compared to the other treatments, including control (Figure 5.2 b,c).

Table 5.2 Two-way Analysis of variance (ANOVA) results from the fertilizer effects on leaf chlorophyll concentration measured on different dates in 2019 and 2020, as shown in Figure 5.2a,b.

Study Year	**Source of Effects**	**df**	**F**	***p***
Vegetative Year (2019)	Measurement Dates	5	82.939	< 0.001
	Treatments	4	5.112	< 0.001
	Dates*Treatments	20	1.094	0.35
Crop Year (2020)	Measurement Dates	5	275.416	< 0.001
	Treatments	8	5.048	< 0.001
	Dates*Treatments	40	1.452	< 0.05

In contrast to the chlorophyll concentration, no significant differences were found in leaf photosynthetic rates (Figure 5.3) among the treatments in the vegetative year (2019). Also, leaf photosynthetic rates (Figure 5.3) in the crop year (2020) were not in agreement with the observed pattern in leaf chlorophyll concentrations (Figure 5.2c) across the applied treatments. The leaf photosynthetic rates were significantly lower in DAP and Seacrop16 treated plots compared to the control and other treatments, and no significant differences were found among all other treatments (Figure 5.3).

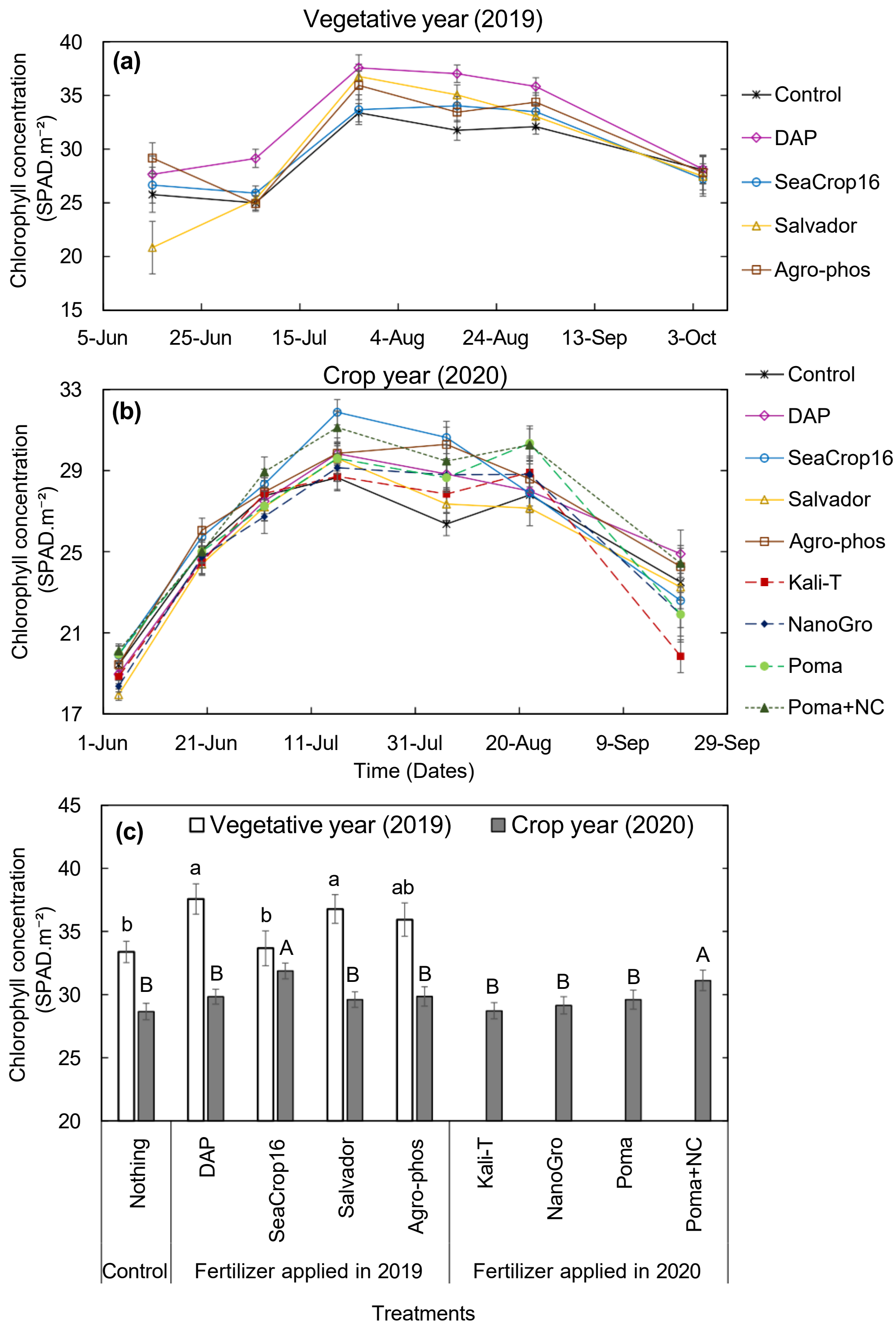

Figure 5.2 Changes in leaf chlorophyll concentration of wild blueberry plants over (a) June to October in 2019 (vegetative year) and (b) June to September in 2020 (crop year) for studied nine different treatments. (c) Comparison in peak leaf chlorophyll concentration of wild blueberry plants in 2019 and 2020 across the nine different treatments. Error bars indicate the standard

error of the mean. Different small letters and capital letters over the bars indicate significant differences among the treatments in 2019 and 2020, respectively at the significance level of $p < 0.05$.

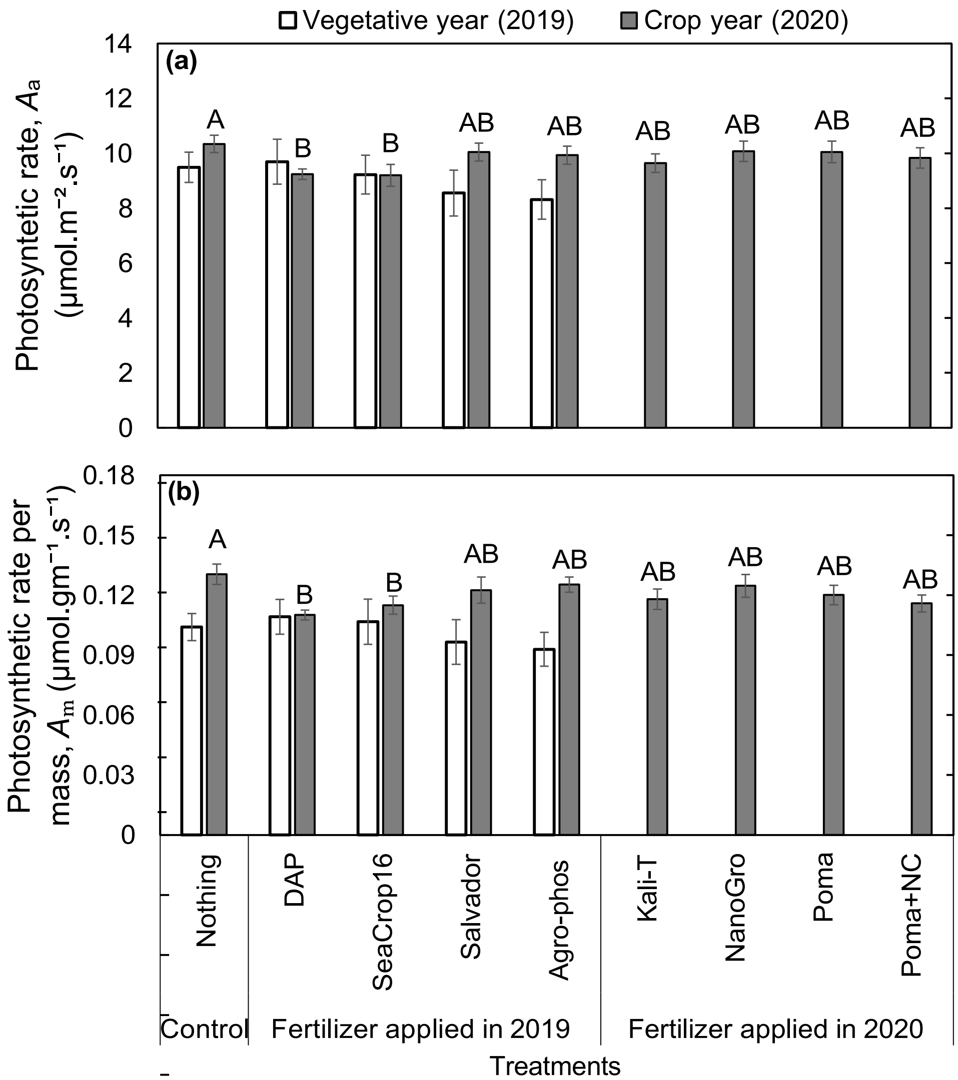

Figure 5.3 Comparison in (a) Photosynthetic rate per leaf area and (b) Photosynthetic rate per leaf mass of wild blueberry plants in 2019 and 2020 across the studied nine different treatments. Error bars indicate the standard error of the mean. No letters over the bars indicate no significant differences among the treatments in 2019, and different capital letters indicate significant differences among the treatments in 2020 at the significance level of $p < 0.05$.

5.4.2. Effects on Wild Blueberry Plant Morphology

No significant differences were found in morphological traits (stem height, leaf area, leaf biomass, and leaf mass per area) of the wild blueberry crops across the treatments in both the vegetative and crop years (Figures 5.4 and 5.5). Final stem heights (Figure 5.4) showed no significant difference among all the treated plots measured in the crop year (2020) right before harvesting the fruit. Overall, leaf area per stem was two times higher, while the leaf mass per stem and leaf mass per area were lower in the crop year (2020) than in the vegetative year (2019). There was no significant difference among different treatments in leaf area per stem, leaf biomass per stem, and leaf mass per area (Figure 5.5). Although not significant, Salvador treated plots had the lowest average leaf area and biomass per stem (Figure 5.5 a,b) among all treatments, including the control. Control plants had the highest average leaf mass per area (Figure 5.5 c) in the vegetative year (2019). In the crop year (2020), higher average leaf area and biomass per stem were found in SeaCrop16, Kali-T, Poma, and Poma + NC treated plots compared to the control, whereas other treatments showed lower leaf area and biomass than the control plot (Figure 5.5 a,b), but not significantly different. SeaCrop16, Salvador, Kali-T, Poma, and Poma + NC treated plots showed higher average leaf mass per area than other treatments, including the control, yet this was not significantly different (Figure 5.5 c).

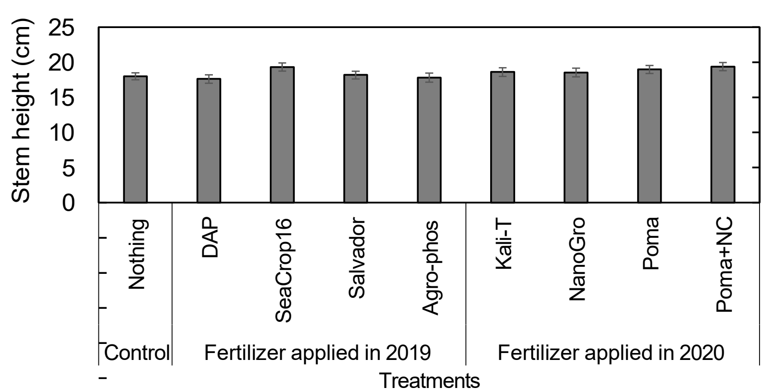

Figure 5.4 Comparison of final wild blueberry stem heights in 2020 (crop year) across the studied nine treatments. Error bars indicate the standard error of the mean. No letters indicate no significant differences among the treatments at the significance level of $p < 0.05$.

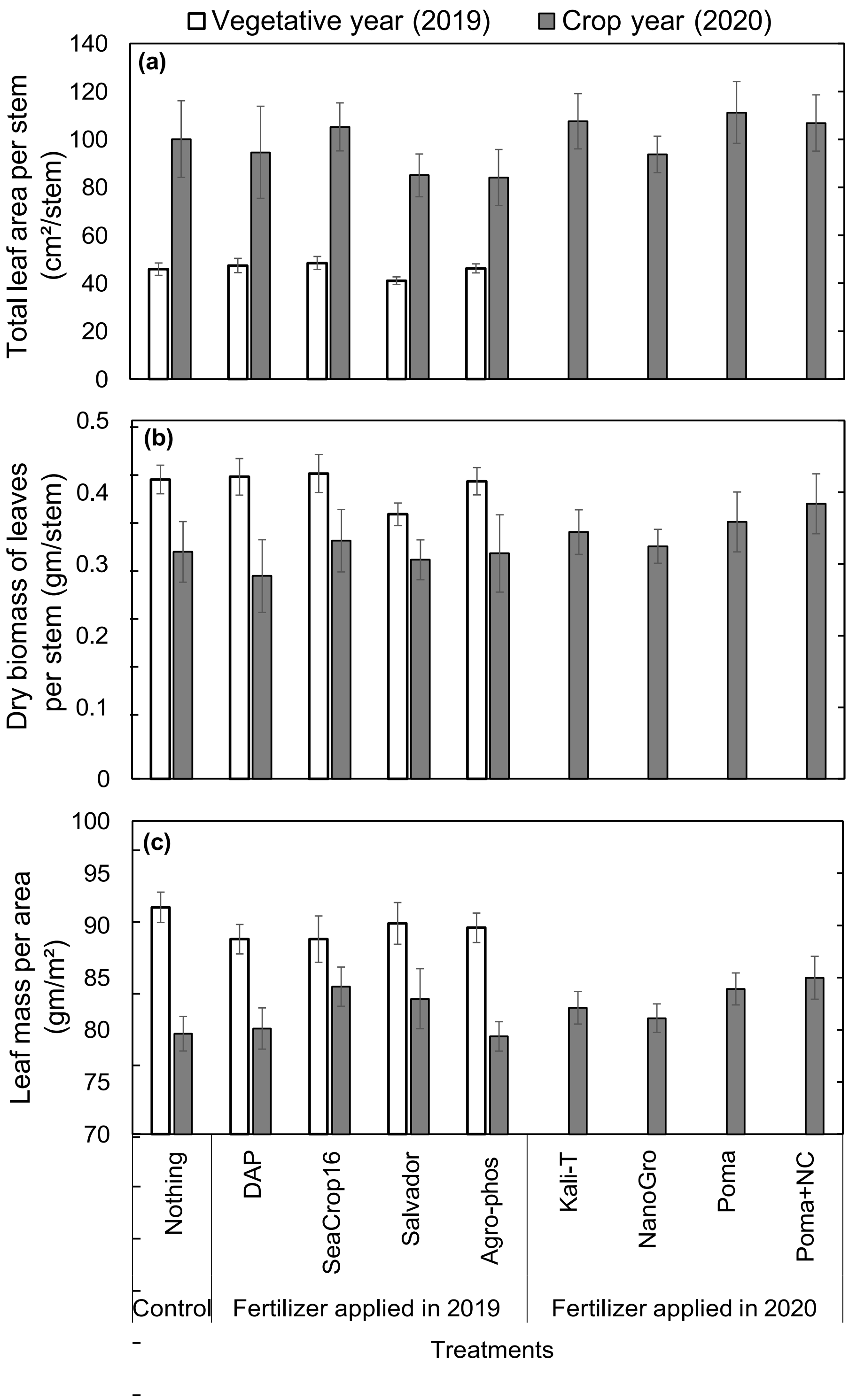

Figure 5.5 Comparison in (a) Total leaf area per stem, (b) Dry biomass of leaves per stem, and (c) Leaf mass per area (LMA) of wild blueberry plants in 2019 and 2020 across the studied nine different treatments. Error bars indicate the standard error of the mean. No letters over the bars indicate no significant differences among the treatments at the significance level of $p < 0.05$.

5.4.3. Effects on Wild Blueberry Leaf Nutrients

In all treatments, leaf nitrogen (N), phosphorus (P), and potassium (K) levels were lower than the established optimum level (Figs. 5.6 a to 5.6 c) for wild blueberry plants in both the vegetative (2019) and crop (2020) years. In contrast, leaf calcium (Ca) (Fig. 5.6 e) and magnesium (Mg) (Fig. 5.6 f) levels were at the optimum level in the vegetative year, and they were higher than the optimum level in the crop year in all the treatments. In terms of differences across the treatments, no significant differences in leaf macro-nutrients (N, P, K, C, Ca, and Mg in Fig. 5.6) in the vegetative year were found. In contrast, in the crop year, significantly higher leaf P (Fig. 5.6 b) was observed in the DAP treatment, yet that was not significantly higher than the control where no fertilizer was applied. However, no significant differences were found in leaf N (Fig. 5.6 a), K (Fig. 5.6 c), and C (Fig. 5.6 d) concentrations across the treatments in crop year whereas significantly lower Ca (Fig. 5.6 e) and Mg (Fig. 5.6 f) concentrations were found in Kali-T treatment compared to others.

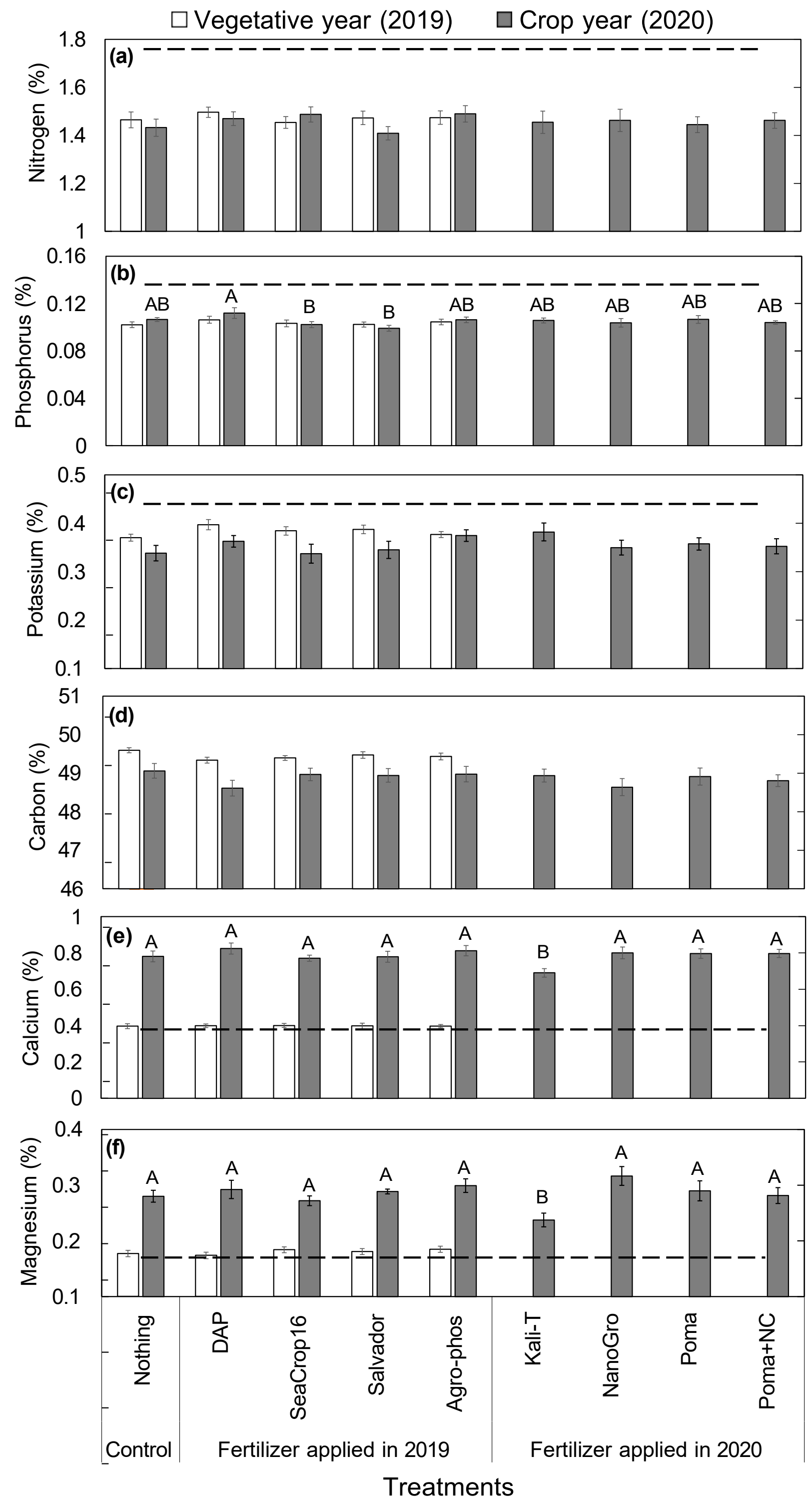

Figure 5.6 Comparison in concentration of macro-nutrient elements per leaf mass of wild blueberry plants in 2019 and 2020 across the studied nine different treatments: (a) Nitrogen, (b) Phosphorus, (c) Potassium, (d) Carbon, (e) Calcium, and (f) Magnesium. Error bars indicate the standard error of the mean. No letters over the bars indicate no significant differences among

the treatments in 2019, and different capital letters indicate significant differences among the treatments in 2020 at the significance level of $p < 0.05$. The dashed lines represent the recommended optimum nutrient levels in wild blueberry leaves (Santiago 2011).

In the vegetative year, leaf micro-nutrients (Figure 5.7) such as Boron (B), Copper (Cu), Iron (Fe), Manganese (Mn), and Zinc (Zn) were at the optimum level in all treatments, whereas only Aluminum (Al) was far below the optimum level required for the wild blueberry plants. These leaf micro-nutrients were close to (Al in Figure 5.7 b) or higher (B in Figure 5.7 a; Cu, Fe, Mn, Zn in Figure 5.7 c–f) than the optimum levels in the crop year. Regarding differences across the treatments, no significant differences were found in micronutrients (Figure 5.7) in the vegetative year (Figure 5.6). In contrast, significant differences among the treatments were found in the crop year for all the micro-nutrients (B, Al, Fe, Mn, Zn in Figure 5.7 a,b,d–f) except for Cu (Figure 5.7 c).

