Controls on Light Inhibition of Leaf Respiration and Water Use Efficiency in a Temperate Broadleaf Deciduous Forest

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Abstract

Water and carbon exchange between the land and atmosphere plays a crucial role in the functioning of ecosystems around the globe. In order to predict how ecosystems will respond to climate change, it is important to understand how they respond to current variation in environmental drivers. Eddy covariance (EC) is a technique that is used extensively to measure net fluxes of carbon dioxide and water vapour. These flux measurements are often partitioned in order to obtain values of the constituent fluxes of photosynthesis, respiration, transpiration, and direct evaporation, which are used to verify Earth-system models. The methods most commonly used to partition carbon and water vapour fluxes are based on assumptions that do not hold under a variety of conditions. In the case of carbon dioxide, the light inhibition of leaf respiration is not taken into account, which results in photosynthesis and respiration fluxes being underestimated in the early part of growing seasons. In the case of water vapour, the most common assumption is that of evaporation being negligible after a few days without rainfall, which can lead to an overestimation of transpiration. In this work I utilise techniques that avoid these assumptions in order to investigate the seasonality of leaf respiration and the response of water use efficiency (WUE)—defined as the ratio of photosynthesis to transpiration—to environmental controls, at a forested site in central Virginia.

I quantify leaf respiration at the ecosystem scale by fitting light response curves to net photosynthesis flux values obtained using the flux variance similarity partitioning method, and find it to be larger in the first half of the growing season than the second, while the sensitivity of leaf respiration to light remains constant throughout the growing season. I measure WUE entirely independently of EC using solar induced chlorophyll fluorescence (SIF), sap flow, and a thermal camera, and compare the results to values derived from EC. There is poor agreement between the two methods, but this work stands as a proof of concept that can be improved upon in future research. I also investigate the relationships of leaf respiration and WUE with the environmental controls of temperature and water availability. Unlike temperature, I find soil water content has no effect on leaf respiration or the sensitivity of leaf respiration to light. When calculated using SIF and sap flow, WUE decreases in magnitude as soil water content increases, but I find no relationship when using WUE values derived from EC measurements. The effect of vapour deficit on WUE differs depending on the measurement method.

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Chapter 1: Introduction

Temperate forests are one of the largest terrestrial carbon sinks globally and play an important role in global water cycles due to their large evapotranspiration fluxes (Falge at al., 2002; Pan et al., 2011; Sanford & Selnick, 2013). Net ecosystem carbon dioxide (CO_2) and water vapour fluxes (F_c and F_q respectively) exchanged between ecosystems and the atmosphere are routinely measured through the use of the eddy covariance technique at locations around the world (Baldocchi, 2008; 2014). F_c is made up of ecosystem respiration and photosynthesis, while F_q is comprised of direct evaporation and transpiration. It is not possible to make direct measurements of each of these components at the ecosystem scale, so to obtain flux values for each of the contributing processes, the measured fluxes must be partitioned. The results of partitioning are often used to validate and improve process-based Earth-system models and remote sensing-based estimates of ecosystem function. These models are used to provide forecasts on how terrestrial ecosystems will respond to and affect a changing climate (Keenan et al., 2019; Stoy et al., 2019).

Both water availability and temperature are drivers of F_c and F_q, and are predicted to alter across the majority of the globe, including in the southeastern United States, as a result of climate change (Mearns et al., 2003; Mitra et al., 2018; Seager et al., 2009; Strzepek et al., 2010; Williams et al., 2013; van der Molen et al., 2011). With the important role that temperate forests play in carbon and water cycles, it is essential to understand how these changes will impact F_c and F_q in the future. In order to do this, it must first be understood how ecosystems respond to these drivers at their current range.

