Progressing Towards Understanding Water Use Efficiency in Southern, Ontario Canada: Quantifying Water Use Efficiency Metrics (WUE) and Investigating Soil and Plant Physiology Influences on WUE

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

ABSTRACT

Climate change, and corresponding temperature increases of 1.4 - 4.8 ºC by the end of the century, are expected to cause shifts in agricultural production. Additionally, shifts in precipitation patterns are expected to cause strains on water resources. Globally, croplands occupy 33-40% of terrestrial land area with only 17-18% of these being irrigated; thus, rainfed croplands will remain important to global food production. This highlights the need to maximize crop resource use while responding to environment shifts and maximizing agricultural production. Water use efficiency (WUE), which measures carbon assimilation per unit water use, has been identified as an important indicator for plant resource use, which has the potential to provide insight into responses of environmental changes. However, there is a paucity of information on differences between crop species WUE or the drivers behind these differences, which are important to consider in climate change scenarios. Furthermore, there are several calculations for WUE, but there is a lack of field-based studies investigating inconsistencies between these calculations.

This thesis addresses these knowledge gaps with the following two objectives: 1) quantify plant water-carbon dynamics of two common forage crops in southern Ontario, maize (*Zea mays L.*) and alfalfa (*Medicago sativa*), and investigate the ecosystem drivers of these differences; and 2) quantify WUE of alfalfa and maize crops using different WUE calculation approaches, and investigate the inconsistencies between these methods. Alfalfa contained greater growing season ecosystem WUE (EWUE) than maize, with daily fluctuations in EWUE being controlled by differences in gross primary productivity (GPP) rather than evapotranspiration (ET). Since these sites were subjected to similar climate and atmospheric variables, and similar soil conditions, differences between the crops were attributed to crop physiology and farming practices which influenced crop growth.

In general, alfalfa had higher growing season “flux-based” EWUE’s, while maize had higher “harvest-based” WUE’s (HWUE’s). Inconsistencies between methods were attributed to processing method, crop physiology, and management influences on crop growth. The importance of timescale was also shown where the typically less efficient C_3 crop (alfalfa) had higher growing season EWUE despite having a lower median half-hourly EWUE The results of this thesis progressed our knowledge of WUE and how crop selection and farming practices influence it. Farming practices that affect crop growth influence these metrics, which can inform future crop selections and aid in adaptation to climate change. This also highlights the importance of considering different variables included in WUE calculations and the need for a more robust approach to crop resource use which accounts for both plant stomatal responses, non-plant ecosystem responses, and biomass production.

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In the spirit of reconciliation, I would like to recognize that the research contained within this thesis was conducted within the traditional territories of the Neutral, Anishnaabeg, and Haudenosauonee peoples. The research sites used for this research lie within, or along the border of the Haldimand Tact, which was promised to the Six Nations of Haudenosauonee peoples (Mohawk, Cayuga, Onondaga, Oneida, Seneca and Tuscarora) under the 1784 Haldimand Treaty. Presently, the Six Nations retain and occupy approximately 5% of the original lands promised to them.

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CHAPTER 1: INTRODUCTION

1.0 Threat of Climate Change to Croplands and Water Resources

Climate change, driven by increases in atmospheric carbon dioxide (CO_2), is expected to cause increases in global surfaces temperature (IPCC, 2014) and changes to precipitation patterns (Dore, 2005; Groisman et al., 2005; Trenberth, 2011). With temperatures expected to rise by 1.4 – 4.8ºC by the end of the century (IPCC, 2014), shifts in ecosystem processes are expected to impact global food production (Nelson et al., 2009; Smith and Gregory, 2013). More specifically, changes in surface temperatures and precipitation patterns are expected to have profound impacts on agricultural carbon and water cycles. Increases in surface temperature stimulates both CO_2 uptake (Shaw et al., 2002) and losses from respiration (Rustad et al., 2001; Shaw et al., 2002). These shifts in carbon cycling are expected to increase net primary productivity in ecosystems, however this can be supressed by shifting precipitation patterns (Gu et al., 2017). That is higher surface temperatures can increase plant transpiration and soil evaporation (Harte et al., 1995; Wan et al., 2002) resulting in lower soil available water promoting water limitations (Niu et al., 2008). As such, agricultural water use is important to future climate scenarios (Kulshreshtha and Grant, 2007) since climate change stressors could affect water availability and therefore plant production (Fuhrer, 2003; Gregory and Ingram, 2000). This is expected to result in an increase in agricultural water use required from irrigation to sustain agricultural demands (Fischer et al., 2007).

There is concern over future water demands from agriculture because of the increasing strain on water resources from the combined effects of climate change, growing population demands, and competition from other economic sectors (Fischer et al., 2007). Globally, croplands and pastures account for between 33% (FAO, 2012) and 40% (Foley et al., 2005; Ramankutty and Foley, 1998) of terrestrial surface coverage. Of these, only 17-18% of these lands are irrigated

(Fischer et al., 2007; Thenkabail et al., 2009), which produce approximately 40% of the world's food supply (Thenkabail et al., 2009) and account for 70-80% of water used by humans (Salmon et al., 2015; Thenkabail et al., 2010). In Canada, agricultural water use is estimated to be approximately 2.34 billion m^3 per year and irrigation accounts for 86% of this (Kulshreshtha and Grant, 2007). With projected stains on water resources combined with projected increases in water demand for irrigation (Gleick, 2003; Ju et al., 2013), rainfed crops remain essential to the sustainability of global food production moving forward (Baig et al., 2013; Rockström et al., 2010). By 2050, there is expected to be a 60% increase in global demand for agricultural production, which will likely need to come from increased productivity rather than expansion of agricultural lands (Alexandratos and Bruinsma, 2012). This highlights the need to improve water management in croplands to maximize yields while reducing unnecessary water losses (Molden, 2007; Rockström et al., 2010).

1.1 Agricultural Response to a Changing Climate – Changes, Adverse Effects, or Resilience?

The Intergovernmental Panel on Climate Change (IPCC, 2014) stipulate that crop production is expected to be impacted by the effects of climate change, with both positive and adverse effects expected. The overall impact remains relatively uncertain at local and regional scales (Porter et al., 2014) because of the complexity of interactions and feedbacks involved (Kirschbaum, 2004). For example, increases in temperature increase vapour pressure deficits (VPD), and consequently, transpiration rates. However, stomata also respond to CO_2 concentration, which could result in decreases in stomatal opening at higher atmospheric CO_2 concentrations (Kirschbaum, 2004). Some regions are expected to see a positive impact of climate change on agricultural production, however, a net negative global impact is expected (Porter et al., 2014; Zhao et al., 2017). Negative impacts from climate change on the agricultural sector are already occurring; for example, global yields of maize and wheat have decreased by 3.8% and 5.5%, respectively, from 1980-2008 (Lobell et al., 2011). In the same study, losses and gains were noted for different regions, which resulted in a balance of losses and gains in soybean and rice (Lobell et al., 2011). In China, these changes are expected to decrease maize, wheat, and rice yields by 2.2–6.7 %, 0.4 %–11.9 %, and 4.3–12.4 %, respectively, by the 2050s (Ju et al., 2013). There is some evidence suggesting that the amount of warming required to see adverse effects from climate change may depend on latitudinal differences, where tropical regions experience a decline in maize and wheat yields at only 1-2 ºC increases, while temperate region yields may not be negatively affected until increases of 3-5 ºC (Porter et al., 2014). Fortunately, the threat of climate change can be reduced through adaptations in practices and increasing agricultural resilience by improving resource use efficiency (Lipper et al., 2014).

1.2 Water Use Efficiency: A means to measure Crop Resource Use

Since water use is of utmost importance when considering agricultural responses to climate change (Kulshreshtha and Grant, 2007), it is important to optimize water use per agricultural production (Evans and Sadler, 2008). Water use efficiency (WUE) is an integrative term to describe the production of plants, or carbon gained, per unit of water used, which is commonly used to indicate vegetation performance (Beer et al., 2009; Ito and Inatomi, 2012; Medlyn et al., 2017). Leaf stomata open and close to assimilate CO_2 for photosynthesis, but this enables water molecules to leave through transpiration (Wright et al., 2003). In most plants, stomata remain open during photosynthetically active daytime and close overnight to conserve water in the absence of photosynthetically active radiation (Eisenach and De Angeli, 2017). Crops function more efficiently when they balance atmosphere gas exchanges to maximize CO_2 uptake for photosynthesis and minimize water loss through transpiration (Lawson and Blatt, 2014). Water use efficiency, therefore, is an important indicator of plant resource use, which has implications for survival strategies under varying water availability and responses to changing environments (Ito and Inatomi, 2012).

1.2.1 Different Calculations of Water Use Efficiency

Due partially to inconsistencies in the definition of WUE, there have been several different attempts to quantify WUE using different carbon assimilation and water use variables. Differences in WUE variables used in calculations can represent different ecosystem processes and mechanisms, which should be considered when interpreting WUE studies (Kang and Kang, 2019). At the ecosystem-canopy scale, carbon assimilation can be measured as gross primary productivity (GPP) while water use is represented by transpiration (T). This measure of WUE captures physiological responses and biochemical functions (Beer et al., 2009; Farquhar and Sharkey, 1982; Ito and Inatomi, 2012) but does not consider the complete water use by an ecosystem. Instead, ecosystem scale WUE can use evapotranspiration (ET) instead of T, which incorporates evaporation – thus providing a holistic ecosystem water use, which could be important for incorporating additional water losses from evaporation and provide better insight into water management adaptation (Goyal and Harmsen, 2014). In agronomy, above ground biomass production per water use is often substituted for carbon assimilation due to the importance of yield production. In some locations, precipitation is used for rainfed systems instead of water use, specifically if water is limiting to crop production (Condon et al., 2002).

1.3 Environmental drivers of water use efficiency

There are two ways to improve WUE: (1) increase carbon assimilation; or (2) decrease water use. In general, there are three categories of environmental drivers that are responsible for field-scale increases and decreases in WUE: climate or atmospheric drivers, edaphic drivers, and vegetation drivers.

Local climates and energy budgets drive atmospheric conditions and weather patterns, which have a profound impact on plant stomatal activity and atmospheric carbon and water exchanges. Carbon assimilation rates are reliant on solar radiation and temperature driven biochemical reactions (Albertson et al., 2001; Yamori et al., 2014). Stomatal opening, which is responsible for GPP and T, is induced by photosynthetically active radiation (PAR) through either a continuous high intensity of light or blue-light dependant responses (Doi et al., 2015; Vavasseur and Raghavendra, 2005). Conversely, stomatal closure is initiated by increases in vapour pressure deficits (VPD; Merilo et al., 2018). Stomatal conductance increases with increasing temperature (Urban et al., 2017), which is also linked to increases in non-stomatal water losses through evaporation (Qiu et al., 1998). However, ET decreases with increases in atmospheric water content (decreasing VPD) (Mitchell et al., 2015). The net interaction between these atmospheric drivers contain numerous feedbacks, making the direct response of WUE variables difficult to isolate. However, WUE has shown significant relationships with VPD (c.f. Jiang et al., 2019; Mitchell et al., 2015), air temperature (c.f. Tong et al., 2014), and PAR (c.f. Medrano et al., 2012).

Edaphic drivers influence WUE predominantly through soil water availability. Plants under water stress can experience reductions in both growth and carbon assimilation (Pugnaire et al., 1999). This occurs at lower soil water content (SWC) since adhesive forces between soil and water particles can exceed the suction force of roots (Allen et al., 1998). Soil water availability has been cited as playing a dominant role in regulating ecosystem carbon and water fluxes (Niu et al., 2008) due to its role in stomatal control. Plant water status is affected by water availability, which then influences the opening and closing of stomata (Passioura, 1988) since stomata rely on maintaining turgidity of guard cells to remain open (Inoue and Kinoshita, 2017; Lawson et al., 2014; Sack and Holbrook, 2006). Moreover, soil water availability has been linked to biomass production (c.f Rossato et al., 2017), GPP (c.f Stocker et al., 2018), ET (c.f Seneviratne et al., 2010), and T (c.f Gardner and Ehlig, 1963). In addition to plant water use, soil moisture also influences the rate of evaporation (Price, 1980) since evaporation processes need to overcome adhesion to soil particles as well.

Vegetation characteristics, which drive WUE and its component variables include control over stomatal apertures, photosynthetic pathway, light interception, and shading. In addition, inter-species differences in hormone regulation (Loveys et al., 2000), plant morphology and hydraulics (Brodribb, 2009), and root morphology and water uptake (Zegada-Lizarazu and Iijima, 2005) also impact WUE. Under limited water supply, stomata can reduce openings and photosynthetic rates by more than 50% to conserve water (Lawson and Blatt, 2014). Aging also influences control over stomata apertures since less mature leaves contain less mature vascular bundles, corresponding with lower hydraulic potential; therefore, as a leaf develops, hydraulic conductance capacity also increases (Brodribb and Holbrook, 2005; Martre et al., 2000). After reaching maximum hydraulic potential, leaf hydraulic conductance begins to decrease due to clotting within xylem, reduced permeability of cell walls and membranes, and other factors, thus initiating senescence (Brodribb and Holbrook, 2005; Sack and Holbrook, 2006; Salleo et al., 2002). Differences in photosynthetic pathway can result in differences in photosynthesis rates (Ghannoum et al., 2011; Hsiao and Acevedo, 1974) due to physical separation of an additional metabolic cycle in C_4 plants, which reduces photorespiration (Way et al., 2014). This lowers stomatal conductance, thereby conserving water (Osborne and Sack, 2012). As such, WUE is typically greater in C_4 plants (Morison and Gifford, 1983; Osborne and Sack, 2012; Rawson et al., 1977; Wang et al., 2018; Way et al., 2014). Leaf area index (LAI) has been identified as relationally important for carbon and water fluxes (Albertson et al., 2001) due to its inherent relationship to canopy development and light interception (Pearce et al., 1965), as well as number of stomata.

The efficiency that plants can use intercepted light in photosynthesis, however, has been shown to vary between species (c.f. Guo and Trotter, 2006). Nonetheless, both T (Ritchie, 1972) and GPP (Gitelson et al., 2014) increase with increases in LAI. At lower LAI, leaf growth contributes to exposed, sunlit leaf area, while increases in LAI at the higher end contributes to increases in shaded leaf area (He et al., 2018). Shaded leaves have lower rates of photosynthesis (Boardman, 1977) because of decreases in energy for electron transport, which lowers stomatal conductance (Wong et al., 1979). However, this lowers T in shaded leaves as well (Nardini et al., 2005). In addition, canopy shading decreases the amount of energy which reaches the ground, lowering the evaporative demand and decreasing soil evaporation (Todd et al., 1991).

1.4 Knowledge Gaps

Hatfield and Dold (2019) identified four factors of climate change that are expected to impact agricultural water use: increasing temperature, more variable precipitation, increasing CO_2, and variations in humidity. However, due to the complexity of interactions between climate, atmosphere, soil, and vegetation, it is difficult to assess how these four factors are going to impact WUE at local and regional scales. Further, in agriculture, farming practices, such as planting in rows, can have a profound impact on plant growth and ecosystem responses to climate. While some of these practices (e.g. row spacing; Barbieria et al., 2012) have been studied on an individual basis, there is a paucity of local scale studies focussing on differences between crop species with different farming practices. Studies that do focus on different species typically focus on bioenergy crop production (c.f. Zeri et al., 2013), which do not emphasize the influence of different growth patterns or farming practices. This highlights a need to further understand the inter crop variations of WUE under current conditions, which could provide insight on adaptation to climate shifts. Moreover, there is a lack of field-based studies investigating the differences between calculations of WUE at local scales, or the influence of crop choice and farming practices on these differences in calculation. Studies investigating differences between WUE calculations typically focus on a couple of calculations at global (c.f. Ito and Inatomi, 2012) or regional (c.f. VanLoocke et al., 2012) scales, but there is a lack of local field-scale studies.

1.5 Research Objectives

The overall research objectives for this thesis were to: 1) quantify plant water-carbon dynamics of two common forage crops in southern Ontario, maize (*Zea mays L.*) and alfalfa (*Medicago sativa*), and investigate the ecosystem drivers of these differences; and 2) quantify WUE of alfalfa and maize crops using different WUE calculation approaches, and investigate the inconsistencies between these methods. The first objective is addressed in Chapter 2 of this thesis while the second objective is addressed in Chapter 3. Forage crops are important to consider in these studies since they occupy approximately 33% of global croplands (FAO, 2012). Maize and alfalfa were selected as study crops because they are two of Ontario's highest yielding forage crops (Bagg et al., 2017; OMAFRA, 2020) and provide an interesting reference point from which to answer these objectives since they contain interesting contrasts in physiology and farming practices. Alfalfa is a perennial crop, which follows the C_3 pathway of photosynthesis (Platt and Bassham, 1978) (Platt and

Bassham, 1978) and contains multiple harvests. Maize, however, is an annual crop, which follows the C_4 pathway of photosynthesis (Taylor, 1996) and is planted in rows.

CHAPTER 2

MANUSCRIPT 1: ECOSYSTEM INFLUENCES ON CARBON AND WATER DYNAMICS OF TWO FORAGE CROPS IN SOUTHERN ONTARIO, CANADA

Overview

Studies of water and carbon exchange are critical to understanding the performance of different crops and evaluating the effects of climate change. Thus, improving our understanding of how water use efficiency (WUE), evapotranspiration (ET), gross primary productivity (GPP) and net ecosystem exchange (NEE) vary across agricultural systems can help farmers better prepare for an uncertain future due to climate change by assessing water requirements for a given crop as a function of current environmental conditions. This study: 1) quantified field-scale carbon-water dynamics of alfalfa (*Medicago sativa*) and maize (*Zea mays*) crops; and 2) identified key environmental drivers of the observed differences between these crops.

A longer growing season yielded higher ET and GPP in alfalfa, resulting in greater WUE than maize. Climate and soil properties were similar between sites, and water availability was not limiting, suggesting that differences in WUE were driven by crop choice. Differences in daily WUE between maize and alfalfa were driven by differences in daily GPP rather than ET. The longer growing season of alfalfa promoted higher GPP production, while multiple harvesting reduced leaf aging effects and promoted periods of rapid growth. In contrast, late seedling emergence and self-shading induced senescence reduced seasonal GPP in maize. The results suggest that alfalfa may further this gap in WUE due longer growing seasons resulting in more cuts, and multiple cuts enabling perennial plants to take advantage of this longer growing season. Earlier establishment of maize could increase WUE at the start of the growing season, however earlier maturation will likely induce earlier senescence effects. Further, lower respiration losses in the C_3 photosynthetic pathway at higher CO_2 concentrations are expected to increase the gap between maize and alfalfa NEE. Future crop selections in this region should consider changes in growing season length as well as crop responses to temperature, CO_2 concentration, and water availability.

2.0 INTRODUCTION

Average global temperatures are expected to rise between 1.4 - 4.8 ºC by the end of the century, which will have major impacts on global food production (IPCC, 2014; Smith and Gregory, 2013). For example, potential evapotranspiration (PET) is expected to increase by 1.5 - 4% per 1ºC increase in temperature (Scheff and Frierson, 2014), which may greatly impact agricultural water and carbon cycles. Both temporal and spatial shifts in precipitation patterns are also expected (Dore, 2005), potentially straining or intensifying local water supplies. Agricultural water use has been identified as an important variable to consider under future climate scenarios (Kulshreshtha and Grant, 2007) since changing environmental and hydrological stressors can affect water availability and plant production (Fuhrer, 2003; Gregory and Ingram, 2000).

Many regions of intense agricultural production exist in semi-arid and sub-humid climates (Greve et al., 2019; Leff et al., 2004), which have been the focal points of research due to concerns over limited water resources (Mo et al., 2017) and expected increases in irrigation demands (Fischer et al., 2007). Therefore, less is known about plant carbon-water relations in humid regions, such as the Great Lakes Region, where an intensification of the hydrological cycle (ie. increased precipitation) is anticipated (Greve et al., 2019; Rojas et al., 2019), which could impact crop production (c.f Riha et al., 1996). Moreover, agricultural water use in these regions differs substantially from drier climates since there is less water limitations to plant growth. The focus of water resource use under future climate scenarios in drier climates tends to focus on water scarcity, whereas increases in temperature may be more important to water use patterns in humid climates.

Water use efficiency (WUE) is a key indicator of the coupling between the carbon and water cycles within terrestrial ecosystems, describing the interplay between water loss via evapotranspiration (ET) and carbon sequestration via photosynthesis (Baldocchi, 1994). At the ecosystem (crop) level, WUE is calculated as the ratio between gross primary productivity (GPP) and ET. Climate change is expected to impact WUE through changes in GPP and ET, as influenced by expected changes in photosynthesis and plant respiration rates (Yamori et al., 2014), soil water availability (Mitchell et al., 2015; Niu et al., 2008), and overall crop yields (Guo et al., 2010; W. He et al., 2018). However, direct responses to climate change are difficult to predict since plant water-carbon responses rely on numerous other factors such as edaphic characteristics (c.f Pugnaire et al., 1999) or shifts in microclimatic conditions (Medrano et al., 2015). More specifically, the impact of these changes are expected to vary regionally since atmosphere-plant exchange rates are affected by biological, physical and chemical processes that behave differently between regions and have varying degrees of influence on plant water-carbon relations (Albertson et al., 2001; Chapin et al., 2011; Guo et al., 2010). In crops, WUE can be used to assess plant carbon-water relations in response to climate change and different management practices (Niu et al., 2011). Moreover, WUE under different regional conditions can provide valuable information to farmers for making crop selections due to species-to-species differences in response to ecosystem controls (c.f. Mbava et al., 2020).

The impact of climate change on plant carbon-water relations is confounded by the complexity of ecosystem level controls on plant-atmosphere interactions. Furthermore, these controls can be impacted by climate drivers, but also influence (ie. amplify or mitigate) the impacts of climate drivers on WUE. Ecosystem level controls that impact the biological, physical, and chemical processes of plant-atmosphere interactions can be broken down into atmospheric influences, edaphic influences, and vegetation influences. Local climates and energy budgets have profound impacts on atmospheric conditions and weather patterns, which have a profound impact on plant stomatal activity and atmospheric carbon and water exchanges. Carbon assimilation rates are reliant on solar radiation and temperature driven biochemical reactions (Albertson et al., 2001; Yamori et al., 2014). Carbon and water fluxes are closely linked to air temperature and vapour pressure deficits (Mitchell et al., 2015; Scanlon and Albertson, 2004; Tong et al., 2014). Soil properties provide important controls on water storage and availability to plants, and soil water content has been identified as an important factor influencing GPP and ET (Mitchell et al., 2015; Niu et al., 2008; Seneviratne et al., 2010; Stocker et al., 2018). From an agricultural perspective, vegetation influences can be either strictly physiological, caused by inter-species variation in response to climate or other environmental controls, or influenced through different management practices. Leaf area index (LAI), which represents the unit leaf area per unit ground area, contains a positive relationship with carbon and water fluxes (Albertson et al., 2001). Crop arrangement and spacing can influence canopy development and LAI, thereby promoting light interception. This can cause shading effects, reducing evaporative demand on soil and leaf exposure to solar radiation (Hatfield and Dold, 2019) which negatively impacts stomatal openings. Thus, crop management practices, such as planting in rows, can influence plant carbon-water dynamics indirectly by impacting crop responses to the environment.