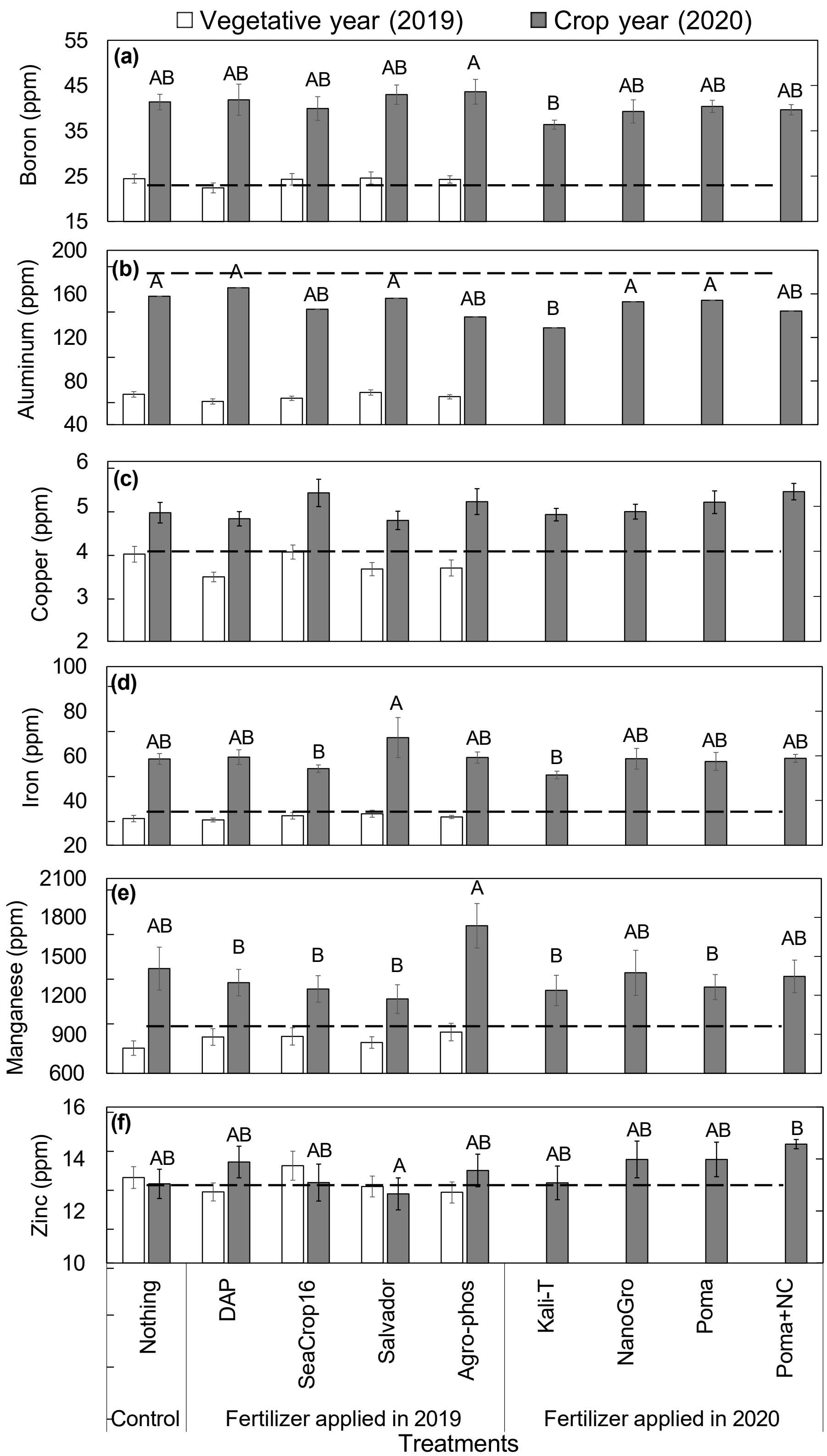

Figure 5.7 Comparison in concentration of micro-nutrient elements per leaf mass of wild blueberry plants in 2019 and 2020 across the studied nine different treatments: (a) Boron, (b) Aluminum, (c) Copper, (d) Iron, (e) Manganese, and (f) Zinc. Error

bars indicate the standard error of the mean. No letters over the bars indicate no significant differences among the treatments in 2019, and different capital letters indicate significant differences among the treatments in 2020 at the significance level of $p < 0.05$. The dashed lines represent the recommended optimum nutrient levels in wild blueberry leaves (Santiago 2011).

5.4.4. Effects on Crop Yield

No significant differences across the treatments were found in harvested wild blueberries at the end of this study in crop year (2020) (Figure 5.8). However, based on the average, although not significant, Poma+NC treated plots followed by NanoGro and DAP treated plots had higher yield than the control, whereas other treated plots had lower yield than the control (Figure 5.8).

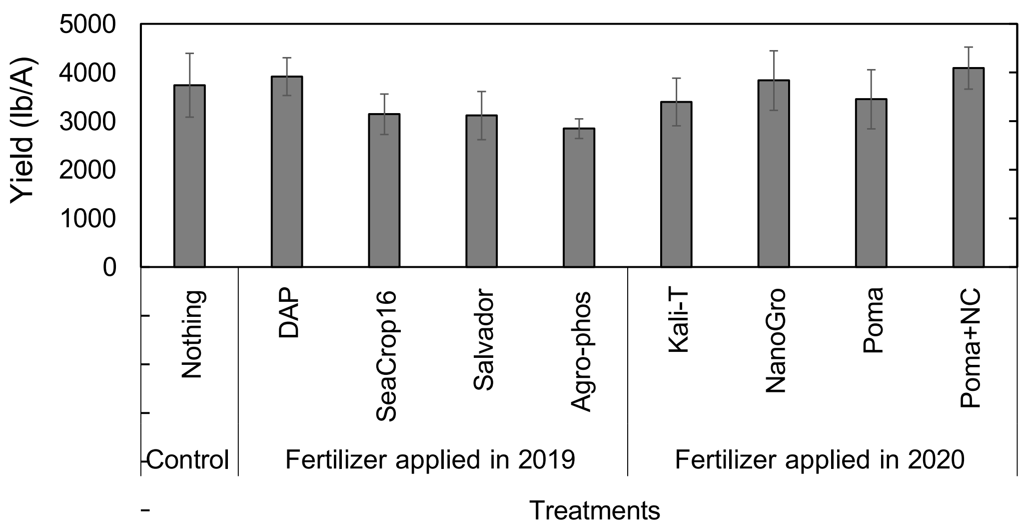

Figure 5.8 Comparison in harvested yield of wild blueberries in crop year (2020) across the studied nine different treatments. Error bars indicate the standard error of the mean. No letters over the bars indicate no significant differences among the treatments at the significance level of $p < 0.05$.

5.4.5. Relationships among All Wild Blueberry Plant Traits and Yield

Based on the principal component analysis of all measured traits when vegetative (traits of vegetative year and crop yield in Figure 5.9 a) and crop (traits of crop year and crop yield in Figure 5.9 b) years are analyzed separately, the vegetative year (Figure 5.9 a) exhibited more closely related traits, especially regarding crop yield, rather than the traits of the crop year (Figure 5.9 b). It is also evident that a higher percentage of the total variance was explained by the first two principal components in the vegetative year (20.8% by PC1 and 14.1% by PC2 in Figure 5.9 a) than in the crop year (17.2% by PC1 and 11.3% by PC2). In fact, all the measured physiological traits, morphological traits, and leaf macro- and micronutrients from the vegetative year significantly ($p < 0.05$) explained 70% of the variation of the yield, whereas those parameters from the crop year explained only 40% (Table 5.3), which is non-significant. Moreover, out of all the measured traits in the vegetative year, the morphological traits such as leaf area per stem, leaf mass per stem, and leaf mass per area were the most important predictive parameters (Table 5.3).

Additional to the bivariate analysis between the measured traits and yield (Table 5.4), we observed that yields are significantly positively related to stem length (Figure 5.10 a) of wild blueberry plants. Leaf area per stem was also positively related to yield according to the multiple regression (Table 5.3), but the bivariate relationship was not significant (Figure 5.10 b). Such relationships indicate that with the increasing stem height and leaf surface area of wild blueberry plants in the vegetative year, an increased yield was observed. The relationship between stem height and yield (Figure 5.10 a) was similar in both vegetative and crop years because stem heights were measured after the tip-die back period in the vegetative year. Therefore, the final stem heights measured in crop year were almost the same as in the vegetative year since the stem heights level off after the tip-die back period passes.

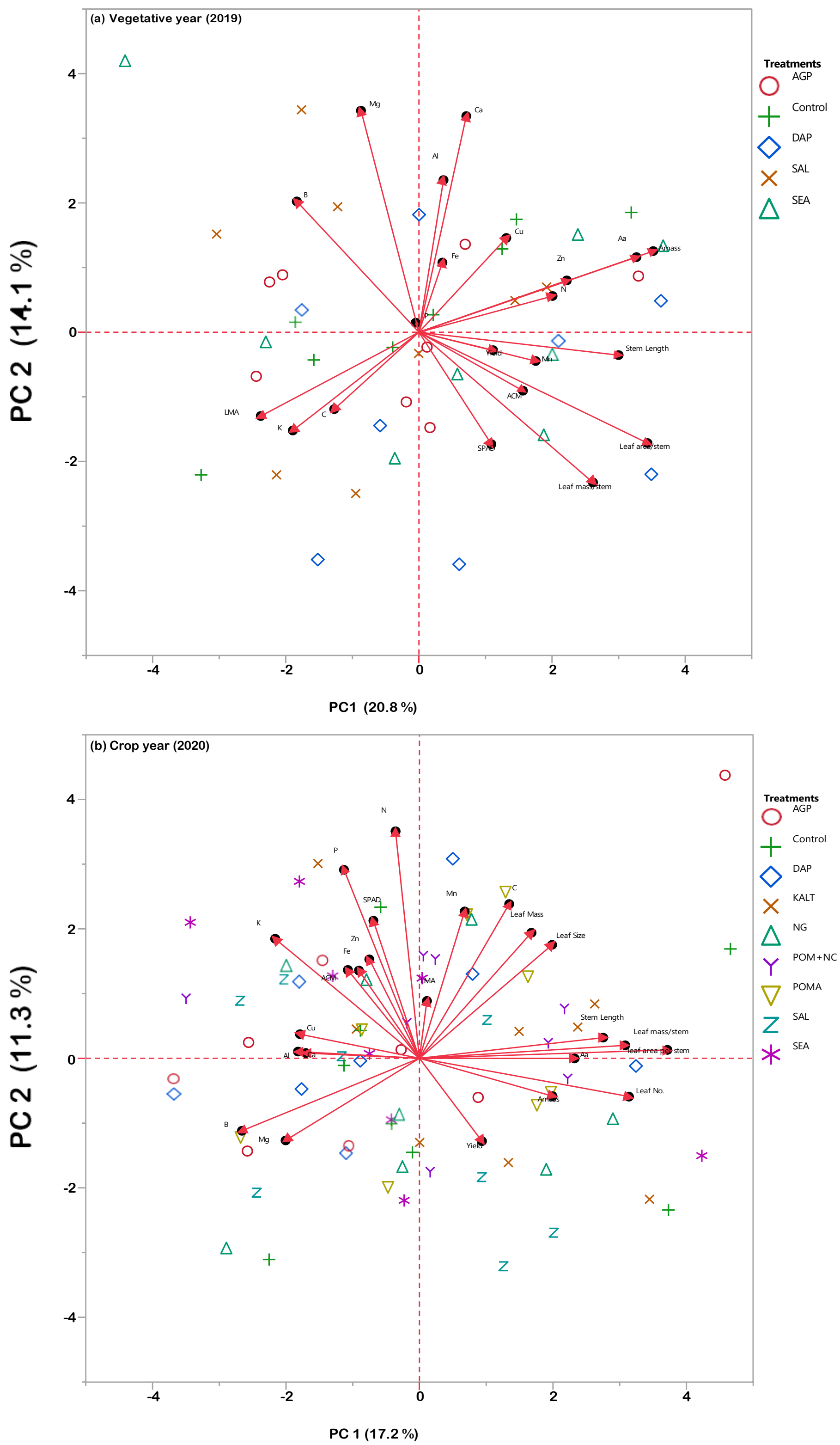

Figure 5.9 Principal Component Analysis (PCA) of physiological traits (SPAD, ACM, Aa, Amass), morphological traits (stem length, leaf size, leaf mass, leaf no., leaf mass per area), major and minor leaf nutrient elements (N, P, K, C, Ca, Mg, B, Al, Cu,

Zn, Mn, Fe) and yield of wild blue-berries in (a) 2019 and (b) 2020. Red arrows indicate the PCA loadings of different traits. Different colored shapes in the background indicate the PCA scores for different studied treatments.

Table 5.3 Multiple linear regression analysis predicting the harvested wild blueberry yield in the crop year using measured physiological traits (SPAD, ACM, Aa, Amass), morphological traits (stem length, leaf area and mass per stem, leaf mass per area), leaf macro- and micro-nutrients (N, P, K, C, Ca, Mg, B, Al, Cu, Zn, Mn, Fe) from both Vegetative year (2019) and Crop year (2020). Bold-red colored values indicate significant parameters at the significance level of $p < 0.05$.

Independent Variables	Dependent variable: Yield					
	Vegetative year (2019)			Crop year (2020)		
	R^2	F	P	R^2	F	P
All	**0.7**	**2.21**	**0.04**	0.4	1.65	0.07

Parameter (independent variables) Estimates				
Independent Variables	**Vegetative year (2019)**		**Crop year (2020)**	
	t	**P**	**t**	**P**
Leaf area per stem	**2.92**	**0.009**	0.1	0.92
Leaf mass per stem	**-2.79**	**0.012**	0.4	0.71
LMA	**4.48**	**0.0003**	0.4	0.66
Stem Length	0.52	0.61	0.8	0.44
A_a	-1.39	0.18	-0.2	0.85
A_{mass}	1.34	0.19	0.25	0.8
SPAD	0.04	0.96	0.2	0.84
ACM	1.17	0.26	-0.5	0.63
N	-0.92	0.37	0.5	0.61
P	-0.05	0.96	-0.53	0.6
K	-1.18	0.25	-1.56	0.12
C	1.09	0.29	-1.71	0.09
Ca	-1.26	0.22	-1.53	0.13
Mg	2.05	0.05	0.54	0.59
Al	0.98	0.34	1.47	0.15
B	-0.3	0.76	-0.01	0.99
Cu	-0.57	0.58	1.07	0.3
Fe	-0.56	0.58	0.65	0.52
Mn	-0.58	0.57	-0.27	0.8
Zn	-0.03	0.97	-0.17	0.86

Table 5.4 Bi-variate linear regression analysis predicting the harvested wild blueberry yield in the crop year using individual measured physiological traits (SPAD, ACM, Aa, Amass), morphological traits (stem length, leaf area and mass per stem, leaf mass per area), leaf macro- and micro-nutrients (N, P, K, C, Ca, Mg, B, Al, Cu, Zn, Mn, Fe) from both Vegetative year (2019) and Crop year (2020). Bold-red colored values indicate significant linear relationships at the significance level of $p < 0.05$. R^2 values with negative signs indicate negative linear relationships between the parameters.

Independent Variables	Dependent variable: Yield			
	Vegetative year (2019)		Crop year (2020)	
	R^2	P	R^2	P
Leaf area per stem	0.055	0.14	0.02	0.27
Leaf mass per stem	0.033	0.26	0.025	0.2
LMA	-0.005	0.67	0.03	0.14
Stem Length	**0.07**	**0.04**	**0.07**	**0.02**
A_a	0.001	0.85	0.02	0.22
A_{mass}	0.004	0.7	0.001	0.75
SPAD	0.02	0.4	0.026	0.17
ACM	0.03	0.3	-0.03	0.17
N	-0.003	0.7	-0.02	0.26
P	-0.01	0.5	-0.003	0.64
K	-0.09	0.06	**-0.113**	**0.004**
C	0	1.0	-0.046	0.07
Ca	-0.003	0.72	-0.02	0.21
Mg	0.016	0.43	0.022	0.21
Al	0.010	0.5	0.04	0.09
B	-0.055	0.14	0	1.0
Cu	0.015	0.45	0.05	0.056
Fe	-0.006	0.63	0.005	0.57
Mn	-0.011	0.5	-0.016	0.3
Zn	0	1.0	-0.02	0.24

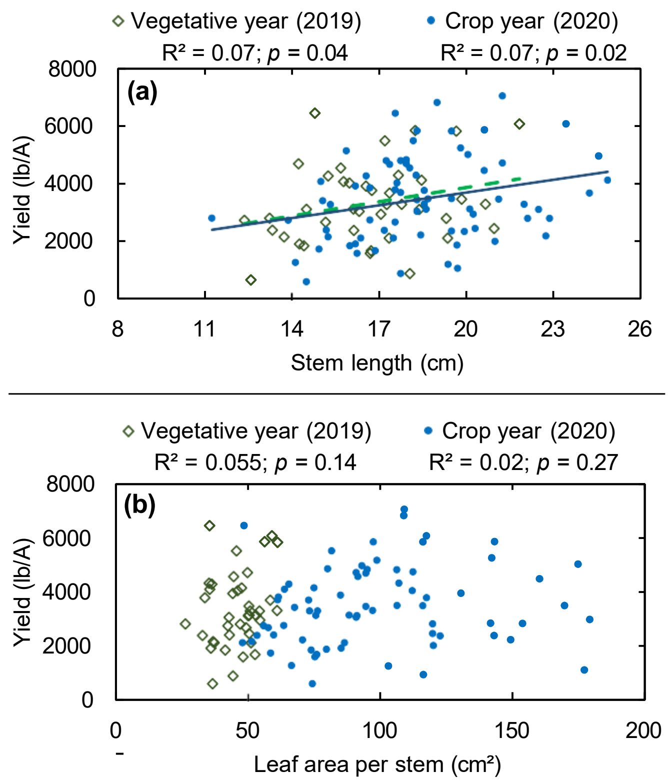

Figure 5.10 Wild blueberry yield in relation to stem length (a) and leaf area per stem (b) in the vegetative year (2019) and crop year (2020). Green dashed line and blue solid line represent significant linear regressions in the vegetative year and crop year, respectively.

5.5 Discussion

We found limited effects of applied fertilizer products on physiological and morphological traits (except leaf chlorophyll concentrations) of wild blueberries in the first (vegetative) year. However, significant differences in most of the traits across treatments were found in the second (crop) year. This could be because wild blueberry leaves need time to absorb nutrients from the applied fertilizers, especially through the existing thick waxy cuticle, and respond slowly. Our results revealed that some mobile macro-nutrients (N, P, K) needed in higher quantities might be more effective if supplied from the soil rather than through foliar products. On the other hand, immobile macro-nutrients such as Ca or plant growth regulators appeared effective when applied through the foliar system. Moreover, if an adjuvant was added to the foliar product, such as the nanocellulose used in this study, wild blueberry leaves would more effectively absorb the nutrients. Our results also revealed that if wild blueberry leaves already contain the optimum level of their most required immobile and micronutrients such as Ca, B, or Mg, it is futile to supply more of those nutrients. In fact, supplying more nutrients above a maximum threshold will not benefit wild blueberry development and production. Rather, it will add an unnecessary cost for the growers. It further implies the importance of testing leaf tissues before supplying any fertilizers to the wild blueberries. Based on the traits measured in both the vegetative and crop years of this study, we established that physiological and morphological performance in the vegetative year rather than the crop year is more likely to decide wild blueberry yield potential.

We found significant effects of applied fertilizers on leaf chlorophyll concentrations in both the vegetative and crop years. In the vegetative year (2019), the high nitrogen content in the DAP (80 lb N/acre) and Salvador (N-P-K: 14-4-6) might be the reason for higher leaf chlorophyll concentrations (Wood et al. 1992a, 1992b, Zhang et al. 2015). Higher leaf chlorophyll concentrations might also help increase the number of flower buds, as found in other studies (Sousa-Souto et al. 2018), that occur almost at the end of summer in the vegetative year for the wild blueberries (Bell et al. 2009). Previous studies have shown that N-P-K and DAP fertilizers are efficient for wild blueberries (Starast et al. 2007, Percival et al. 2002, Percival and Sanderson 2004, Smagula 1993). Although leaf chlorophyll concentration has been shown to be a strong determinant of photosynthesis for other plants (Evans 1985, Seemann 1987), it might not be true for wild blueberries. This is because leaf photosynthetic rates measured in this study did not follow the trend of the measured leaf chlorophyll concentration, and hence there was no correlation or relationship between these two physiological traits. Two treatments (DAP, SeaCrop16) showed lower, and other treatments showed similar leaf photosynthetic rates to the control plot, which might be because of high variation in photosynthetic capacity across genets (Tasnim and Zhang 2021). Our study observed leaf photosynthetic rate variation of 1 to 3 $\mu mol.m^{-2}.s^{-1}$ across different treatments with different genets in the studied field. This is consistent with the previous study (Tasnim and Zhang 2021) conducted in the same field, which has shown high variation (range of 2 to 5 $\mu mol.m^{-2}.s^{-1}$) in leaf photosynthetic capacity across different wild blueberry genets. Average leaf surface area per stem was higher in SeaCrop16 and

DAP treatments (although not significant), possibly because of the cell division regulator hormone (cytokinin) in SeaCrop16 and the high nitrogen supply from the DAP fertilizer (Taiz et al. 2015, Zhang et al. 2015). Since wild blueberry is a slow-growing plant that needs time to uptake and metabolize the applied nutrients, especially because of the acidic soil environment (Yarborough 2012), more significant effects from all the treatments were found in the crop year. However, significant differences were not found in morphology or fruit yield in the crop year. A possible explanation is that the wild blueberry leaves already contained the recommended levels of their most important nutrients [Santiago 2011, Calderwood et al. 2020) in the vegetative year, most of which increased beyond the optimum ranges after fertilizer application. This implies that if wild blueberry leaves already contain optimum nutrient levels, it is unnecessary to apply more as they can be toxic rather than helpful to the crop (Taiz et al. 2015), not to mention extravagant for growers. For instance, excessive B and Ca can be harmful to the wild blueberry plant (Calderwood et al. 2020), which is possibly why there were no significant differences in yield across the treatments as the plants already had much more B and Ca than they required. Still, Poma+Nanocellulose containing Ca and adjuvant showed some promising results, such as higher leaf chlorophyll concentration, but Poma and Poma+Nanocellulose did not significantly increase the amount of leaf Ca. In terms of applying such foliar nutrients (Ca), an adjuvant like the cellulose nanofibril (CNF) appears to help the nutrient element get through the waxy coating of the wild blueberry leaf (Zhang et al. 2022, Fernández and Eichert 2009, Schönherr 2001). Adjuvants such as the cellulose nanofibril (CNF) might help the foliar nutrients disperse well and get into the leaf stomata and stick to the leaves for a comparatively longer time during windy and rainy weather (Zhang et al. 2022, Fernández and Eichert 2009, Schönherr 2001). However, further investigation is needed to explore the use of different adjuvants for foliar nutrient adsorption by the wild blueberry plants because adjuvants vary widely (Zhang et al. 2022, Fernández and Eichert 2009, Schönherr 2001).