To date, a majority of studies that use eddy covariance derived fluxes have partitioned using a small subset of available methods. One of the drawbacks of often used CO_2 flux partitioning methods (e.g. Reichstein et al., 2005; Lasslop et al., 2010) is that they rely heavily on the temperature-respiration relationship being unchanging between the daytime and night-time, and over a range of timescales. This assumption does not account for the light inhibition of leaf respiration, known as the Kok effect. The Kok effect has been shown to result in daytime ecosystem respiration being lower than night-time ecosystem respiration during the early stages of the growing season in temperate deciduous forests, despite average temperatures being higher during the day (Keenan et al., 2019; Wehr et al., 2016). Wehr et al. (2016), and subsequently Keenan et al. (2019), characterised the relative size of the resulting overestimation by comparing the results of their less widely used partitioning methods with those of the more popular techniques, and described how their findings differ to widely accepted models of diel and seasonal CO_2 flux cycles. Building on this work, in Chapter 2 of this thesis I use a partitioning method that makes no assumptions about the light inhibition of leaf respiration to quantify leaf respiration at the ecosystem scale for the first time. I find that leaf respiration is higher in the first half of the growing season than the second. I also describe the seasonality of leaf respiration and the Kok effect, and investigate how each responds to temperature and soil water content, environmental variables known to influence respiration rates.

Water use efficiency (WUE) is a measure of how much carbon dioxide is fixed by plants through photosynthesis per unit of water lost through transpiration, and therefore links the carbon and water cycles of an ecosystem (Beer et al., 2009). WUE is most often calculated at the ecosystem scale through the use of partitioned fluxes measured using the eddy covariance technique (e.g. Moncrieff et al., 1997; Beer et al., 2007; Beer et al., 2009; Lu & Zhuang, 2010; Ruehr et al., 2012; Keenan et al., 2013). In addition to the assumptions made in order to perform CO_2 flux partitioning, further assumptions are required for the partitioning of F_q into evaporation and transpiration. For example, it is often assumed that the contribution of evaporation to F_q is negligible when there has been no precipitation for a couple of days. Whether this assumption holds depends upon ecosystem type and the climatic conditions at the location (Stoy et al., 2019). In other partitioning techniques, WUE is used as a basis for partitioning of both water vapour and CO_2 fluxes (Scanlon et al., 2019; Stoy et al., 2019). To date, there have been few attempts to validate flux partitioning derived values of WUE. In Chapter 3 I calculate a proxy for WUE using a novel combination of measurement techniques entirely independent of eddy covariance measurements, and compare the results to values derived from eddy covariance measured fluxes. I also investigate the relationship between WUE and water availability, both in the form of soil water content and canopy vapour deficit.

In Chapter 4 I provide a summary of this work and discuss implications of my findings. I also suggest potential future research avenues.

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Chapter 2: Quantifying the Seasonality of Light Inhibition of Leaf Respiration

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Abstract

Photosynthesis and respiration are the processes through which terrestrial ecosystems exchange carbon dioxide with the atmosphere. Accurate prediction of climate change therefore relies on understanding the controls on these fluxes. It is not possible to measure these fluxes directly, so they must be partitioned from net carbon dioxide flux measurements. Recent studies have shown that standard partitioning methods do not accurately capture the seasonal dynamics of the two carbon fluxes, as they fail to account for the light inhibition of leaf respiration, known as the Kok effect. In broadleaf deciduous forests, the Kok effect has been shown to inhibit daytime ecosystem respiration by up to 30% in the early growing season, before reducing to a lower level in the later growing season. However, the seasonal dynamics of leaf respiration and its inhibition in the light remain unclear, as it is yet to be isolated from other contributing sources to total ecosystem respiration. Here, light response curves of photosynthesis are used to quantify leaf respiration at the ecosystem scale, and provide insight into how the behaviour of the Kok effect varies seasonally in a temperate broadleaf deciduous forest. It is found that leaf respiration is higher in the first half of the growing season than the second, while the potency of the Kok effect remains the same, indicating that the higher percentage of inhibition in the early growing season is simply due to there being more leaf respiration to inhibit. It is suggested that leaf respiration may play a more significant role in ecosystem carbon cycles that previously thought. Environmental controls on leaf respiration are also investigated; unexpectedly, leaf respiration and its sensitivity to the Kok effect are found to have negative correlations with air temperature, while soil water content has no impact. The findings of this study add a further level of detail to the current understanding of seasonality of land-atmosphere carbon exchange.