The multitude of variables that influence plant carbon-water relations make it difficult to isolate the effects of environmental drivers on WUE, and plant carbon-water dynamics in general. However, shifts in climate and precipitation patterns are expected to cause changes in these environmental drivers. This highlights a need to quantify seasonal variations in carbon-water dynamics of agricultural ecosystems and determine the influences of different drivers on these variations. Understanding the influence of drivers on seasonal variability of WUE, GPP and ET could therefore provide insight into crop responses to shifts in environmental variables and promote agricultural adaptation. This study aims to investigate seasonal GPP, ET and WUE for two common forage crops in southern Ontario. The objectives of this study are to: 1) quantify field-scale plant-water-carbon dynamics for silage maize (*Zea mays L.*) and alfalfa (*Medicago sativa*) crops in Southern Ontario, Canada; and 2) identify the differences in plant carbon-water dynamics between these two crops and relate these differences to ecosystem drivers. For the purposes of this study, ecosystem drivers are defined as the climate or atmospheric, edaphic, and vegetation variables which directly or indirectly influence plant carbon-water relations in agricultural systems. For example, photosynthetic pathways (vegetation variable) directly impact stomatal opening and closing which influences carbon assimilation and transpiration rates. This study will then use the information gathered from these two objectives to infer potential responses of these crops to climate change.

2.1 METHODS

2.1.1 Site Description

Forage crops occupy 33% of croplands globally (FAO, 2012) and therefore represent significant importance to the agriculture industry with maize and alfalfa being at the forefront of the industry in Ontario. Alfalfa is commonly grown in Ontario for its high-quality forage, crop rotation value due to nitrogen fixation, and nutritional value for livestock (Li and Brummer, 2012; Bagg et al., 2017). Alfalfa is an herbaceous perennial legume that follows the C_3 pathway of photosynthesis, which is typically seeded with a random distribution and contains multiple harvests throughout the growing season. Unlike alfalfa, maize is annually planted and follows the C_4 pathway of photosynthesis (Taylor, 1996), which is planted in rows that results in linear canopy development. Maize is frequently chosen for forage due to its quick growth and high yields. In Ontario, forage maize occupies approximately 16 % of fodder land use, while producing 62 % of fodder production by mass in 2018 (OMAFRA, 2020).

Alfalfa (9.9 ha) and maize (22.9 ha) study sites were located in the Hopewell Creek Watershed near Maryhill, Ontario, Canada [43.5 º N, 80.425º W] (Figure 2.1c), approximately 4.4 km apart. Thirty year (1981-2010) monthly mean temperatures from May to September, range from 12.5 to 20.5 ºC, with average May-September precipitation of 435.0 mm and annual precipitation of 917 mm (Waterloo Wellington A, Waterloo, Ontario, Canada, Climate ID:6149387, ~10 km from study sites; Environment and Climate Change Canada, 2019). Both fields are undulating, as is typical for the region (maize 327.9 - 333.8 masl, (Figure 2.1b); alfalfa 330.6 - 332.1 masl (Figure 2.1a)). Soils in the watershed contain a compact layer of till (~ 2m below the surface) that restricts drainage, necessitating the use of tile drainage in both fields. As such, fields are tile-drained at 90 - 100 cm below ground surface. Tile laterals and mains on the different fields drain directly into drainage ditches. No irrigation was used at either site.

The silage maize field received one pass of vertical conservation tillage before being planted in 80 cm spaced rows in early May (2018). Maize was fertilized with liquid dairy manure, 112 kg ha^{-1} nitrogen, 67 kg ha^{-1} phosphorus, 90 kg ha^{-1} potassium, and a 47 kg ha^{-1} starter fertilizer. The maize plants sprouted shortly before May 29 (2018) when it was observed shorter than 10 cm, and harvesting began on September 11 (2018). In contrast, alfalfa was randomly seeded with oats, triticale, and peas as nurse crops in April 2016. The field was cut in July 2016 leaving only alfalfa crop on the field. Fertilization of the alfalfa field occurred in September 2017 (392 kg ha^{-1} 0-12-44 NPK granular fertilizer). Alfalfa sprouted shortly after snowmelt and was cut four times throughout the season, dried, and then bailed for hay. Alfalfa was cut on 7 June (2018), 6 July (2018), 13 August (2018), and 21 September (2018).

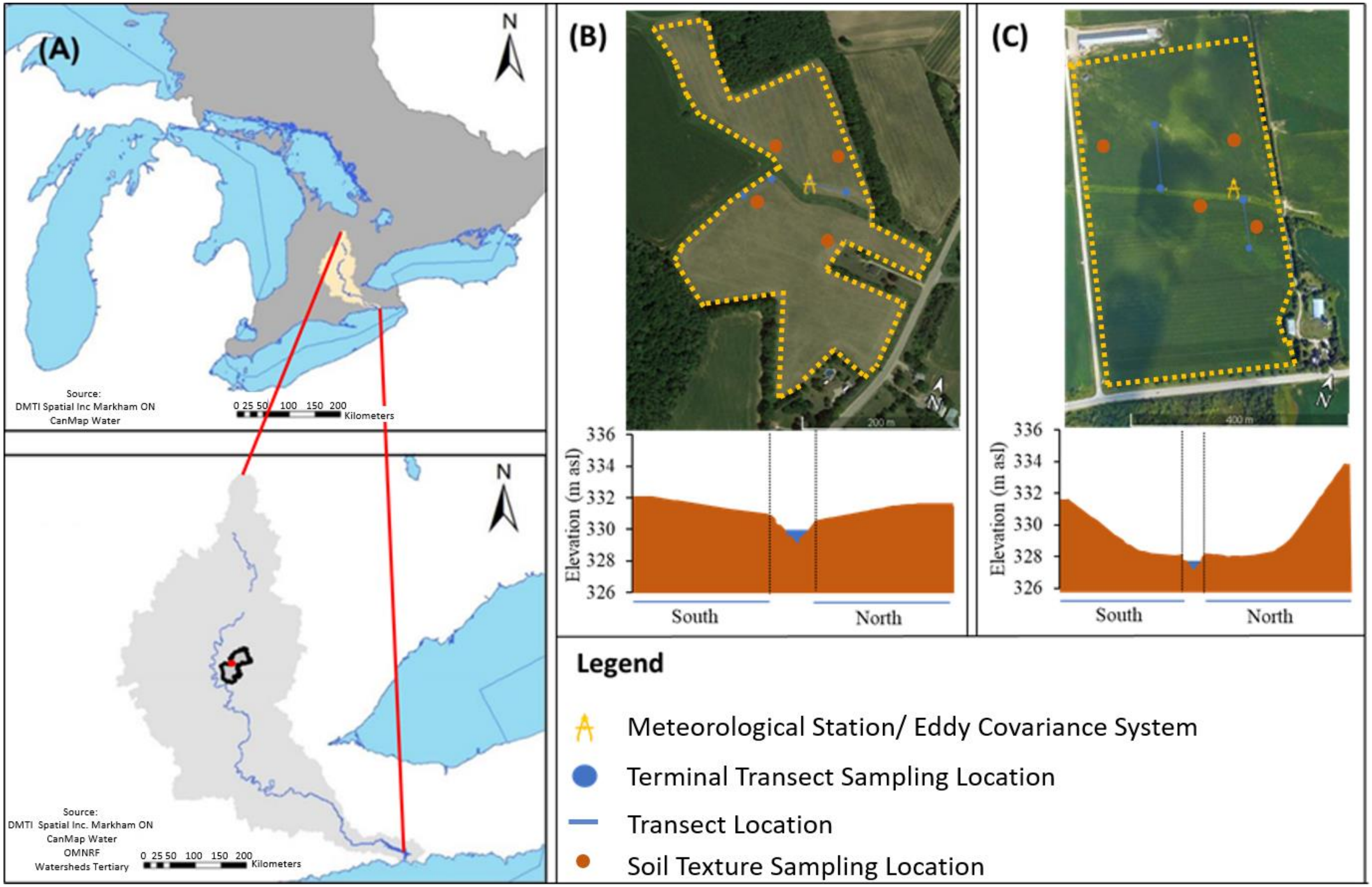


*Figure 2.1 (A) is adapted from Irvine (2018) and satellite imagery of (B) and (C) were extracted from Google Earth (2020) using imagery from July 7, 2018 and August 9, 2018 respectively. The dotted lines in relief gradients in (B) and (C) show discontinuation, where the transect ends and the other begins on the other side of the creek.

Figure 2.1 Alfalfa (B) and maize (C) fields with location of transects, soil pits and eddy covariance / meteorological tower. Maize and alfalfa fields are part of Hopewell Creek Watershed (A), within Southern Ontario

2.1.2 Data Collection and Methods

2.1.2.1 Meteorological Data Collection

Meteorological stations at each site contained: a 4-component net radiometer (3.75 m above ground; CNR4, Kipp & Zonen, Netherlands), two soil heat flux plates (5 cm below ground; HFP01, Hukseflux Thermal Sensors, Netherlands), four soil temperature probes (108 thermistors, Li-Cor Inc, Nebraska, USA; at 5, 10, 25 and 50 cm depths), three soil moisture probes (EC-5, Decagon Devices, Inc., Washington, USA; at 5, 25 and 50 cm depths), and an air temperature and relative humidity probe (HMP 155, 2.5 m above ground; Vaisala Oyj, Finland). Additional air temperature and relative humidity sensors (HOBO U23 Pro v2, Onset Hobo, Massachusetts, USA) were installed at 1.0 and 3.5 m above ground level for quality control. Precipitation was measured using a tipping bucket rain gauge (RG3, Onset Hobo, Massachusetts, USA) installed 1.5 m above ground. All data was sampled at 10 s intervals, averaged every 30-minutes on a XLite 9210B data logger (Sutron Corporation, Virginia, USA).

2.1.2.2 Carbon, Water and Energy Fluxes

Eddy covariance (EC) systems were installed at both sites on a tripod mast 4 m above the ground surface to measure fluxes of latent heat (from which ET is computed), sensible heat and carbon dioxide (i.e. NEE, from which Re and GPP are computed). The systems included a closed-path infrared gas analyzer (LI-7200/RS, Li-Cor Inc., Nebraska, USA) and a three-dimensional ultrasonic wind anemometer (Windmaster Pro, Gill Instruments, UK), and are controlled by the LI-7550 Analyzer Interface Unit (Li-Cor Inc., Nebraska, USA). At maximum crop heights, the sensor heights were > 3 m above alfalfa canopy and > 1 m above the maize canopy. Changes in canopy height over the course of the season were accounted for in EC data processing.

Twenty Hz raw data was processed into 30 minute average fluxes by EddyPro® software (v7.0.4, Li-Cor Inc, Nebraska, USA; Fratini and Mauder, 2014; LI-COR Biosciences, 2017) where density (Webb et al. 1980) and coordinate rotations were applied (Kaimal and Finnigan 1994). The half hourly fluxes from EddyPro® were further processed as follows: to ensure sufficient number of samples were used in calculating the half-hourly means, data was filtered to ensure that each 30 minute timestamp has at least 80% of records per each half-hour analyzed (n>0.80*36,000). Additional quality checks and assurances on the data were completed using a rolling 5 half-hour and 10 half-hour windows. Means and standard deviations were calculated for these windows and the half-hour records were first checked to be within 3.5 times the standard deviation of the 5 half-hours mean. If those were not available, the half-hour records were checked using the 10 half-hour mean and visually checked to be within the expected physically probable values for the sites. A flux footprint analysis following Kljun et al. (2015) was performed to ensure that all fluxes originated from within 80% of the area of interest. Filtered net ecosystem exchange (NEE) fluxes were gapfilled and partitioned into GPP and R_e following the methods of Wutzler et al. (2018) and using their REddyProc, R-script. This script estimated the u* threshold based on (5%, 50%, and 95%) confidence intervals as well as a user-defined threshold of 0.15 m/s, to account for periods of low turbulence. Bowen ratio and energy balance closure were estimated from the energy fluxes for each site. Fluxes used in this analysis accounted for energy budget closure (Barr et al., 2006). Ground heat flux was gap-filled as 10% of net radiation. Potential evapotranspiration (PET) was calculated using meteorological data and Priestly-Taylor (Priestley and Taylor, 1972) equation for each half hour for each site. A relationship was established between actual evapotranspiration (AET) (obtained from filtered non-gap-filled latent heat fluxes) and PET (AET : PET), which was used to scale PET to AET in order to gap-fill ET when ET was unavailable. Daily and seasonal WUE was calculated at the ecosystem scale as the ratio of GPP to water lost through ET. Daily Bowen ratio was calculated as the ratio between daily Q_e and Q_h.

2.1.2.3 Vegetation Analysis

Each site had two transects for measuring plant heights, stomatal resistance, and leaf area index (LAI). Maize transects were 96 m (south of creek) and 127 m (north of creek) in length and expanded along topographical gradients covering 327.9 to 331.5 masl, and 327.9 to 333.8 masl, respectively (Figure 2.1b). The alfalfa field had limited access due to potential trampling damage so 60 m transects were created along paths created by machinery and measurements were taken approximately 1.5 m off the track to minimize disturbance (Figure 2.1a).

Height, LAI, and stomatal conductance were collected approximately every 3-4 days along these transects at ~10 m intervals. Three plants were randomly selected at each interval and heights were measured from ground surface to top of plant using a standard measuring tape. One plant was randomly selected for stomatal resistance measurements using an open chamber leaf porometer (SC-1, Decagon Devices, Washington, USA). Three leaves at each of the low (bottom third of stem), middle (middle third of stem), and upper sections (top third of stem) of the canopy, were measured. LAI measurements were conducted mid-day, using a LI-COR 2200C with a 90º cap (Li-Cor Inc., Nebraska, United States). Three measurements were taken at each transect location on days when sky conditions were suitable (clear or consistently overcast). For alfalfa, these measurements were taken from random points below the canopy. Maize measurement points were taken at approximately ¼, ½, and ¾ of row spacing and averaged to better represent row-crop canopy. LAI data was processed using the FV2000 software (Li-Cor Inc, Nebraska, USA), accounting for scattering correction. In addition to measurements taken, growing degree days (GDD) were calculated,

$$GDD = [(T_{air}^{max} + T_{air}^{min}) / 2] – T_{air}^{base} \tag{2}$$

where T_{air}^{max} and T_{air}^{min} are the maximum and minimum daily air temperatures in Fahrenheit, respectively, and T_{air}^{base} is the base temperature required for maize and alfalfa growth. For maize, T_{air}^{base-} was 50 ºF (10ºC), and for alfalfa, T_{air}^{base-} was 41 ºF (5ºC; Mahanna et al., 2017).

2.1.2.4 Soil Analysis: Texture and Surface Hydraulic Properties

At four randomly selected locations, three soil cores were extracted within each field for soil texture determination (Figure 2.1a and 2.1b). The three soil cores (5 cm in length by 10 cm diameter PVC) were extracted at 5 - 10 cm, 25 - 35 cm, and 45 - 50 cm depths in maize, and 5 - 10 cm, 20 - 25 cm, and 40 - 45 cm in alfalfa. The 25 - 35 cm sampling depth in the maize field varied between soil pits based on shifts in soil colour shade (darker to lighter shade): three samples were taken from 25 - 30 cm and one sample was taken from 30 - 35 cm. No visible changes in soil were detected in the alfalfa field so samples were taken from 20 - 25 cm for consistency. The average clay, silt, and sand components of all soil samples from each of the sites were characterized using a laser diffraction particle size distribution analyzer (LA-950V2, Horiba Scientific, Japan).

Soil hydraulic properties were determined for near surface soil (0 - 10 cm) using 10 cm diameter PVC pipe cores, which were pressed into the ground and extracted at 10 m intervals along the transects. These cores were saturated in water for 48 hours and weighed prior to gravity draining on a rack for 24 hours and weighing again. Specific yield (S_y) was calculated,

$$S_y = V_{WD} / V_T \tag{2}$$

where V_{WD} is the volume of water drained (cm^3) during 24-hour gravity drain and V_T is total core volume (cm^3). Samples were then oven dried for 72 hours and weighed for dry mass. Bulk density (ρ_b) was then calculated,

$$\rho_b = M_{DRY} / V_T \tag{3}$$

where M_{DRY} is dry mass of soil (g) after drying period. Porosity was also calculated,

$$n = ((M_{WET} – M_{DRY})/\rho_w)/V_T = V_v / V_T \tag{4}$$

where V_v is volume of voids (cm^3). Volume of voids was determined from saturated (M_{WET}) and dry masses (M_{DRY}) of these soil cores. Water losses were assumed representative of void volume after accounting for water density (ρ_w).

2.1.2.5 Soil Water: Soil Water Content, Water Retention Curve and Saturated/Unsaturated Hydraulic Conductivity

Soil moisture (volumetric water content; VWC) was measured at 5, 25 and 50 cm depths using soil moisture probes (EC-5, Decagon Devices, Inc., Washington, USA). To confirm that soil moisture measured at the meteorological station were consistent with in field soil moisture, VWC was measured three times at each transect interval using a handheld measuring device calibrated for mineral soils (Integrated surface 7 cm; WET-2 Sensor, Delta-T Devices, Cambridge, UK; data shown in Appendix A Figure 2.8).

Water retention curves were determined for each 5 cm core used for soil texture analysis according to van Genuchten (1980), and then averaged by depth. Soil texture data (%clay, %silt, %sand) was used to estimate van Genuchten parameters through the ROSETTA computer program (Schaap, Leij & van Genuchten, 2001). These parameters were used to model water retention curves using HYDRUS-1D (Šimůnek et al., 2013). Unsaturated hydraulic conductivity (K_{UNSAT})

functions were determined according to Anlauf (2014), which combines the van Genuchten (1980) formulation for water retention curve with unsaturated hydraulic conductivity function from Mualem (1976). Saturated vertical hydraulic conductivities (K_{SAT}) were determined at depths of 20 and 40 cm using a Guelph permeameter (2800K1 Guelph Permeameter, Soil Moisture Corp, Santa Barbara, California) following the methods of Reynolds (1986). Single head tests with shape factors determined according to Zhang et al. (1998) were used.

2.2 RESULTS

2.2.1 Temporal Variability in Plant Carbon-Water Dynamics

Growing season (2018) totals for evapotranspiration (ET, Figure 2.2c), gross primary productivity (GPP) and water use efficiency (WUE) were greater for alfalfa (ET 614mm; GPP 1891.6 g of C m^{-2}; WUE 3.11 mg of C g^{-1} of H_2O) than maize (ET 545.9 mm; GPP 1390.2 g of C m^{-2}; WUE 2.58 mg of C g^{-1} of H_2O). Although seasonal differences in ET existed, daily ET rates were similar for maize and alfalfa (Figure 2.2b). Temporal variation in ET was driven by climate (Figures 2.3, 2.4), with dips in ET corresponding to rain events or heavy cloud cover days. These dips corresponded with spikes in daily WUE. Daily WUE differed between maize and alfalfa, but the differences were not consistent throughout the growing season (Figure 2.2a). For example, early and late season WUE was greater for alfalfa than maize, whereas maize WUE was greatest in the peak season. Seasonal changes in daily WUE also differed between crops. For example, maize (annually seeded) WUE was very low at the start of the season, increased until it peaked and then decreased towards the late season. In contrast, alfalfa (perennial) did not have a seasonal pattern of peaking and instead reflected the crop management pattern. As a result, WUE was high in the early season, dropped abruptly following each cut, increased following harvests, and stabilized before subsequent cuts.

There were also large differences in temporal patterns of daily carbon fluxes between crops. Similar to WUE, maize GPP was very low at the start of the season, increased until it peaked, and decreased until harvest (Figure 2.2d). Alfalfa also followed a similar trend in GPP as WUE, but daily GPP continued to decrease for a few days after harvesting during cuts 2 and 3, which was not observed in WUE (Figure 2.2d). Net ecosystem exchange (NEE) indicated that both crops behaved similarly as carbon sinks (+) when considering entire growing season (alfalfa 194.9 g C m^{-2}; maize 187.8 g C m^{-2}; Figure 2.2g), but fluctuated between source (-) and sink (Figure 2.2f). Maize was a source early in the growing season but transitioned to a sink in mid-June, remaining as such until near the end of the season when it began to fluctuate between source and sink. The NEE of alfalfa, however, declined following harvests and behaved as a source briefly before increasing. Following the 3rd cut, alfalfa remained a source for an extended period (~ 11 days) and then varied between source and sink until final cut.

Figure 2.2 Daily water use efficiency (WUE) (a), evapotranspiration (ET) (b), gross primary productivity (GPP) (c), and net ecosystem exchange (NEE) (d) for alfalfa and maize crops over the 2018 growing season. Cumulative evapotranspiration (c), gross primary productivity (e), and net ecosystem exchange (g) over the 2018 growing season are also presented for both alfalfa and maize.

2.2.2 Climate differences between maize and alfalfa fields

Annual precipitation in 2018 was ~763 mm (Waterloo Wellington A, Waterloo, Ontario, Canada, Climate ID:6149387, ~10 km from study sites; Environment and Climate Change Canada, 2019). Growing season precipitation was 263 mm at the alfalfa site, and 248 mm at the maize site. There were no significant differences in daily fluctuations of relative humidity (rH; Pearson correlation, $r = 0.98$, $p < 0.001$; Figure 2.3b), vapour pressure deficit (VPD; Pearson correlation, $r = 0.97$, $p < 0.001$; Figure 2.3c) or mean air temperature (T_{air}; Pearson correlation, $r = 0.99$, $p < 0.001$, Figure 2.3a) between the two study sites. In late May, VPD and mean daily T_{air} were higher in maize. Similarly, there was no significant difference in surface energy balance (SEB) between the alfalfa (Figure 2.4a) and maize (Figure 2.4b) for most of the season except for late may to early June. Daily net radiation (Q*), however, did not differ between crop sites (Pearson correlation, $r = 0.97$, p-value < 0.001) meaning it was the allocation of available energy that caused differences during these periods. In this case, maize had a significantly higher Bowen ratio (Figure 2.4c) suggesting higher proportions of energy were being put towards sensible heat during that period. Outside of this period, however, latent heat accounted for most Q* in the SEB demonstrating the strong control solar energy had over ET throughout the season.