Lastly, in this study, the physiological and morphological performance of wild blueberries in the vegetative year were better indicators of yield compared to those in the crop year. Specifically, stem heights and leaf surface area appeared to play important roles in yield prediction. In the vegetative year, wild blueberry plants need sufficient nutrients, such as N, P, K, to build the stems and leaves for carbohydrate production, transportation, and storage (Calderwood et al. 2020). For such processes to occur, they especially need nitrogen to invest in building leaf chlorophyll to produce carbohydrates (Taiz et al. 2015, Wood et al. 1992a, 1992b, Zhang et al. 2015) and hence flower buds (Sousa-Souto et al. 2018). These flower buds will become fruit in the crop year as long as they receive enough pollination (Bell et al. 2009, Drummond 2019, Qu and Drummond 2018, Obsie et al. 2020, Qu et al. 2021). As a perennial and clonal shrub plant, wild blueberry has a large underground energy and carbohydrate reservoir in the rhizomes and roots. The carbohydrates produced in the vegetative year could be crucial for the flower development in the crop year when leaves are still young. In the crop year, more vital factors such as pollination, pest pressure, and soil moisture during fruit set and maturation determine actual fruit production (Yarborough and Smagula 2013, Drummond 2019, Qu and Drummond 2018, Obsie et al. 2020, Qu et al. 2021).

CHAPTER 6

INTERACTIONS OF CELLULOSE NANOFIBRILS WITH A FOLIAR FERTILIZER AND WILD BLUEBERRY LEAVES: POTENTIAL TO ENHANCE FRUIT YIELD

6.1 Abstract

Nanocellulose is trending for its promising application in agriculture. A few studies have reported the use of nanocellulose as an adjuvant with foliar-applied pesticides and fertilizers. Yet, the mechanism of how the cellulose nanofibrils (CNFs) interact with foliar fertilizers and leaves is still not well-understood. Therefore, we studied the effects of CNFs on leaves of two wild lowbush blueberry species (*Vaccinium angustifolium* and *Vaccinium myrtilloides*). Our study showed that the CNF addition significantly affected the surface wettability and water loss of the V. myrtilloides leaves but not the V. angustifolium leaves. The difference could be related to denser trichomes in V. myrtilloides leaves. Our study also revealed that the CNFs assisted the foliar fertilizer to disperse into smaller particles on the leaf surface of V. angustifolium species, which might have resulted in a higher average fruit yield, inviting further study.

6.2 Introduction

Nanomaterials have been widely studied for their agricultural applications, for example, growth regulators, nanopesticides, nanofertilizers, nanoantimicrobial agents, biosensors, etc. (Hu and Xianyu 2021, Pestovsky and Martínez-Antonio 2017). The use of nanomaterials is promising for improving agrochemical efficiencies, plant growth, and fruit yields. Meanwhile, two major concerns hindered large applications of those materials in agricultural fields: cost and safety (Kumar et al. 2019). Nanocellulose is a group of nanomaterials mainly derived from plant biomass. Also, the production cost of nanocellulose is fairly low compared to that of conventional nanomaterials (e.g., carbon nanotubes). Moreover, nanocellulose is nontoxic and biodegradable, causing much less impact on human health and the environment (Kangas and Pitkänen 2016). The agricultural applications of nanocellulose are emerging

(Alhamid et al. 2018, Zhang et al. 2022, Xiang et al. 2019, Alonso-Díaz et al. 2019). However, little research has investigated the interactive effect of nanocellulose, agrochemicals, and leaf structural features on plant physiology and fruit yield. In agriculture, fertilization by soil-applied fertilizers and foliar-applied fertilizers is a common practice where foliar fertilization has been trending in recent years (Haytova 2013, Fageria et al. 2009). Soil applications of chemical and/or organic fertilizers are common practices to maintain soil fertility and to achieve high yields (Souri and Sooraki 2019). However, under certain environmental conditions, foliar application of fertilizers performs better than soil application in terms of efficiency and environmental footprint (Aghaye Noroozlo et al. 2019). Foliar fertilizers are defined as liquid fertilizer products specifically designed to be applied directly to crop leaves (Alexander and Schroeder 1987). Foliar fertilization could avoid the negative impacts of continuous soil fertilization such as environmental contamination, water eutrophication, groundwater pollution, and wastage of fertilizers (Congreves and Van Eerd 2015, Kleinman 2017). Also, foliar fertilization is more efficient when required nutrients are not available for plants such as in acidic soils (Taiz et al. 2015). For instance, wild lowbush and highbush blueberries in North America have been thriving and growing in acidic soils (soil pH of 4−5.5) (Hart et al. 2006, Yarborough and Bhowmik 1993) which could be benefitted from foliar fertilization. Several previous studies proved that foliar fertilization is beneficial in supplying micronutrients and improving the yield for highbush blueberries (*Vaccinium corymbosum* L.) (Hart et al. 2006, Karlsons and Osvalde 2019, Wach and Błazewicz-Wo´zniak 2012). In contrast, contradictory results were reported regarding the efficacy of foliar fertilization in wild lowbush blueberries (Chen et al. 1998, Eaton and Ju 2007, Tasnim et al. 2022). Also, foliar applied nutrients could take a longer time to be absorbed by the blueberry leaves because of their thick waxy leaf cuticle, while those nutrients might be washed away by rain and wind (Hart et al. 2006, Wach and Błazewicz-Wo´zniak 2012). In such cases, nanocellulose has been trending to help foliar-applied pesticides and fertilizers remain on the leaves and be absorbed by them (Zhang et al. 2022, Tasnim et al. 2022, Schönherr 2001). A recent study reported the detailed mechanisms in which an eco-friendly cellulose nanofiber (CNF) strengthened the adhesion between pesticide droplets and plant leaves, which prolonged the pesticide retention on leaves (Zhang et al. 2022). Also, a similar nanocellulose material (CNF) has been found to be a potentially promising adjuvant for foliar fertilizers for wild blueberries (Tasnim et al. 2022). However, it is still unclear how CNFs interacted with the wild blueberry leaf surface and foliar-applied products, which calls for further research. The interaction between nanomaterials and leaves depends on the physical properties of the material and structural features of the leaf (Zhang et al. 2022, Tasnim et al. 2022, Schönherr 2001). Leaf surface features, for example, leaf cuticle thickness, trichome density, and their wettability, play important roles in leaf water uptake, storage, and losses (Berry et al. 2019, Pan et al. 2021). These processes regulate the foliar nutrient uptake in a plant (Berry et al. 2019, Pan et al. 2021, Fernández et al. 2021). Therefore, such interactions among the CNFs, foliar fertilizers, as well as leaf surfaces need to be explored to understand how CNFs could help certain plants efficiently absorb the foliar-applied fertilizers. Specifically, it is vital to understand how the wild blueberry leaf surface wettability, water uptake, and loss would change in reaction to the added CNFs. To this end, our objectives were to (1) characterize the interactions between CNFs, leaf surfaces, and a foliar fertilizer, as well as the effects of the CNFs on the wild blueberry leaf surface wettability, water storage, and water loss, and to (2) understand the interacted effects of a promising foliar fertilizer and CNFs on wild blueberry fruit yield (Tasnim et al. 2022). By advancing the understanding of the nanocellulose−fertilizer−leaf interactions, our study will open a new avenue of using a sustainable material such as nanocellulose to improve fertilizer use efficiency and berry production.

6.3 Materials and Methodology

6.3.1 Materials

We studied the wild lowbush blueberry in Maine, USA, where common lowbush (*Vaccinium angustifolium* Aiton) and velvet-leaved lowbush (*Vaccinium myrtilloides* Michx) blueberries coexist in the field. The leaves of both the Vaccinium species have trichomes (leaf hairs) on both adaxial and abaxial surfaces, where they are denser and more visible in V. myrtilloides leaves than in V.

angustifolium leaves (https://gobotany.nativeplanttrust.org/species/vaccinium/angustifolium; https://gobotany.nativeplanttrust.org/species/vaccinium/myrtilloides/). In any given wild blueberry field, 90% of the blueberry plants are V. angustifolium (common lowbush) and the rest of the plants are V. myrtilloides. We used a foliar fertilizer named "Poma" that was found to be effective for wild blueberries in a recent study (Tasnim et al. 2022) and CNFs. Poma was from Agro-100 Global Inc., Quebec, Canada, and contained 6% calcium (Ca) that works as an osmo-regulator and stabilizes plant cell membranes to prevent drought and frost damage (Taiz et al. 2015). Also, Poma (Ca) helps berry development, especially the development of berry skin. Poma also contains adjuvants (e.g., salicylic acid) helping the calcium to stay in the applied solution longer on the leaf surface without being washed away and to penetrate the leaf easily according to Agro-100 Global Inc. To test whether Poma alone is sufficient or additional adjuvant is needed with such foliar products, we studied CNFs, which could potentially help any foliar product to effectively remain on the leaves (Zhang et al. 2022). The CNFs are derived from pulp fibers, which were further refined to obtain a nanoscale diameter (a nominal fiber width of 50 nm and lengths of up to several hundred microns), produced and provided by The University of Maine Process Development Center (PDC), Orono, ME, USA, which is capable of producing CNFs at one ton per day.

6.3.2 CNF Effects on Leaf Water Holding, Water Release, and the Interaction between Water and Wild Blueberry Leaves

We collected stems of similar heights (30cm, 12 in.) from different genets of V. angustifolium and V. myrtilloides plants at the Blueberry Hill Farm, Jonesboro, Maine, USA. Note that the stems had alternate leaves on them, and we visually made sure to select stems with leaves of similar sizes. Typically, stem heights, leaf sizes, and leaf masses do not significantly differ within one wild lowbush blueberry genet (Tasnim et al. 2020, 2021). Then we sprayed three stems with only water and the other three stems with CNFs+water (Table 6.1) in the laboratory. To ensure uniform spraying, we sprayed each stem separately by holding it vertically, and the leaves of the stem received the treatment uniformly because of their alternate arrangements. Then we kept the stems in the refrigerator for 24 h and then rehydrated them for 1 h by cutting a small portion of the stem under distilled water (20 °C). From each stem, we detached 13 matured leaves and weighed them ($mass_i$, mg) in a precision balance (0.0001 g), which represents the saturated leaf weight ($mass_{sw}$, mg) at that initial stage before starting the natural dehydration process. Then we placed the leaves on a laboratory bench for natural dehydration, and we weighed the leaves every 15 min for the first 10 h and then every 2 h until reaching 18 h of natural dehydration to quantify the relative water content (RWC) over time. After 18 h, we dried the leaves in an oven at 70 °C for 72 h to a constant weight and weighed the dry leaves ($mass_{dry}$, mg). We quantified the water storage capacity of those leaves by calculating the mass-based saturated water content, MSWC ($mg \cdot mg^{-1}$) = $[(mass_{sw} - mass_{dry})/mass_{dry}]$. For this drying process, we kept the 13 matured leaves of each stem from each genotype separately in a weighing dish and considered each of them as one replicate to make sure each dish contained similar sizes and mass of leaves. Then we calculated the changes in RWC (%) from the changes in leaf weight ($mass_i$, mg) every 15 min for the first 10 h and then every 2 h until reaching 18 h of natural dehydration. RWC of the water-saturated leaves was considered as 100% (where $mass_i = mass_{sw}$), so the leaf water loss was zero when we weighed the leaves right after rehydrating the stems. Then we obtained the leaf water loss curves of the two studied species with two different treatments (water and CNFs) by plotting changes in RWC against time (minutes) where RWC (%) = $[\{(mass_i - mass_{dry})/(mass_{sw} - mass_{dry})\} \times 100]$. We also determined the time (T_{70}) to dry from saturated leaves to 70% RWC as the leaf water loss tolerance and compared it across the treatments because 70% RWC is the physiological threshold of natural plants (Hao et al. 2010, Zhang et al. 2015). Further, we quantified the contact angle of a deionized water droplet on both adaxial and abaxial leaf surfaces of three matured leaves from the rehydrated stems by a KRUSS mobile surface analyzer (Matthews, NC, USA).

Table 6.1 Chemical Properties, Application Rates, and Frequencies of the Applied Treatments of This Study in the Conventional Wild Blueberry Field at the Blueberry Hill Farm, Jonesboro, Maine, USA, on a Crop Year (2021)

Foliar Treatments	**Type**	**Content**	**Application**	
			Rate per Acre (rate per 4046.9 m^2 area)	**Frequency**
Water	Liquid (spray on leaves)	Fresh water	242 gal (916 L)	3 times (27 May, 3 June, 20 June)
Poma		6% Ca + adjuvant	0.5 gal (1.9 L) Poma + 242 gal (916 L) water	
Cellulose Nanofibrils (CNFs)		CNF slurry (3% solid content, derived from wood -fibers)	847 gm CNF slurry + 242 gal (916 L) water	
Poma + Cellulose Nanofibrils (CNFs)		6% Ca + adjuvant + 3% CNFs	0.5 gal (1.9 L) Poma + 847 gm CNFs + 242 gal (916 L) water	

6.3.3 Interaction among CNFs, Foliar Fertilizer, and Wild Blueberry Leaves

We conducted a field experiment in a wild blueberry crop field at the Blueberry Hill Farm, Jonesboro, Maine, USA, where we selected six genets of *V. angustifolium*, and within each genet, we selected four plots of 2 feet × 2 feet (0.61 m × 0.61 m) area. Since 90% of the blueberry plants in the wild blueberry field are *V. angustifolium* (common lowbush), we conducted our initial field experiment on *V. angustifolium* only. We sprayed four different foliar treatments on the leaves in those plots (Table 1) at a recommended rate by Agro-100 Global Inc. in their Poma foliar product label. We collected leaves after the last foliar application, and we took scanning electron microscopy (SEM) images (TM 3000, Hitachi High-Technologies Corporation, Tokyo, Japan) without sputter coating at an accelerating voltage of 15 kV on both sides of the leaves to explore how the applied foliar products disperse on the leaf surface. Fourier transform infrared (FTIR) characterization was performed to further understand the interaction between the Poma and CNFs. Four samples were prepared for this purpose, CNF, Poma, CNF-Poma unwashed (without washing fertilizer), and CNF-Poma washed (with filtration washing of fertilizer using an excess of DI water). CNF-Poma samples were prepared using the same ratio mixture mentioned in Table 6.1. FTIR spectra of samples were measured on a PerkinElmer UATR 2 diamond crystal spectrometer. A total of 60 cumulative scans in absorption mode were taken, with a resolution of 1 cm^{-1} in the frequency range of 4000−450 cm−1. The Ca^{2+} on unwashed and washed CNF/Poma sample surfaces was further analyzed with a SEM (Cube II, EMCRAFTS, Korea) with an energy-dispersive analyzer.

6.3.4 Foliar Fertilizer and CNF Effects on Wild Blueberry Leaves and Yield

From the field experiment, we collected one stem from each treatment at midday (∼12:30 h solar time) on 22 June 2021 to measure midday leaf water potential (LWP) by a leaf pressure chamber (PMS Inc., Albany, OR, USA). When fruits were mature, we collected stems to measure leaf characteristics and harvested the fruits to measure the yield on 22 July 2021. We measured the leaf area using a LI-3000A area meter (Li-Cor, Lincoln, NE, USA), and then we oven-dried the leaves at 70 °C to a constant mass and weighed them to quantify leaf mass per area (LMA) as leaf dry mass divided by leaf area. Then we ground those dried leaves and sent them to the University of Maine Soil and Plant Tissue Testing Laboratory in Orono, Maine, for leaf tissue nutrient testing (Table D1).

6.3.5 Data Analysis

We conducted statistical analyses in JMP Pro 16.2 (SAS Institute Inc., Cary, NC, USA) using Analysis of Variance (ANOVA) based upon a randomized complete block design and general linear models to compare the parameters across different treatments. These analyses were followed by a Tukey's pairwise comparison and least significant difference posthoc test ($\alpha = 0.05$) where treatments were fixed factors and genets were random factors. Bonferroni confidence interval adjustment was applied, and data were transformed by the square root prior to analysis if and where necessary. Bivariate linear regression analyses were conducted to predict the yield using LMA (Figure D1).

6.4 Results

6.4.1 CNF Effects on the Interaction between Water and Wild Blueberry Leaves

While comparing water loss curves of the two blueberry species (Figure 6.1a), *V. myrtilloides* leaves were losing water significantly ($p < 0.05$) faster than *V. angustifolium* leaves (Figure 6.1a,b). In particular, *V. myrtilloides* leaves treated with CNFs were losing water faster than the leaves without CNFs, but the difference was not always significant during the drying process. In contrast, there were no differences in water loss between *V. angustifolium* leaves with and without CNFs. Specifically, the time to reach 70% RWC (Figure 6.1b) was 85−105 min for *V. myrtilloides* leaves without CNFs and 70−80 min for *V. myrtilloides* leaves with CNFs. For *V. angustifolium*, it was 110−135 min for both leaves with and without CNFs. In agreement, mass-based saturated water content was found to be significantly higher in *V. angustifolium* leaves compared to that in *V. myrtilloides* leaves (Figure 6.1c) regardless of the CNF treatment. However, MSWC did not differ between *V. angustifolium* leaves treated with CNFs and those without CNFs. In contrast, MSWC was found to be significantly higher in *V. myrtilloides* leaves treated with CNFs compared to that in the leaves without CNFs (Figure 6.1c).

Both abaxial (Figure 6.2a) and adaxial (Figure 6.2b) leaf surfaces of the two blueberry species were hydrophilic as the contact angles of the water droplets on those surfaces were found to be less than 90°. The addition of CNFs to *V. angustifolium* leaves did not affect their wettability as the contact angles of the water droplets on both surfaces were as same as the leaves without CNFs. In contrast, the addition of CNFs significantly increased the hydrophilicity of *V. myrtilloides* leaves as the contact angles of the water droplets were significantly lower on both surfaces of those leaves compared to that of the leaves without CNFs.

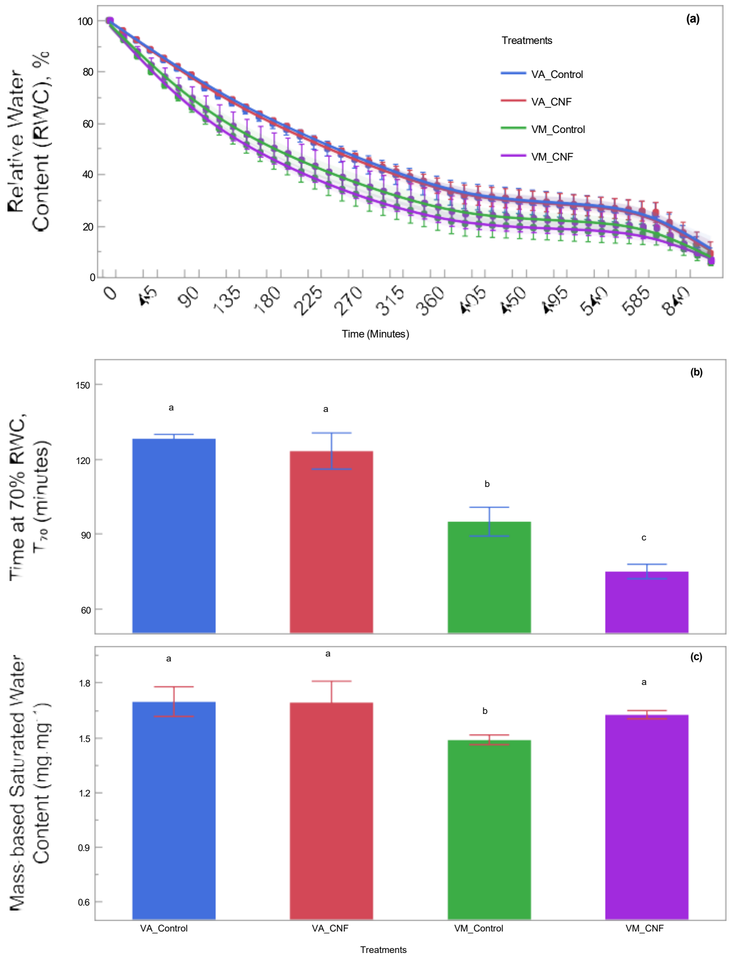

Figure 6.1. Comparisons in (a) leaf water loss over time, (b) time reaching 70% relative water content from saturation point, and (c) mass-based saturated water content of two wild blueberry species among applied treatments. In (a), data points indicate the mean of relative water content (%) of the studied leaves at a particular time; error bars indicate the standard error of the mean (n = 3), and shaded areas indicate a 95% confidence interval. Different letters in (b,c) indicate significant differences at the significance level of $p < 0.05$. Here, "VA" indicates *Vaccinium angustifolium*, and "VM" represents *Vaccinium myrtilloides*.