1. Introduction

Net ecosystem carbon dioxide (CO_2) fluxes (F_c) are routinely measured in a variety of environments, including temperate forests, through the use of the eddy covariance technique (Baldocchi, 2008; 2014). F_c is the difference between the positive (upward) flux due to respiration and the negative (downward) flux of photosynthesis. It is not possible to make direct measurements of these flux components at the ecosystem scale, so to obtain them, the net CO_2 flux must be partitioned. The results of partitioning are often used to validate and improve process-based Earth-system models and remote sensing-based estimates of ecosystem function (Keenan et al., 2019).

There are two commonly used carbon flux partitioning methods (Keenan et al., 2019). One uses night-time fluxes, comprised of respiration only, to develop a respiration-temperature relationship, which is then extrapolated to daytime temperatures (Reichstein et al., 2005). The second method fits light response curves to daytime F_c, and night-time respiration is estimated from the ordinate intercept. A respiration-temperature relationship is then developed to estimate daytime respiration (Lasslop et al., 2010). In both methods, once daytime ecosystem respiration has been estimated, F_c can be subtracted from it to find ecosystem photosynthesis (gross ecosystem productivity, GEP). It is important to note that both partitioning methods assume respiration is a function of temperature only. Recent studies have shown that these methods may not capture the diurnal and seasonal cycles of photosynthesis and respiration due to them not taking into account the light inhibition of leaf respiration, known as the Kok effect (Keenan et al., 2019; Kok, 1949; Wehr et al., 2016). The Kok effect has been found to occur at the leaf level in many plant species, and can inhibit up to 100% of leaf respiration (Heskel et al., 2013). Using an isotopic partitioning approach in a temperate broadleaf deciduous forest, Wehr et al. (2016) found that failing to account for this phenomena may lead to an overestimation of both GEP and daytime ecosystem respiration, especially early in the growing season. Using standard partitioning techniques, GEP and daytime respiration is observed to peak early in the growing season and then steadily decline. Contrary to this, Wehr et al. (2016) found that GEP remained relatively constant throughout the growing season, and daytime ecosystem respiration was lower during the first half of the growing season than the second. In addition, partitioning using the isotopic method showed that the response of GEP to absorbed photosynthetically active radiation (APAR) remained stable throughout the growing season, whereas standard partitioning methods showed a decrease between the first and second halves (Wehr et al., 2016).

Keenan et al. (2019) quantified light inhibition of leaf respiration by taking the difference between daytime and night-time respiration calculated using each of the commonly used partitioning techniques. When applied to data from multiple FLUXNET sites in a variety of ecosystem types, this method revealed widespread inhibition, the magnitude of which differs between ecosystem types. This suggests that global photosynthesis and respiration fluxes may be overestimated.

Both Wehr et al. (2016) and Keenan et al. (2019) focussed on Harvard Forest for at least part of their studies. While the diurnal CO_2 flux cycles they found agree to a certain extent, there are large margins of error associated with the isotopic flux partitioning method, and there are various uncertainties to be considered with the approach of Keenan et al. (2019). These uncertainties could be at least partly addressed by comparing results between partitioning methods based on different assumptions.