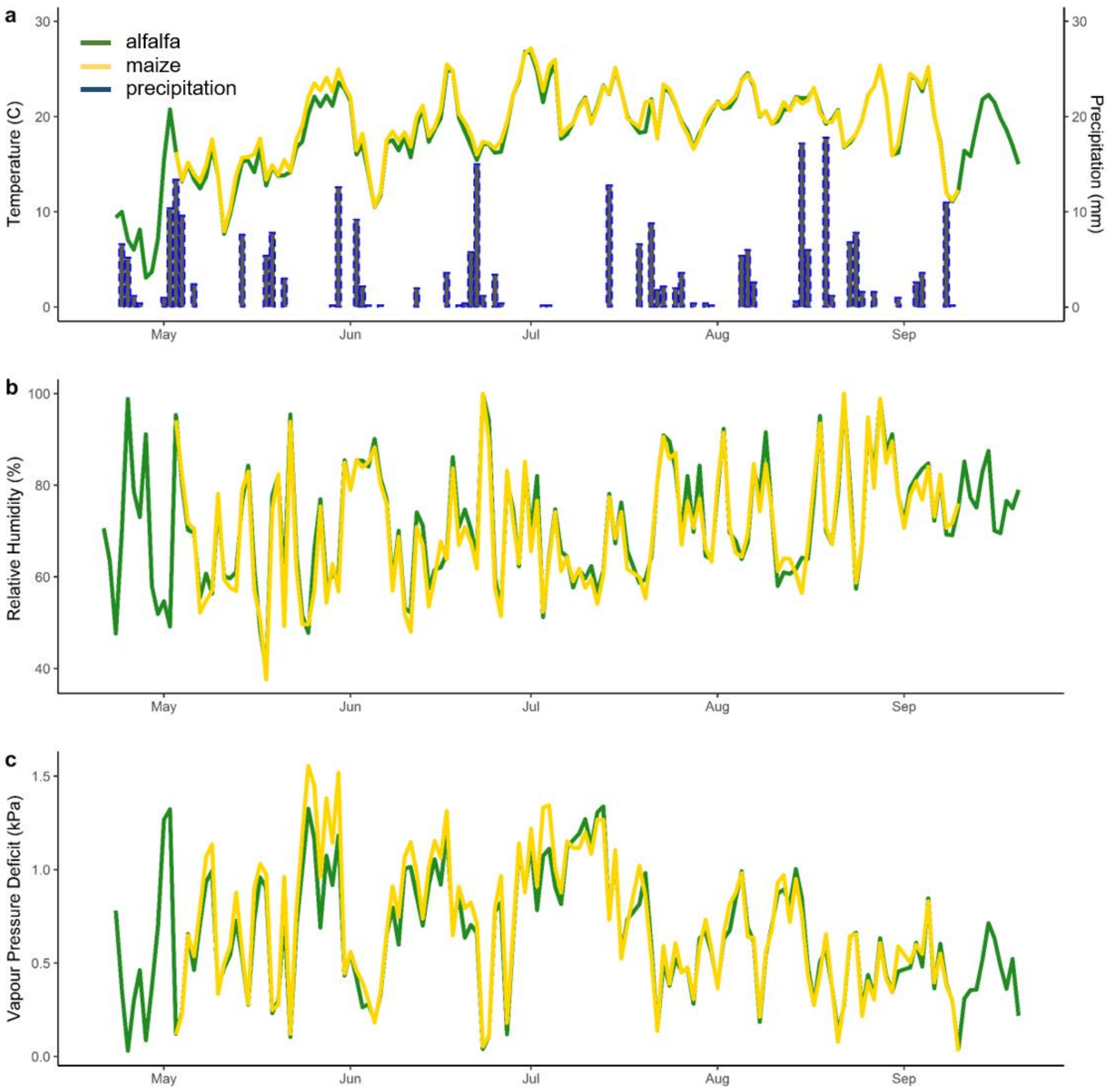
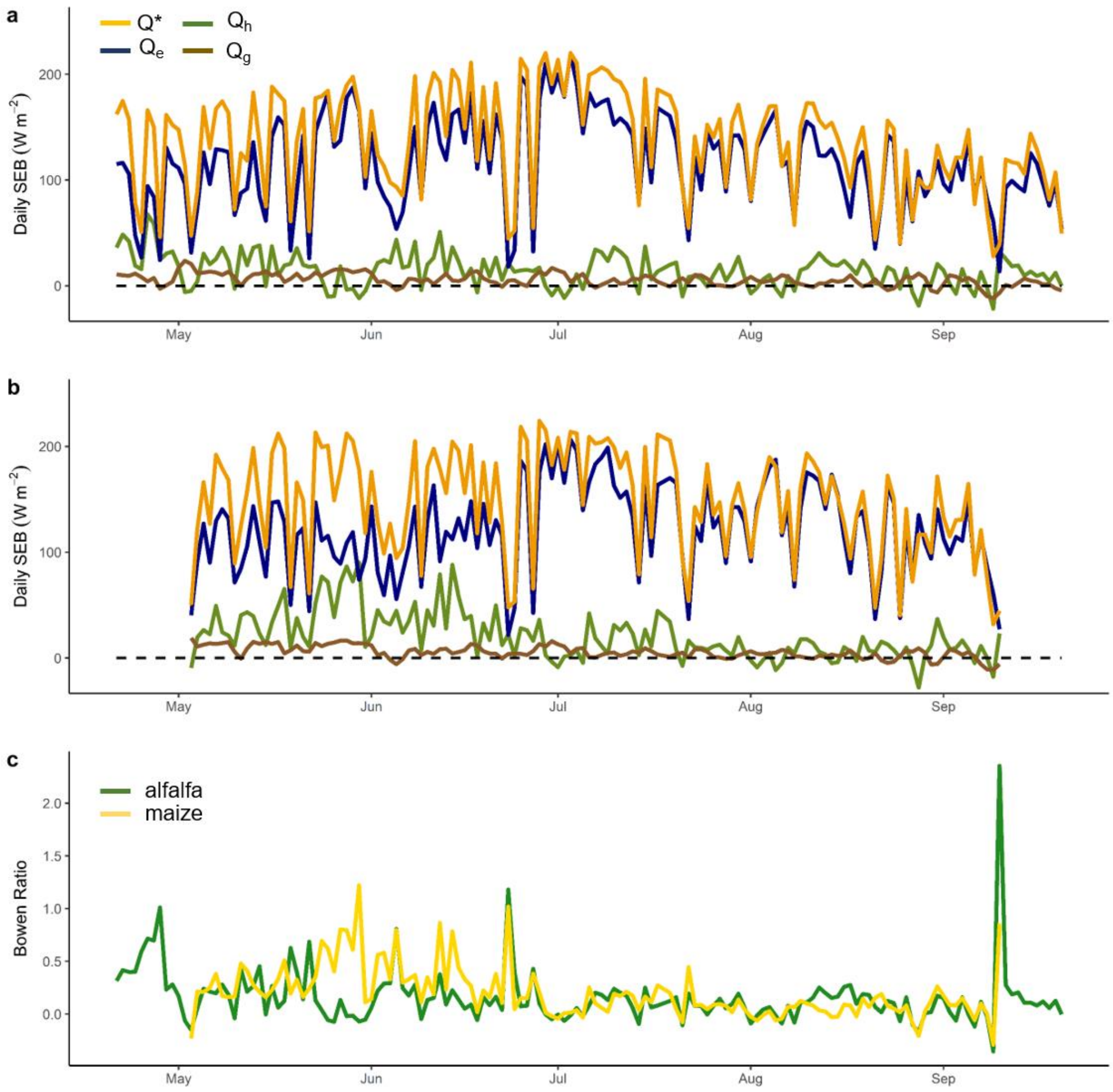


Figure 2.3 Daily (a) mean air temperature and total precipitation, (b) mean relative humidity, and (c) mean vapour pressure deficit for alfalfa and maize sites, 2018 growing season.



Surface energy balance is composed of net radiation (Q), latent heat (Q_e), sensible heat (Q_h), and ground heat (Q_g).

Figure 2.4 Surface energy balance (SEB) for alfalfa (a) and maize (b) sites, and Bowen ratio (c) across the 2018 growing season.

2.2.3 Soil differences between maize and alfalfa fields

Soils at both sites were classified as silt loam, with maize containing more clay and sand, and alfalfa more silt (Appendix A, Figure 6.2, Table 6.1). However, this did not translate into remarkable differences in surface hydraulic soil properties (Table 2.1) between maize and alfalfa sites with differences in means for bulk density, porosity, and specific yield falling within one standard deviation of each other.

Table 2.1 Bulk density, porosity, specific yield, and saturated hydraulic conductivities of soils for alfalfa and maize sites. Bulk density, porosity and specific yield were calculated for the top 10 cm of soil while K_{sat} was determined at 20 and 40 cm depths.

	Depth	**Alfalfa**	**Maize**
Bulk Density	0 - 10 cm	1.36 ± 0.09 g cm^{-3}	1.43 ± 0.11 g cm^{-3}
Porosity	0 - 10cm	0.47 ± 0.05	0.44 ± 0.06
Specific Yield	0 - 10cm	0.09 ± 0.02	0.07 ± 0.02
Saturated Conductivity	20 cm	2.15×10^{-6} m s^{-1} ± 2.11×10^{-6} m s^{-1}	2.23×10^{-6} m s^{-1} ± 1.78×10^{-6} m s^{-1}
	40 cm	2.04×10^{-6} m s^{-1} ± 2.50×10^{-6} m s^{-1}	2.31×10^{-6} m s^{-1} ± 1.97×10^{-6} m s^{-1}

* Values are the average ± standard deviation of measurements.

Soil hydraulic properties did not differ greatly between sites. Maize saturated conductivity (K_{SAT}) at 20 cm and 40 cm depths was marginally higher, but not statistically significant. Both alfalfa and maize maintained relatively similar unsaturated hydraulic conductivities (K_{UNSAT}) at moistures up to field capacity (Figure 2.5a), which occurs at pF = 2.5 or -33 kPa (Richards and Weaver, 1944). At field capacity, volumetric water content (VWC) ranged from 0.28 - 0.30 at different depths of the alfalfa site, while maize ranged from 0.26 - 0.29. Wilting point VWC, which occurs at approximately pF = 4.2 or -1580 kPa (Richards and Weaver, 1943; Román Dobarco et al., 2019), was 0.05 - 0.07 in alfalfa soil and 0.06 - 0.07 in maize (Figure 2.5b). Water between field capacity and wilting points is considered available for plant use. Plant available water was greater in alfalfa at all depths by 2.6% at 5 - 10 cm (cf. alfalfa 0.24 vs maize 0.23), 12.9% at 20 - 35 cm (cf. alfalfa 0.23 vs maize 0.21) and 12.0% at 40 - 50 cm (cf. alfalfa 0.21 vs maize 0.19).

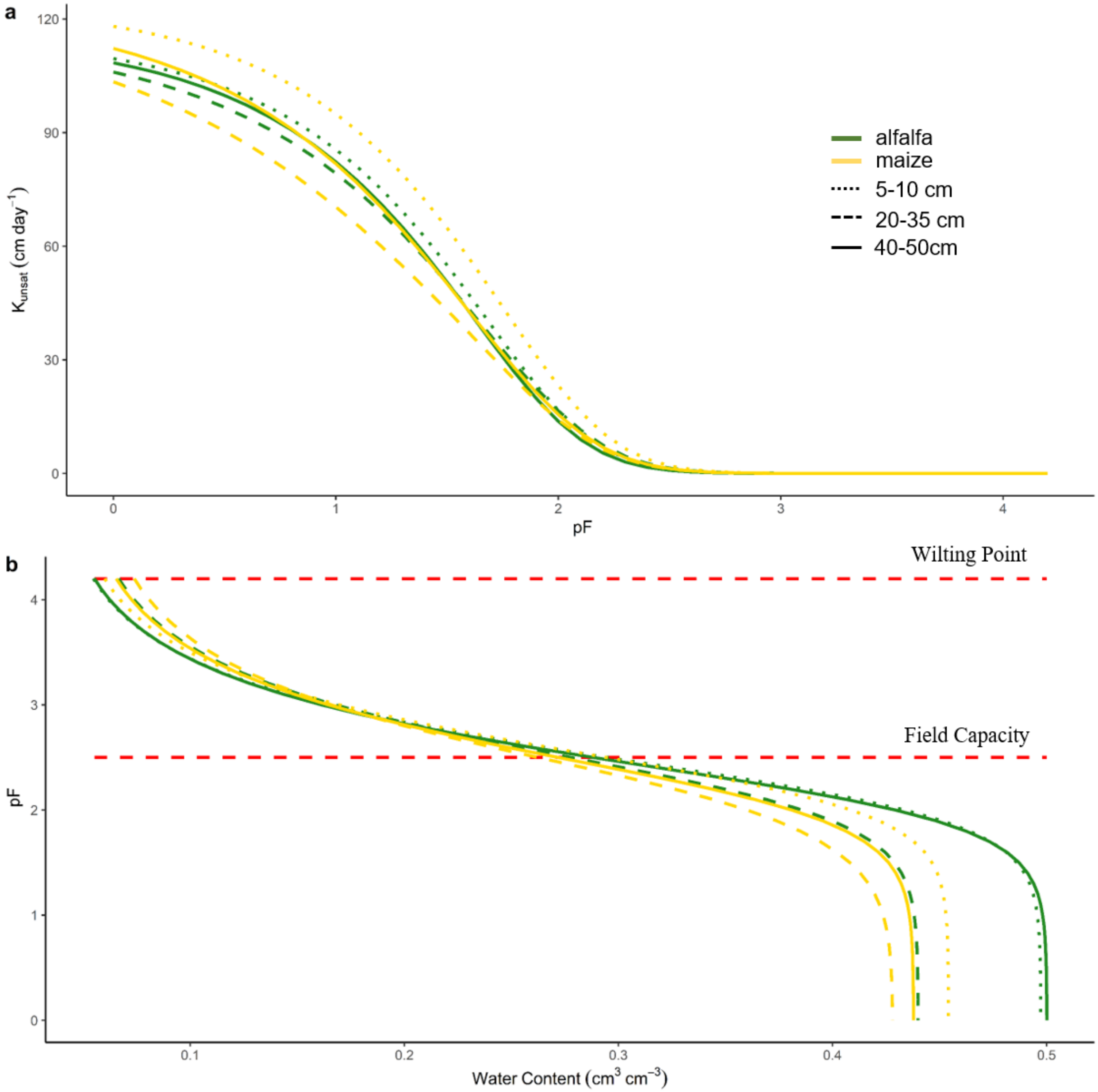


Figure 2.5 Unsaturated hydraulic conductivity (K_{UNSAT}) at different suction pressures (a) and van Genuchten (1980) water retention curves (b) for alfalfa and maize soil textures.

Neither site reached wilting point this year (Figure 2.6), despite lower annual and growing season precipitation. At 5 cm depth, the lowest observed soil VWC was approximately 0.11, 0.10 and 0.09 at the maize site in late May, mid-June, and mid-July, respectively (Figure 2.6a). At 25 cm (Figure 2.6b) and 50 cm depth (Figure 2.6c), there was no indication of water stress. Maize VWC was lower than alfalfa at 5 cm until mid-July, at which point VWC was similar at both sites until the end of the season. At 25 cm, maize soil water content dropped below alfalfa at the end of May until the middle of June, which coincided with rapid maize growth. At 50 cm, alfalfa VWC was greater than maize from the start of season until mid-July, after which there was a steep drop in VWC at 50 cm in alfalfa where it remained lower than maize for the rest of the season.

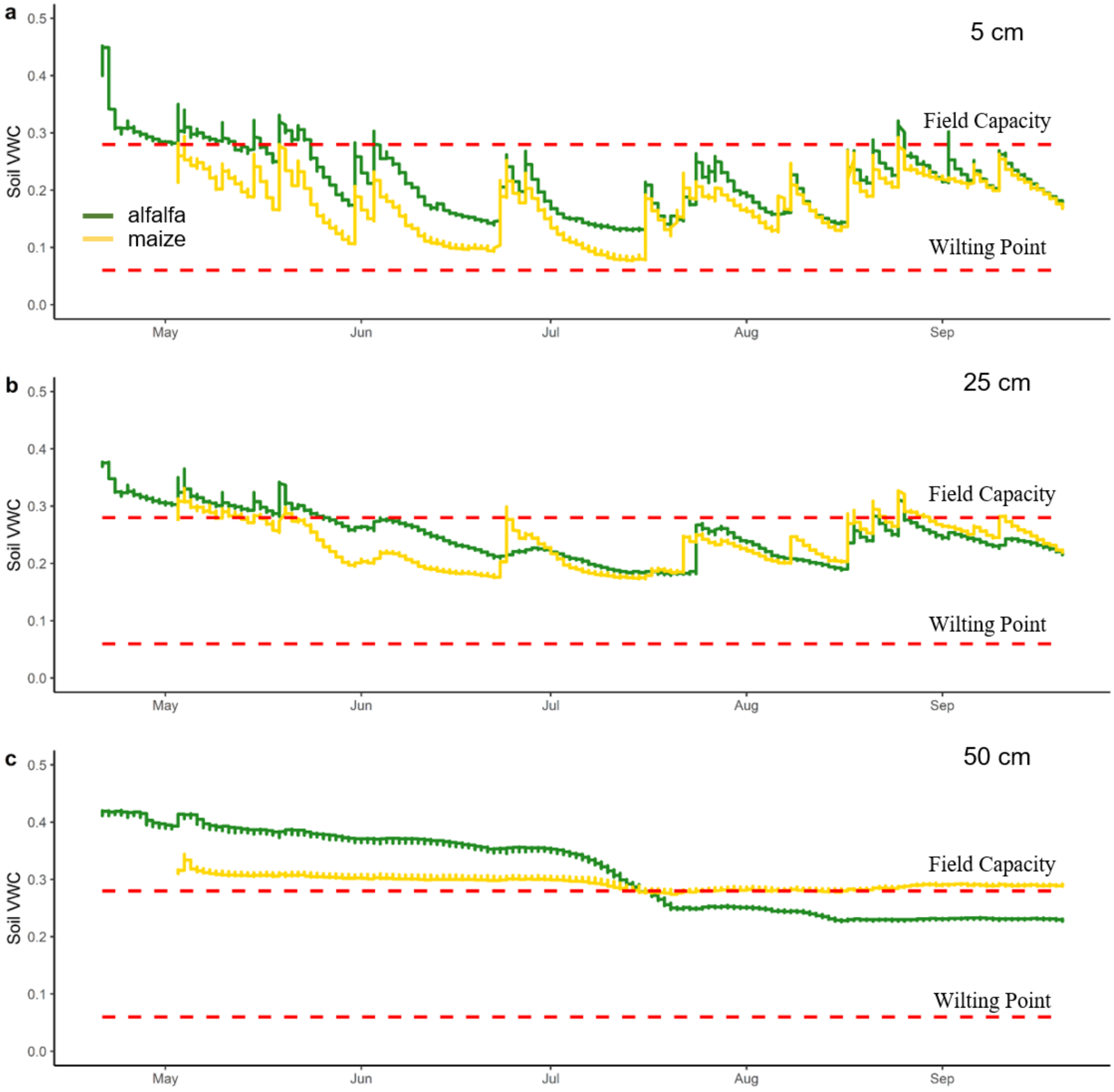
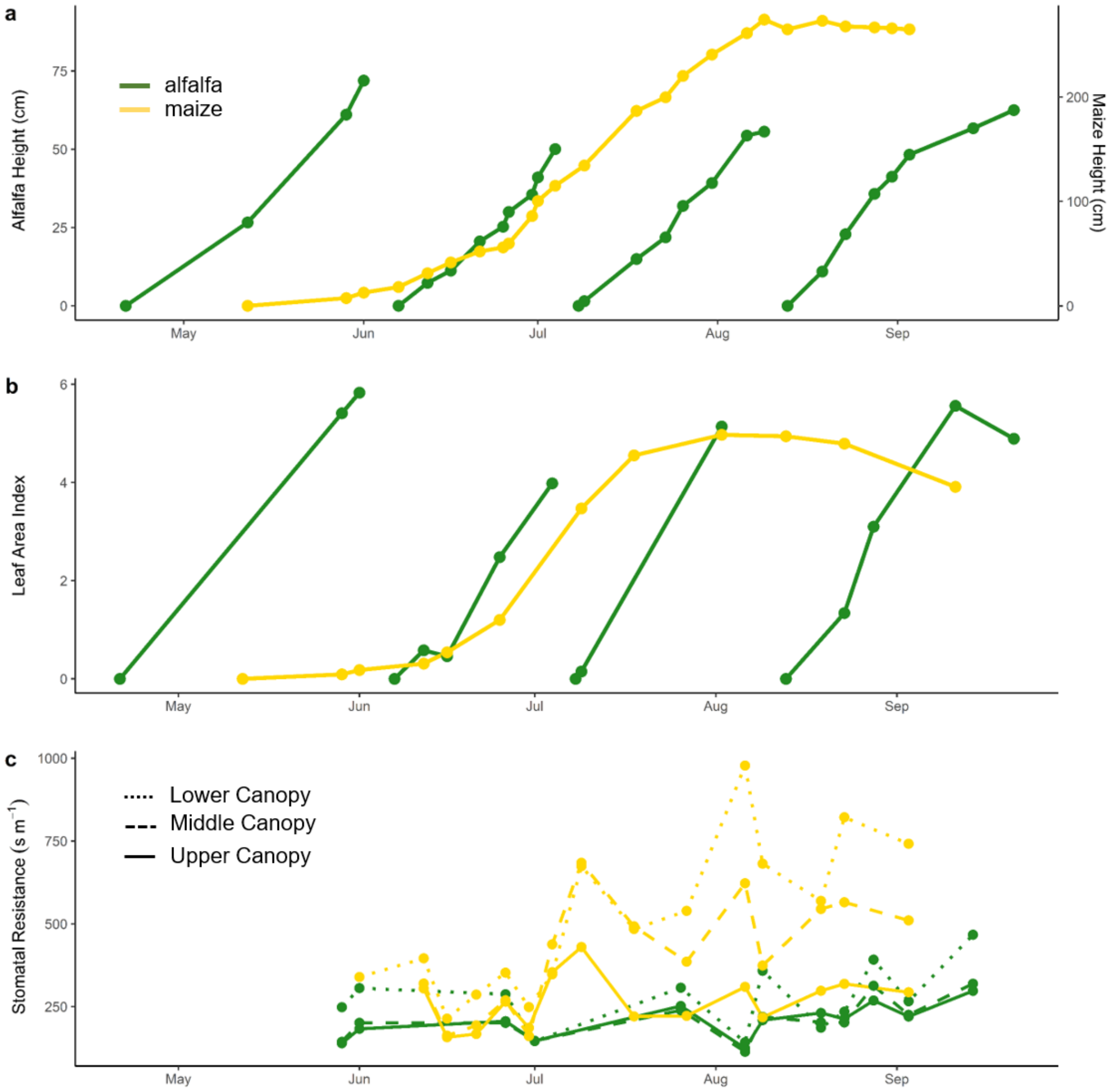


Figure 2.6 Soil volumetric water content (Soil VWC) measured at the meteorological station for alfalfa and maize over the 2018 growing season at 5cm (a), 25 cm (b) and 50 cm (c) below ground surface.