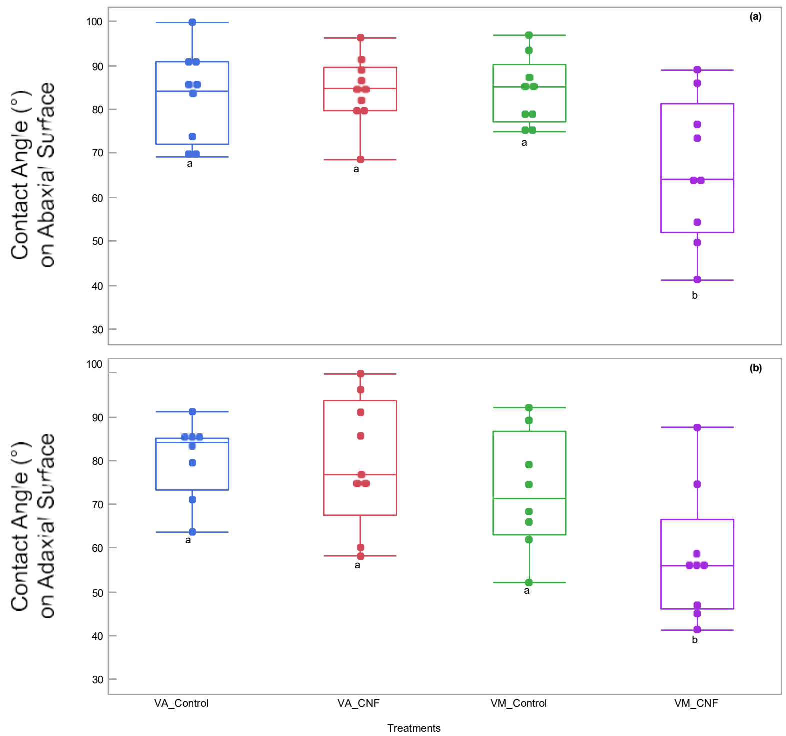

Figure 6.2. Comparisons in the contact angle using deionized a water droplet on (a) abaxial and (b) adaxial leaf surfaces of two wild blueberry species among the applied treatments. Different letters indicate significant differences at the significance level of $p < 0.05$. Here, "VA" indicates *Vaccinium angustifolium*, and "VM" indicates *Vaccinium myrtilloides*.

6.4.2. Interaction among CNFs, Foliar Fertilizer, and Wild Blueberry Leaves.

Based on the observations from the SEM images (Figure 6.3) of the leaves, Poma fertilizer formed large aggregates on both adaxial (Figure 6.3a) and abaxial (Figure 6.3c) leaf surfaces when applied alone. In contrast, when CNFs were applied with the Poma fertilizer, the fertilizer was dispersed on both adaxial (Figure 6.3b,e) and abaxial (Figure 6.3d) leaf surfaces rather than forming large aggregates. In fact, the dispersed fertilizer also broke down into smaller particles around the leaf stomata of the abaxial surface (Figure 6.3d). FTIR spectra of CNFs showed characteristic peaks corresponding to mainly three major bands, which included the large hydroxyl group band at around 3350 cm^{-1}, a C−H carbohydrate stretching band at 2900 cm^{-1}, and a sharp band corresponding to characteristic pyranose C−O−C stretching present in the CNF carbohydrate ring structure at around 1050 cm^{-1} (Figure 6.4) (Parit et al. 2020). CNF-Poma unwashed and CNF Poma washed samples also showed the presence of these peaks (Figure 6.4).

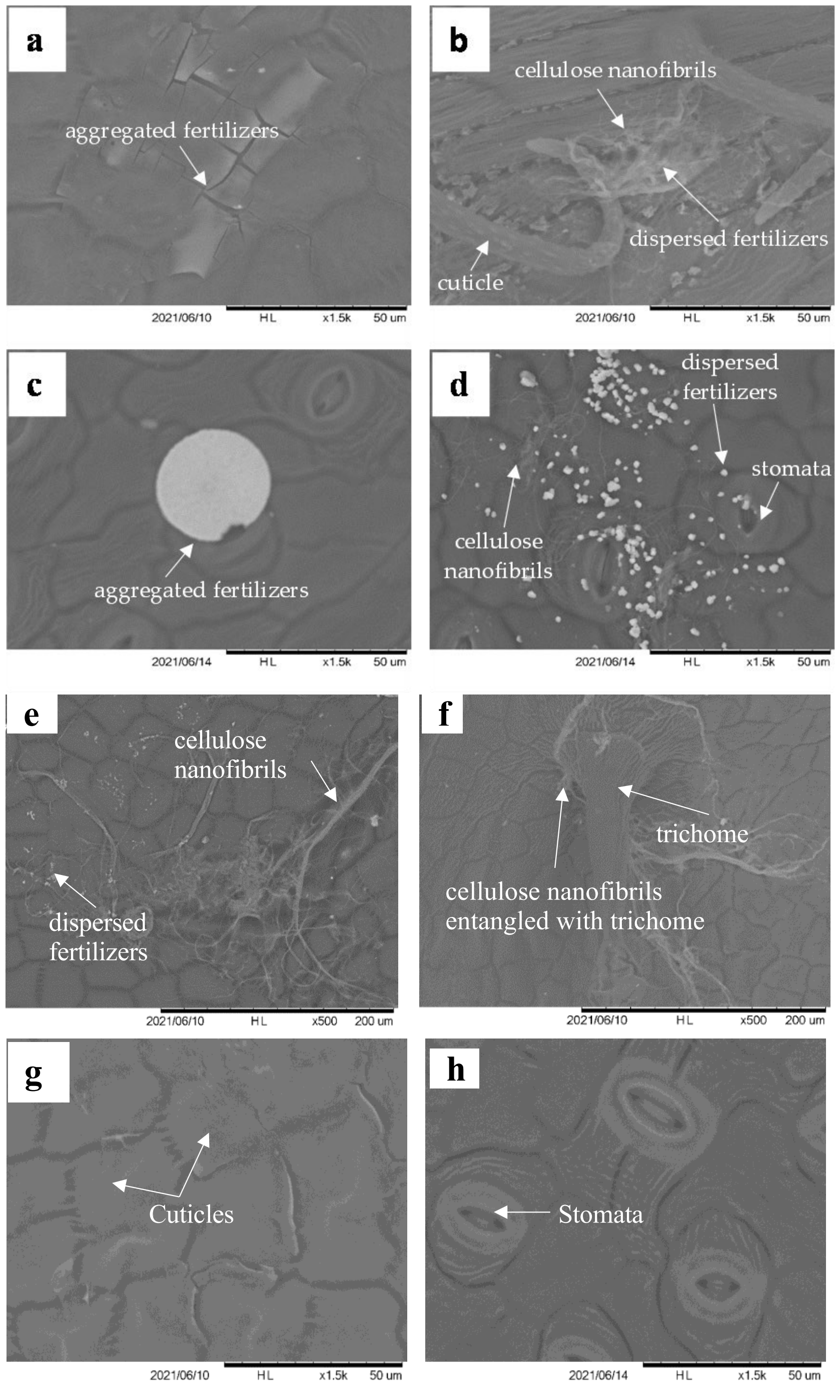

Figure 6.3. Interactions of nanocellulose with the foliar fertilizer on wild blueberry leaves (Vaccinium angustifolium): (a) aggregated fertilizers on the adaxial leaf surface; (b) entangled CNFs with trichomes on adaxial leaf surface; (c) aggregated

fertilizers on abaxial leaf surface; (d) dispersed fertilizers with smaller particle size on abaxial leaf surface; (e) dispersed smaller fertilizers on adaxial leaf surface enabled by cellulose nanofibrils; (f) nanocellulose entangles with trichomes of leaves; (g) adaxial leaf surface; and (h) abaxial leaf surface.

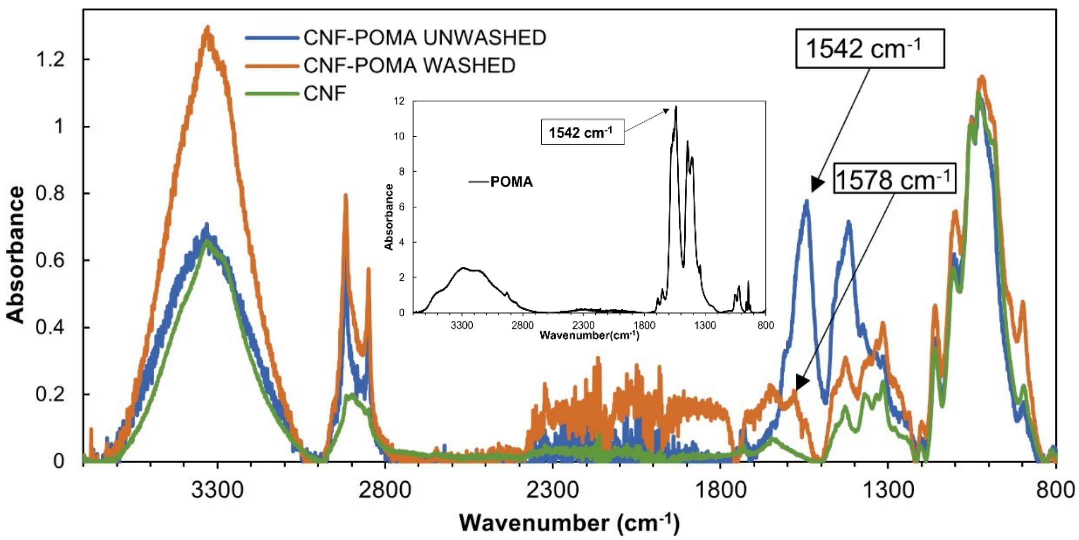

Figure 6.4. FTIR spectra of CNF, CNF-Poma washed, CNF-Poma unwashed, and Poma (inset).

In the case of Poma, the strong peaks were observed at 1542 and 1444 cm^{-1} that were associated with its acetate functionality (https://webbook. n i s t . g o v / c g i / c b o o k . c g i ? I D =B6007911&Mask=80). In CNF-Poma unwashed samples, these characteristic peaks were still prominent due to the presence of a high concentration of Poma, whereas for CNF-Poma washed samples, the characteristic Poma peaks were still observed but with reduced intensity, suggesting a medium retention of CNFs toward the Poma fertilizer. The percentage of calcium on the CNF surface was quantitatively measured by SEMEDX. For an unwashed sample, calcium is about 9%. After being washed, the calcium content dropped to around 2% (Figure D2).

6.4.3. CNF and Foliar Fertilizer Effects on Wild Blueberry Leaves and Yield

Wild blueberry plants showed higher water deficits under the CNF and Poma treatments as their Leaf Water Potentials (LWPs) were lower than that of the control, and LWP was the lowest under Poma + CNF treatment, but the differences were not significant due to limited replicates (Figure 6.5a). At the end of the season during harvesting in July 2021, leaf size was similar under all of the treatments (Figure 6.5b), whereas Leaf mass per area (LMA) was lower in plants treated with Poma + CNF compared to other treatments (Figure 6.5c). In contrast, the average fruit yield in the Poma + CNF treated plants tended to be higher than that in the plants treated with other treatments, but the difference was not statistically significant in this early stage study (Figure 6.5d). This is possibly due to high variations among genets and the fact that only *Vaccinium angustifolium* species were investigated. In agreement with these results, a significant negative linear relationship between LMA and yield was found in this study (Figure D1).

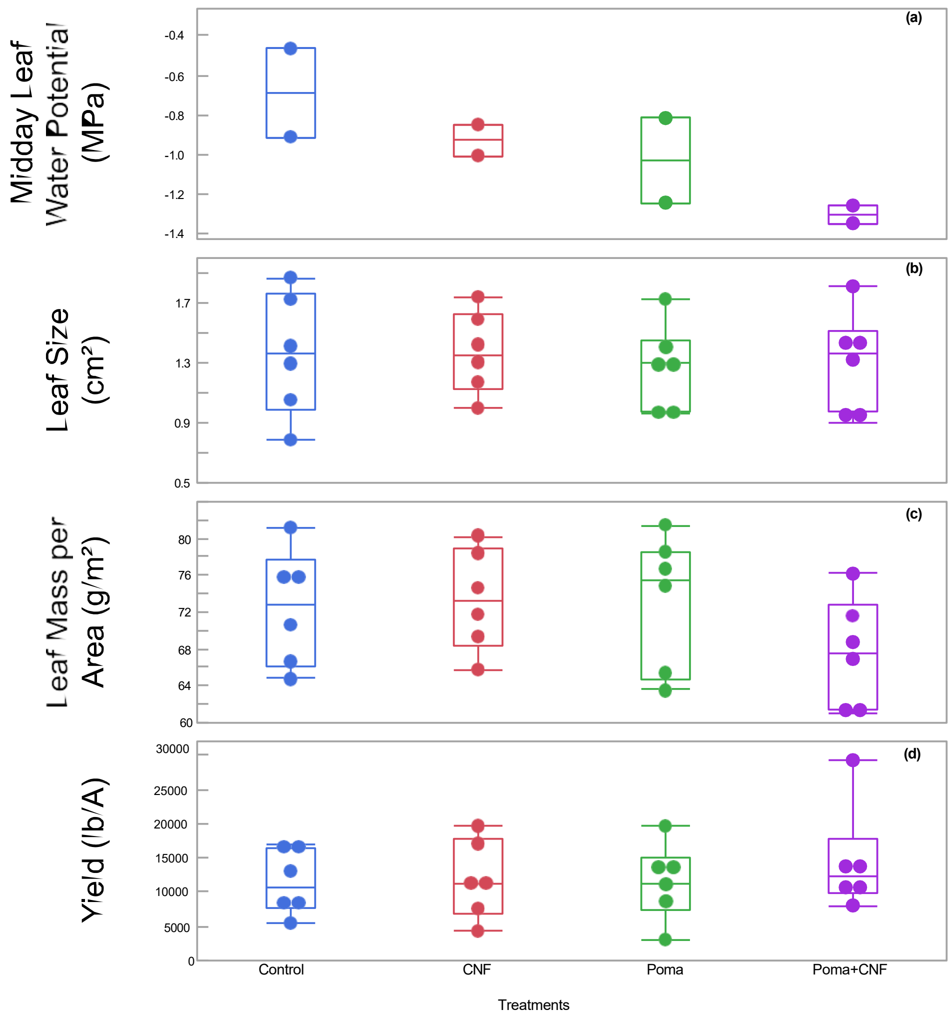

Figure 6.5. Comparisons in (a) Midday leaf water potential (LWP), (b) Leaf size, (c) Leaf mass per area (LMA), and (d) Yield of wild lowbush blueberries (*Vaccinium angustifolium*) among different applied treatments. No letters indicate no significant differences among the treatments at the significance level of $p < 0.05$.

6.5 Discussion

Our results suggested significant interactions among CNFs, wild blueberry leaves, and the foliar fertilizer POMA, which are trichome-dependent. The CNFs helped the retention of fertilizer, as suggested by the FTIR experiment, possibly due to the van der Waals interaction between CNFs and calcium acetate. Surface modification on CNFs can potentially improve the retention of fertilizers during rainwash. CNFs also had significant effects on the leaf hydrophilicity, water storage, and water loss for *V. myrtilloides* leaves but not for *V. angustifolium* leaves. CNFs helped the *V. myrtilloides* leaves to increase their water uptake by increasing their hydrophilicity, but those leaves also dehydrated faster. This could be because of the interaction between the CNFs and denser visible trichomes on *V. myrtilloides* leaves (https://gobotany.nativeplanttrust.org/species/vaccinium/myrtilloides/) compared to those on V. angustifolium leaves (https://gobotany.nativeplanttrust.org/species/vaccinium/angustifolium). The trichomes of *V. myrtilloides* might have facilitated the foliar water uptake but at the same time allowed faster water loss (Berry et al. 2019, Pan et al. 2021, Grammatikopoulos and Manetas 1994, Schmitt et al. 1989). CNFs could entangle with trichomes to create a much larger hydrophilic surface area (Figure 6.3b,f), facilitating the water storage and release. This effect is visible with the SEM images, but due to the low density of trichomes on *V. angustifolium* leaves (Figure 6.3f), it could be undetectable in leaf-level wettability and water loss measurements. These phenomena suggest a potential trade-off between the water absorption and conservation (Grammatikopoulos and Manetas 1994, Schmitt et al. 1989) which could be altered by the addition of CNFs on leaves. Moreover, with the faster foliar water uptake, those CNF treated leaves could also rapidly uptake the nutrient molecules and solutes dissolved in water (Zhang et al. 2022, Schönherr 2001, Berry et al. 2019, Pan et al. 2021). In contrast, CNF addition did not affect the hydrophilicity and water loss in *V. angustifolium* leaves possibly because of the smaller density of trichomes for CNFs to anchor on.

Further, leaf water potential values agree with the statement that CNFs facilitated water loss of the wild blueberry leaves, as indicated by lower leaf water potentials in Poma + CNF treated plants. Interestingly, those treated plants invested less in their leaf robustness/biomass, as indicated by the similar leaf size but lower average LMA under the Poma + CNF treatment compared to controls. The physiological mechanisms behind that need further studies. Moreover, the significant negative linear relationship between LMA and yield suggests a trade-off between leaf mass production and berry yield. Thus, the Poma + CNF treated plants invested their energy more in their fruits than the leaves during their flowering and fruit maturation period compared to controls (Tasnim et al. 2022). In agreement with a recent study (Tasnim et al. 2022), our results suggest that the CNF addition with the Poma foliar fertilizer together can be promising for the wild blueberries rather than their separate applications. In the FTIR of the washed CNF-Poma mixture, the Poma peak at 1542 cm^{-1} has been shifted to 1578 cm^{-1}, which could be due to the interaction between CNFs and Poma components. There was a possible van der Waals interaction between CNFs and calcium acetate of Poma. Additionally, the entrapment of Poma by CNFs (Figure 6.3b,e) due to their large surface area and networked structure would result in its retention when used in conjunction with the CNFs (Arbatan et al. 2012, Keller and Luner 2000). Such interactions between CNFs and Poma enabled the foliar nutrient elements to disperse well on the leaf surface by preventing them from forming large aggregates. The resulting smaller nutrient particles could get into the leaf fast when water/moisture is present, which would be more beneficial and efficient for the plants without wasting foliar fertilizers.

Lastly, the addition of CNFs showed a detectably higher amount of fruit yield (but not significant) in this study for trichome-deficient *Vaccinium angustifolium* species. Our results imply that switching the research subject to trichome-rich *Vaccinium myrtilloides* species or a higher amount of CNF addition might provide significant effects on fruit yield. This is because the CNFs successfully interacted with the foliar nutrients as well as with the trichomes on the *Vaccinium myrtilloides* leaves. However, further investigations are still needed to quantify the trichome density of these two blueberry and other crop species with different trichome densities, and test different amounts of CNFs on them to determine the promising rate of CNF application. Also, this CNF application potentially reveals a new avenue for research on how it can alter the crop foliar water and nutrient uptake, water conservation and evaporation in a future with warmer climates and more drought events.

CHAPTER 7

CONCLUSIONS, LIMITATIONS, AND RECOMMENDATIONS

In Chapter 2, I found differences in the increase in growing season maximum temperature (T_{max}), minimum temperature (T_{min}), average temperature (T_{avg}), and total precipitation (P_{total}) between the studied fields in Downeast, Maine and the overall spatial average for the region (state of Maine), as well as among the blueberry fields. The maximum, minimum, and average temperatures of the studied 26 wild blueberry fields in Downeast, Maine showed higher rates of increase than those of the entire region during the last 40 years. Fields closer to the coast showed higher rates of warming compared with the fields more distant from the coast. Consequently, potential evapotranspiration (PET) has been also increasing in wild blueberry fields, with those at higher elevations showing lower increasing rates. An optimum T_{max} and PET for EVI_{max} at 22.4 °C and 145 mm/month suggest potential negative effects of further warming and increasing PET on crop health and productivity. These climate change patterns and associated physiological relationships, as well as threshold values, could provide important information for the planning and development of optimal management techniques for wild blueberry fields experiencing climate change.

In Chapter 3, I found that the temperatures in summer, fall, and winter consistently rose in the wild blueberry barrens regardless of location over the past 41 years, whereas precipitation was relatively stable. Moreover, rates of temperature increase were faster during nighttime than daytime, and during the fall and winter seasons than in summer. The barrens located towards the south-west (Hancock to York) warmed up at a slower rate than the barrens located towards the north-east (Piscataquis and Washington). Such temporal and spatial temperature change will likely impact wild blueberry barrens positively in some years and negatively in other years. This unpredictable variation calls for further research on the responses of wild blueberry plants to a climate with warmer days and nights, and warmer summer, fall, and winter seasons. Further research is also needed to understand the effect of climate extremes on this crop in recent years. For instance, heatwaves and rainfall anomalies, heavy precipitation events, and decreased snow cover during days with extremely low temperatures may cause larger and irreversible damage to this crop than the changes seen from overall averages. Thus, novel management techniques need to be developed to enhance the capacity of this crop production system in buffering the negative effects of climate extremes.

Chapter 4 concludes that the wild blueberry crops might grow better under warmer climates if sufficient soil moisture condition prevails. Wild blueberry plants growing under the warmer environment in this study exhibited higher photosynthetic capacity, stomatal conductance, chlorophyll concentration, and berry production. Higher organic matter and/or other soil amendments like biochar-compost application on the soil surface can be beneficial for wild blueberries in drier summers to conserve soil moisture. This could further minimize water usage leading to comparatively lower irrigation costs for wild blueberry growers in the long run. As maintaining good chronic water status of soils is important for maintaining the yield of wild blueberries (Barai et al. 2021), irrigation or soil amendment to enhance soil water holding will be important to secure wild blueberry production in a future with increasing climate variability. This study provides a possible solution and opportunity to use sustainable materials like biochar and compost to save the crucial future of the wild blueberry industry under the predicted warmer and drier summers. However, this study also has limitations that need to be addressed prior to providing concrete recommendations to the wild blueberry growers. This study was conducted for only one crop cycle in a single wild blueberry production region on six genotypes.

In Chapter 5, our result implies that vegetative growth in the vegetative year is important to guarantee a high yield in the following season. Proper fertilization management according to leaf tissue nutrient content in the vegetative year after the tip-die back need to be conducted to reach its yield potential and to manage the wild blueberry farms economically. In terms of fertilization, foliar products might be a better option to correct for deficiencies of immobile and micro-nutrients, whereas an adjuvant might also help for better utilization of such foliar products. Since our studied plants did not show any micro-nutrient deficiency and I only studied a few foliar products, there are more opportunities for further investigations. Research for more than one crop cycle will be required to identify when the nutrient deficiencies occur in wild blueberry plants, and how foliar products with adjuvants like nanocellulose materials will help manage them efficiently and economically.