Carbon and water cycles are linked through stomatal and non-stomatal processes. Stomata are small pores on a leaf surface that mediate gas exchange between the inside of a leaf and the outside environment. They allow CO_2 to enter a leaf down a concentration gradient, and the carbon is then assimilated through carboxylation. Water vapour simultaneously follows a concentration gradient out of the leaf, and is lost as transpiration (Berry et al., 2010). The non-stomatal processes of ecosystem respiration and evaporation are also linked, though on longer timescales. Reductions in water availability, and therefore evaporation, as a result of drought, can lead to reduced ecosystem respiration (Van der Molen et al., 2011). Flux variance similarity (FVS) based partitioning relies on the correlation of non-stomatal fluxes, stem and soil respiration and evaporation (F_r and F_e respectively), and the anti-correlation of stomatal fluxes, net photosynthesis and transpiration (F_p and F_t respectively) (Scanlon et al., 2019). This theoretical basis makes no assumptions about light inhibition of leaf respiration. Net photosynthesis is defined as carboxylation minus photorespiration and leaf respiration (often referred to as "dark respiration" or "mitochondrial respiration") (Wohlfahrt & Gu, 2015). Due to the subtraction of leaf respiration, net photosynthesis takes into account the effect that light inhibition will have on CO_2 flux through the stomata. F_p and F_r calculated using FVS are smaller than GEP and ecosystem respiration calculated using standard partitioning approaches, with the difference being leaf respiration (Scanlon et al., 2019). Application of the FVS technique may allow further analysis of the seasonality of photosynthesis and daytime respiration, and what impact the Kok effect has on this.

The objectives of this work are to (1) quantify leaf respiration at the ecosystem scale, (2) explore how the Kok effect impacts the seasonality of leaf respiration and photosynthesis, and (3) determine the effect of environmental variables on leaf respiration and photosynthesis at the ecosystem scale. This will be the first example of leaf respiration being isolated from all other respiration fluxes and quantified at the ecosystem scale. The intent of this is to shed light on how the proportions of contributing sources of respiration within a forest ecosystem vary seasonally, helping to explain the seasonal behaviour of total ecosystem respiration and photosynthesis.

2. Methods

2.1. Site Description

The Virginia Forest Research facility (37.9229 N, 78.2739 W) is located in a secondary growth mixed deciduous forest in Fluvanna County, central Virginia. The facility includes a 40m tower, around which the forest extends >1 km in all directions. The forest is dominated by oaks (Quercus spp.), Virginia pine (Pinus virginiana), red maple (Acer rubrum), hickory (Carya spp.), American beech (Fagus grandifolia) and tulip tree (Liriodendron tulipifera) (Chan, 2011)

The 30-year average temperature of the growing season (May to September) in Fluvanna County is 20.5 °C, with an average rainfall of 59.1 cm. The average annual precipitation is 114.2 cm. The 2019 growing season, which will be studied here, experienced an average temperature of 22.1 °C and 55.5 cm of rainfall. Total annual precipitation for 2019 was 107.1 cm (NOAA National Centers for Environmental Information, Climate at a Glance: County Time Series, https://www.ncdc.noaa.gov/cag/). Volumetric soil water content varied between 0.26 and 0.50 over the growing season (Figure 1).

2.2. Data Collection and Processing

CO_2 and water vapour concentrations were measured with an open-path infrared gas analyser (IRGA; LI-7500, LI-COR, Lincoln, NE, USA), and wind velocities measured with a sonic anemometer (CSAT3, Campbell Scientific, Logan, UT, USA). Measurements were taken at a frequency of 10 Hz at a height of 32 m, above the 24 m canopy. Shortwave and longwave radiation was measured using a net radiometer (CNR4; Kipp & Zonen, Delft, Netherlands), measurements of incoming photosynthetically active radiation (PAR) were taken every minute (LI-190R, LI-COR, Lincoln, NE, USA) and were averaged to 30 minute values, and independent measurements of air temperature and relative humidity were made using an air temperature and relative humidity sensor (HMP45C, Vaisala, Vantaa, Finland).