2.2.4 Vegetation Influences on Plant Water-Carbon Relations

Crop choice had three primary influences on water-carbon dynamics: (1) growing season length (as driven by interaction between climate and crop); (2) growth habits; and (3) harvesting practices.

Alfalfa had a longer growing season by 22 days (21 April to 21 September 2018) than maize (3 May to 11 September 2018). Due to differences in growing season length and growth temperatures (maize emergence requires 10 ºC (Sánchez et al., 2014) while alfalfa begins growing at 3.5-5 ºC (Sharratt et al., 1989)), maize experienced lower GDD (~2450) than alfalfa (~4170). This meant that alfalfa was subjected to more favourable plant development temperatures than maize. Further, since alfalfa was planted in April 2016, it sprouted and began growing earlier than maize as it did not need to invest in root development (Figure 2.7). Maize grew slowly at the start of the growing season, increased until the end of June before it grew more rapidly reaching a maximum height (274.2 cm), stabilizing until harvested (Figure 2.7a). Maize LAI followed the same trends, other than decreasing after reaching its maximum (LAI = 5.0) until harvest (LAI = 3.9; Figure 2.7b). Alfalfa cuts eliminated non-growth periods, promoted more consistent growth rates, and reduced LAI decreases from senescence. LAI and height followed similar trends with only cut 4 experiencing a decrease prior to harvesting. Stomatal resistances were relatively similar between both crops until July (Figure 2.7c). Beginning in July, stomatal resistance was generally higher in maize than alfalfa. Alfalfa stomatal resistance did not vary greatly based on the distribution of leaf area, with lower canopy exhibiting only slightly greater stomatal resistances. However, maize stomatal resistance began varying with canopy height in early July with lower and middle canopies showing greater resistances. In the middle of July, the lower canopy stomatal resistance further increased from the middle canopy until the end of the season.



*In Figure 2.7a, maize height is presented on the right axis while alfalfa height is presented on the left axis. Lower canopy stomatal resistance values were collected from the bottom third of stalk, middle canopy from middle third and upper canopy from the most exposed leaves at the top of stalk.

Figure 2.7 Height (a), leaf area index (LAI) (b), and stomatal resistance (c) for alfalfa (green) and maize (yellow) sites over the 2018 growing season.

2.3 DISCUSSION

2.3.1 Carbon-Water-Dynamics

2.3.1.1 Comparison of carbon-water fluxes with other studies

Seasonal and intra-seasonal GPP, ET, and NEE were similar to results elsewhere under similar environmental conditions for maize (Suyker and Verma, 2010; Verma et al., 2005; Wagle et al., 2018). Suyker and Verma (2010) reported peak daily GPP of 20.3 to 24.6 g m^{-2} day^{-1} in rainfed maize over a 6 year period in Mead, Nebraska, when peak daily ET ranged from 5.6–6.3 mm day^{-1}, which were lower values than reported here (7.14 mm day^{-1}). These differences are likely related to climate variations, which delivered significantly less precipitation (average annual precipitation ~747 mm (1981-2010)) in Mead, Nebraska (HPCC, 2020). Wagle et al. (2018) reported similar seasonal trends in daily GPP, NEE and ET in a study in Texas with peak ET, GPP and NEE of 7.3 mm day^{-1}, ~24 g m^{-2} day^{-1}, 14.78 g C m^{-2} day^{-1}. Verma et al. (2005) demonstrated slightly larger carbon fluxes of rainfed maize in Nebraska with seasonal GPP of ~1550 g C m^{-2}. Compared to a study in Michigan, maize WUE here (2.58 mg C g^{-1} H_2O) was significantly lower than their range reported over a four year interval (3.5-4.5 mg C g^{-1} H_2O; Abraha et al., 2016). However, Abraha et al. (2016) reported peak daily ET values of 7 mm day^{-1} and peak daily GPP of 22-23 g C m^{-2} day^{-1}, which are close to results here. This discrepancy in seasonal WUE is likely attributed to the dates used in WUE calculation, where the start of the growing season selected in these studies is based on net carbon gain or losses, while this study considered the start of growing season by planting date.

Similarly, alfalfa carbon and water fluctuations observed in this study were comparable to those recorded elsewhere. Saliendra et al. (2018) reported seasonal GPP of 1283-1454 g C m^{-2} from 2010-2013 in North Dakota, which was significantly lower than this seasonal GPP (1891.6

g C m^{-2}). Wagle et al. (2019a) reported peak 8-day average daily NEE and GPPs of 8.17 and 16.69 g C m^{-2} day^{-1}, respectively, in Oklahoma for rainfed alfalfa. Daily peak GPP was higher in this study (22.23 g C m^{-2} day^{-1}), however, peak NEE was comparable (7.70 g C m^{-2} day^{-1}). Wagle et al. (2019a) also recorded similar seasonal trends of daily GPP and NEE following harvests, which peak in daily ET at 6.9 mm day^{-1} (compared to 7.57 mm day^{-1} here), peak daily WUE ~4 mg C g^{-1} H_2O, and seasonal WUE 2.38 and 2.57 mg C g^{-1} H_2O. In contrast, this study contained significantly greater daily peak WUE (14.65 mg C g^{-1} H_2O) and seasonal WUE (3.11 mg C g^{-1} H_2O), which is likely attributed to the higher GPP reported here.

2.3.1.2 ET, GPP and WUE of maize and alfalfa

ET directly drives local carbon cycles through the coupling of transpiration (T) and GPP at stomatal surfaces, and also indirectly influence the carbon cycles through soil water availability to plants (Pielke et al., 1998). In this study, daily ET did not vary significantly between maize and alfalfa (Figure 2.2b) since both fields had similar SEB (Figure 2.4a,b). Daily ET differences between alfalfa and maize were minimal, even with immature maize plants, since the evaporation component of ET increases with soil exposure, particularly when soil is wet (Villalobos and Fereres, 1990). When SEB was different between crops, and maize had higher Bowen ratios (Figure 2.4c), ET was lower in maize, likely because of lower surface soil water availability (Figure 2.6a) and alfalfa root maturity, which was able to draw water from deeper reservoirs.

Differences in daily WUE between maize and alfalfa (Figure 2.2d) was driven primarily by changes in GPP due to only small differences in crop ET, consistent with other research where GPP drove seasonal variation in WUE of grasslands (Hu et al., 2008). When GPP is similar or does not vary, ET drives differences in WUE (Ponton et al., 2006). This was seen in this study when daily changes in GPP were minimal, but daily changes in WUE fluctuated with ET (Figure 2.2). This suggests that although WUE is sensitive to daily changes in ET, which is evident by spikes and dips in daily WUE corresponding with dips and spikes in ET, differences between crop species are driven by differences in GPP under similar energy and water availability. This has implications for crop selections under future climate scenarios with shifts in energy (Allan et al., 2014) and water regimes (Lorenz et al., 2010; Wild and Liepert, 2010) expected to accompany CO_2 and temperature rises. More specifically, this could suggest that local crop selection should maximize GPP under specific water and energy regimes to maximize WUE.

In this study, temporal variation in carbon sequestration was observed between sites, which appears to be predominantly driven by plant maturity. More mature plants have a greater LAI (Figure 2.7b), which typically corelates with higher GPP (Figure 2.2d) and a net carbon sink (Figure 2.2f) over the growing season (Gitelson et al., 2014). Immature plants typically have a lower LAI with lower GPP, which corresponds with negative NEE (Figure 2.2f). This suggests that selecting and managing crops that maximize GPP could be important for increasing carbon sink behaviour in agricultural systems. However, although alfalfa GPP exceeded that of maize (Figure 2.2e), there was a much smaller difference between seasonal NEE (Figure 2.2g). This occurred because maize follows the C_4 pathway of photosynthesis, which reduces photorespiration losses by saturating rubisco with CO_2 in a physically separated metabolic cycle (Edwards et al., 2010; Way et al., 2014). As such, maize carbon sequestration contained a higher GPP ratio, which could be important when considering resource use efficiency under changing climates. More specifically, this suggests that optimizing crop selection for GPP may not result in greater yields since there will be differences in photorespiration between crop choices.

2.3.2 Drivers of plant carbon-water differences between alfalfa and maize fields

At the ecosystem scale, there is a link between carbon and water cycling through stomatal openings, where more efficient crops optimize carbon uptake (GPP) with less water use through transpiration (Lawson and Blatt, 2014). Further, water use also incorporates evaporation due to its important link to biological functions through influence over atmospheric water content, which drives water transport in plants (Mahajan et al., 2008; Zhang et al., 2017) and influences soil water content (Brutsaert, 2014). To address our second objective, determining environmental drivers of variability in alfalfa and maize carbon-water relations, three spheres of potential influence (climate and atmosphere, edaphic factors, and vegetation/biological controls) are considered.

2.3.2.1 Differences in climate and atmospheric drivers

In this study, variations in solar radiation did not likely influence differences between maize and alfalfa plant-carbon relations directly (Figure. 2.4a,b). However, the partitioning of Q* into ground (Q_g), latent (Q_e), and sensible (Q_h) heat fluxes (i.e the SEB) differed between sites in late May to early June, when maize had a higher Bowen ratio (Figure 2.4c). This is a result of soil moisture near the surface being lower in maize than alfalfa (observed at 5 cm and 25 cm depths; Figure 2.6), which would have resulted in less free water for evaporation. This was the only period of noticeable difference in daily ET; however, these differences were overshadowed by larger differences in GPP meaning the lower ET did not contribute substantially to differences in WUE between alfalfa and maize. Likewise, this was the only period of differences in mean daily mean daily T_{air} (Figure 2.3a) and VPD (Figure 2.3c). VPD responds to shifts in relative humidity (rH) and T_{air}, which can control stomatal behaviour (Merilo et al., 2018). However, during the period of VPD difference, LAI was low in maize and the contribution to differences in stomatal regulation between crops was minimal. Thus, the effect of climate and atmosphere drivers on plant carbon-water relations is negligible in this study.

Precipitation can be a strong driver of changes in WUE at some locations (Sun et al., 2018; Zhang et al., 2016) due to water limitation; and is most prominent in arid and semi-arid regions (Niu et al., 2011). Zhang et al. (2018) investigated the effects of water addition (10 mm added weekly) and observed a higher increase in WUE when there was lower precipitation. This occurred because the water promoted plant activity, which had an overall greater increase in primary production than ET. However, this study also stipulated WUE increases were less when there was more natural precipitation since there was less water limitation (Zhang et al., 2018). Higher precipitation does not always result in higher WUE since precipitation does not always contribute to water availability to plants. Parkin et al. (1999) estimated that average annual water surpluses, which contribute to deep drainage and run off, are approximately 42% for a nearby field (Guelph, Ontario), which suggests that precipitation may not be a good indicator of plant carbon-water relations in this humid region. In other climates where water limitations are normal, precipitation metrics would be more fitting since water surpluses would be much lower. Moreover, precipitation was similar for both crop fields in this study meaning it could not contribute to variation in WUE at these sites. Rather, soil water availability could be a better indicator of water limitation induced influence on these plant carbon-water relationships.

2.3.2.2 Differences in soil drivers

Soils at the alfalfa and maize sites were similar in texture, surface hydraulic properties (Table 2.1), saturated hydraulic conductivities (K_{SAT}; Table 2.1), and unsaturated hydraulic conductivities (K_{UNSAT}; Figure 2.5a). Thus, variation in VWC (Figure 2.6) are likely attributed to factors other than soil properties. At 5 cm depth, maize had lower VWC until the middle of July when the crop reached mature height (Figure 2.7a) due to soil evaporation (Figure 2.6a). While alfalfa grew quickly and re-established quickly after cuts, maize was planted as a row-crop and was slow growing at the start of the growing season. Canopies provides shade, which reduces evaporative demand on surface soils (Hatfield and Dold, 2019). Once the maize canopy matured, this difference in the 5 cm VWC disappeared suggesting surface soil evaporation decreased. The 25 cm VWC in maize dropped below alfalfa VWC in late May until the end of June (Figure 2.6b), which corresponded with the period of maize growth from sprouting to mature height (Figure 2.7b). Water consumption demands from maize increase significantly during plant growth as root density increases (Sharp and Davies, 1985), and is greatest in the 0 - 30 cm depth due to higher root density (Djaman and Irmak, 2012). Maize develops two rooting systems: seminal roots, which assists with establishment of young plants; and nodal roots, which develop as the plant grows, with new nodes developing at the top of the previous node (Feldman, 1994; Hoppe et al., 1986). Innermost nodes emerge when the plant has approximately three leaves and continue to appear successively approximately every two leaves, resulting in a high density of roots developed at shallow soil depths (Hoppe et al., 1986). Lower VWC observed here at 25 cm in May and June is a result of higher root densities and water extraction from this area prior to maize reaching maturity. After reaching their maximum heights, maize VWC did not differ, as root systems were matured and accessing deeper water. At 50 cm, soil moisture was higher in alfalfa from the beginning of the growing season until the middle of July when it suddenly decreased. Prior to this, there was a lack of precipitation and alfalfa likely accessed deeper water stores using deep tap roots (Johnson et al., 1998; McIntosh and Miller, 1981; Weaver, 1926), decreasing VWC at 50 cm. Prior to this, the water table was likely close to the surface and capillary effects caused VWC to be higher than field capacity at 50 cm in the alfalfa field.

Soil water content never exceeded wilting point for the entire 2018 growing season; however, crops can experience moisture stress before soil water availability approaches wilting point at rooting depth since water is more strongly bound to soil particles at lower concentrations and become more difficult for roots to extract (Allen et al., 1998). Allen et al. (1998) estimated that readily available water represents 55% of total available water from the rooting zones, when ET demands were around 5 mm day^{-1} for both maize and alfalfa. Total available water was not measured in this study since rooting depths were not recorded and soil moisture was only measured at 5, 25 and 50 cm. This 55% threshold was not reached at the 25 and 50 cm depth, suggesting there should be water available in this zone. However, VWC was frequently below this threshold at the 5 cm depth at both sites. In maize, soil water extraction, or water uptake through roots, follows an approximate conical 40%, 30%, 20% and 10% water consumption pattern from the first, second, third and fourth quarter of rooting depths, respectively (Kranz et al., 2008). Typical rooting depths range from 1.5 - 1.8 m for maize (Feldman, 1994; Weaver, 1926) 1.0 - 2.0 m for alfalfa (Allen et al., 1998), putting 25 cm VWC measurements in the top quarter of rooting depth and 50 cm in either of the first or second quarter where 40% and 30% of water uptake occurs, respectively. In this study, alfalfa was planted in the previous year meaning the rooting system was established with mature rooting depths.

2.3.2.3 Vegetation drivers of differences between WUE

Daily WUE differences between alfalfa and maize fields were primarily driven by GPP rather than ET, which was influenced by crop maturity and LAI (Figure 2.7). Zhu et al. (2015) found that 65%

of the spatial variability of WUE between ecosystems in China was accounted for by LAI and altitude. At higher LAI, more photosynthesis can occur since more PAR is intercepted by the canopy. However, there is a diminishing return on rate of change in photosynthesis due to mutual shading within the canopy (Hatfield and Dold, 2019). For row crops, mutual shading diminishing GPP production occurs around LAI > 4 (Ritchie, 1972). In this study, maize GPP increased with increasing LAI up to a value of 4 in early July (Figure 2.7b), when daily GPP peaked (30 June 2018; Figure 2.2d). This suggests that self-shading from canopy development had occurred at the maize site, which is supported by increases in stomatal resistances in lower and middle canopies beginning in July (Figure 2.7c). At lower light intensities, light-dependant genes become less expressed and photosynthesis proteins and chlorophyll stop being produced, initiating senescence (Thomas, 1978). The effects of senescence and leaf aging were evident in maize, which resulted in decreases in daily GPP following July (Figure 2.2d). As leaves age, decreases in WUE occur due to shifts in stomatal conductance (Lin and Ehleringer, 1982; Warren, 2006; Wullschleger and Oosterhuis, 1989). However, alfalfa did not experience extended periods of these reductions in GPP since it was harvested multiple times throughout the growing season. These harvests resulted in immediate drops in GPP (Figure 2.2d) and corresponding WUE (Figure 2.2a), and restarted the regrowth process. Alfalfa stomatal resistance only experienced slight deviation in the lower canopies (Figure 2.7c), suggesting self-shading only had a slight impact at the lowest canopies. However, this did not translate to significant differences in daily GPP increases as alfalfa grew (Figure 2.2d).

Growth patterns also influenced plant carbon-water dynamics resulting in stark contrasts in daily GPP patterns. Maize spent more time in its seed emergence and seedling state in early growing season compared to alfalfa (Figure 2.7a). After establishment, maize grew rapidly until it reached maturity, while alfalfa established shortly after the beginning of the growing season and after each subsequent cut. This occurred because alfalfa did not invest resources into its rooting systems since it already had established roots. Near the beginning of the 2018 growing season, however, maize was developing both rooting systems and above ground biomass, which resulted in a much slower start to the growing season. Maize growth is also reduced at temperatures less than 20 ºC, which could be partly responsible for the later development of the crop (Greaves, 1996) and could have contributed to lower seasonal WUE in maize (Figure 2.2a). However, maize underwent rapid growth following this period when it grew at a much quicker rate than alfalfa until it reached maximum height (Figure 2.7a). During this period, maize exceeded alfalfa daily GPP (Figure 2.2d) suggesting that crop growth could promote higher GPP and simultaneously, higher WUE. Further, crop development is limited by temperatures; silage maize was harvested at ~2450 GDD which is typically around the time kernel denting occurs in silage maize, shortly before physiological maturity (Mahanna et al., 2017). While alfalfa development can also be linked to its GDD, multiple cuts in a season result in alfalfa re-setting physiological development. Alfalfa's seasonal GPP, therefore, is not limited by GDD.

2.3.3 Implications for Climate Change Adaptation in Southern Ontario

Results from this study provide insight into implications of climate change on current agricultural crop choices and management practices in southern Ontario under non-water limiting conditions. Since both crop sites were subjected to very similar climate and atmospheric conditions, we can isolate differential responses to these variables on daily scales. Moreover, soil was similar between sites, which allows us to isolate vegetation carbon-water responses to changes in environmental variables. These responses can infer potential implications under future climate normals (i.e. not considering extreme weather events) in silt loam soils.

The effects on crop carbon-water relations expected from climate change is due to expected increases of atmospheric CO_2, increased temperature, changes to precipitation, and variations in humidity (Hatfield and Dold, 2019). The increase in temperature will increase atmospheric water demand, thereby increasing ET from crops and reducing soil water (Hatfield and Dold, 2019). However, this increase in atmospheric water demand could be mitigated by expected increases in precipitation in this region (IPCC, 2014) and higher water holding capacity of silt loams (De Jong and Shields, 1988). Since water was not limiting in this study, which was a dry year, and water inputs are expected to increase, water stress will likely continue to be minimal in these fields.

The IPCC (2014) has stated that increases of atmospheric CO_2 by 100 ppm since the start of the industrial revolution has enhanced WUE of C_3 plants due to increased CO_2 concentrations increasing photosynthetic capacity of C_3 plants because of increased Rubisco saturation (Gornall et al., 2010). However, C_4 plants are not expected to see the same increases under non-water stressed conditions since they maintain higher rubisco saturation already (Edwards et al., 2001). This could reduce the higher WUE in maize under peak growing season conditions (Figure 2.2a) when temperature is highest (Figure 2.3a). Under current conditions, alfalfa WUE was not significantly lower than maize and CO_2 fertilization could promote even greater differences in seasonal WUE of alfalfa and maize. In addition, this would reduce photorespiration losses of CO_2 in alfalfa and promote higher NEE, increasing the difference between maize and alfalfa carbon sequestration.

Higher growing season temperatures are expected to impact agricultural activity in several ways. Specifically, at mid and high latitudes, crop productivity is projected to increase, especially with cereal crops (Maracchi et al., 2005; Olesen et al., 2007). For maize, this is particularly important in the early growing season (c.f. Chen et al., 2011) since maize growth in reduced below 20 ºC (Greaves, 1996). This could promote earlier seedling emergence in maize, which could increase daily WUE at the beginning of the growing season. However, it is difficult to assess whether this would improve seasonal WUE since this could also result in earlier maturation, shading induced senescence, and aging effects. Maize maturation time differs from region to region (Kiniry, 1991) due to differences in temperature with optimal growing conditions for maize between 21 and 27 ºC (Shaw, 1977) suggesting that increases in daily temperature could have a negative effect should temperatures exceed this regularly. However, increases in temperature is unlikely to benefit maize over the growing season since it will reach maturation sooner and not benefit from the higher GDD thereafter. Moreover, timing of maize maturation is important for crop success since earlier maturation can result in heat stress and adversely affect pollination success (Harrison et al., 2011). In contrast, alfalfa WUE could benefit from increasing temperatures in this region. With frost-free growing season lengths expected to continue to rise (Environmental Commissioner of Ontario, 2017; Kunkel et al., 2004; Zhong et al., 2017), perennial crops will begin growing earlier and the multiple cuts of alfalfa will prevent senescence and leaf aging effects from limiting the benefits of a longer growing season. Moreover, there is evidence that increased temperatures could reduce the number of days between cuts (Ruget et al., 2012) and increase the number of cuts made in a season (Jing et al., 2014). This would promote higher seasonal GPP and transpiration since there would be increased periods of rapid growth, with the cumulative effect expected to benefit WUE in alfalfa.