Chapter 6 concluded that there were significant interactions among nanocellulose (CNFs), wild blueberry leaves, and the foliar fertilizer POMA, which are trichome-dependent. The CNFs helped the retention of fertilizer. CNFs also had significant effects on the leaf hydrophilicity, water storage, and water loss for *V. myrtilloides* leaves but not for *V. angustifolium* leaves because of different leaf trichome densities. The addition of CNFs also showed a detectably higher amount of fruit yield (but not significant) in this study for trichome-deficient *Vaccinium angustifolium* species. Our results imply that switching the research subject to trichome-rich *Vaccinium myrtilloides* species or a higher amount of CNF addition might provide significant effects on fruit yield. This is because the CNFs successfully interacted with the foliar nutrients as well as with the trichomes on the *Vaccinium myrtilloides* leaves. However, further investigations are still needed to quantify the trichome density of these two blueberry and other crop species with different trichome densities, and test different amounts of CNFs on them to determine the promising rate of CNF application. Also, this CNF application potentially reveals a new avenue for research on how it can alter the crop foliar water and nutrient uptake, water conservation and evaporation in a future with warmer climates and more drought events.

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APPENDICES

APPENDIX A

Supporting Information for Chapter 2

A1. Supplementary Methods: Workflow and Analysis in Arc GIS Pro 2.4.2 Software [1]

A1.1 Analysis of Climate Variables and Maximum EVI using "Geoprocessing" tools

Wild blueberry polygons (as a KMZ file) were imported to Arc GIS Pro [1] and converted into a Shapefile using the "KML to Layer" tool. Then the shapefile was projected in the coordinate system "NAD 83 UTM Zone 19N" using the "Project" tool, as were all of the shapefiles used in our analysis. This file had several thousand polygons of wild blueberry fields including small patches of wild blueberry plants. From those polygons, 26 wild blueberry fields (polygons) of 1 km^2 and larger area were selected from the Downeast region of Maine using the "Select by Attributes" tool. The selected polygons were further converted into points inside the polygons (Fig. 1) using the "Feature to Point" tool for further GIS analysis. Then, the Latitude, Longitude, and Elevation data of those wild blueberry field locations as well as the Vertical Distance of those fields from the coast were calculated and extracted.

After acquiring 40 Raster layers of 40 years (1980 – 2019, 1 layer per yr) for each climate variable (maximum temperature, minimum temperature, and precipitation), all 120 raster layers were projected in the same coordinate system using the "Project" tool. Each of those raster layers for each year contains 12 Bands representing 12 months from January to December, each month containing daily average maximum temperature (T_{max}), or daily average minimum temperature (T_{min}), or total monthly precipitation (P_{total}). In order to obtain a summary of these climate variables during the growing season (May to September), for each raster layer (each year) of 1980 - 2019, average T_{max}, average T_{min}, and P_{total} from May (Band 5) to September (Band 9) were calculated using the "Raster Calculator" tool. Then, for each of the climate variables (T_{max}, T_{min}, and P_{total}), those calculated 40 raster layers of 40 years (1980 - 2019) were stacked from 1980 to 2019 sequentially using the "Composite Bands" tool to get 3 final individual raster layers of average T_{max}, average T_{min}, and P_{total}. The average T_{max}, average T_{min}, and P_{total} of the 26 wild blueberry fields (points) over 40 years (1980 - 2019) were taken from each of those 3 final raster layers individually and were extracted into attribute tables using the "Zonal Multi-value to points" tool. The attribute tables were finally copied into standalone tables using the "Copy Rows" tool.

Similarly, for the Maximum Enhanced Vegetation Index (EVI_{max}), after acquiring 17 raster layers over 17 years (2001 - 2017) for Downeast Maine, the layers were projected in the same coordinate system using the "Project" tool. Then, from those projected 17 raster layers of 17 years (2001 - 2017), the EVI_{max} of the 26 wild blueberry fields were extracted into 17 attribute tables individually using the "Zonal Statistics as Table" tool, followed by copying into 17 standalone tables using the "Copy Rows" tool.

Finally, all the extracted final standalone tables of average T_{max}, average T_{min}, P_{total}, and EVI_{max} were then transferred from Arc GIS Pro to an Excel spreadsheet using the "Table to Excel" tool. In Excel, the average temperature was calculated from the maximum and minimum temperature over 40 years. Furthermore, the comparisons and trendlines of historical climate change for the entire state of Maine and the 26 wild blueberry fields as well as relationships of the climate variables with the EVI_{max} were explored in Excel.

A1.2 Analysis of Potential Evapotranspiration using "Geoprocessing" tools

After acquiring 12 raster layers (representing 12 months from January to December) of Monthly Global Potential Evapotranspiration (PET) averaged over 1970 to 2000, all 12 layers were projected in the same coordinate system using the "Project" tool. Then those projected 12 raster layers of 12 months (January – December) were stacked from January to December sequentially using the "Composite Bands" tool to get one final raster layer. From that final layer, the monthly PET averaged over the 1970 - 2000 period for the wild blueberry fields from January to December were extracted into an attribute table using the "Zonal Multi-value to points" tool, followed by copying into a standalone table using the "Copy Rows" tool.

Again, after acquiring 12 raster layers (representing 12 months from January to December) for each year over 15 years (2000 - 2014) of Monthly Global PET, all those 180 raster layers were projected in the same coordinate system using the "Project" tool. Then, for each year from 2000 to 2014 individually, the projected 12 raster layers of 12 months (January – December) were stacked from January to December sequentially using the "Composite Bands" tool to get one final raster layer for each year hence, to get 15 final raster layers for 15 years (2000 - 2014). From those final 15 layers, the monthly PET of 15 years (2000 - 2014) for the wild blueberry fields from January to December were extracted into an attribute table using the "Zonal Multi-value to points" tool, followed by copying into a standalone table using the "Copy Rows" tool.

Finally, the two extracted final tables of Monthly PET (one table for 1970 – 2000 period, one table for 2000 – 2014 period) of the wild blueberry fields were then transferred from the Arc GIS Pro software environment to an Excel spreadsheet. Further, the comparisons of the PET among the two different time periods for the wild blueberry fields as well as the relationship between PET and EVI_{max} were explored in Excel.

Reference

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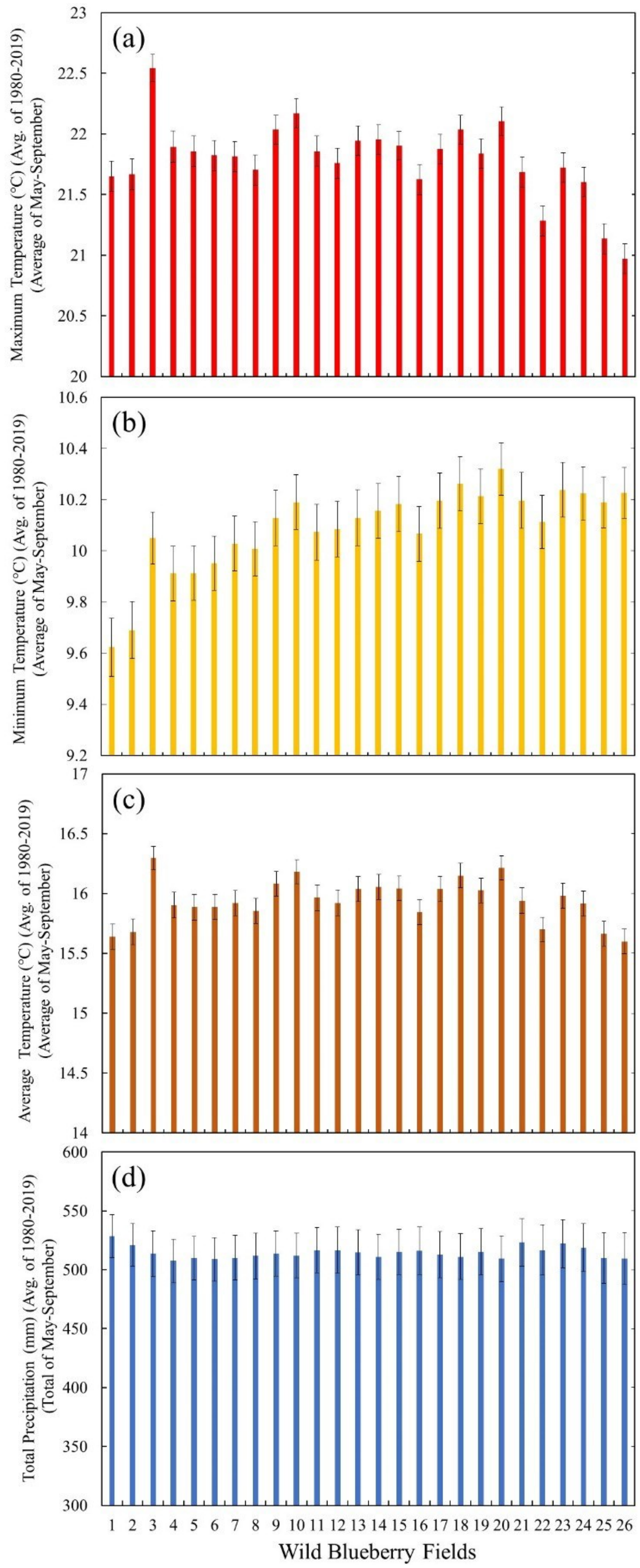


Figure A.1. Comparison of (a) maximum temperature (average of May - September), (b) minimum temperature (average of May - September), (c) average temperature (average of May - September) and (d) precipitation (total of May - September) from 1980 to 2019 among wild blueberry fields in Downeast

Maine. The climate variables are represented as the Mean ± Standard error (n = 40; where 40 represents the years from 1980 to 2019).

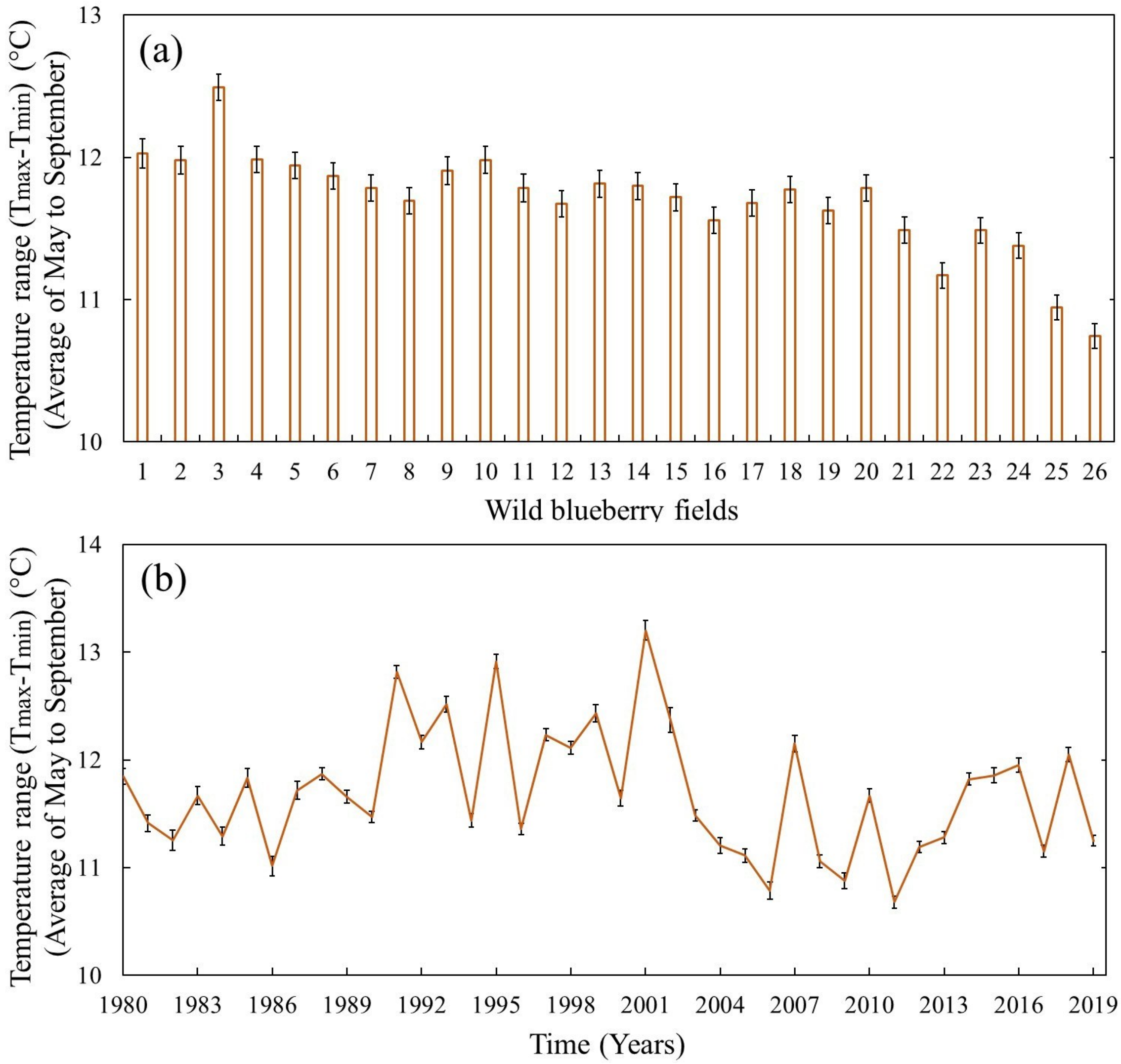


Figure A.2. (a) Comparison of temperature range (T_{max} - T_{min}) (average of May - September) from 1980 to 2019 among wild blueberry fields in Downeast Maine. The climate variables are represented as the Mean ± Standard error (n = 40; where 40 represents the years from 1980 to 2019). (b) Historical (1980 to 2019) changes in temperature range (T_{max} - T_{min}) (average of May - September) at the 26 wild blueberry fields (shown in Fig. 2.1) in Downeast Maine. The climate variables from the fields are represented as the Mean ± Standard error (n = 26).

Table A.1. Changes (increasing or decreasing) in climate variables (T_{max}, T_{min}, T_{avg}, and P_{total}) over last 40 years from 1980 to 2019 at the studied 26 wild blueberry fields in Downeast, Maine as well as at the overall state of Maine. T_{max}, T_{min}, and T_{avg} represent the maximum, minimum, and average temperatures, respectively averaged from May to September; P_{total} represents the total precipitation from May to September.

Wild blueberry fields in Fig. 1	**Changes [Increase (+) or Decrease (-)] over 40 years from 1980 to 2019**			
	T_{max} (°C)	**T_{min} (°C)**	**T_{avg} (°C)**	**P_{total} (mm)**
1	+1.30	+1.70	+1.55	+30
2	+1.40	+1.70	+1.55	+30
3	+0.75	+1.30	+1.00	+25
4	+1.45	+1.55	+1.55	+30
5	+1.40	+1.55	+1.50	+30
6	+1.30	+1.58	+1.50	+38
7	+1.25	+1.60	+1.55	+30
8	+1.34	+1.60	+1.50	+44
9	+1.00	+1.60	+1.30	+40
10	+1.00	+1.60	+1.30	+35
11	+1.30	+1.70	+1.50	+55
12	+1.35	+1.65	+1.50	+50
13	+1.20	+1.70	+1.45	+50
14	+1.20	+1.60	+1.40	+55
15	+1.00	+1.60	+1.30	+50
16	+1.35	+1.70	+1.53	+46
17	+1.25	+1.65	+1.40	+58
18	+1.10	+1.55	+1.35	+43
19	+1.25	+1.65	+1.45	+60
20	+1.05	+1.50	+1.28	+50
21	+1.25	+1.70	+1.48	+75
22	+1.45	+1.55	+1.50	+70
23	+1.20	+1.65	+1.42	+75
24	+1.30	+1.62	+1.45	+78
25	+1.50	+1.55	+1.53	+80
26	+1.50	+1.45	+1.48	+80
State of Maine	+0.95	+1.25	+1.10	+60

Table A.2. Regression analysis between climate variables (T_{max}, T_{min}, T_{avg}, P_{total} and Rainfall anomaly) and Maximum Enhanced Vegetation Index (EVI_{max}) at the studied 26 wild blueberry fields in Downeast, Maine. T_{max}, T_{min}, and T_{avg} represent the maximum, minimum, and average temperatures, respectively averaged from May to September averaged over 40 years (1980 – 2019); P_{total} represents the total precipitation from May to September. P_{total} and Rainfall anomaly are averaged over 40 years (1980 – 2019) [Numbers are representing the Coefficient of determination (R^2) values of the linear or quadratic relationship with the significance of difference: P < 0.001***; P < 0.01**; P < 0.05*]

Wild blueberry fields (Fig. 1)	T_{max} Vs EVI_{max}	T_{min} Vs EVI_{max}	T_{avg} Vs EVI_{max}		P_{total} Vs EVI_{max}	Rainfall anomaly Vs EVI_{max}	
	Quadratic	Linear	Linear	Quadratic	Quadratic	Linear	Quadratic
1	0.13	0.24*	0.075	0.092	0.081	0.009	0.081
2	0.28	0.055	0.009	0.078	0.199	0.151	0.199
3	0.22	0.002	0.073	0.08	0.458*	0.385**	0.458*
4	0.10	0.09	0.000	0.083	0.489**	0.254*	0.489**
5	0.076	0.33*	0.051	0.053	0.476*	0.394**	0.476*
6	0.196	0.06	0.009	0.164	0.217	0.216	0.217
7	0.143	0.004	0.001	0.032	0.203	0.014	0.203
8	0.006	0.25*	0.05	0.068	0.157	0.04	0.157
9	0.018	0.03	0.000	0.033	0.166	0.122	0.166
10	0.223	0.008	0.072	0.077	0.363*	0.271*	0.363*
11	0.018	0.055	0.01	0.01	0.208	0.053	0.208
12	0.07	0.052	0.003	0.032	0.079	0.047	0.079
13	0.04	0.054	0.001	0.002	0.249	0.16	0.249
14	0.18	0.012	0.048	0.053	0.351*	0.269*	0.351*
15	0.277	0.026	0.07	0.111	0.199	0.071	0.199
16	0.063	0.135	0.001	0.005	0.315	0.273*	0.315
17	0.044	0.155	0.008	0.008	0.319	0.294*	0.319
18	0.066	0.04	0.004	0.01	0.251	0.204	0.251
19	0.034	0.094	0.007	0.026	0.212	0.191	0.212
20	0.004	0.019	0.011	0.035	0.04	0.03	0.04
21	0.163	0.000	0.066	0.068	0.086	0.086	0.086
22	0.116	0.001	0.027	0.073	0.122	0.113	0.122
23	0.051	0.068	0.000	0.002	0.288	0.108	0.288
24	0.007	0.123	0.02	0.036	0.232	0.16	0.232
25	0.083	0.153	0.142	0.215	0.115	0.057	0.115
26	0.15	0.003	0.072	0.12	0.019	0.017	0.019

APPENDIX B

Supporting Information for Chapter 3

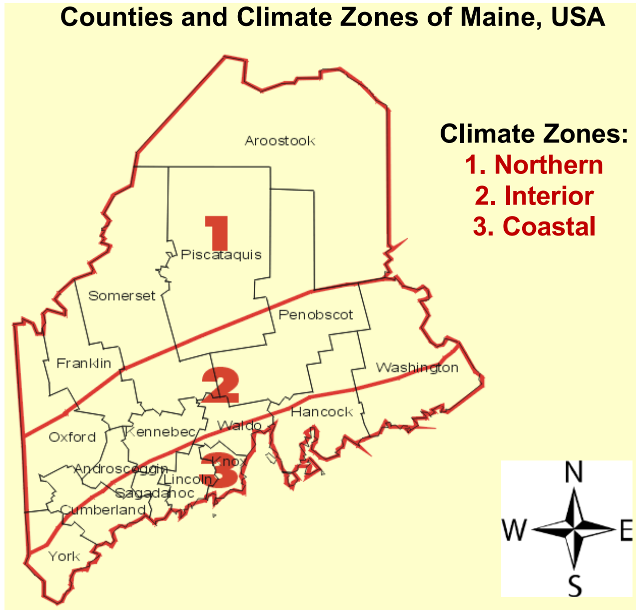


Figure B.1. A map showing different counties and climate regions of Maine. (The map was acquired from NOAA National Weather Service, NOAA Center for Weather and Climate Prediction (website: https://www.cpc.ncep.noaa.gov/products/analysis_monitoring/regional_monitoring/CLIM_DIVS/maine.gif; accessed on 22 March 2022).

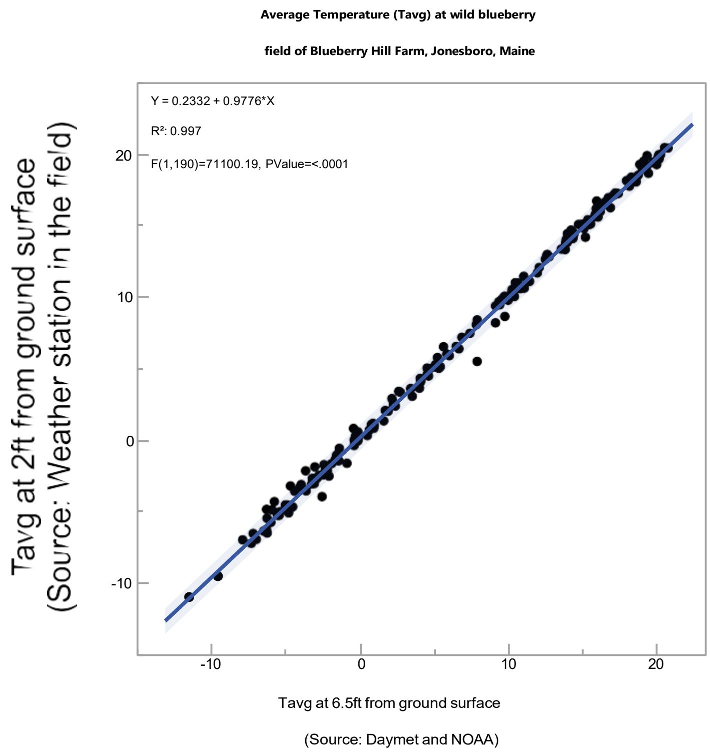


Figure B.2. The relationship between average temperature recorded by different weather stations at 2 ft and 6.5 ft from the ground surface in a wild blueberry field at the Blueberry Hill Farm, Jonesboro, Maine. Here, each point represents monthly average temperature calculated from the recorded daily maximum and minimum temperature by the deployed weather stations. The solid line represents a linear relationship fitted to the data by linear regression analysis (p < 0.0001) and the shaded region represents a 95% confidence interval.