Half-hourly net carbon and water vapour fluxes (F_c and F_q respectively) were calculated using EddyPro software (LI-COR, Lincoln, NE, USA). Flux variance similarity (FVS) based partitioning, as described by Scanlon et al. (2019), was used to partition F_c and F_q into their constituent parts; F_q is comprised of direct evaporation from soil and vegetation surfaces (F_e) and transpiration via stomata (F_t), and F_c is comprised of net photosynthesis (F_p), defined as carboxylation minus photo-respiration and leaf respiration (Wohlfahrt and Gu, 2015), and stem and soil respiration (F_r). FVS-based partitioning requires five input parameters: F_c and F_q, standard deviations in CO_2 and water vapour concentrations, and the correlation coefficient between those concentrations. The correlation coefficient is not included in the output of EddyPro, so was calculated through analysis of the 10 Hz eddy covariance data using MATLAB (version 9.8.0.1323502 (R2020a), The MathWorks, Inc., Natick, MA, USA). Before calculation of the required variables, the following standard eddy covariance pre-processing steps were performed: Only half-hour sections where at least 75% of the possible 18000 time points were present were used. Any half-hour sections of the 10 Hz data that occurred during night-time, when average incoming shortwave radiation is < 10 Wm^{-2}, were discarded. Those that remained were despiked using a modified version of the method described by Vickers and Mahrt (1997), a double axis rotation was performed (Wilczak et al., 2001; Cheynet, 2018), and the Webb-Pearman-Leuning (WPL) correction (Detto and Katul, 2007) applied. Further data quality assessment was performed using the flagging policy of Foken (2003) generated by EddyPro for F_c and F_q.

An estimate of water use efficiency is also required for FVS-based partitioning, and was found using the optimization theory of Scanlon et al. (2019). This requires values of vapour deficit and CO_2 concentration at the canopy level (c_a). c_a was found using a logarithmic profile to project CO_2 concentration down from the measurement height to the canopy. Similar flux profiles of water vapour concentration and temperature were used to find water vapour pressure, and saturation water vapour pressure respectively. Canopy vapour deficit was calculated as the difference between these two values.

Volumetric soil moisture content was measured using two 30 cm water content reflectometers (CS616, Campbell Scientific, Logan, UT, USA). Measurements were taken every 30 minutes and an average of the two values was used.

2.3. Analysis

Moncrieff et al. (1997) described daytime F_c as following a rectangular hyperbola with respect to PAR. This was used to investigate the relationship between F_p and PAR, with the relationship taking the general form,

$$F_p = \frac{aI_p}{\frac{a}{b} + I_p} + c \tag{1}$$

where F_p is net photosynthesis flux, I_p is incident photosynthetic radiation (PAR), c is the net photosynthesis value when $I_p = 0$ (i.e. dark leaf respiration), $a + c$ is the saturation value of F_p, and b is the slope of the hyperbola when $I_p = 0$, which describes the sensitivity of leaf respiration to PAR. The point at which the hyperbola crosses the PAR axis is where leaf respiration plus photorespiration equals carboxylation. The PAR value at which this occurs (I_{p0}) can be found by setting $F_p = 0$ and rearranging (1) for I_p as follows,

$$I_p = \frac{-ca}{b(a + c)} \; . \tag{2}$$

Outliers were removed by setting defined thresholds, before equation (1) was fitted to data over a 5-day moving window using a non-linear least squares fit. PAR values below 200 μmol m^{-2} s^{-1} were removed to prevent the partitioning requirement of $F_p \leq 0$ from artificially lowering the curve at low PAR values. Parameters a, b, and c were further screened for outliers by setting boundaries on the span of the 95% confidence intervals. The parameters were then normalised by the leaf area index (LAI) MODIS Land Product (Myneni and Park, 2015; ORNL DAAC, 2018) which was interpolated to get 30 minute values and smoothed by removing days with cloud cover (Figure 1). When examining the relationships between variables, a significance threshold of $p < 0.05$ was used.