2.4 CONCLUSIONS

The objectives of this study were to quantify field-scale plant water-carbon dynamics of maize (*Zea mays*) and alfalfa (*Medicago sativa*) crops in Southern Ontario, Canada, to identify differences in plant carbon-water dynamics and contributions to these differences from ecosystem drivers. Seasonal differences in daily WUE between maize and alfalfa was driven by changes in GPP rather than differences in ET. While climate, atmosphere, and soil factors influence ecosystem processes, which impact plant carbon-water relations, vegetation factors appear to be the dominant driver of these differences in the humid study region. Growth rates, LAI, leaf aging, and growing season length drove plant physiological processes responsible for the seasonal trends observed. Multiple harvesting in alfalfa promoted more periods of rapid stem elongation and reduced the effects of leaf aging, while summer planting prior to the 2018 growing season enabled an earlier start to the growing season. In these non-water limiting regions, future forage crop selection should consider how increased growing season lengths impact crops. Perennial crops such as alfalfa are better suited to take advantage of longer growing seasons since they can grow earlier and contain multiple cuts, which can take advantage of the longer growing season and reduce senescence effects. Annually seeded crops such as maize may be less suited to take advantage of climate change shifts resulting in earlier start to the growing season since it will mature earlier, senesce earlier and not benefit from longer growing seasons. However, more research is needed to further comprehend how topographic, edaphic, and atmospheric variables influence plant carbon-water dynamics at larger spatial scales. Close monitoring of these ecosystem drivers and how they adjust to shifts in climate and precipitation patterns is essential to promote higher production crop selection.

CHAPTER 3

MANUSCRIPT 2: IMPLICATIONS OF ESTIMATION METHODS, PLANT PHYSIOLOGY AND MANAGEMENT INFLUENCES OVER CROP GROWTH ON DISCREPENCIES BETWEEN DIFFERENT WATER USE EFFICIENCY CALCULATIONS IN ALFALFA AND MAIZE

Overview

Water use efficiency (WUE) can be calculated using a range of methods that differ in their carbon uptake and water use variable selection. Consequently, inconsistencies arise between WUE calculations due to complex physical and physiological interactions. The purpose of this study was to quantify and compare WUE estimates (harvest or flux-based) for alfalfa (C_3 plant) and maize (C_4 plant) and determine the effects of input variables, plant physiology and management influences over crop development on estimates. Four WUE calculations were investigated: two "harvest-based" methods that used above ground carbon content and either precipitation or evapotranspiration (ET), and two "flux-based" methods, which used gross primary productivity (GPP) and either ET or transpiration. WUE estimates differed based on method used at both half-hourly and seasonal scales. Input variables used in calculations affected WUE estimates, and plant physiology led to different responses in carbon assimilation and water use variables. Plant physiological responses differed in alfalfa and maize due to inherent crop traits and management influences over development, leading to different crop responses. WUE estimates also differed due to different eddy covariance processing methods, even when the same carbon assimilation and water use variables were considered. This study demonstrates that our ability to compare WUE estimates across studies is hampered by inconsistencies between WUE calculations, and highlights a need to develop a metric of measuring cropland carbon-water coupling that accounts for all water use components, plant carbon responses, and biomass production. Until such a metric is available, we suggest that water use and carbon assimilation variables be independently investigated to avoid inconsistencies in the broader scientific community.

3.0 INTRODUCTION

Water use efficiency (WUE) represents an important indicator of plant resource use, with implications for local, regional, and global carbon and water cycle responses to changing environments (Ito and Inatomi, 2012; Knauer et al., 2018). WUE is the ratio of plant production (carbon assimilation) per unit of water use, and is commonly used to indicate vegetation performance (Beer et al., 2009; Ito and Inatomi, 2012; Kuglitsch et al., 2008; Medlyn et al., 2017). Plants function more efficiently when they balance atmospheric gas exchanges to maximize carbon dioxide uptake for photosynthesis and minimize water use through transpiration (Lawson and Blatt, 2014).

The WUE of plants can be quantified using several approaches that use different calculations of carbon assimilation and water use. “Harvest-based,” approaches rely on above ground biomass (AGB) measurements as an indicator of carbon assimilation, whereas “flux-based” approaches use measured exchanges of gross primary productivity (GPP) carbon from eddy covariance instrumentation (Baldocchi, 2003). Harvest and flux-based approaches can be further subdivided based on their water use variables. Harvest-based approaches can use ET, which accounts for water use within an ecosystem, or precipitation (P), which presumes water use is related to water input. Flux-based WUE approaches can use evapotranspiration (ET), which indicate ecosystem water use, or transpiration (T), which only considers canopy water use. Flux-based WUE approaches are highly dependant on vegetation cover and short-term variation (half-hourly; daily) in meteorological conditions (Jiang et al., 2019; Kuglitsch et al., 2008). At longer timescales (seasonal, interannual), the variation in WUE caused by meteorological conditions decreases (Kuglitsch et al., 2008); however, this may not be true for agricultural crops, which are characterized by drastic changes in canopy development over the growing season. Therefore, variation in seasonal WUE at incremental timescales (ie. half-hourly) may be important to agricultural WUE trends, reflecting changes due to plant canopy structure and development (Hatfield and Dold, 2019).

WUE of plants is also affected by environmental conditions, which play important roles in rates of both carbon assimilation and water use. Environmental drivers can have varying degrees of influence on carbon assimilation and water use variables (Albertson et al., 2001; Chapin et al., 2011). Indeed, the variables of water use and carbon uptake may be affected differently by climate, soil, vegetation, and hydrological factors. As such, variable climate and hydrological regimes have a large impact on crop resource-use, and consequently, the prediction of field-scale changes in WUE is complicated by the numerous environmental interactions (Hatfield and Dold, 2019).

The varying influences of environmental controls can result in different patterns of WUE depending on the calculation method used. For example, Kang and Kang (2019) identified nine different equations for WUE, including seven ecosystem scale equations, which resulted in inconsistencies in WUE estimates across the different methods. Moreover, the carbon and water variable used in different WUE calculations provide different insight into plant-carbon-water dynamics, each of which have different advantages or disadvantages. For example, WUE calculations using T and GPP consider physiological responses and biochemical functions of vascular plants (Beer et al., 2009; Farquhfrowthgar and Sharkey, 1982; Ito and Inatomi, 2012). Calculations that use ET, however, consider physical responses (evaporation) from the environment as well as biological, offering a more complete picture of water cycles for ecosystems (including agricultural) that are important for water management (Goyal and Harmsen, 2014). Methods that use above ground biomass provide insight into yield per water use, which is important in agriculture for maximizing production. Further, in drier environments, precipitation is sometimes used due to its importance to agricultural production in water-limited environments (Condon et al., 2002).

The method of determining water and carbon variables can also influence WUE calculations. Although comparisons of field-based harvest and ecosystem flux approaches have been reported in the literature (ex. Maleski et al., 2019), the magnitudes of these differences vary regionally (VanLoocke et al., 2012). Kimball et al. (2019) investigated 29 models that used different approaches to calculate ET for two maize fields and found significant inter-seasonal and intra-seasonal variations in model performance due to soil exposure, seasonal aridity, and model parameterization. This demonstrates that some ET models can produce vastly different results under the same conditions due to their method of calculation and variables considered. Furthermore, differences in the handling of eddy covariance (EC) data filtering and processing (ex. McMillen, 1988; Moncrieff et al., 1997; Skaggs et al., 2018; Wutzler et al., 2018), foot printing (ex. Arriga et al., 2017; Foken and Leclerc, 2004; Vesala et al., 2008), and gap-filling techniques (ex. Falge et al., 2001; Zhao and Huang, 2015) can also result in ET and GPP calculation discrepancies. Thus, although determinations of WUE are relevant to understanding the functionality of plants in a field setting, estimates can vary substantially depending on how WUE and its input variables are determined. This complicates the ability to compare WUE estimates across different studies.

Although attempts have been made to compare different calculations of WUE, these have typically focussed on large regional scales (ex. VanLoocke, Twine, Zeri, & Bernacchi, 2012) or global scales (ex. Ito & Inatomi, 2012) and there is a paucity of observational studies that investigate field-scale discrepancies in calculation of WUE. Moreover, the few studies that have been conducted have focussed on bioenergy crops, often switchgrass (Eichelmann et al., 2016;

Maleski et al., 2019), and have not specifically investigated inconsistencies in patterns between calculations. Forage crops represent 70% of agricultural land globally (FAO, 2010) and are therefore important to include in WUE comparison studies. Thus, there is a need to quantify the WUE of forage crops, and a need to understand if and how this varies with the method of WUE calculation. Thus, the objectives of this study are to: 1) quantify and compare growing season WUE of two forage crops, using harvest-based and flux-based methods; 2) investigate how the choice of input variables to calculate WUE impacts estimates; and 3) investigate if and how plant physiology and management influences on crop development impact discrepancies between WUE estimates at both seasonal and shorter timescales.

3.1 METHODS

3.1.1 Site Description

The study crop (alfalfa and maize) fields were in the Hopewell Creek Watershed, Mayhill, Ontario, Canada (Figure 3.1a). The maize site [(43.525º N, 80.425º W)] was located 4.4 km WSW of the alfalfa site [(43.549º N, 80.381º W)]. The elevation at the maize site (Figure 3.1c) ranged from 327.9 to 333.8 masl, while the elevation at the alfalfa site (Figure 3.1b) ranged from 330.6 to 332.1 masl. Thirty year (1981-2010) monthly mean average temperatures for this area (Waterloo Wellington A, Waterloo, Ontario, Canada, Climate ID:6149387, 9.8 and 11.1 km from study sites) between May and September, ranged from 12.5 to 20.5º C with an average precipitation of 435.0 mm (Canada, 2019). Both sites had silt loam soils with porosities (± standard deviations) of 0.46 (± 0.05; alfalfa) and 0.44 (± 0.06; maize), and bulk densities (± standard deviations) of 1.36 (± 0.09 g cm^{-3}; alfalfa) and 1.43 (± 0.11 g cm^{-3};maize). Both fields were tile-drained at depths of 90 - 100 cm below the ground surface and crops were not irrigated. Originally, alfalfa was randomly seeded with nurse crops (oats, triticale, and peas) in April 2016. In July 2016, the field was cut and only alfalfa continued to grow thereafter. The alfalfa received 0-12-44 NPK granular fertilizer at 392 kg ha^{-1} in September of 2017. Maize was planted in rows with 80 cm spacing in early May (2018) after the field received one pass of vertical conservation tillage. The maize field was fertilized with liquid dairy manure,112 kg ha^{-1}nitrogen, 67 kg ha^{-1} phosphorus and 90 kg ha^{-1} potassium dry fertilizer. Starter fertilizer was also applied at 47 kg ha^{-1}. During the 2018 growing season, sprouting of alfalfa was observed shortly after snowmelt (late April) while maize sprouting was not observed until May 29.

Maize (*Zea. mays)* and alfalfa (*Medicago sativa*) crops were used as they are two of the most prominent forage crops in Southern Ontario, Canada (Great Lakes Region). Differences between these two crops are highlighted by physiological aspects including photosynthetic pathway and growth patterns, as well as farming techniques that influence plant growth. Alfalfa is an herbaceous perennial legume that follows the C_3 pathway of photosynthesis and is an important protein source (Platt and Bassham, 1978). Typical farming techniques in southern Ontario include random summer seeding in the previous year, which results in an equally distributed canopy, undisturbed growth until flowering occurs, and, harvesting before flowers develop. Multiple harvests are made in one growing season. In contrast, maize is an annual crop that follows the C_4 pathway of photosynthesis (Taylor, 1996), and is planted in rows, resulting in linear canopy growth and increased soil exposure prior to canopy closure. Maize is harvested once at the end of the growing season.

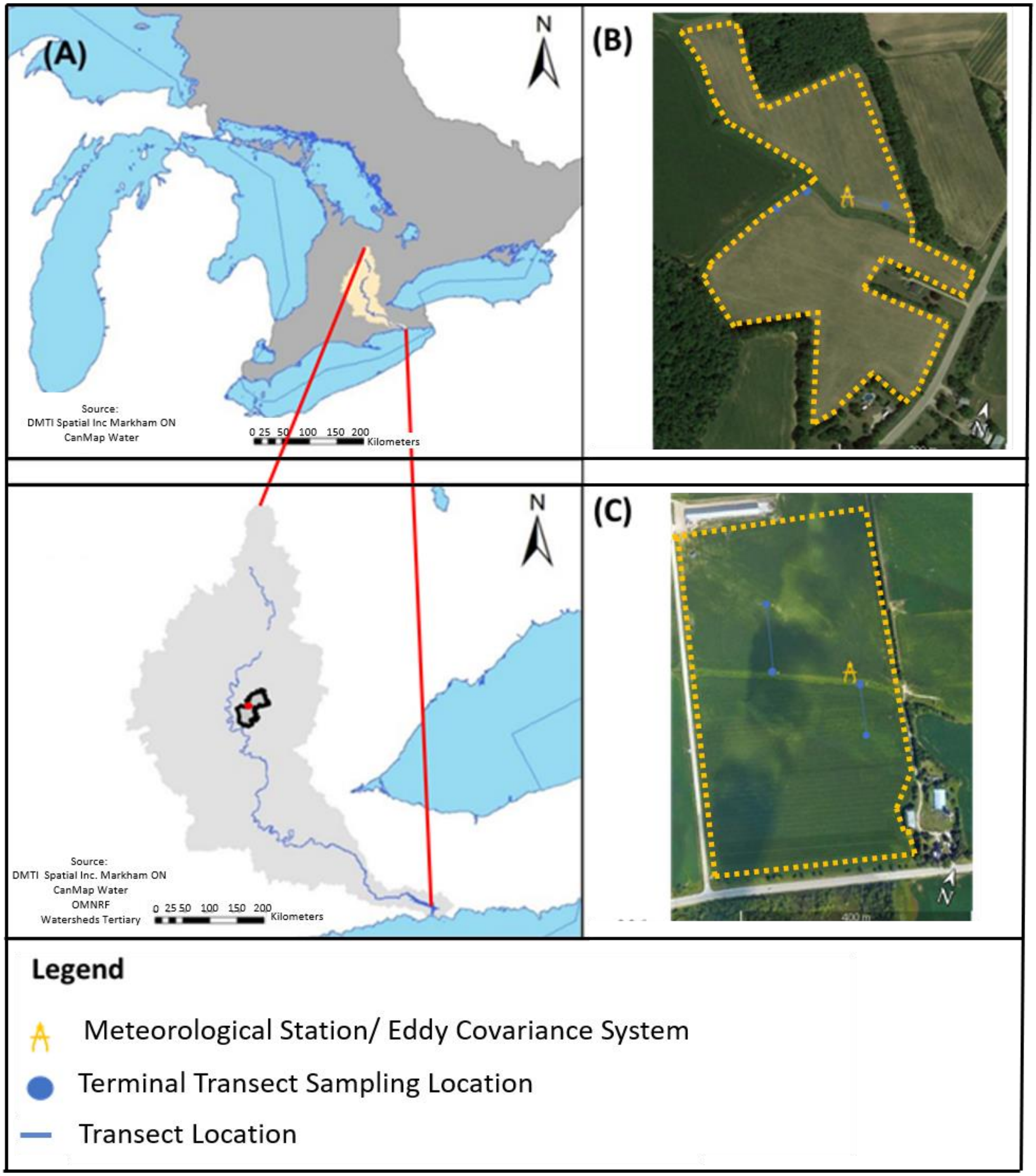


*Figure 1 (A) is adapted from Irvine (2018) and satellite imagery of (B) and (C) were extracted from Google Earth (2020) using imagery from July 7, 2018 and August 9, 2018 respectively. The dotted lines in relief gradients in (B) and (C) show discontinuation, where the transect ends and the other begins on the other side of the creek.

Figure 3.1 Location of (A) study sites and Hopewell Creek Watershed within Southern Ontario, and alfalfa (B) and maize (C) fields with location of transects, and eddy covariance / meteorological tower.

3.1.2 Data Collection

3.1.2.1 Vegetation

At each site, two transects were established where plant heights, stomatal resistance, and leaf area index (LAI) were measured every 3-5 days, at ~10 m intervals (Figure 3.1b,c). Alfalfa transects were approximately 50 m long with relief varying from 330.56-331.97 masl. The maize site included transects on the north (127 m) and south (96 m long) sides of the creek, ranging 327.94-333.78 masl and 327.90-331.49 masl, respectively. Coordinates and relief were determined using a Differential Global Positioning System device (Viva GS14 GNSS RTK, Leica Geosystems, Switzerland; ± 0.5 cm vertical accuracy).

On each sampling date, three plants were selected randomly at each 10 m interval and heights were measured from ground to top of plant using a standard measuring tape. In addition, a single plant was randomly selected at each 10 m interval for stomatal resistance measurements using an open chamber leaf porometer (SC-1, Decagon Devices, Washington), where three leaves in the upper, middle, and lower canopy were measured. The upper canopy was characterized by little to no shading from sun exposure, while the middle canopy represented the middle third of the stem and was characterized by some shading, and the lower canopy experienced significant amounts of shading. LAI measurements were conducted using a LI-COR 2200C (Li-Cor Inc., Nebraska, United States) when sky conditions were clear or consistently overcast. LAI data was run through FV2000 software (Li-Cor Inc., Nebraska, United States), which accounted for scattering correction. Dates when the instrument reported unrealistic, or non-values, were discarded; 12 dates were included (2 discarded) from the alfalfa site and 11 dates (3 discarded) from the maize site.

Samples of alfalfa and maize were harvested four times throughout the season to quantify biomass accumulation for periods within the growing season. A 50 x 50 cm quadrat was placed at three randomly selected locations along transects. Within each quadrat, vegetation was harvested for above-ground biomass (AGB). Samples were dried at 80 ^{o}C for 72 hours before being weighed for dry biomass. At the maize site, 40 x 50 cm sampling areas were used to better represent 80 cm crop row spacing. Alfalfa cumulative biomass was calculated using a linear regression equation ($R^2 = 0.88$) between height and biomass measurements collected at each harvest. The use of this linear regression was justified despite a low sample size since height measurements at harvest dates occurred prior to stem elongation ending and "cut at first flower" techniques were implemented. Some thickening occurs during stem elongation, which would be accounted for in height-biomass relationship, but lignification of phloem and xylem, which would be a significant source of deviation to measured height-biomass relationship, occurs after stem elongation has completed (Engels and Jung, 1998). In Pittman et al. (2015), measured canopy height was the most effective parameter at explaining variation in alfalfa biomass, which accounted for 68.5% of variation under a cut at 10% flower scenario.

Carbon contents were determined using a 4010 Elemental Analyzer (Costech Instruments, Italy) coupled to a Delta Plus XL (Thermo-Finnigan, Germany) continuous flow isotope ratio mass spectrometer (CFIRMS) at the Environmental Isotope Laboratory at the University of Waterloo in Waterloo, Ontario, Canada. Three grams of dry leaf matter was ground into fine powder and mixed to ensure sample homogeneity. As determined in a practice trial, approximately 0.7 mg from each alfalfa sample and 1.0 mg from each maize sample were encased in 3 x 5 mm tin capsules and run in the CFIRMS. The device converts the plant matter to gas through combustion and analyzes for ^{13}C.

3.1.2.2 Hydrometric Data Collection

A meteorological station was installed at each site that included a net radiometer (3.75 m above ground; CNR4, Kipp & Zonen, Netherlands), two soil heat flux plates (5 cm below ground; HFP01, Hukseflux Thermal Sensors, Netherlands), four soil temperature probes (108 thermistors, Li-Cor Inc, Nebraska, USA) at 5, 10, 25 and 50 cm depths, and a temperature and relative humidity probe (2.5 m above ground; HMP 155, Vaisala Oyj, Finland) where all data was averaged every 30-minutes to the data logger (XLite 9210B, Sutron Corporation, Virginia, USA). Additional temperature and relative humidity sensors (HOBO U23 Pro v2, Onset Hobo, Massachusetts, USA) were installed at 1 and 3.5 m above ground level for quality control. Precipitation was measured using a tipping bucket rain gauge (RG3, Onset Hobo, Massachusetts, USA) installed 1.5 m above ground. An eddy covariance (EC) system, which included a closed-path infrared gas analyzer (4 m above ground surface; LI 7200, Li-Cor Inc, Nebraska, USA) paired with a three-dimensional ultrasonic wind anemometer (4 m above ground; Windmaster Pro, Gill Instruments, UK) sampling at a rate of 20 Hz, was installed. The sensor heights were > 3 m above maximum canopy of alfalfa and > 1 m above maximum canopy of maize, where canopy dependant calculations varied depending on measured canopy height throughout the season.

3.1.2.3 Carbon and Water flux processing

Two different methods of carbon and water flux processing were done in this study: one using EddyPro (Fratini and Mauder, 2014; LI-COR Biosciences, 2017) and REddyProc (Wutzler et al., 2018), and another using Fluxpart software (Skaggs et al., 2018). For the EddyPro/REddyProc EC method, the 20 Hz high-frequency data was processed into 30-min average fluxes via the EddyPro software (v7.0.4, Li-Cor Inc, Nebraska, USA). Fluxes were corrected for density, sensor separation (Leuning and Judd, 1996; Webb et al., 1980) and coordinate rotation (double rotation; Tanner & Thurtell, 1969). This flux data was further subject to filtering to ensure sufficient samples per 30-min (n>0.85*36,000) were available. In addition, a suite of statistical filtering was completed that first determined an acceptable growing season range. Measurements which exceeded the mean of the dataset plus ± 3.5 times the standard deviation of the entire dataset were excluded. Additionally, the growing season datasets were grouped by each 30-min diurnal timestamp (00:30-24:00), where each half hour was filtered if it was out of the range of the mean plus the ± 3.5 times the standard deviation for that 30-min group. Furthermore, a moving window (± 2.5 hour and 5 hours, depending on record set greater than 80%) was applied where values larger than 3.5 times the standard deviation were removed. A final inspection was completed with a manual inspection where data was filtered based on physically realistic site values. Finally, a flux footprint analysis was performed which ensured that all fluxes originated from within the area of interest (Kljun et al., 2015), followed by a frictional velocity (u*) threshold of u* = 0.1 m s^{-1} filter, which was used to remove periods with low turbulence (Goulden et al., 1996). Net ecosystem exchange (NEE) was gapfilled and partitioned into gross primary productivity (GPP) and respiration (R_e) following the methods of (REddyProc; Wutzler et al., 2018). ET was calculated from a closed energy balance (Wilson, 2002) and gapfilled using a site-specific Priestley-Taylor (Priestley and Taylor, 1972) PET to ET relationship.