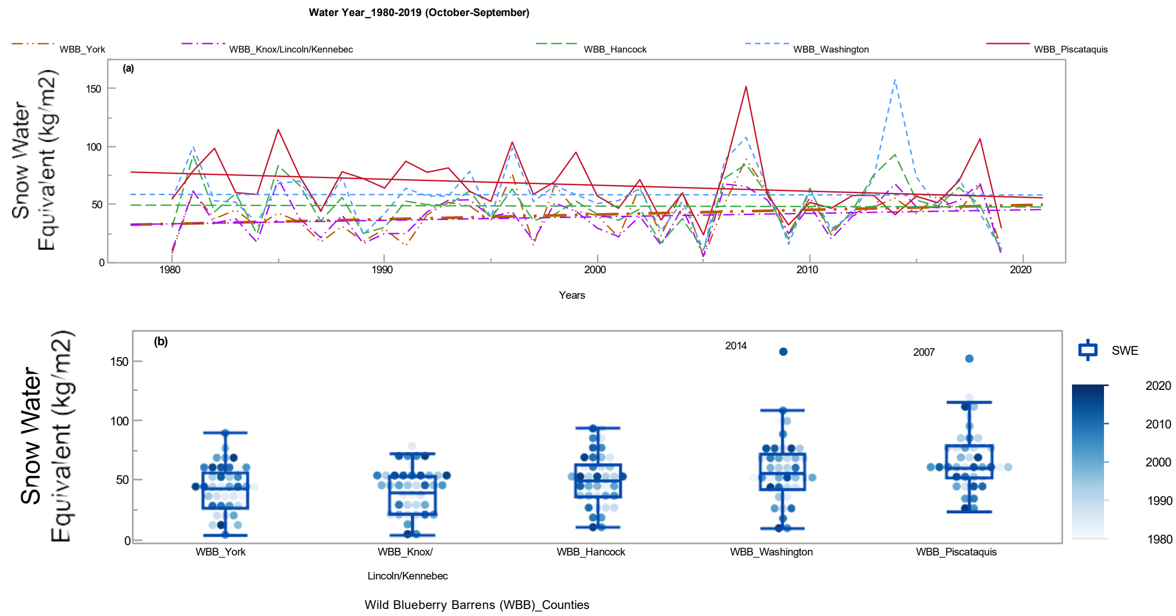


Figure B.3. (a) Historical (water year: 1980 to 2019) changes with fitted linear regression trendlines for the snow water equivalent (SWE) throughout the studied wild blueberry barrens (WBB) and (b) Comparison of historical (water year: 1980 to 2019) snow water equivalent (SWE) among the studied wild blueberry barrens (WBB) at different counties from North-Central (Piscataquis) and North-East (Washington, Hancock) to South-West (Knox, Lincoln, Kennebec, York) of Maine as shown in Fig. 3.1 and B.1. Here, 1980 water year indicates October 1980 to September 1981, and 2019 water year indicates October 2019 to September 2020.

Table B.1. Historical trend analysis of Seasonal climate variables using Mann–Kendall test, and comparison of a linear regression fitted slopes using slope t-test among the studied wild blueberry barrens (WBB) at different counties from North-Central (Piscataquis) and North-East (Washington, Hancock) to South-West (Knox, Lincoln, Kennebec, York) of Maine (shown in Fig. 3.1) from 1980 to 2020. Bold parts indicate significant strength in historical climate trends. Different letters associated with the "Slope rate" and "°C/year" indicate significant differences among the counties at a significance level of $p < 0.05$.

Season	Climate variables	Mann-Kendall & Slope t-test	Wild Blueberry Barrens (WBB)_Counties				
			WBB_ Piscataquis	WBB_ Washington	WBB_ Hancock	WBB_Knox/ Lincoln/Kennebec	WBB_ York
Summer	T_{max} in Fig. 3.5a	Kendall's tau	**0.27**	**0.36**	0.14	0.12	0.05
		p-value	**0.01**	**0.0008**	0.21	0.24	0.64
		Trend	**Increasing**			Increasing	
		Slope rate, °C	1a	1.5b	0.6c	0.6c	0.2d
		°C/year	0.024a	0.036b	0.015c	0.015c	0.005d
	T_{min} in Fig. 3.5b	Kendall's tau	**0.4**	**0.47**	**0.44**	**0.38**	**0.5**
		p-value	**0.0002**	**<0.0001**	**<0.0001**	**0.0004**	**<0.0001**
		Trend			**Increasing**		
		Slope rate,	1.7ab	1.8a	1.7ab	1.4b	1.9ac

		°C					
		°C/year	0.04ab	0.044a	0.04ab	0.034b	0.046ac
	T_{avg} in Fig. 3.5c	Kendall's tau	**0.39**	**0.48**	**0.35**	**0.24**	**0.31**
		p-value	**0.0003**	**<0.0001**	**0.0013**	**0.02**	**0.004**
		Trend			**Increasing**		
		Slope rate, °C	1.3ab	1.6b	1.1a	1a	1a
		°C/year	0.03ab	0.04b	0.027a	0.024a	0.024a
	P_{total} in Fig. 3.5d	Kendall's tau	0.02	0.0073	0.0073	0.06	0.07
		p-value	0.8	0.94	0.94	0.56	0.53
		Trend			No change		
	T_{max} in Fig. 3.7a	Kendall's tau	0.21	0.21	0.16	0.15	0.04
		p-value	0.05	0.05	0.12	0.17	0.67
		Trend			Increasing		
		Slope rate, °C	1.3a	1ab	0.9b	0.8b	0.4c
		°C/year	0.03a	0.024ab	0.022b	0.02b	0.009c
	T_{min} in Fig. 3.7b	Kendall's tau	**0.26**	**0.24**	**0.28**	**0.23**	**0.28**
		p-value	**0.02**	**0.026**	**0.009**	**0.03**	**0.008**
		Trend			**Increasing**		
		Slope rate, °C	2.1a	2a	2a	1.9a	2a
Winter		°C/year	0.051a	0.049a	0.049a	0.046a	0.049a
	T_{avg} in Fig. 3.7c	Kendall's tau	**0.25**	**0.23**	**0.22**	0.19	0.18
		p-value	**0.02**	**0.03**	**0.04**	0.07	0.08
		Trend		**Increasing**		Increasing	
		Slope rate, °C	1.7a	1.5ab	1.5ab	1.3b	1.3b
		°C/year	0.04a	0.036ab	0.036ab	0.032b	0.032b
	P_{total} in Fig. 3.7d	Kendall's tau	0.15	**0.22**	0.19	0.16	0.18
		p-value	0.15	**0.04**	0.08	0.14	0.09
		Trend	Increasing	**Increasing**		Increasing	
		Slope rate, mm	20-50 mm increasing rate (no significant changes among counties)				
		mm/year			0.5-1.2 mm increasing rate per year		
	Summer T_{max} - Winter T_{min} in Fig. 3.8	Kendall's tau	-0.17	-0.13	**-0.24**	**-0.21**	**-0.26**
		p-value	0.11	0.23	**0.02**	**0.048**	**0.02**
		Trend	Decreasing			**Decreasing**	
		Slope rate,	3a	1.5b	3a	2.9a	3.6c

		°C					
		°C/year	0.073a	0.036b	0.073a	0.07a	0.088c
Fall	T_{max} in Fig. 3.10a	Kendall's tau	**0.31**	**0.35**	**0.28**	**0.26**	0.13
		p-value	**0.004**	**0.0014**	**0.01**	**0.01**	0.21
		Trend	**Increasing**				Increasing
		Slope rate, °C	2.2a	2.1a	1.5b	1.5b	0.9c
		°C/year	0.053a	0.051a	0.036b	0.036b	0.022c
	T_{min} in Fig. 3.10b	Kendall's tau	**0.42**	**0.41**	**0.44**	**0.48**	**0.56**
		p-value	**<0.0001**	**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
		Trend	**Increasing**				
		Slope rate, °C	2.5a	2b	2.5a	2.4a	2.9c
		°C/year	0.06a	0.049b	0.06a	0.058a	0.07c
	T_{avg} in Fig. 3.10c	Kendall's tau	**0.41**	**0.39**	**0.37**	**0.38**	**0.38**
		p-value	**0.0002**	**0.0003**	**0.0007**	**0.0004**	**0.0005**
		Trend	**Increasing**				
		Slope rate, °C	2.35a	2a	2a	2a	1.9a
		°C/year	0.057a	0.049a	0.049a	0.049a	0.046a
	P_{total} in Fig. 3.10d	Kendall's tau	0.14	0.13	0.11	0.18	0.19
		p-value	0.18	0.21	0.3	0.09	0.07
		Trend & Rate	Increasing 20-50 mm over 1980-2020				
		mm/year	0.5-1.2 mm increasing (no significant changes among counties)				
Spring	T_{max} in Fig. 3.12a	Kendall's tau	0.03	0.08	-0.04	-0.03	-0.11
		p-value	0.77	0.46	0.68	0.77	0.3
		Trend	No significant changes				
	T_{min} in Fig. 3.12b	Kendall's tau	0.05	0.07	0.06	0.002	0.05
		p-value	0.64	0.5	0.56	0.98	0.6
		Trend	No significant changes				
	T_{avg} in Fig. 3.12c	Kendall's tau	0.03	0.06	-0.02	-0.05	-0.05
		p-value	0.78	0.57	0.87	0.65	0.65
		Trend	No significant changes				
	P_{total} in Fig. 3.12d	Kendall's tau	-0.002	-0.024	-0.012	-0.12	0.02
		p-value	0.98	0.82	0.91	0.26	0.84
		Trend	No significant changes				

Appendix C

Preliminary Study for Chapter 4: The future of wild blueberries: Testing warming impacts using open-top chambers

Abstract: Wild blueberries are one of the most economically and culturally important crops in Maine. Global climate change has already changed the growth pattern of crops in this region. However, it is not known how wild blueberries (*Vaccinium angustifolium*) will respond to warming. Here we present the methodology we used to investigate warming effects on wild blueberries and preliminary results to foster interdisciplinary and collaborative research. A 2.6 m^2 open-top chamber (OTC) was designed and built to accommodate wild blueberry plants in the field. In order to test the most effective method of heating the chamber, both passive and active heating was tested. The active heating system consistently maintained air temperature 3 to 5ºC higher than the ambient control. The passive heating system increased the temperature during the day but decreased the temperature at night. Under the active heating treatment, the photosynthetic rate and water-use efficiency of the wild blueberry plants decreased. Plants also produced smaller and thinner leaves with lower leaf mass per area under active heating compared to the control (ambient temperature) treatment. Moreover, whole plant CO_2 assimilation rate also decreased (32% to 40% lower) under warming. These preliminary results indicate that warming had a negative effect on both leaf and plant level performance of wild blueberries. While these results need to be tested on a larger scale and over longer terms, we suggest the importance of studying climate change effects on crops. We also suggest actively heated OTC as a sound method of studying ground-level response to warming. This tool could be used for further studies on the responses of wild blueberries and other crops to warming in Maine. This information is crucial for developing techniques and policies that allow farmers to adapt to climate change.

Keywords: Warming, Wild blueberry, Open-top chamber, Crop physiology and growth.

Introduction:

Anthropogenic climate change brings relatively quick changes in climate conditions and increased climate variability, which pose great challenges to worldwide agricultural systems (Bita & Gerats 2013). Atmospheric temperatures have increased significantly in the past century and will continue to climb in the future because of greenhouse gas emission. Atmospheric temperatures are predicted to increase by 1.8 to 4ºC at the end of this century (IPCC 2014). In Maine, USA, temperatures are expected to increase 2 to 6 ºC more by the end of this century (The University of Maine Climate Institute 2015). Global warming will significantly influence the physiology, growth, and yield of crops since crop production may be stimulated by 1 to 3ºC increases in temperate regions whereas crop production might be hindered by any degree of warming in tropical and subtropical regions (Easterling et al. 2007; Hatfield et al. 2011). Different plants and ecosystems have distinct responses to warming with different ranges of temperatures for physiological performance and growth (Hatfield et al. 2011; Zhang et al. 2016). Both positive and negative effects of warming on crop yield have been reported (Easterling et al. 2007; Hatfield et al. 2011; Semenov & Shewry 2011), but it is still unclear how the physiological performances of temperate crops, such as Maine's wild blueberry, will benefit from warming.

Warmer temperatures due to climate change affect the development of temperate fruits and vegetables by impacting various plant processes such as photosynthesis, respiration, and water and nutrient transport (Magan et al. 2011). Warming has been found to have negative effects on a variety of temperate crops (Lobell & Field 2007). For example, temperate crops such as maize and wheat show increasing leaf photosynthetic rates due to increasing temperature up to 33ºC and lower final biomass (Reynolds et al. 2000; Badaruddin et al. 1999). Reynolds et al. (2000) also observed lower chlorophyll content and total canopy photosynthetic rate in temperate crops under warm environments.

However, under controlled warming experiments on temperate crops, studies from various regions reported contradictory observations under different warming temperatures. Mitchell et al. (1995) reported that winter wheat phytomass and yield declined by 20 to 30% when ambient temperature was warmed by 4ºC. Xiao et al. (2010) reported that winter wheat yields increased by 6% when ambient temperature was warmed by 2.2ºC, whereas, Fang et al. (2010) reported that winter wheat yields declined by 27% when ambient temperature was warmed by 2.5ºC. To our knowledge, the effects that future warming will have on small fruit crops, specifically wild blueberry (*Vaccinium angustifolium*), and other crops in Maine, USA has not been studied.

Wild blueberry production is a unique semi-natural agricultural system. Wild blueberry plants are initially established from seeds naturally (not planted) and then underground stems (rhizomes) develop (Fig. C1). Roots then grow directly off of rhizomes creating a tightly woven mat across fields. Rhizomes grow within approximately 10 cm of the soil surface and produce upright stems above the soil surface. An individual wild blueberry plant, with its spreading rhizome system, is referred to as a genet (Bell et al. 2009) (Fig. C1). Each genet is genetically different from neighboring plants which together create a complex mixture of genotypes in the wild blueberry field providing natural resilience to pests and a rich diversity of flavors to consumers.

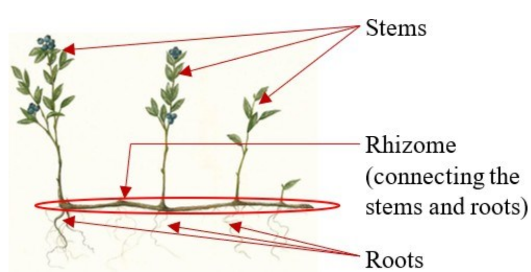


Figure C1. Wild blueberry stems with roots growing from the rhizome, creating one individual wild blueberry plant referred to as one genet in the wild blueberry field.

Here, we present the methodology used to measure warming in the field using open-top chambers and preliminary wild blueberry plant response to 3 to 5°C warming inside the chambers. We used a representative wild blueberry genet to address the following questions: 1) Is passive or active heating the most effective way to heat an open-top chamber to study warming effects on the wild blueberry crop? and 2) Will warming enhance or reduce physiological and growth performances of wild blueberry? A systematic approach was used to study the response of morphological, structural, and physiological processes to warming. We sought to establish the mechanistic linkages among different physiological processes including water use, nutrition, and carbon assimilation using a representative genet, which will be tested further with a larger and long-term experiment including more genets. Anyone interested in using our system to study climate change impacts on crops, pests, micro-organisms, and human nutrition could contact the authors.

Materials and Methods

Experimental Site

The study site was located at the Blueberry Hill Farm in Jonesboro (Longitude: -67.6495° N, Latitude: 44.6454° W), Maine, USA which is the only university-based (The University of Maine) wild blueberry research facility in the United States. The average annual temperature in Jonesboro, Maine is 6.3ºC with an annual low monthly average temperature of 0.9ºC and annual high of 11.7ºC. The average annual precipitation and snowfall are 1298 mm and 158 cm (Climate data for Jonesboro, Longitude: -67.6495° N, Latitude: 44.6454° W; average weather Jonesboro, ME - 4648 - 1981-2010 normal). The experiment was conducted in a field that was in the crop cycle (second year cycle) in 2018. Wild blueberries are managed on a two-year cycle: during the first year the plants are pruned and grow vegetatively and in the second year the plants flower and produce a fruit crop. The soil at the site is classified as a Colton gravelly sandy loam which is excessively drained and has a depth to water table of greater than 203 cm (Hunt et al. 2008). The experiment was conducted from June to September 2018. During this period, no additional irrigation or fertilizer was applied in the experimental field. Notably, only one large representative genet (~10 m^2) was selected to test the chamber system and conduct the preliminary trial.

Experimental Design

Three areas were selected within the same wild blueberry genet to compare among three different treatments: one area was warmed using the Open-Top Chamber (OTC)-cable system (Fig. C2a & C2b) which is indicated as AH (Active heating) treatment. The OTC-cable system with a 288 W power rating consisted of a standard International Tundra Experiment (ITEX) six-sided OTC (Bokhorst et al. 2008), an RKF Series Silicone Heating Tape, and a heat resistant PEX-tube. The design of the OTC and heating system followed Sun et al. (2013). The OTC was constructed with a LEXAN polycarbonate sheet (glass substitute) of the following dimensions: 3 mm thickness with a 100 cm base, 70 cm top, and 55 cm sides cut at an angle of 60°. These sheets feature high light transmittance (86%) and high infrared transmittance (~85%). A 12 m waterproof silicone heating tape with an 20 Wm^{-1} and 240 W power rating (Briskheat, Columbus, OH, USA) was coiled around a heat resistant PEX tubing (2.5 m in length and 3.5 cm in diameter), after which the tube was reshaped into a circle, wrapped with an aluminum tape, and fixed inside the OTC (using timber stakes) at a height of 15 cm. PVC pipe distance to plants was 10 to 15 cm while distance to OTC inner walls was 15 cm. This was to prevent compromising the OTC or risk burning the plants. Another OTC without the heating tape system was installed in a second area (Fig. C2c) and represented the Passive heating (PH) treatment. The third area (Fig. C2d) was staked out as a control treatment (NC) under the ambient weather condition with no chamber or heating system installed.

Environmental Variables

Atmospheric temperature and relative humidity (Fig. C3) were instantaneously recorded every 10 minutes using Watchdog 1000 series micro stations (Spectrum Technologies, Inc, Aurora, IL 60504) in all three area from June 2018 to September 2018. Vapor pressure deficit in air was calculated using the atmospheric temperature and relative humidity data. Soil temperature and volumetric water content were measured by a Fieldscout TDR 150 Soil Moisture Meter (Spectrum Technologies, Inc, Aurora, IL, USA) from six random places within each of the treatment areas.

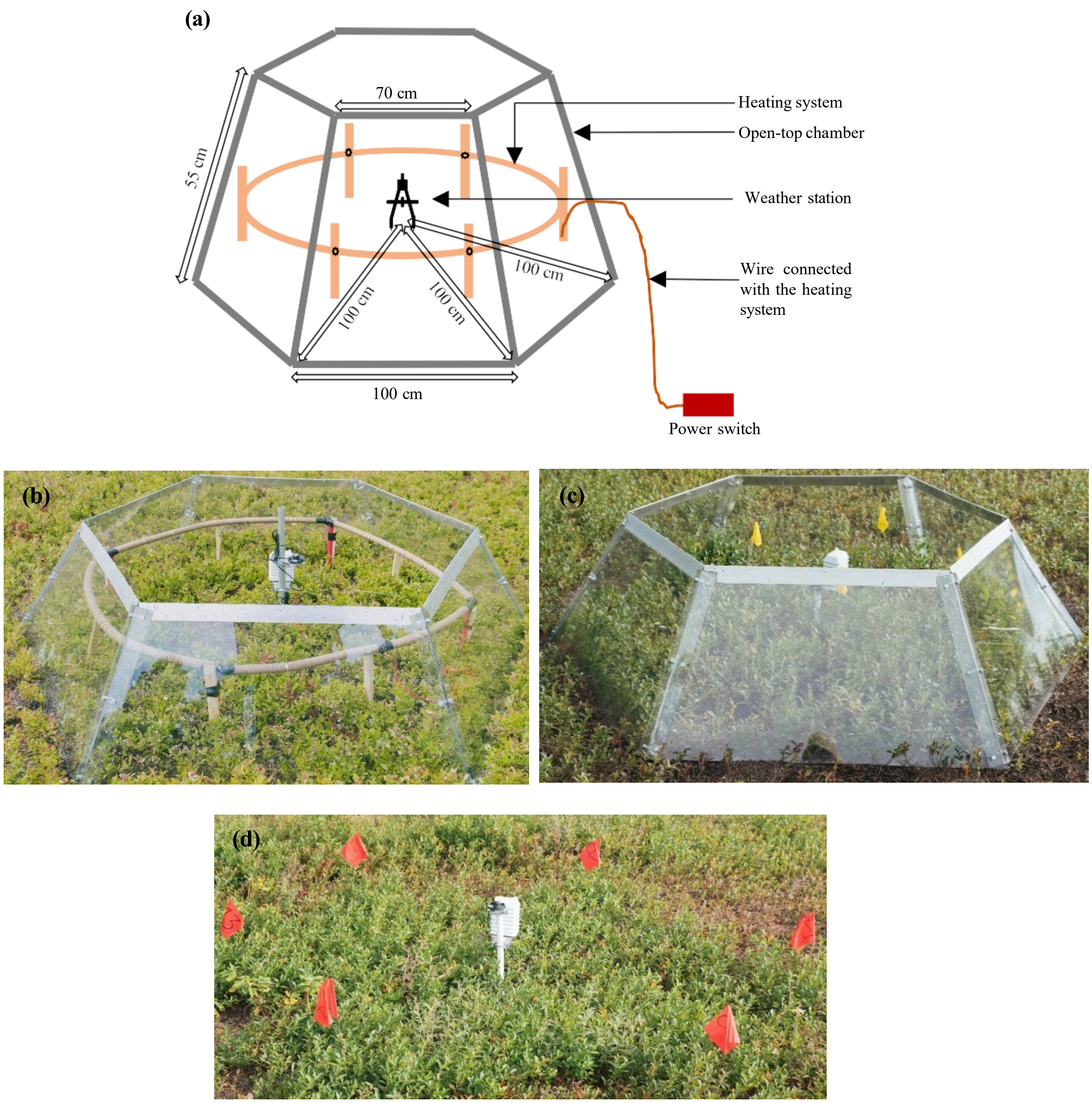


Figure C2. The open-top chambers (OTC) and control plot used for this study in the wild blueberry field: (a) Schematic diagram of OTC with heating (to supply additional heat) [Hexagon dimensions: 100 cm (ground); 55 x 70 cm (top); 100 cm (radius)]; (b) AH (active heating): OTC with heating; (c) PH (passive heating): Only OTC (no additional heating); (d) NC (control): Open plot marked with the red flags referred as "No Chamber (NC)" (similar to the space of the OTC).

Leaf and stem structural traits

Six wild blueberry stems were randomly selected from each treatment plot and their leaf and stem structural traits (stem length and diameter, leaf number, leaf size, total leaf area and dry biomass) were measured. Samples were collected in August 2018 at the end of the growing season of wild blueberry plants. Leaf area was determined using a LI-3000A area meter (Li-Cor, Lincoln, NE, USA), then the leaves were oven-dried at 70ºC to constant mass and weighed. Leaf mass per area (LMA) was determined as leaf dry mass divided by leaf area (g m^{-2}). Stem volume was measured using water displacement method (Archimedes' principle) with a precision balance (0.0001gm). The stems were oven-dried at 70ºC to constant mass and weighed and the wood density was determined as stem dry mass divided by stem volume (g cm^{-3}).

Leaf water status and Chlorophyll concentrations

Six wild blueberry stems were randomly selected from each treatment area to measure midday leaf water potential and chlorophyll content. Leaf water potential was measured by a leaf pressure chamber (PMS Inc., Albany, OR, USA) on samples collected at midday 12:30 h solar time. Chlorophyll content per leaf area was measured by a SPAD Chlorophyll Meter (SPAD 502; Minolta Corp., Osaka, Japan). Chlorophyll content per leaf mass was determined as chlorophyll content per leaf area divided by LMA.

Plant physiological traits

Leaf stomatal conductance, photosynthetic rate, and intercellular CO_2 concentration from twelve randomly selected wild blueberry stems in each treatment area were measured by a portable photosynthetic measurement system (li-6400; Li-Cor Biosciences, Lincoln, NE, USA) on a typical sunny day (17 August 2018) between 11:00 and 14:00 h solar time at a photosynthetic photon flux density of 1500 µmol m^{-2} s^{-1}. Pre-dawn Fv/Fm (maximum potential quantum efficiency of photosystem II) was measured from six randomly selected wild blueberry stems in each treatment area by a portable photosynthetic measurement system (li-6400; Li-Cor Biosciences, Lincoln, NE, USA). During the measurements, the atmospheric temperatures, relative humidity and leaf temperatures ranged from 25ºC to 30ºC, 50% to 60%, and 27 to 31ºC, respectively. Leaf photosynthetic rate per mass was determined as the photosynthetic rate divided by LMA. Water use efficiency of wild blueberry stems was determined as the photosynthetic rate divided by the stomatal conductance of leaves. CO_2 assimilation rate per stem was determined as the photosynthetic rate (area based) multiplied by the total leaf area of an individual stem.

Leaf nutrient concentrations

Leaf nutrition was assessed by excising leaves from 12 randomly selected wild blueberry stems from each treatment. In order to get enough dry mass for nutrient concentration measurements, leaf samples from the 12 stems per treatment were pooled into two samples and sent to the University of Maine Soil and Plant Tissue Testing laboratory in Orono, Maine. One individual stem in treatment did not have enough dry mass for leaf nutrient concentration determinations.

Data Analyses

Statistical analyses were applied using SPSS V21 (IBM Corp., Armonk, NY, USA). ANOVA analyses followed by Tukey's post-hoc HSD test were conducted to determine the statistical significance of the data among the three treatments (Control, Passive heating and Active heating) at a significance level of 0.05. Our assumption in using ANOVA in this non-replicated treatment experiment was that the between stem variance was as great as between treatment variance within the genet, and therefore used stems within treatments as replicates. In a heavily replicated experiment on leaf and flower node removal in lowbush blueberry, variance among stems within a genet was found to be as high as among treatment plots within genets for 50% of the measured physiological responses, suggesting that stems within a genet respond similarly whether they are spatially proximal or not, despite the rhizome structure underground that connects stems within a genet (Bajcz & Drummond 2017). This was true even when rhizomes were severed among stems. We found in a preliminary variance components analysis of our data with one morphological measure (LMA) and two physiological measures (chlorophyll concentration and photosynthetic rate) that in two of three measures the overall stem within genet variance was no different than variance among the locations within the genet. Only with LMA, the variance among locations within the genet was greater than the variance between stems within the genet. Therefore, our assumption holds for at least some of our measures. Since only one genet was used here and stems were the only basis of replication, the probability of a Type I error might be biased toward a low probability level.

Results

During June-September' 2018 (Fig. C3a), in the passive heating (PH) and active heating (AH) treatments, the average temperature was approximately 0.5ºC and 3.5ºC higher, respectively, compared to the control (NC). Over the same period, the relative humidity (RH) (Fig. C3b) in the PH and NC treatment was not significantly different from each other but in the AH treatment it was 10% lower compared to the NC and PH treatments. The average air vapor pressure deficit (VPD) (Fig. C3c) in the NC and PH treatments was similar, 0.6 to 0.8 kPa. However, in the AH treatment, the VPD was 1.26 kPa which is 0.5 kPa higher compared to the NC and PH treatments.

On a typical sunny day (17 August 2018), the atmospheric temperature was 17.6 ± 0.3ºC, 18.1 ± 0.5ºC and 21.8 ± 0.4ºC in the NC, PH and AH treatments, respectively (Fig. C3d). On average, the temperature was approximately 0.5ºC higher and 4ºC higher in the PH and AH treatments, respectively, compared to the NC treatment. However, the air temperature was lower inside the PH chamber during the night than the ambient air temperature. On the same day, the atmospheric RH (Fig. C3e) in the NC and PH treatments was similar, 86.8 ± 1% and 87.3 ± 1.2%, respectively but in the AH treatment, the RH was 72.6 ± 0.7% which was 13% lower compared to the NC and PH treatments. Consequently, the VPD was 0.33 ± 0.03 kPa, 0.4 ± 0.04 kPa and 0.8 ± 0.04 kPa in the NC, PH and AH treatments, respectively (Fig. 3f). Based on the 0.5 to 4ºC higher atmospheric temperature and 13% lower RH, the VPD in AH treatment was approximately two times higher compared to the NC and PH treatments.

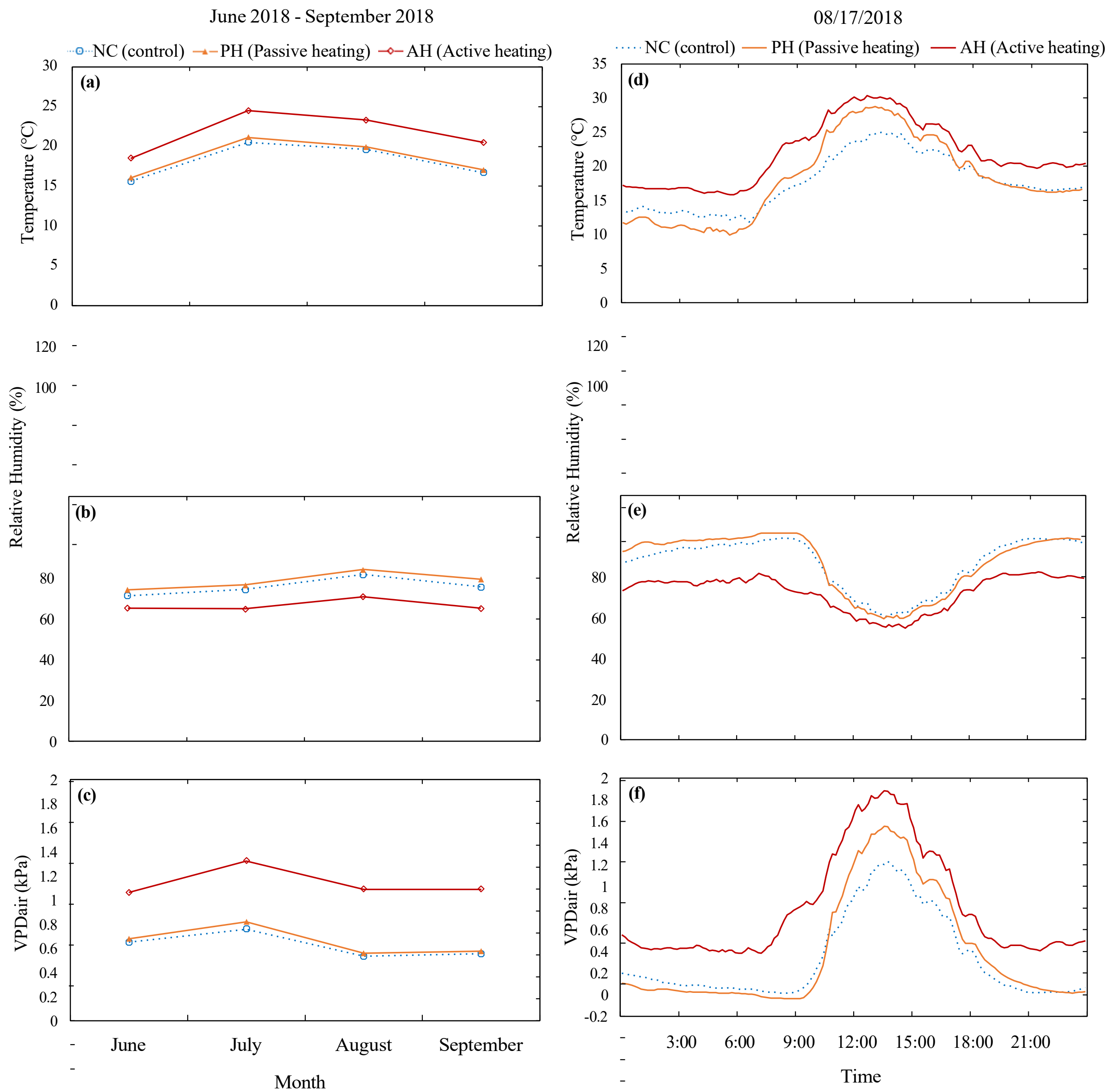


Figure C3. Monthly averages under control, active heating and passive heating treatments in (a) Atmospheric Temperature, (b) Relative Humidity, and (c) Vapor Pressure Deficit during June 2018 to September 2018; Diurnal changes in (d) Atmospheric Temperature, (e) Relative Humidity, and (f) Vapor Pressure Deficit on a typical sunny day (17 August 2018).

Soil temperature was significantly higher in the AH treatment compared to the other two treatments, which was expected. In contrast, soil temperature was slightly, but significantly lower in the PH treatment compared to the NC treatment (Fig. C4a). Consequently, the volumetric water content (VWC) in soil was significantly lower in the AH treatment compared to the NC and PH treatments (Fig. C4b). There was no significant difference between the NC and PH treatments in soil VWC. The average leaf water potential (LWP) (Fig. C4c) in the AH treatment was approximately 0.5 MPa lower than the LWP in the PH treatment and approximately 0.9 MPa lower than the control.

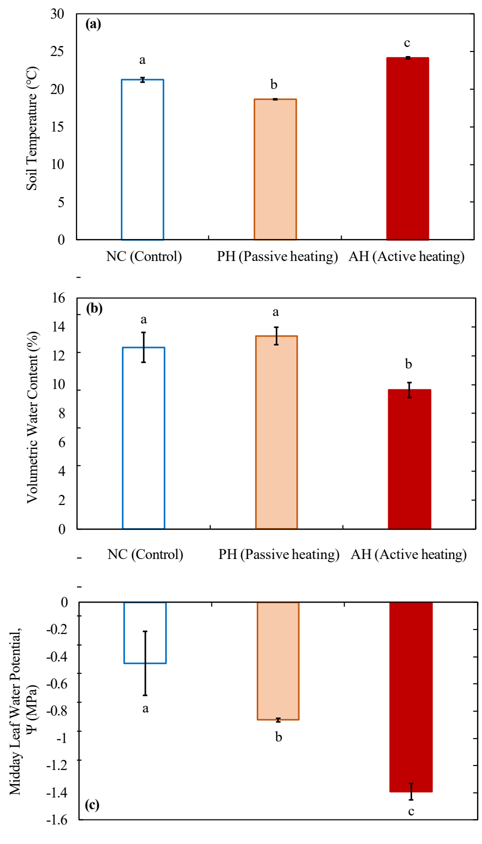


Figure C4. (a) Soil Temperature, (b) Volumetric Water Content in soil, and (c) Leaf Water Potential of wild blueberry stems under three different treatments (Control, Active Heating and Passive Heating) in a typical sunny day (17 August 2018). Data are averages ± S.E. (n = 6); Bars topped by the same letter do not differ significantly ($P < 0.05$).

Photosynthetic rates per leaf area of wild blueberry stems were significantly lower in both PH and AH treatments compared to those of the control (Fig. C5a). However, the observed photosynthetic rate per leaf mass of wild blueberry stems was similar among the NC, PH and AH treatments (Fig. C5b). No significant difference in maximum quantum efficiency of Photosystem II of the wild blueberry stems (Fv/Fm) were detected among the NC, PH and AH treatments (Fig. C5c). Similarly, the observed stomatal conductance (Fig. C5d) of wild blueberry stems showed no significant differences among different treatments.

In accordance with the lower photosynthetic rate, the leaf intercellular CO_2 concentration (Fig. C5e) of wild blueberry stems was significantly higher in AH treatment compared to the NC and PH treatments. The AH treatment also significantly decreased water use efficiency of wild blueberry stems compared to those of the NC but not significantly higher than that of the PH treatment (Fig. C5f). At a similar level of stomatal conductance for all three treatments (Fig. C5d), the photosynthetic rate per leaf area was significantly (Fig. C5a) lower for the PH and AH treatments compared to the control.

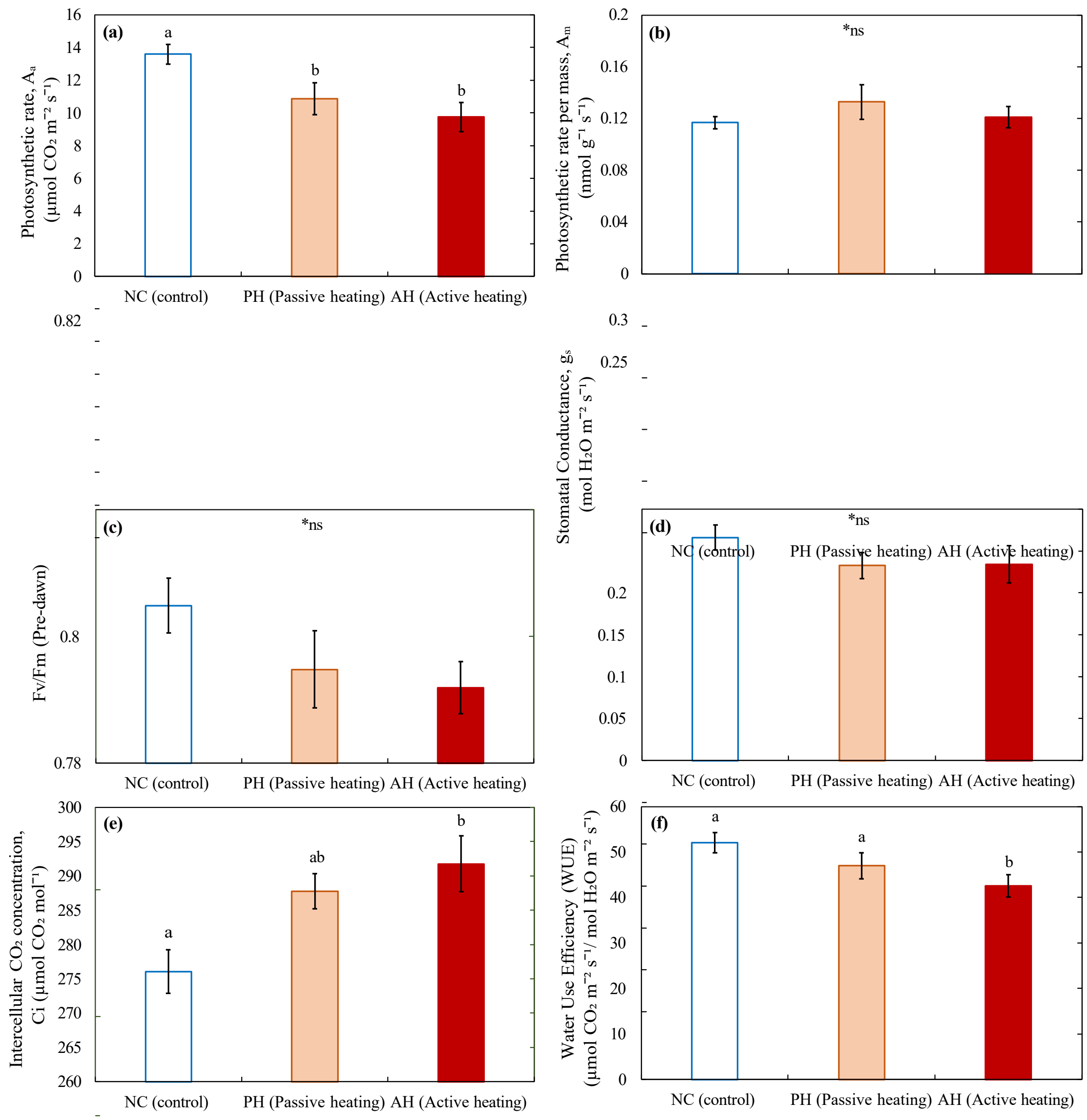


Figure C5. (a) Photosynthetic rate per leaf area, (b) Photosynthetic rate per mass, (c) Pre-dawn Fv/Fm (maximum potential quantum efficiency of photosystem II), (d) Stomatal conductance, (e) Leaf intercellular CO_2 concentration, and (f) Water use efficiency of wild blueberry stems growing under three treatments (Control, Passive Heating and Active Heating) on 17 August 2018. Data are averages ± S.E. (n = 12). Bars topped by the same letter do not differ significantly ($P < 0.05$).

The PH treatment had significantly lower chlorophyll per leaf mass than the AH or NC treatments (Fig. C6a). Chlorophyll concentration per leaf area was significantly lower in PH and AH treatments (Fig. C6b) and had 20% lower total nitrogen concentration (Fig. C6c and C6d) compared to the NC treatment. This agrees with the observed lower photosynthetic rate per leaf area (Fig. C5a). In the AH treatment, a 40% higher phosphorous concentration per leaf mass was observed compared to the NC and PH treatments (Fig. C6e) whereas only a slight difference was observed in phosphorous concentration per leaf area (Fig. C6f) among the NC, PH, and AH treatments. However, no difference was observed in total carbon concentration per leaf mass among the treatments (Fig. C6g), whereas, a higher carbon concentration per leaf area was observed (Fig. C6h) in the NC treatment compared to those of the AH and PH treatments.