Fluxpart (Skaggs et al., 2018) is a program that implements flux variance similarity (FVS) partitioning theory (Scanlon and Kustas, 2010; Scanlon and Sahu, 2008). ET is calculated directly from water component fluxes according to,

$$\mathrm{ET} = \{w'q'\} \tag{1}$$

where *w* represents vertical wind velocity, *q* represents water vapour concentration, {} indicate temporal means over a 30-minute interval, and the prime (′) represents deviations from the 30-minute mean. Similarly, NEE is calculated directly from carbon dioxide component fluxes according to,

$$\mathrm{NEE} = \{w'c'\} \qquad (2)$$

where *c* represents carbon dioxide concentration. Briefly, FVS partitions ET and NEE into component water (transpiration (T) and evaporation (E)) and carbon (GPP and R_e) fluxes based on deviations from Monin-Obukhov similarity theory (Monin and Obukhov, 1954; Scanlon and Sahu, 2008). Monin-Obukhov similarity theory suggests that water vapour and carbon dioxide concentrations exhibit perfect correlation when measured from the same spot within a homogenous atmospheric layer. Disturbances to the perfect correlation can be attributed to the presence of multiple sources and sinks of these fluxes. The FVS technique relies on the degree of disturbance to infer the relative amounts of stomatal (T and GPP) and non-stomatal (E and R_e) fluxes present (Skaggs et al., 2018; Scanlon and Sahu, 2008). The contribution of stomatal components to water and carbon fluxes is calculated through a series of algebraic equations and assumptions outlined in Skaggs et al. (2018), based on work by Scanlon and Sahu (2008). This requires the input of a leaf-level WUE (LWUE) value for each half-hour interval, which is used to determine the variance of photosynthesis CO_2 concentration, and correlation coefficient for photosynthesis and respiration CO_2 concentrations before solving for GPP and T. LWUE can be input manually into Fluxpart or calculated according to,

$$\mathrm{LWUE} = 0.625 \times \frac{\{c_a\} - \{c_i\}}{\{q_a\} - \{q_i\}} \qquad (3)$$

where c_a and q_a represents ambient CO_2 and water vapour concentrations, and c_i and q_i represent intercellular CO_2 and water vapour concentration, respectively (Skaggs et al., 2018; Scanlon and Sahu, 2008). The 0.625 value represents molecular diffusivities for water vapor and CO_2 (Massman, 1998). Ambient concentrations, $\{c_a\}$ and $\{q_a\}$, are extrapolated from tower measurements, while $\{q_i\}$ is equal to relative humidity vapour concentration at a given leaf temperature, and $\{c_i\}$ is estimated based on photosynthetic pathway (C_3 or C_4). Since LWUE was not continuously measured, equation 3 was used for this study.

3.1.3 Data Analysis

A total of five methods were used to calculate water use efficiency (WUE) in this study (Table 3.1). Two methods of calculating "harvested" WUE (HWUE) were used in this study: $HWUE_{ET}$, which considers water use through ET, and $HWUE_P$, which considers water input (precipitation) as the sum of water uses. "Ecosystem" WUE (EWUE) approaches were computed in three ways in this study: $EWUE_S$, $EWUE_F$ and $EWUE_C$. $EWUE_S$ was calculated using GPP and ET values derived from the Eddypro/REddyProc analysis (GPP_S and ET_S), while $EWUE_F$ was calculated using GPP and ET derived from Fluxpart's FVS partitioning method (GPP_F and ET_F). $EWUE_C$ also used flux data derived from Fluxpart and represented canopy-level dynamics, by using T instead of ET for the water use variable in WUE calculations.

WUE was computed at three temporal scales: 1) Half-hourly (flux-based methods only); 2) Growing Season ($EWUE_S$ and HWUE methods); and 3) Cuts (alfalfa) or Growth Stages (maize) (all WUE methods). EWUE from FVS partitioning ($EWUE_F$ and $EWUE_C$) was not possible to gap-fill and therefore was not available at seasonal timescale. Growing season length (GSL) was 153 days for alfalfa (21 April– 21 September 2018) and 131 days for maize (3 May– 11 September

2018). The four alfalfa cut periods extended from: 1) 21 April– 7 June 2018; 2) 8 June– 6 July 2018; 3) 7 July– 13 August 2018; and 4) 14 August– 21 September 2018. Maize growth stages were determined by visible changes in crop growth, wherein biomass sampling occurred, and classified by their growing degree days (GDD): growth stage 1 (GS1) represented planting until 12 leaves when ear formation begins (GDD = 841: 3 May– 26 June 2018), growth stage 2 (GS2) represented the period between ear formation and silking, when pollination first occurs and maize reaches maximum height (GDD = 1359 : 27 June– 18 July 2018), growth stage 3 (GS3) represented the period between silking and kernels in dough stage (GDD = 1925: 19 July– 13 August 2018) and growth stage 4 (GS4) represented the period between kernels in dough stage until harvest, which occurs during after kernel denting in silage maize (GDD = 2452: 14 August– 11 September 2018). For simplicity, GS1 will be referred to as early growing season stage, GS2 silking stage, GS3 as ear maturation stage, and GS4 as harvest stage. GDD was calculated as,

$$GDD = [(T_{air}^{max} + T_{air}^{min}) / 2] - T_{air}^{base} \tag{1}$$

where T_{air}^{max} and T_{air}^{min} are the maximum and minimum daily air temperatures (in Fahrenheit), respectively, and T_{air}^{base} is the base temperature required for growth. For maize, T_{air}^{base-} was 50 ºF, or 10ºC (Mahanna et al., 2017).

Analysis was completed using data from the whole growing season and/or different sub-periods of the growing season, as defined above. The half-hourly WUE values used in this study were not available for harvest-based techniques since biomass sampling at this timescale would be impractical. Only non-gapfilled, QA/QC filtered for outliers, footprint, u*, as well as for daytime, half-hourly values were used in comparisons between the flux-based methods since Fluxpart estimates could not be gap-filled due to data insufficiency. Likewise, seasonal values from Fluxpart’s were not possible. For each crop type, EWUE$_S$ and EWUE$_{F,}$ and EWUE$_F$ and EWUE$_C$ values were compared using a 1:1 relationship and linear regressions, whenever corresponding half-hourly values were available for both crop species. Each sample set was tested for normality using the Shapiro-Wilk normality test (Shapiro and Wilk, 1965) and non-parametric Spearman’s correlation analysis was used to determine the correlation between WUE components. All statistical analyses were done using R Statistics software (R Core Team, 2018).

Table 3.1 Five methods of determining water use efficiency (WUE) used in this study including their formula, variables used, advantages, and disadvantages to method.

Method	Timescale	Formula	Variables	Advantages	Disadvantages
$HWUE_{ET}$	Season, Cuts (alfalfa), Growth Stage (maize)	$HWUE_{ET} = \frac{BioC}{ET}$	AGB carbon content[a] EddyPro/REddyProc ET[b]	*Yield is important to agricultural production, helpful with irrigational needs and water saving.*[1]	*Does not consider below ground carbon storage.*[2]
$HWUE_P$	Season Cuts (alfalfa) Growth Stage (maize)	$HWUE_P = \frac{BioC}{P}$	AGB carbon content[a] Precipitation	*Yield is important to agricultural production and plant water use is linked to precipitation in agriculture because there is no other water source.*[3] *Does not require expensive equipment.*	*Does not consider below ground carbon storage.*[2] *Evaporation and soil water depletion not measured.*[3] *Frequency and intensity of precipitation (not considered) can impact water availability.*[4]
$EWUE_S$	Season Cuts (alfalfa) Growth Stage (maize) Half-hourly	$EWUE_S = \frac{GPP}{ET}$	EddyPro/REddyProc GPP[c] EddyPro/REddyProc ET	*Direct measurement of carbon and water exchanges between ecosystem and atmosphere.*[5] *Intra-seasonally variation in WUE*[e] *quantifiable.*	*Carbon assimilation and transpiration are not directly quantified.*[5] *Requires additional inputs from meteorological data to partition NEE*[f] *to GPP*[c] *and* R_e[g,6].
$EWUE_F$	Half-hourly	$EWUE_F = \frac{GPP}{ET}$	Fluxpart* GPP[c] Fluxpart* ET[b]	*Does not require as much equipment to partition NEE*[f] *into GPP*[c] *and* R_e[g,6]. *Intra-seasonally variation in WUE*[e] *quantifiable.*	*Relatively new program still requiring validation across various environmental/meteorological conditions.*[7] *Continuous estimation of leaf scale WUE*[e] *required*[6].
$EWUE_C$	Half-hourly	$EWUE_C = \frac{GPP}{T}$	Fluxpart* GPP[c] Fluxpart* T[d]	*Stomatal components provide better measure of physiological responses.*[7]	*Relatively new program still requiring validation across various environmental/meteorological conditions.*[8] *Continuous estimation of leaf scale WUE*[e] *required*[6] *and stomatal fluxes sensitive to it.*[6,9]

*Fluxpart determines fluxes based on Monin-Obukhov similarity theory, for explanation of partitioning method differences see section 3.1.2.3 Carbon and Water flux processing

[a] Above-ground biomass (AGB) was converted to biomass carbon content (BioC) based on carbon contents from carbon isotope analysis to enable comparison between flux-based and harvest-based approaches carbon assimilation [b]ET = evapotranspiration [c]GPP = gross primary productivity [d]T = transpiration [e]WUE = water use efficiency [f]NEE = net ecosystem exchange [g]R_e = respiration

[1](Deng et al., 2006). [2](VanLoocke et al., 2012). [3](Varvel, 1995). [4](Miranda et al., 2011). [5](Beer et al., 2009). [6](Sulman et al., 2016). [7] (Beer et al., 2009; Farquhar and Sharkey, 1982; Ito and Inatomi, 2012). [8](Skaggs et al., 2018). [9](Klosterhalfen et al., 2019b)

3.2 RESULTS AND DISCUSSION

3.2.1 Influence of Approach and Crop Type on WUE Estimates

Growing season WUE for maize and alfalfa are summarized in Table 3.2. Seasonal values reported in this study are within range of those reported elsewhere for both maize $HWUE_{ET}$ (Hussain et al., 2019; VanLoocke et al., 2012; Zwart and Bastiaanssen, 2004) and $EWUE_S$ (Suyker and Verma, 2010; Wang et al., 2018), and alfalfa $HWUE_{ET}$ (Jefferson and Cutforth, 2005; Mueller et al., 2005). Alfalfa $EWUE_S$ was greater than values recorded elsewhere (Wagle et al., 2019b), which is attributed to lower ET found here.

Differences in WUE were observed at the maize and alfalfa sites during the 2018 growing season (Table 3.2); however, these differences were inconsistent when different methods of calculating WUE were used. For example, when growing season harvested WUE ($HWUE_P$ and $HWUE_{ET}$) was employed, the WUE of maize was greater than that of alfalfa. In contrast, when growing season $EWUE_S$ was employed, the WUE of alfalfa exceeded that of maize. This is notable given literature suggests that C_4 pathway (maize) of photosynthesis typically exhibits greater ecosystem WUE than the C_3 pathway (alfalfa) (Ghannoum et al., 2011; Hsiao and Acevedo, 1974; Morison and Gifford, 1983; Osborne and Sack, 2012; Rawson et al., 1977; Wang et al., 2018; Way et al., 2014) due to physical separation of an additional metabolic cycle, which reduces photorespiration (Way et al., 2014), and physiological differences in the hydraulic pathway (Kocacinar et al., 2008; Osborne and Sack, 2012). The inconsistency between HWUE and EWUE of maize and alfalfa is likely related to differences in plant physiology where alfalfa invests more carbon into below ground biomass. Comparatively, alfalfa has substantially greater root components with shoot: root ratios estimated at 1.33-1.37 in year one (Bolinder et al., 2002;

Skuodienė and Tomchuk, 2015a), 0.80-0.87 in year two (Bolinder et al., 2002; Skuodienė and Tomchuk, 2015a), 0.58 in year three (Skuodienė and Tomchuk, 2015a), and 0.34 in the fourth growing season after seeding (Li et al., 2019). In contrast, maize has higher above ground biomass accumulation, where maize only stores 24-29% of carbon below ground (Amos and Walters, 2006; Hirte et al., 2018). The conversion of GPP to above ground biomass is captured in HWUE; however, the conversion of GPP into below ground biomass is not. Thus, GPP: above ground biomass ratios are responsible for most of the differences in HWUE and EWUE. Further differences can be attributed to lower photorespiration in maize due to its C_4 pathway of photosynthesis (Way et al., 2014), resulting in lower carbon losses through stomata.

Not only are there inherent differences between HWUE and EWUE methods, there are also inconsistencies observed within EWUE and HWUE methods themselves. These differences can be attributed to their input variable selection, which can be impacted differently by the physiological components of different crops. In this study, climatic differences or soil factors did not differ significantly between the two sites, which suggests that inconsistencies between species was driven by crop physiology. Linear regressions of half-hour intervals of air temperature (R^2=0.98, $p < 0.001$, n=6336) and relative humidity (R^2=0.96, $p < 0.001$, n=4264) measured at the two fields demonstrate 1:1 relationships, indicating that conditions were comparable at the sites (data not shown). In addition, another study using these study fields found that water availability in the soil was not limiting at rooting depths (see Chapter 2) and soil textures are similar at the two sites. These inconsistencies are investigated in the following sections.

Table 3.2 Growing season $HWUE_p$. $HWUE_{ET,}$ and $EWUE_S$ and median $EWUE_S$, $EWUE_F$ and $EWUE_C$ of maize and alfalfa for the 2018 growing season.

		Date	$HWUE_p$[a]	$HWUE_{ET}$[b]	$EWUE_S$[c]	Median $EWUE_S$[d]	Median $EWUE_F$[e]	Median $EWUE_C$[f]
Alfalfa	Cut 1	21 Apr – 7 June	1.51	0.81	3.11	3.79	18.17	31.46
	Cut 2	8 June – 6 July	1.80	0.66	2.84	3.35	12.87	19.31
	Cut 3	7 July – 13 Aug	1.14	0.65	3.02	3.66	16.65	20.54
	Cut 4	14 Aug – 21 Sept	1.43	1.04	3.57	4.85	19.42	27.86
	Growing Season	21 Apr – 21 Sept	1.45	0.78	3.11	3.91	16.81	24.48
Maize	Growth Stage 1	3 May – 26 June	0.42	0.25	1.26	2.06	15.97	21.55
	Growth Stage 2	27 June – 18 July	5.24	1.73	3.42	4.01	12.16	16.05
	Growth Stage 3	19 July – 13 Aug	4.01	2.24	3.46	5.18	17.47	20.39
	Growth Stage 4	14 Aug – 11 Sept	4.76	3.96	3.21	5.83	17.17	22.50
	Growing Season	3 May – 11 Sept	3.01	1.73	2.58	4.05	16.18	20.57

[a] $HWUE_P$ = Harvested water use efficiency using precipitation for water use variable [b]$HWUE_{ET}$ = Harvested water use efficiency using evapotranspiration for water use variable [c]$EWUE_S$ = Ecosystem water use efficiency using evapotranspiration as water use variable processed by EddyPro/REddyProc [d]Median $EWUE_S$ = Median half-hourly ecosystem water use efficiency using evapotranspiration as water use variable processed by EddyPro/REddyProc [e]Median $EWUE_F$ = Median half-hourly ecosystem water use efficiency using evapotranspiration as water use variable processed by Fluxpart [f]Median $EWUE_C$ = Median half-hourly ecosystem water use efficiency using transpiration as water use variable processed by Fluxpart

* Alfalfa site was divided by cut periods, while maize was divided into growth stage periods of biomass sampling points. The growth stages for maize represented early growing season (1), silking (2), ear maturation (3) and harvest stages (4). All WUE values are presented in mg C g^{-1} H_2O.

3.2.2 Importance of Input Variables and Processing Methods on WUE Estimates

The differences between the various methods used to estimate WUE mainly lie in how the two key input variables (carbon uptake and water use) of this ratio are derived (Table 3.3; see Table 3.1 for information on how these variables were calculated).

Table 3.3 WUE input variables for maize and alfalfa sites for the 2018 growing season. Alfalfa site was divided into subsections of cuts (1-4), while maize was divided into growth stages (early growing season, silking, ear maturation, harvest stages).

				WATER VARIABLE				**CARBON VARIABLE**			
		Date	**P**[a]	**ET$_S$**[b]	**Median ET$_S$**[b]	**Median ET$_F$**[c]	**Median T$_F$**[d]	**BioC**[e]	**GPP$_S$**[f]	**Median GPP$_S$**[f]	**Median GPP$_F$**[g]
Alfalfa	Cut 1	21 Apr – 7 June	0.93	1.75	2.21	1.15	0.548	1.41	5.45	1.25	3.95
	Cut 2	8 June – 6 July	0.54	1.48	3.57	2.74	1.76	0.98	4.21	2.01	5.38
	Cut 3	7 July – 13 Aug	0.95	1.67	3.30	2.68	1.69	1.09	5.04	1.82	4.68
	Cut 4	14 Aug – 21 Sept	0.90	1.24	2.22	1.87	1.11	1.29	4.42	1.57	4.11
	Growing Season	21 Apr – 21 Sept	3.30	6.14	2.65	1.97	1.15	4.77	19.1	1.68	4.44
Maize	Growth Stage 1	3 May – 26 June	1.22	2.05	4.20	1.93	0.990	0.51	2.58	0.712	3.42
	Growth Stage 2	27 June – 18 July	0.38	1.15	9.60	5.37	3.93	1.99	4.00	3.76	6.34
	Growth Stage 3	19 July – 13 Aug	0.68	1.22	5.47	4.22	3.56	2.73	4.21	3.02	6.79
	Growth Stage 4	14 Aug – 11 Sept	0.89 .	1.07	3.46	2.54	2.12	4.24	3.44	2.17	4.59
	Growing Season	3 May – 11 Sept	3.16	5.50	4.76	3.46	2.46	9.48	14.2	1.76	5.37

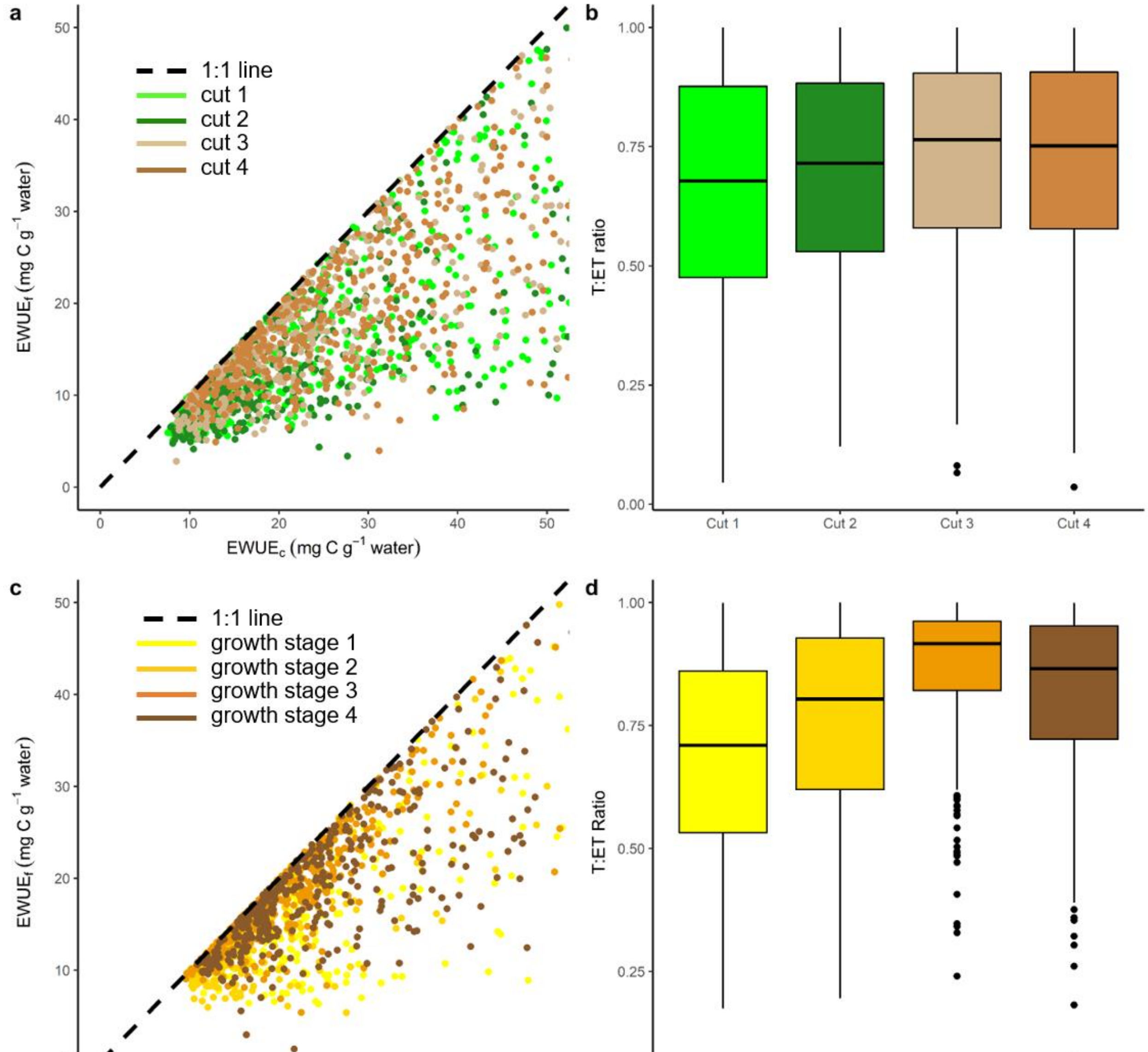
[a]P = precipitation, [b]ET$_S$ = evapotranspiration from EddyPro processing methods [c]ET$_F$ = evapotranspiration from Fluxpart processing methods [d]T$_F$ = transpiration from Fluxpart processing methods [e]BioC = biomass carbon content [f]GPP$_S$ = gross primary productivity from EddyPro/REddyProc processing method [g]GPP$_F$ = gross primary productivity from Fluxpart processing methods.

* Precipitation (P) and evapotranspiration from EddyPro/REddyProc method (ET$_S$) are presented in x10^5 g H_2O m^{-2} , while median half-hourly evapotranspiration by EddyPro/REddyProc (MED ET$_S$) and flux variance similarity (FVS) (ET$_F$), and transpiration by FVS (T$_F$) are presented in x10^{-2} g H_2O m^{-2} s^{-1}. Biomass carbon content (BioC) and gross primary productivity (GPP) from EddyPro/REddyProc method (GPP$_S$) were presented as x10^5 mg C m^{-2} over the stated period, while median GPP by EddyPro/REddyProc (MED GPP$_S$) and FVS (GPP$_F$) were presented as x10^{-1} mg C m^{-2} s^{-1}.

3.2.2.1 Importance of Water Use Variable

Harvested techniques are similar in that they both consider above ground biomass, but the two methods differ in their water input variables, where one uses precipitation (P; $HWUE_P$) and the other ET ($HWUE_{ET}$). However, P and ET do not always correlate, as is the case in this region since it is a humid region with frequent rain events (Ecoregions Working Group, 1989). This is because ET is limited by both energy and water supply (Yang et al., 2016) and frequent rain events increase water storage. Since maize and alfalfa draw from groundwater (due to a higher water table) and access this water storage (see chapter 2) to meet water demands, ET was limited by energy supply, creating a discrepancy between these two methods. Moreover, there is no consistency in this discrepancy. Throughout the growing season, $HWUE_P$ was greater than $HWUE_{ET}$, irrespective of crop, but these differences were inconsistent between sub periods (Table 3.2). For example, $HUWE_P$ was highest in alfalfa during cut 2, followed by cut 1, but $HWUE_{ET}$ was highest during cut 4. Similarly, $HWUE_P$ was highest during growth stage 2 in maize but $HWUE_{ET}$ was highest in growth stage 4. Due to the lack of agreement between these methods, it is difficult to directly compare WUE estimates made by different HWUE techniques.

Differences between canopy scale EWUE ($EWUE_C$) and field scale EWUE ($EWUE_F$) occur because they use different water use variables, T and ET, respectively (Figure 3.2a,c). This results in inconsistencies when comparing these techniques since different crops can have different T:ET ratios throughout the growing season. For example, in this study, median half-hourly $EWUE_F$ did not vary greatly (less than 4% difference) between species, however, median $EWUE_C$ was significantly greater in alfalfa (19%). This occurs because maize has a higher T:ET ratio over the growing season (Figure 3.2a,c). Moreover, when examined at smaller timescales, differences between T and ET methods are greater among the different alfalfa cuts than they are for different growth stages in maize (described in more detail in a following section).



*Alfalfa was separated by cuts 1 (light green, n=678), 2 (dark green, n=548), 3 (beige, n=541), and 4 (brown, n=594), while maize was divided into the four growth stages: early growth (Stage 1; yellow, n=296), silking stage (Stage 2; light orange, n=257), ear maturation stage (Stage 3; dark orange, n=518), and harvest stage (Stage 4; brown, n=442).

Figure 3.2 Half-hourly $EWUE_F$ (mg C g^{-1} H_2O m^{-2} s^{-1}) versus half-hourly $EWUE_C$ (x axis) and transpiration: evapotranspiration ratios for alfalfa (a,b) and maize (c,d) over the 2018 growing season.

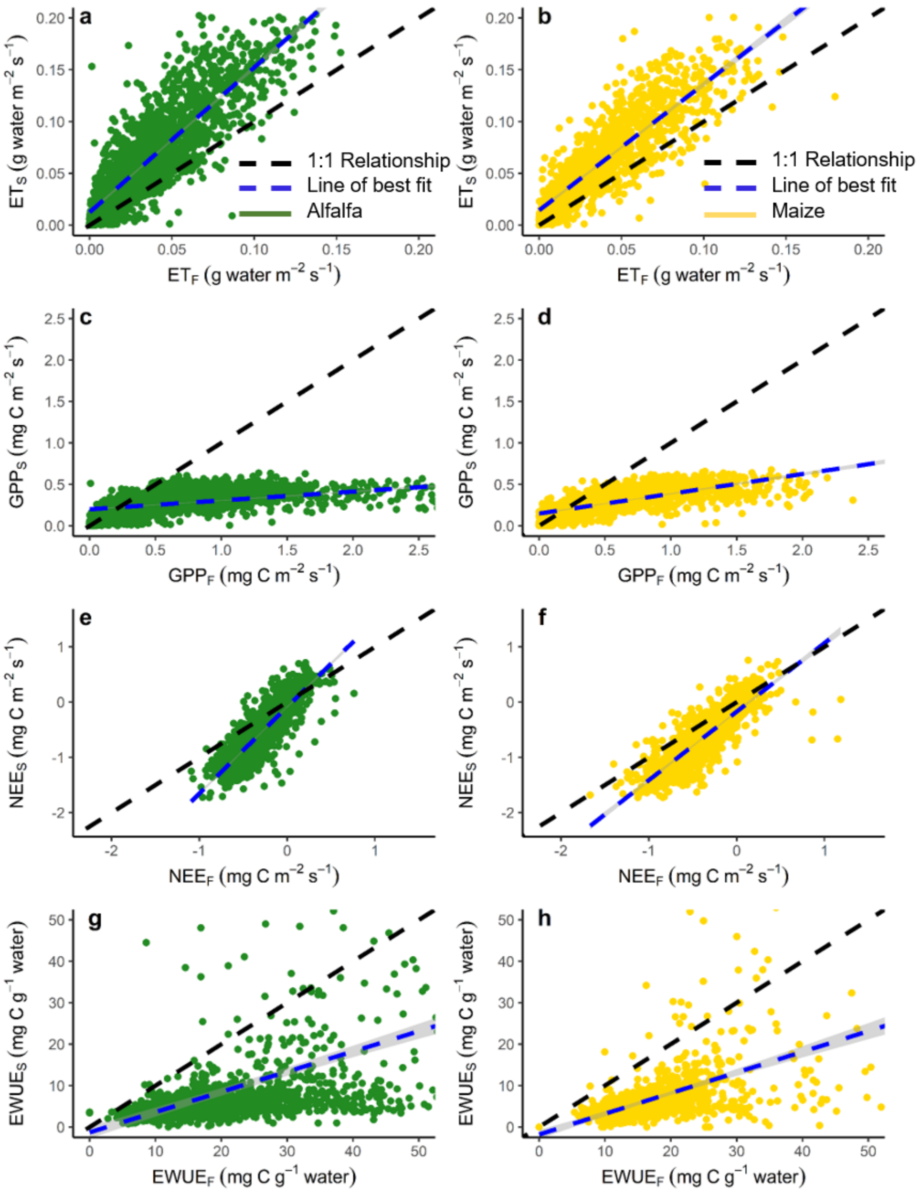
3.2.2.2 Importance of Carbon Input Variables on Differences between Harvest Water Use Efficiency and Ecosystem Water Use Efficiency

HWUE and EWUE techniques also vary due to the differences in assimilated GPP and accumulated above-ground biomass carbon content (BioC) because of carbon allocation and photorespiration. Even with the same water use variable, there are discrepancies in magnitude of differences measured between crops; alfalfa's $HWUE_{ET}$ estimate was 75% lower than its $EWUE_S$ value, while maize's $HWUE_{ET}$ estimate was 33% lower than $EWUE_S$ (Table 3.2). This provides inherent inconsistencies in techniques since some crops, such as maize, produce more biomass per carbon assimilation (i.e higher carbon use efficiencies) than other crops (c.f Choudhury, 2001). These differences in carbon use efficiencies are compounded by the carbon allocation issue mentioned earlier, where alfalfa invests significantly more into below-ground biomass than maize.

3.2.2.3 Importance of Processing Method for Ecosystem Water Use Efficiency Method

In addition to physical variable selection, processing method for determining these variables is also important to consider. EddyPro/REddyProc and FVS processing (Fluxpart) approaches use the same calculation variables for $EWUE_S$ and $EWUE_F$, and the same high frequency data files, but there is disagreement between these methods where half-hourly $EWUE_S$ values were substantially lower (Figure 3.3g-h). The discrepancies between these techniques is caused by lower calculated magnitudes of ET (Figure 3.3a-b; Spearmen correlation for alfalfa: R=0.87, p-value < 0.001, n=2104; for maize: R=0.88, p-value < 0.001, n=1304), and higher, more variable, GPP (Figure 3.3c-d; Spearmen correlation for alfalfa: R=0.73, p-value < 0.001, n=2056 ; maize: R=0.77, p-value < 0.001, n=1314) under FVS processing. Previous studies have shown that Fluxpart results coincide with agricultural flux trends that are expected when accounting for vegetation dynamics and harvesting (Wagle et al., 2020), which was also observed in this study despite the magnitude of these fluxes differing from EddyPro/REddyProc methods. Fluxpart is dependant on leaf WUE estimates, which is frequently not available and therefore estimated by the program (Anderson et al., 2017; Klosterhalfen et al., 2019a; Palatella et al., 2014; Sulman et al., 2016). Perez-Priego et al. (2018) tested a few LWUE parameterization scenarios using FVS partitioning and found that component fluxes can be biased by up to 30% based on the accuracy of estimated internal leaf-to-ambient CO_2, with the poorest performing model being the one that used a constant for internal leaf-to-ambient CO_2 concentration. In this study, internal leaf CO_2 was assigned a default constant, based on photosynthetic pathway (Skaggs et al., 2018), which may have been partly responsible for the inconsistencies between $EWUE_S$ and $EWUE_{F.}$

The magnitude of disagreement between these two programs also differed between crops. $EWUE_S$ ranged from 21-26% of corresponding median half-hourly $EWUE_F$ for each cut of alfalfa but was 30-34% of corresponding median half-hourly $EWUE_F$ estimates during GS2-4 in maize. This could be a limitation in FVS partitioning since it has been reported elsewhere that lower measurement height-canopy height ratio improves results produced, while immature shorter stature plants tend to reduce accuracy (Klosterhalfen et al., 2019a). This is further supported by this study since Fluxpart was unable to produce consistent estimates in the early growing season of maize (n=258), particularly before sprouting was observed (n=19).



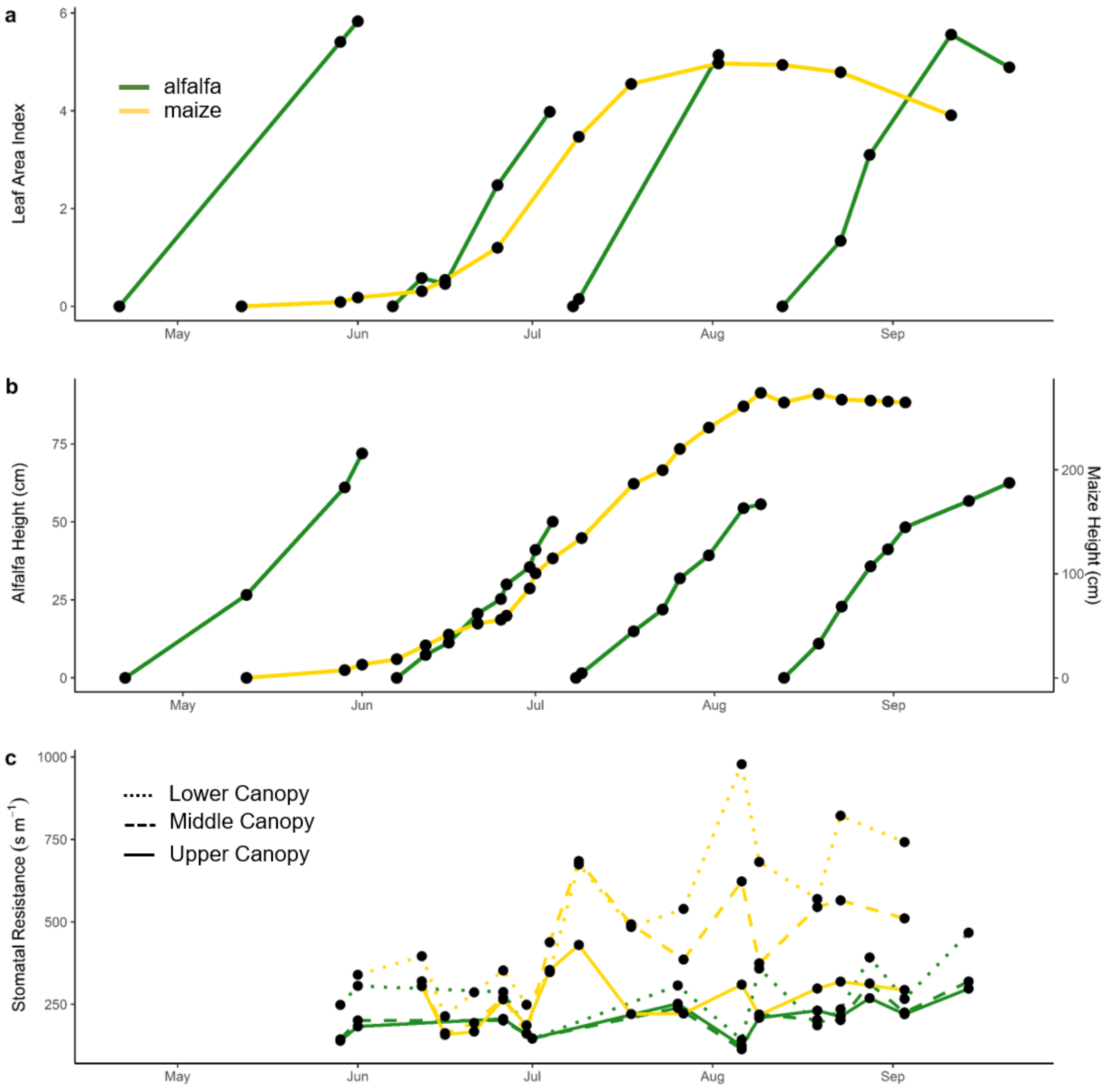
*Line of best fit and 1:1 line were included where R^2 = 0.75 (a, n=2104), R^2 = 0.74 (b, n=1304), R^2 = 0.28 (c, n=2056), R^2 = 0.50 (d, n=1314), R^2 = 0.75 (e, n=2065), and R^2 = 0.69 (f, n=1315). Only half-hour intervals where a value for both flux components or EWUE values existed prior to gap-filling were used.

Figure 3.3 Half-hourly evapotranspiration from EddyPro processing ($ET_{S)}$ versus Fluxpart processing (ET_F; a, b), gross primary productivity from EddyPro/REddyProc processing (GPP_S) versus Fluxpart processing (GPP_F; c,d), net ecosystem exchange from EddyPro processing (NEE_S) versus Fluxpart processing (NEE_F; e, f), and ecosystem water use efficiency from EddyPro/REddyProc processing ($EWUE_S$; mg C g^{-1} H_2O) versus Fluxpart processing ($EWUE_F$; mg C g^{-1} H_2O) for alfalfa (green; a, c, e, g) and maize (yellow; b, d, f, h) over the 2018 growing season.

3.2.3 Impact of Physiological Stage and Management Influences on Growth on WUE methods at both seasonal and shorter timescales

While input variable selection is responsible for differences between WUE estimation methods, crop physiological components drive inconsistencies between these calculations. WUE observed at different time scales and the discrepancies between the methods are going to differ due to physiology and farming practices, which influence crop growth. Thus, the timescales at which WUE is quantified can result in inconsistencies between methods, and these can differ from what is observed at the seasonal or growing season timescale. For example, growing season $EWUE_S$ was greater in alfalfa whereas median half-hourly $EWUE_S$ was greater in maize (Table 3.2). Since alfalfa is a perennial plant in its third year, it began growing immediately following snowmelt, which promoted a longer growing season (22 days longer than maize). This resulted in greater seasonal EWUE estimates since growing crops under cooler conditions can improve WUE due to lower evaporative demand (Craufurd et al., 1999; Sinclair et al., 1984). Maize, on the other hand, took a while to establish after planting, but did experience higher median $EWUE_S$, which is likely attributed to C_4 plants containing greater WUE at higher temperatures (Ehleringer et al., 1997; Osborne and Sack, 2012). At lower temperatures, the energy costs of trapping the carbon internally are greater than the photorespiratory costs making the C_4 pathway less efficient (Edwards et al., 2010). Over the course of the growing season, this C_4 pathway advantage did not overcome that of alfalfa due to its earlier start in cooler conditions, but did result in greater median half-hourly $EWUE_S$.

The time in the growing season that measurements are made is also relevant due to physiological mechanisms in the crops considered. This is a result of physiological changes at different growing stages in crops, which is especially apparent for single harvest crops such as maize. Alfalfa, however, did not experience drastic growth stage changes since it is a perennial crop and multiple harvests interrupting lifecycle. As such, this study divided alfalfa into different cuts, which contained relatively steady growth rates (Figure 3.4b; 1.5-1.6 cm day^{-1} average per cut) while maize was divided by growth stages, which exhibited drastically different growth rates (Figure 3.4b). These growth rates were 1.9 cm day^{-1}, 5.7 cm day^{-1}, and 4.0 cm day^{-1} for GS1-3, respectively. Growth stage 4 did not experience height growth since it was directing growth into reproductive organs and experienced senescence (see LAI; Figure 3.4a). This complicates direct comparison of HWUE methods for sub-periods in the growing season, whereas EWUE is based on meteorological measurements and can therefore be more easily compared.



*The left y-axis in 3.6b presents alfalfa heights while the right y-axis presents maize heights. Stomatal resistances were divided into upper canopy, middle canopy, and lower canopy. The sample sizes for dates (dots) were 29 and 25 for alfalfa and maize height, respectively, 16 and 12 for alfalfa and maize LAI, respectively, and 12 and 15 alfalfa and maize stomatal resistances, respectively.

Figure 3.4 Leaf area index (a), height (b), and stomatal resistance (c) of maize and alfalfa sites across the 2018 growing season.

Naturally, alfalfa and maize have different growth patterns due to their different maturation rates, sprouting times, and since one is annual (maize) and the other perennial (alfalfa). This impacts our ability to compare different WUE methods. For example, there were differences observed between alfalfa cuts within the season, but these differences were consistent when comparing harvested versus ecosystem WUE techniques ($HWUE_{ET}$ was 22-29% of $EWUE_S$ during each cut; Table 3.2). Alfalfa underwent four cuts during the growing season and as a result it did not experience a reproductive phase or stem thickening, whereas maize is an annual crop that has a stem that thickens throughout the season and undergoes biomass accumulation into reproductive components. Alfalfa biomass accumulation patterns were consistent between cuts, which led to consistency in carbon use efficiency (conversion of assimilated carbon into biomass). In turn, this resulted in the differences between $HWUE_{ET}$ and $EWUE_S$ also being consistent across cuts.

In contrast, there were differences in trends for the growth stages of maize and these differed based on the WUE method used. Maize is an annual crop with prolonged pre-sprouting period, followed by rapid growth, tasseling and ear development. In contrast to alfalfa, maize undergoes stem elongation, thickening and development of reproductive components. Additionally, maize established a root system during early growing season while alfalfa contained an established rooting system from previous years. This results in discrepancies between EWUE and HWUE since the relationship between GPP and above ground biomass accumulation is not consistent across the season. Thus, the two methods provide inconsistent results for differences between EWUE and HWUE throughout the season, when smaller timescales within a season are used. For example, during GS1, estimates of $HWUE_{ET}$ were 80% lower than of $EWUE_S$ estimates (Table 3.2). In subsequent growth periods, this gap decreased and during GS4, $HWUE_{ET}$ estimate was 23% greater than $EWUE_S$. This makes it more challenging to compare WUE during different growth stages for maize when studies use different WUE methods. This is seldom an issue as most studies simply look at growth season harvesting and seldom explore growth stage; however, it was shown earlier that the maize and alfalfa experienced different discrepancies between these methods when considering the growing season as well. If HWUE methods are used for different stages of the growing season, they should be used with caution.

For EWUE methods, $EWUE_F$ (uses ET) and $EWUE_C$ (uses T) are compared for the different stages of alfalfa and maize. Discrepancies were observed between these methods for alfalfa, but varied among the four cuts throughout the growing season. More specifically, median half-hourly $EWUE_C$ was 73%, 57%, 23% and 43% greater than $EWUE_F$ for cuts 1-4, respectively (Table 3.2). The variability between these two methods is likely caused by crop physiological responses to changes in temperature and microclimate conditions. At higher temperatures in C_3 photosynthesis, greater CO_2 losses occur since rubisco fails to distinguish between CO_2 and O_2 (Edwards et al., 2010). The uptake of both CO_2 and O_2 therefore results in higher photorespiration, which in turn results in greater transpiration losses (Osborne and Sack, 2012). This was seen in T:ET ratios, where there was higher T:ET ratios later in the season whereas cut 1 experienced lower T:ET ratio.

Although $EWUE_C$ was always greater than $EWUE_F$, the magnitudes of the differences between the 2 methods were smaller in maize than were observed for alfalfa. Some of this occurred because the FVS partitioning program (Fluxpart) did not produce frequent estimates when maize was in its early growing season when the difference between these two methods was expected to be greatest, and is therefore a limitation of this study. However, half-hourly $EWUE_S$ indicates that there should be a large increase in median half-hourly $EWUE_F$ between GS1 and GS2 that is not captured by this data limitation. Moreover, since crop heights were lower, and soil was exposed (not shaded) due to planting in rows, and evaporation components of ET would have been much higher than T during GS1 (Todd et al., 1991). A previous study has shown that reducing spacing between rows, enabling earlier canopy closure, can increase EWUE by up to 17% (Barbieria et al., 2012). Following canopy development, less soil is exposed and differences between the two methods are lessened (Hatfield and Dold, 2019; Todd et al., 1991). Moreover, higher leaf coverage results in more T per unit area (Ritchie, 1972). Thus, the difference between these two methods should have been lower during GS2 than GS1, when it was approximately 32%, and even smaller during GS3 (17%) when it reached maximum height and LAI (Figure 3.4). However, differences increased again during GS4 (31%) due to self-shading, and deterioration of leaves due to aging and senescence. As LAI increases, more light is intercepted; however, this can also have self-shading effects on lower canopy positions, where self-shading can be the dominant factor in determining the rate of change in photosynthesis and T, which are affected disproportionally (Hatfield and Dold, 2019). This is complicated by leaf aging, which can cause decreases in WUE due to shifts in stomata conductance (Lin and Ehleringer, 1982; Warren, 2006; Wullschleger and Oosterhuis, 1989) explaining the larger variance in GS4. Comparatively, alfalfa did not experience as much soil exposure due to random sowing techniques or leaf aging effects due to multiple harvest practices.

3.3 CONCLUSIONS

This study quantified growing season WUE of maize and alfalfa crops, which resulted in higher harvest WUE (HWUE) estimates in maize but higher ecosystem WUE (EWUE) in alfalfa. In addition, it shows the importance of input variable choice, as well as the timescale over which WUE is determined, when interpreting WUE due to the inconsistencies observed between these methods. Patterns observed between methods do not necessarily correlate because above ground biomass and GPP estimates used as WUE calculation inputs are influenced differently by plant physiology and human influences over crop development. For example, above ground biomass carbon content did not correlate with GPP due to differences in photorespiration associated with C_3 and C_4 photosynthetic pathways, and allocation of carbon to below ground carbon storage. Similarly, ET and T are influenced disproportionately by plant physiology and management influences over crop development due to differences in soil exposure, canopy development, aging effects, and growth rates. This was a result of differences in physiology between species, maize being planted in rows, and alfalfa undergoing four cuts throughout the season. Furthermore, two input-data processing methods were used in this study to produce the same flux-based WUE calculation, which provided substantially different results and results using the same method showed that the timescale data is collected from is important when making comparisons. Thus, inter-method comparisons of WUE calculations should be done with caution. Due to the complexity of physical and physiological mechanisms involved in these WUE estimates, it is recommended that future studies focus on the individual water use and carbon uptake variables rather than the seemingly arbitrary WUE measure.