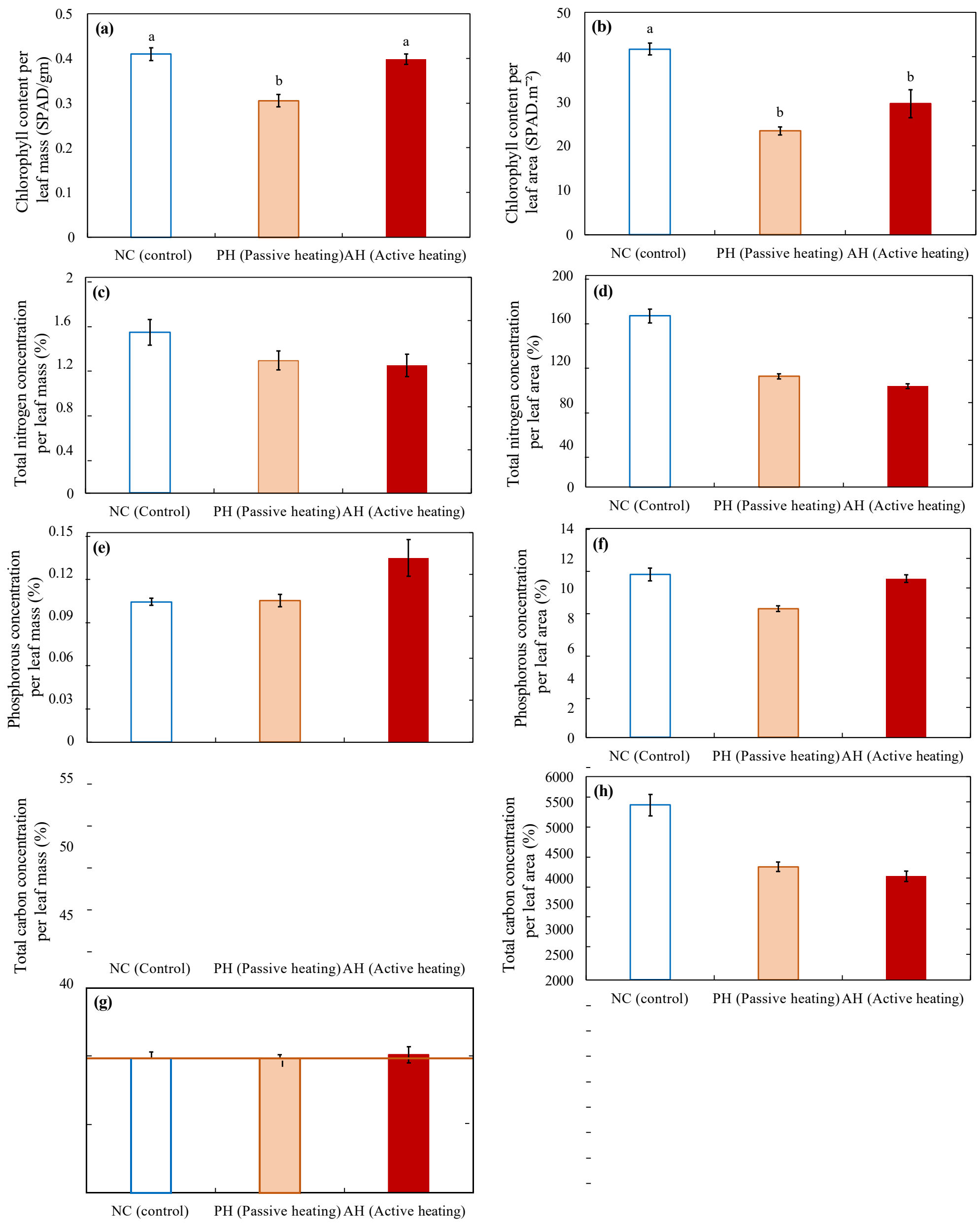
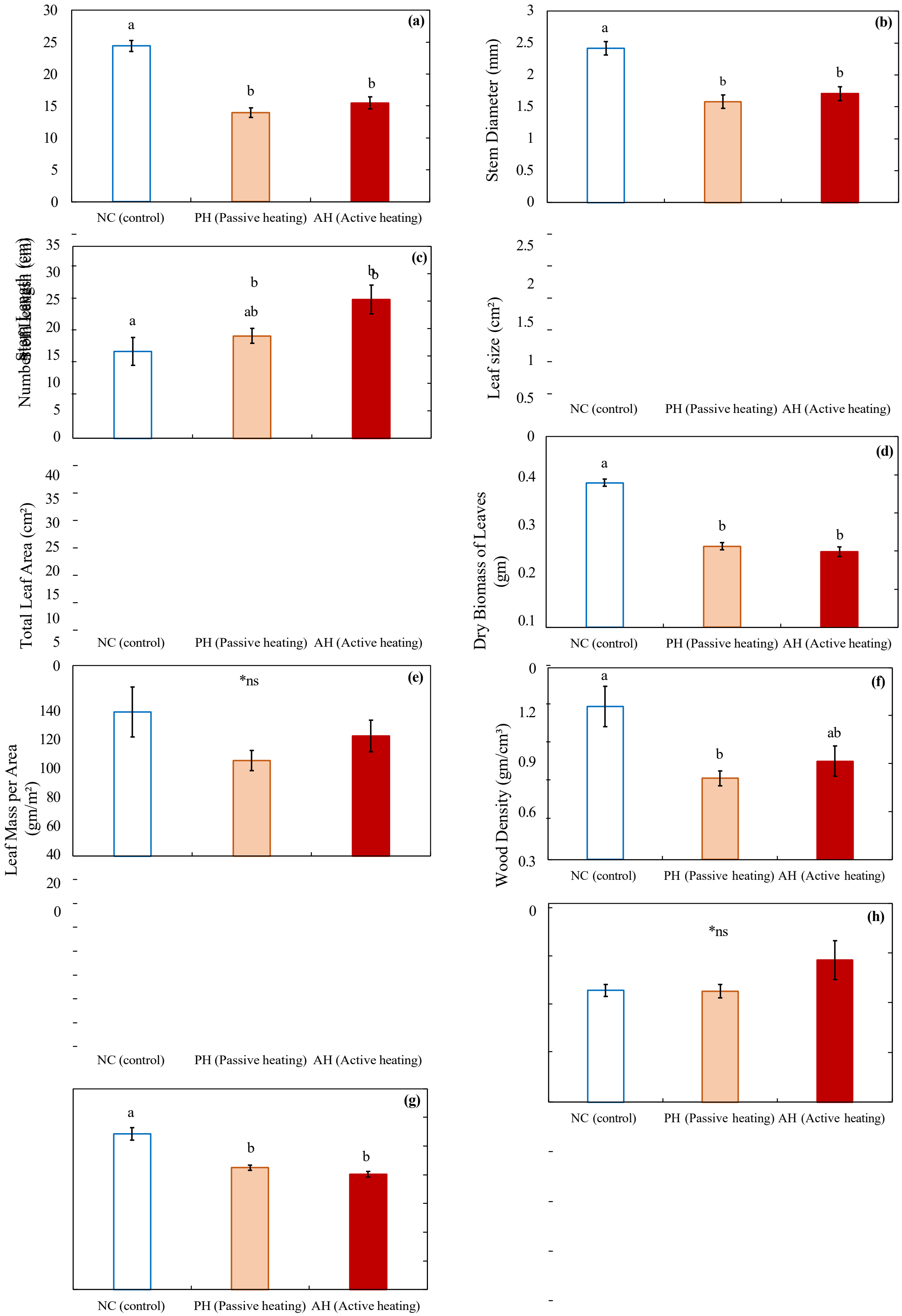


Figure C6. (a) Chlorophyll content per leaf mass (n = 6), (b) Chlorophyll content per leaf area (n = 6), (c) Total nitrogen concentration per leaf mass (n = 2), (d) Total nitrogen concentration per leaf area (n = 2), (e) Phosphorous concentration per leaf mass (n = 2), (f) Phosphorous concentration per leaf area (n = 2), (g) Total carbon concentration per leaf mass (n=2), and (h) Total carbon concentration per leaf area (n = 2) in leaves of the wild blueberry stems growing under three different treatments (Control, Active Heating and Passive Heating). Data are averages ± S.E. Bars topped by the same letter do not differ significantly ($P < 0.05$).



(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Stem Length (cm)
Stem Diameter (mm)
Number of Leaves
Leaf size (cm²)
Total Leaf Area (cm²)
Dry Biomass of Leaves (gm)
Leaf Mass per Area (gm/m²)
Wood Density (gm/cm³)
NC (control)
PH (Passive heating)
AH (Active heating)
*ns

Figure C7. Leaf and stem structural traits of the wild blueberry stems growing under different treatments (Control, Active heating and Passive heating). (a) Stem length, (b) Stem diameter, (c) Number of leaves, (d) Leaf size, (e) Total leaf area, (f) Dry biomass of leaves, (g) Leaf mass per area (LMA), and (h) Wood density. Data are averages ± S.E. (n = 6). Bars topped by the same letter do not differ significantly ($P < 0.05$).

For the stem structural traits, stem length (Fig. C7a) and diameter (Fig. C7b) were significantly smaller in the PH and AH treatments compared to the control. In contrast, from the leaf structural traits of wild blueberry plants, it was observed that the number of leaves was significantly higher in the AH treatment compared to the control (Fig. C7c) whereas the leaf size of wild blueberry stems was significantly smaller in both heating treatments compared to the stems in the control (Fig. C7d). Hence it is observed that, with the increasing atmospheric temperature wild blueberry stems were developing more leaves with smaller surface area per leaf. The average total leaf area per stem was smaller in the heating treatments compared to the control (Fig. C7e), but the difference was not significant. Dry biomass of leaves (Fig. C7f) was lower (although not significant) and leaf mass per area (Fig. C7g) was significantly lower in PH and AH treatments compared to the control. In contrast, no significant difference was observed in wood density among the treatments (Fig. C7h). The CO_2 assimilation rate per stem was significantly (32% to 40%) lower in the AH and PH treatments compared to the control (Fig. C8).

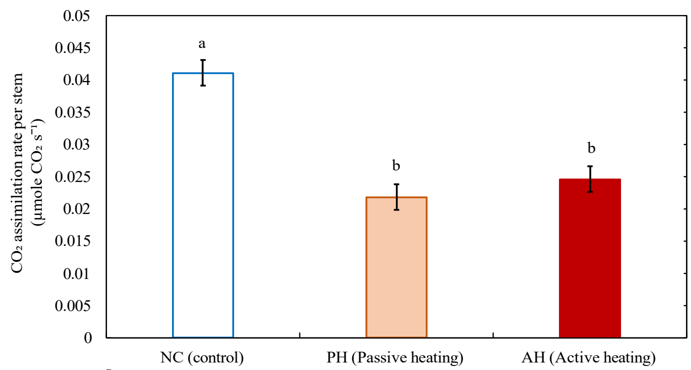


Figure C8. Estimated CO_2 assimilation per individual stem of the wild blueberries growing under three different treatments (Control, Passive heating, and Active heating). Data are averages ± S.E. (n = 12). Bars topped by the same letter do not differ significantly ($P < 0.05$).

Discussion

Our results indicate that the open-top chamber (OTC) with active heating (AH) system is an efficient and feasible way to manipulate plant canopy and soil temperatures. We found that we were able to successfully measure warming effects on physiological development of wild blueberry plants in the field. The passive heating (PH) chamber produced warmer temperatures during the day, but cooler temperatures during the night inside the chambers. This pattern introduced uncertainties and confounding factors to the experimental design. By increasing temperatures of 3 to 5ºC, the AH chamber effectively altered plant morphology, structure, and physiology. Warming decreased leaf photosynthetic rates and water use efficiency of wild blueberries. Warming by both the AH and PH also decreased leaf sizes but increased total leaf numbers. The cost of building an AH chamber including the weather station for monitoring temperature, RH, and soil moisture was ~$1,800.

Many temperate crops are expected to benefit from 1 to 3ºC warming of ambient temperature (Easterling et al. 2007; Hatfield et al. 2011). Photosynthetic rate of plants generally is expected to increase with increasing temperature between 13ºC and 25ºC assuming that no other limiting factors such as light, nutrient, and CO_2 concentration changes occur (Curtis & Clark 1950). Some temperate crops (i.e., wheat, maize) exhibit the maximum photosynthetic rate at 33ºC, but with photosynthesis declining after the temperature reaches more than 33ºC (Reynolds et al. 2000; Badaruddin et al. 1999). Forsyth & Hall (1965) observed that the rate of apparent photosynthesis in wild blueberry crops increased with increasing leaf temperature from 13ºC to 29.5ºC under laboratory conditions. However, in our study, photosynthesis of wild blueberry declined when air temperature increased to more than 25ºC and leaf temperature increased to more than 28ºC. Forsyth & Hall (1965) only treated submerged leaf disks under different temperatures for 10-15 minutes. Therefore, they could not capture developmental changes in leaf structure and physiology under warmer temperatures, as well as stomatal responses to VPD.

Photosynthetic rate could be limited by stomatal conductance over a wide range of environmental conditions (Xu et al. 2016; Verhoef & Egea 2014). However, the negative impact of warming on the photosynthesis of wild blueberries cannot be explained by stomatal limitation as there was no decline in stomatal conductance under warming conditions (Fig. 5d). High leaf-intercellular CO_2 concentration under warming conditions also suggests that decline in photosynthesis is not a result of increased stomatal limitation. Instead, the decline in photosynthetic rate (Fig. 5a) and water use efficiency (Fig. 5f) can be explained by the decline in chlorophyll (Fig. 6a & 6b) and nitrogen (N) concentration (Fig. 6c & 6d) which were lower in PH and AH treatments where the air temperature was higher than 25ºC (Fig. 6c & 6d). This implies that physiological performance of the wild blueberry genotype that we studied could decline after reaching the air temperatures of approximately 24 to 25ºC. Leaf nitrogen (N) is one of the most important determinants of photosynthesis (Wright et al. 2004; Reich et al. 1999). Lower leaf N concentration could be because of increased soil water deficits (Fig. 4b) that restrict plant nutrient uptake. Since N is supplied to leaves through the mobile NO_3^- or NH_4^+ that are taken up by the roots from the soil mainly through water/mass flow (Barber 1995, Jungk 1996), decreased soil water content can inhibit N uptake. However, the average Fv/Fm values of the plants which was below 0.8 under warming treatments (both PH and AH) imply that the wild blueberry stems in the AH and PH chambers could maintain physiological function under long-term stress (Maxwell & Johnson 2000).

Warming also changed leaf and stem structural traits. Generally, wild blueberry stems under warming had more leaves that were smaller without changes in total leaf surface area. Stems were smaller, shorter, and with denser wood under warming treatments. All of these suggest higher water deficits under warming treatments. Air humidity (Fig. 3b & 3e), soil water content (Fig. 4b), and leaf water potential (Fig. 4c) were lower under warming. The increased water deficits can decrease cell turgor and consequently leaf and stem development. Additionally, LMA of wild blueberry stems was significantly lower under warming compared to the control. This indicates that leaves were thinner or less dense under warming conditions. High LMA is related to plant adaptation to drought (Gratani et al. 2009, Villar et al. 2013, Zhang et al. 2017). Smaller LMA under warming conditions implies that the wild blueberry plants might not be efficient in enhancing their drought tolerance associated with increased temperatures.

Overall, whole stem CO_2 assimilation rate in wild blueberries (Fig. 8) under warming were 32% to 40% lower compared to the control, suggesting warming has negative effects on stem level physiological performance. Declined whole stem CO_2 assimilation also suggests that warming may also decrease the yield, which needs to be further studied over a diversity of genotypes.

Conclusions

We suggest by proving in our study that the active heating (AH) open-top chamber is an effective system to study warming effects on wild blueberries in the field. The passive heating chambers, however, introduced some uncertainties including the confounding effects of cooler nights. The AH system successfully manipulated atmospheric warming and can also be applied to other crops like potatoes in Maine. Our results, while preliminary and based upon only one genotype, suggest a potential negative effect of warming on wild blueberry growth by decreasing soil water and nutrient availabilities. Consequently, warming will make summer drought worse, which itself is predicted to increase in frequency and severity in Maine (The University of Maine Climate Institute 2015). Thus, to sustain wild blueberry production in a future with warmer and drier summers, techniques to mitigate these effects should be developed and tested. Potential sustainable solutions, e.g. effective irrigation, mulching, and nutrient management could be tested with the AH system presented here. Our system could also be used to investigate different responses of different wild blueberry genotypes and other crops to climate change. Additionally, our system can be used to study the response of weeds, pathogen disease, pollinators, herbivores, micro-organisms, and fruit quality to warming. Therefore, it could be a platform fostering interdisciplinary research. Those who are interested in studying climate change effects on wild blueberries, other crops, or microorganisms in the field can contact the authors for collaborative research or chamber construction guidelines.

List of Abbreviations

AH: Active Heating

PH: Passive Heating

OTC: Open-Top Chamber

NC: No Chamber

IPCC: International Panel of Climate Change

LMA: Leaf Mass per Area

LWP: Leaf Water Potential

N: Nitrogen

VPD: Vapor Pressure Deficit

VWC: Volumetric Water Content

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Appendix D

Supporting Information for Chapter 6

Table D.1. Macro- and micro-nutrients in leaf tissues of wild blueberry plants treated with different treatments in Experiment 1. The optimum recommended nutrient levels in wild blueberry leaf tissue are also reported here for comparison. Red colored values indicate that they are higher than the optimum level.

Nutrient elements in leaf tissue		Optimum level	Treatments			
			Control	CNF	Poma	P+CNF
Macro-nutrient elements	Carbon (%)	N/A	48.11±0.2	48.3±0.16	48.5±0.18	48.24±0.14
	Nitrogen (%)	1.76	1.54±0.06	1.535±0.05	1.45±0.04	1.49±0.06
	Phosphorus (%)	0.136	0.12±0.006	0.112±0.0023	0.113±0.006	0.116±0.007
	Potassium (%)	0.44	0.41±0.034	0.37±0.026	0.36±0.02	0.37±0.03
	Calcium (%)	0.38	0.77±0.04	0.76±0.05	0.69±0.03	0.74±0.05
	Magnesium (%)	0.17	0.26±0.02	0.26±0.012	0.24±0.02	0.236±0.02
Micro-nutrient elements	Boron (ppm)	23	36.8±4.5	35.47±3.5	32.9±4.9	34.7±2.8
	Iron (ppm)	35	41.7±2	42±1.28	36.27±1.16	42.75±1.17
	Manganese (ppm)	963	1484±304	1225±258	1257±177	1627±258
	Zinc (ppm)	13	13.45±1.1	12.26±1.02	11.8±0.85	12.46±0.98
	Copper (ppm)	4	3.74±0.24	3.4±0.3	3.53±0.2	3.65±0.25
	Aluminum (ppm)	179	106.2±9.16	98.65±8.95	88.97±2.4	114.05±6.9

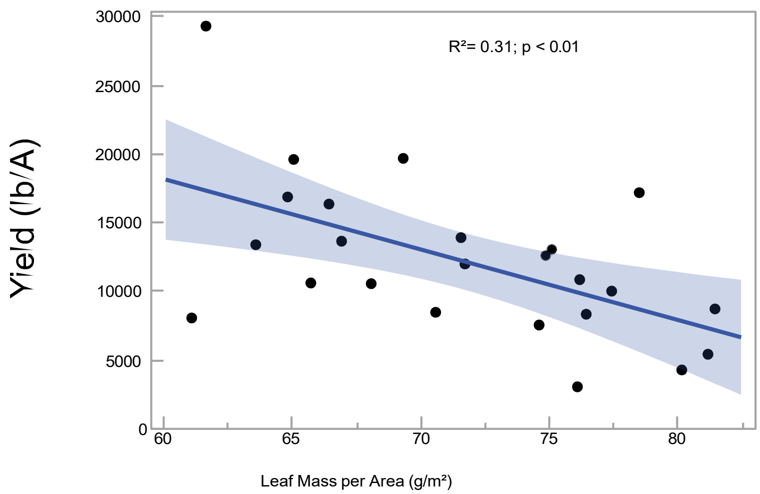

Figure D1. Relationship between leaf mass per area (LMA) and yield of studied wild blueberries. The solid line represents a linear relationship fitted to the data by linear regression analysis ($p < 0.01$) and the shaded region represents a 95% confidence interval.

Quant Results (unwashed)

Element	Weight %	MDL	Atomic %	Error %	Net Int.	R	A	F
C K	67.50	70.16	76.60	89.47	0.17	0.9086	0.4690	1.0000
N K	16.13	260.44	15.47	99.99	0.01	0.9418	0.0872	1.0000
O K	7.02	115.20	5.90	99.99	0.01	0.9460	0.1434	1.0000
Ca K	9.35	80.45	3.14	99.99	0.07	0.9808	0.9853	1.0196

Quant Results (washed)

Element	Weight %	MDL	Atomic %	Error %	Net Int.	R	A	F
C K	64.17	5.37	69.86	28.31	0.38	0.9423	0.4498	1.0000
N K	15.57	17.44	14.54	99.99	0.03	0.9472	0.0994	1.0000
O K	18.32	26.26	14.97	74.00	0.00	0.9611	0.1712	1.0000
Ca K	1.94	6.39	0.63	99.99	0.01	0.9830	0.9894	1.0307

Figure D2. EDAX results of calcium percentage before and after filtration washing.

Appendix E

Supporting Information for Chapter 4

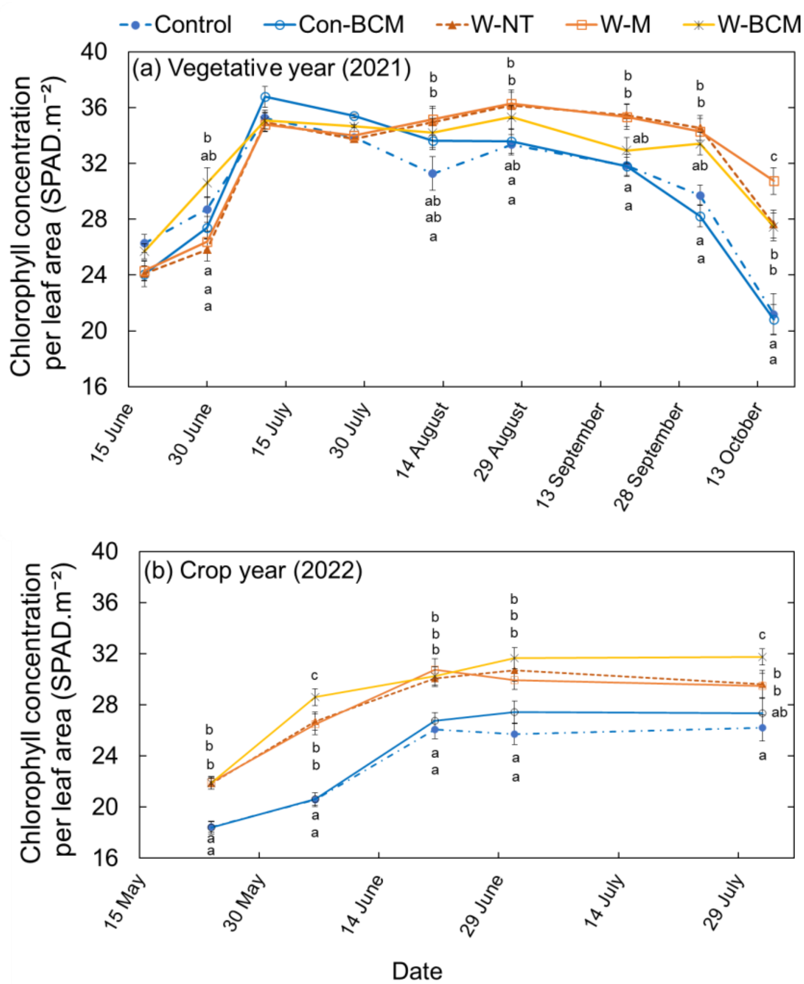

Figure E1. Seasonal changes in chlorophyll concentration per leaf area in the wild blueberry leaves across five different treatments on (a) vegetative year (June to October 2021) and (b) crop year (May to early August 2022). Error bars indicate the standard error of the mean. Different letters corresponding with different treatments on most of the measurement dates indicate significant differences and no letters on few measurement dates indicate no significant differences among the treatments at the significance level of $p < 0.05$.

BIOGRAPHY OF THE AUTHOR

Rafa Tasnim was born and raised in Dhaka, Bangladesh. She earned her bachelor's degree (B.Sc. in Civil Engineering) from the Military Institute of Science & Technology, Dhaka, Bangladesh in 2016. Then she moved to Hong Kong and earned her master's degree (M.Phil. in Civil Engineering) from the Hong Kong University of Science & Technology, Hong Kong in 2018 where she conducted her research on soil-plant-climate interaction. Being enthusiastic about research on plants, Rafa then joined Dr. Yong-Jiang Zhang's plant physiology lab at the University of Maine, Maine, USA to further conduct doctoral research on plants in 2019. Rafa is a candidate for the Doctor of Philosophy degree in Ecology and Environmental Science from the University of Maine in August 2024.