The results of this study affirm that hastened canopy development is important to reducing water losses from evaporation by reducing soil exposure (Todd et al., 1991), which could be important under strained water resources and work to increase canopy EWUE. This is particularly important for row-crops, such as maize, which have larger evaporation due to the exposure of bare soil in rows gaps. Selecting crops that undergo multiple harvests, such as alfalfa, could reduce senescence effects and increase ecosystem and canopy level EWUE, especially under lengthened growing seasons. However, this does not equate to improved HWUE since GPP and AGB were not well correlated. This complicates future agricultural responses to changing climate since the results of this study suggest that selecting crops which accumulate greater biomass, such as maize, would be beneficial to optimize HWUE. This highlights the need for a more robust approach to quantify water resource use which accounts for both plant (stomata) responses (GPP, T), abiotic field responses (ET), and agricultural production (AGB). Future research into the agricultural water use resources must consider all three of these components to promote a holistic approach to climate change agricultural adaptability.

CHAPTER 4: CONCLUSIONS AND LIMITATIONS

Chapter 2 quantified the plant carbon-water dynamics of maize and alfalfa crops in southern Ontario, Canada, and investigated environmental drivers of observed differences in daily evapotranspiration (ET), gross primary productivity (GPP), net ecosystem exchange (NEE), and ecosystem water use efficiency (EWUE; GPP/ET). It was suggested that future crop selections, which may be subjected to longer growing seasons, should consider more efficient crop resource use. This can be promoted by perennial crops that take less time to establish and have multiple harvests within a growing season. Chapter 3 quantified WUE of alfalfa and maize according to four different equations and investigated inconsistencies between these methods. Results of this chapter show that WUE according to one calculation may not correlate to WUE produced by another equation due to inherent differences in crop species and management influences on growth. This suggests that conclusions drawn from one calculation may not correspond with conclusions from another: thus, highlighting the need for a more robust approach to agricultural carbon-water relations.

The information presented in this thesis works to further our understanding of water use efficiency (WUE) in rainfed croplands. More specifically, it addresses knowledge gaps wherein there was a lack of field-scale studies, which investigated differences between crop species due to physiology and farming practices influencing plant growth. It showed that C_3 pathway plants could be more efficient than typically more efficient C_4 plants. Moreover, it showcased how plant physiology and farmer practices influencing plant growth can impact different calculations of WUE and that these metrics do not always agree with one another. Through progressing the knowledge of current WUE dynamics and drivers of differences between crop WUE, this research can aid in adapting to climate change. For example, if growing seasons expand because of increased surface temperatures, the results of this thesis suggest that making crop selections that establish earlier in the season and provide multiple harvests throughout the season could be beneficial to water resource use. In addition, the results of this thesis show that different metrics of WUE provide different insight into crop resource use and suggests that there needs to be a more well-rounded approach to quantifying resource use which accounts for carbon assimilation, biomass production and all components of the water cycle.

There were two main sources of limitations to the study in chapter 2: (1) it only considered one growing season of the perennial alfalfa crop; and (2) continuous soil data was collected between the field and riparian zone at each site. Multi-year studies of alfalfa have shown that alfalfa production year-to-year increases in productivity from the first-second-third years (c.f Skuodienė and Tomchuk, 2015), which was not accounted for in this single year study. In addition to the limitations outlined for chapter 2, there were limitations to this study since a leaf-level WUE (LWUE) was calculated using a default internal leaf carbon dioxide (CO_2) concentration in Fluxpart. Other research has indicated that Fluxpart is sensitive to LWUE and using the default constant internal leaf CO_2 concentration can cause variations of up to 30% (Perez-Priego et al., 2018). In addition, gap-filling was not possible for Fluxpart outputs since gaps were too large and the early growing season was almost entirely missing in maize, which meant it was not possible to compare $EWUE_S$ and $EWUE_F$ across the entire growing season. Furthermore, the results of this research did not consider nutritional components, which can influence WUE (Raven et al., 2004) or other benefits such as crop rotations or crop specific benefits such as nitrogen fixing or soil aeration. Future research is needed to address these limitations as well as other factors which could influence plant carbon-water dynamics. For example, differences in maize heights were observed along a hillslope, though it is unclear what caused this to occur (supplementary material).

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APPENDIX

6.1 Chapter 2 Supplementary Graphs

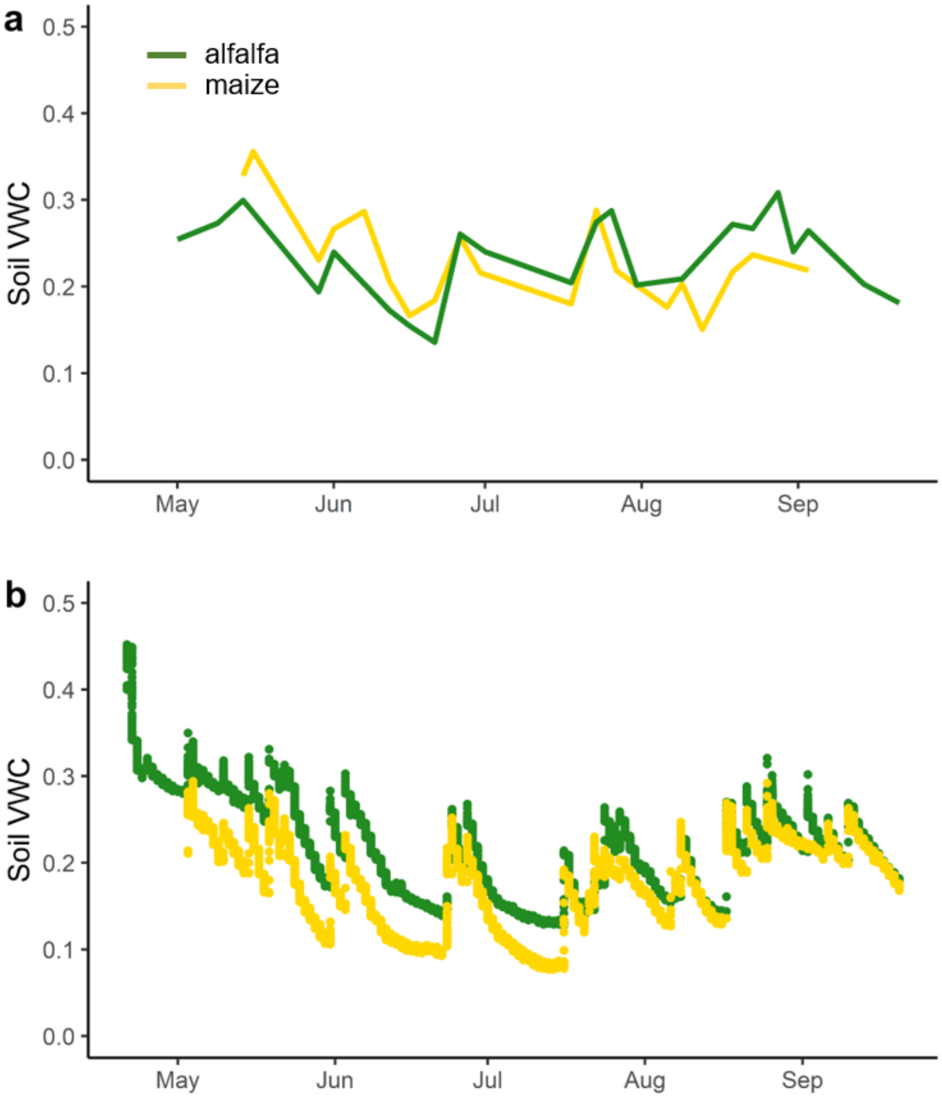


Figure 6.1 Within field surface soil volumetric water content (Soil VWC) (a) validation that soil moisture measurements at 5 cm depth of meteorological station (b) are representative of fields.

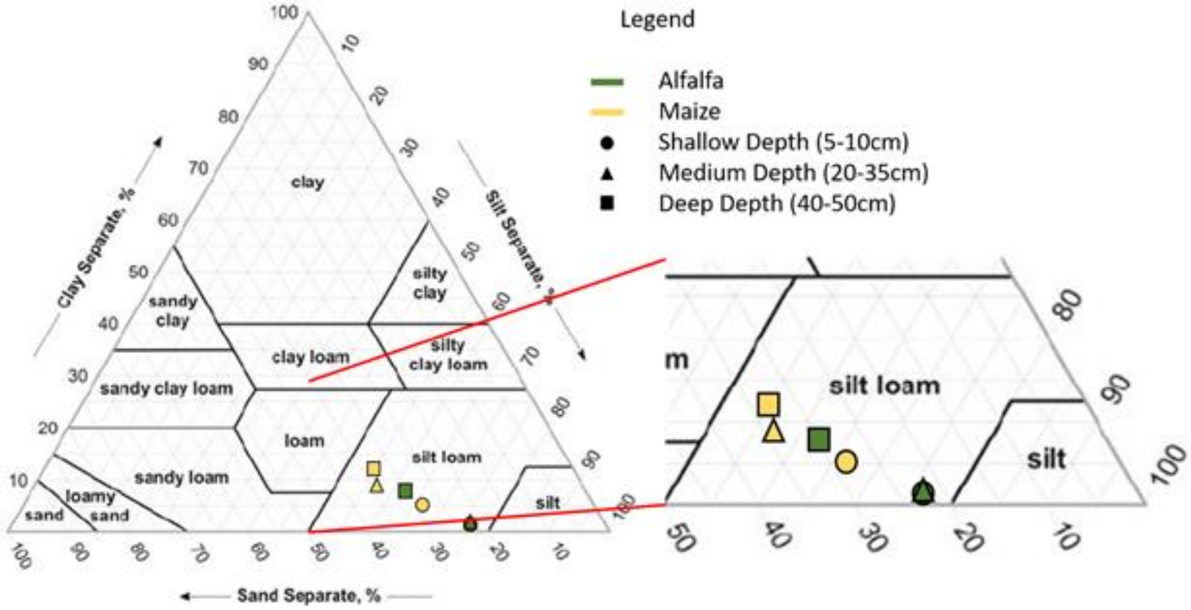


Figure 6.2 Soil texture classification for maize and alfalfa sites at shallow (5-10 cm), medium (20 - 25 cm for alfalfa; 25 - 35 cm for maize) and deep (40 - 50cm) depths, according to the Canadian Soil Classification System. (n=4 per point/symbol).

Table 6.1 Soil texture analysis summary for alfalfa and maize fields at different depths.

Depth	**Texture**	**Alfalfa**	**Maize**
5-10cm	Clay (%)	1.0 ± 2.0	5.0 ± 4.2
	Silt (%)	75.8 ± 6.3	66.8 ± 8.0
	Sand (%)	23.3 ± 5.5	28.2 ± 5.5
20-30cm[a]	Clay (%)	0.8 ± 2.0	7.1 ± 5.8
	Silt (%)	75.4 ± 8.6	58.1 ± 18.3
	Sand (%)	23.8 ± 9.4	34.8 ± 13.7
40-50cm[b]	Clay (%)	7.6 ± 5.1	12.7 ± 7.1
	Silt (%)	62.9 ± 12.4	54.4 ± 16.5
	Sand (%)	25.9 ± 29.4	33.0 ± 11.1

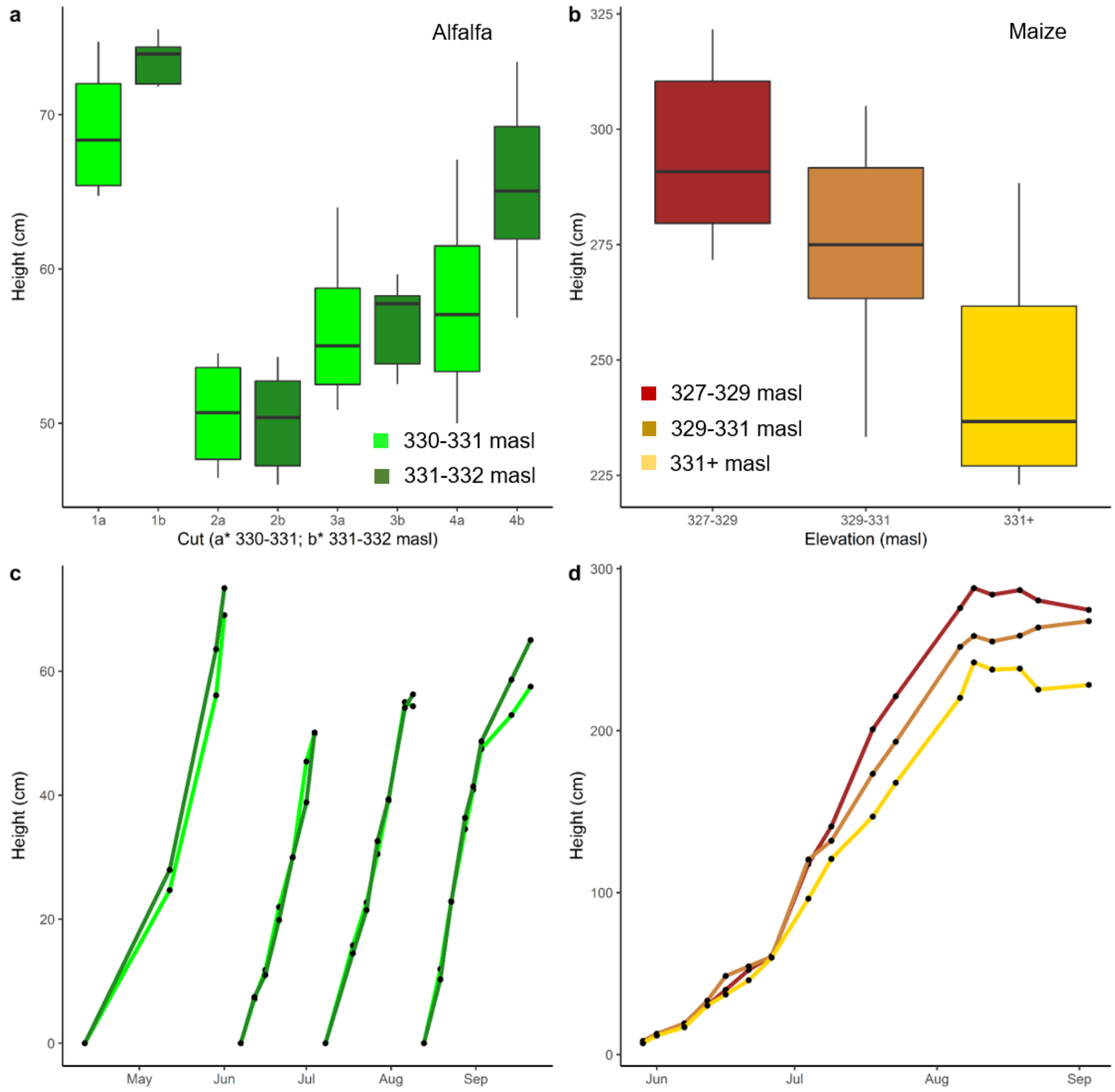
* Values are the average ± standard deviation of measurements

[a] Three maize samples were taken from 25 - 30 cm and one sample was taken from 30 - 35 cm. Alfalfa soil cores collected from 20-25 cm as there was no change in appearance.

[b] Maize was collected from 45-50cm while alfalfa was collected from 40-45cm.

6.2 Spatial Heterogeneity of Vegetation and Surface soil moisture regimes at research sites

Spatial variation in vegetation and soil were assessed along the established transects. Maximum plant heights vs elevation was plotted for each 10 m interval along the transects wherein there was no significant relationship between elevation and maximum heights of alfalfa cuts (Figure 6.1a). Maize maximum heights, however, showed a negative linear relationship (R^2=0.50, p-value=0.0002, n=22), where maximum crop height decreased with increasing elevation (Figure 6.1b). The 10 m intervals along transects were grouped by similar elevations and average heights were plotted across the entire growing season. Alfalfa height did not differ based on elevation categories except for cut 4 starting 14 September when alfalfa plants at elevation 331-332 masl were slightly shorter than plants at elevation 330-331 masl (Figure 6.1c). At the maize site, crop height was impacted by elevation beginning in late June, when crops located at elevations greater than 331 masl were shorter than other elevations (Figure 6.1d). This continued for the remainder of the growing season, with crops located at 329-331 masl containing significantly shorter height than plants located between 327-329 masl beginning mid-July.

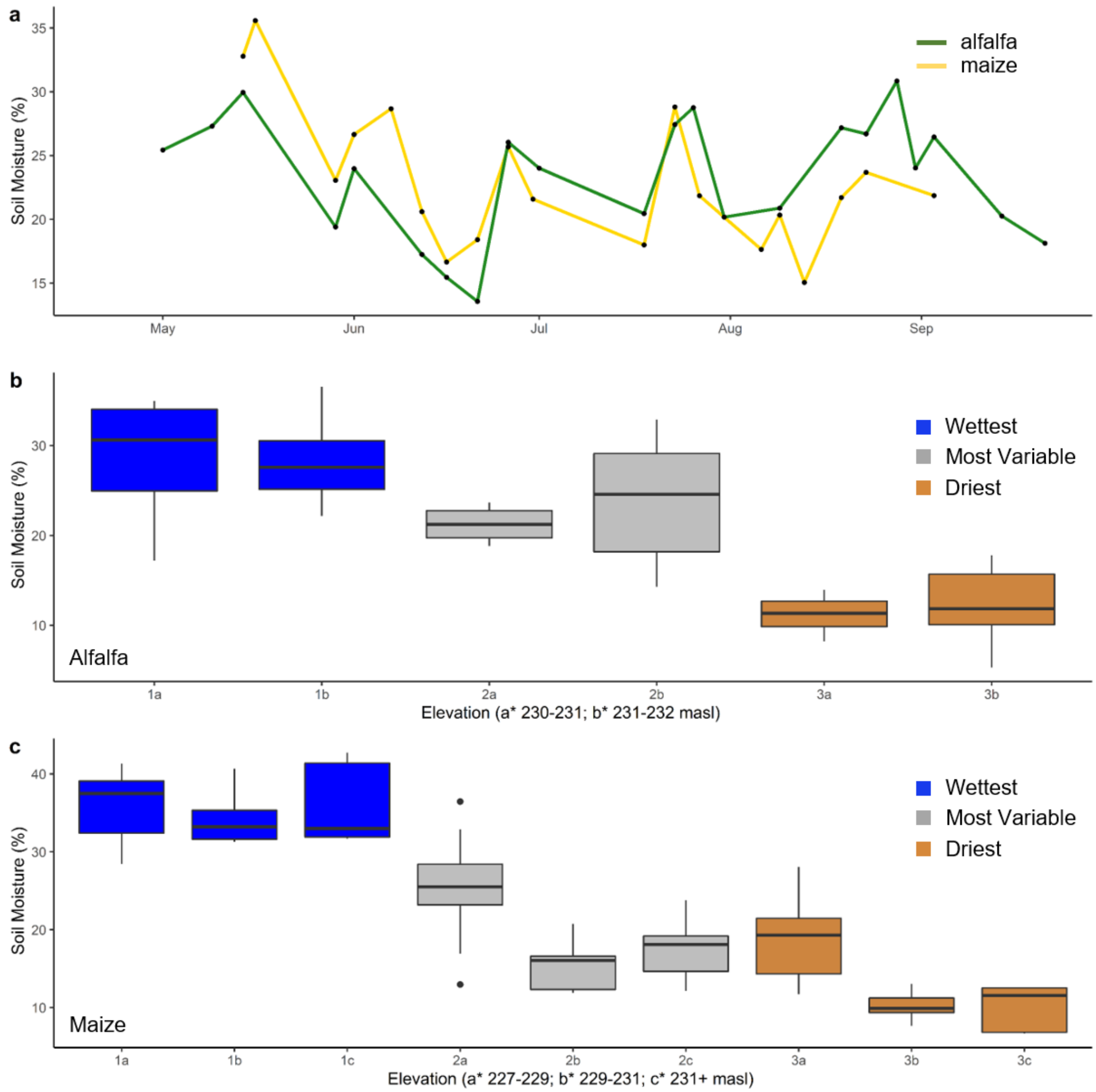


*Elevation categories for alfalfa were 330-331 masl (n=4) and 331-332 masl (n=8) while maize elevation categories (d) were 327-329 masl (n=12), 329-331 masl (n=5), and 331+ masl (n=4).

Figure 6.3 Maximum crop height for alfalfa (a) and maize (b) by elevation, and crop height by elevation category for alfalfa (c) and maize (d) over the 2018 growing season.

Surface soil moisture from manual within field measurements did not vary substantially based on elevation at the alfalfa site during wettest (28 August 2018) or driest (21 June 2018) measurement days. The date with the highest standard deviation in soil moisture (most variable in figure 6.2b,c) for alfalfa (19 August 2018) was also similar. In contrast, although there were similar soil moistures at differing elevations on the wettest day for maize (16 May 2018), both the driest (13 August 2018) and most variable (27 July 2018) soil moisture dates had negative linear relationships with elevation (Driest: R^2=0.47, p-value=0.0002, n=22; Variable: R^2=0.41, p-value=0.0006, n=22; Figure 6.2c). The maize most variable day occurred after several days of rainfall, which was preceded by a period of low precipitation.

Overall, there was no topographical differences in soil moisture or vegetation height at the alfalfa site, but significant differences were observed at the maize site. The affect of slope on maize growth aligned with research elsewhere that found decreases in height with increasing location along the slope (Changere and Lal, 1997). In Changere & Lal (1997), they found that maize height was 13.5% greater at lower slope positions ten weeks after planting along a 5-6% slope (compared to 3-4% on south transect, 5% on north transect). Furthermore, they found that yields were 3.14-3.70 times greater in these lower positions during dry years. Hillslope moisture has been observed by others to contain more variability under dry conditions (Zhao et al., 2015) due to runoff. Lower near surface soil moisture at higher elevations along the slope during drier conditions was evident in this study. However, it was not possible to assess spatial variability soil moisture regimes at deeper soil depths. Moreover, soil moisture was higher at 25 and 50 cm depths and may not have been limiting or affected by slope and other factors such as differences in light interception could have played a role as well. As such, it is recommended that future studies focus on the potential influence of slope on plant carbon-water dynamics which was beyond the scope of this thesis.



*Elevation categories for alfalfa were 330-331 masl (n=4) and 331-332 masl (n=8) while maize elevation categories (d) were 327-329 masl (n=12), 329-331 masl (n=5), and 331+ masl (n=4).

Figure 6.4 Average Delta T WET-2 Sensor within field soil moisture for the surface 7 cm over the 2018 growing season (a), and soil moisture by elevation category for the wettest, driest and most variables soil moisture records for alfalfa (b) and maize (c).