3. Results

Table 1 | Average values of hyperbola parameters for the 2019 growing season. All values have been normalised by leaf area index. Displayed uncertainties represent one standard error about the mean.

	Early growing season	Late growing season
a (μmol CO$_2$ m^{-2} s^{-1})	-8.6 ± 0.3	-7.3 ± 0.5
c (μmol CO$_2$ m^{-2} s^{-1})	3.3 ± 0.3	2.1 ± 0.5
$a + c$ (μmol CO$_2$ m^{-2} s^{-1})	-5.3 ± 0.4	-5.2 ± 0.7
b (μmol CO$_2$ μmol PAR^{-1})	$(-4.5 \pm 0.4) \times 10^{-2}$	$(-3.7 \pm 0.7) \times 10^{-2}$
I_{p0} (μmol PAR m^{-2} s^{-1})	126 ± 10	50 ± 31

Time series of a, b, c, and I_{p0} show no clear trends (Figure 2), due to the large uncertainties resulting from removing points at low PAR values before curve fitting, and compounded by gaps in the data. The size of the uncertainties could also have been increased by high rates of photosynthesis occurring at PAR values in the range of 200–600 µmol m^{-2} s^{-1} in response to diffuse radiation (Knohl & Baldocchi, 2008). This produced collections of points below the curve of the hyperbola typically seen on clear days, but were not removed as outliers as they represent a real physical response. The negative values of c (Figure 2) are most likely due to an uneven distribution of points within a window, most often taking the form of few points at lower PAR values, resulting in an atypical curve fit.

Averages of a, b, and c were taken for the early (May, June, July) and late (August, September) periods of the growing season, and used to generate light response curves (Figure 3). Compared to the late growing season, the early growing season has a higher rate of dark leaf respiration (c) (Table 1). There is no significant difference ($p = 0.32$) in the sensitivity to light at low PAR values (b) between the early and late growing season. The early growing season has a higher PAR value at which the rate of carboxylation is greater than the rate of leaf respiration and photorespiration (I_{p0}), as expected from c being higher in the early growing season than the late, while b remains constant. The light saturation of net photosynthesis ($a + c$) is essentially the same (Table 1) between each half of the growing season, which is a similar result to the relationship between GPP and absorbed photosynthetic radiation (APAR) found by Wehr et al. (2016). Due to the relatively high LAI and its subtle variability throughout the growing season (Fig. 1), the use of PAR instead of APAR in this study would not significantly affect the observed relationships. APAR can be related to PAR based on an LAI-dependent exponential relationship between incident PAR and APAR (Baret & Guyot, 1991), which indicates that APAR remains at 93-94% of PAR throughout the growing season at this site. This is also supported by the findings of Wehr et al. (2016) at Harvard Forest, where there was no significant difference between measured PAR and APAR over the course of the growing season.

None of the hyperbola parameters exhibit statistically significant relationships with soil moisture during the growing season (Figure 4). The relationships for a, b, and c are significant with respect to air temperature ($0.33 < |\rho| < 0.36$). When averaged over the two halves of the growing season, the average daytime temperature varied by only 1.30 °C between the early and late growing season, so this relationship is unlikely to have a substantial impact on the seasonality of the light response curves (Figure 1). However, the relationship does show that a, b, and c are partially dependent on temperature on a day-to-day basis.

4. Discussion

Recent studies have shown that light inhibition of foliar mitochondrial respiration, known as the Kok effect, inhibits daytime ecosystem respiration (DER) to the degree that DER is lower than night-time ecosystem respiration. In deciduous broad-leaf forests, the difference between day- and night-time respiration is most pronounced in late spring and early summer, with apparent inhibition reaching ~30% before reducing to near zero for the remainder of the growing season (Keenan et al., 2019; Wehr et al., 2016). This understanding is contrary to the frequently used theory of diel respiration cycles, based on standard partitioning techniques, where ecosystem respiration is higher during the day than at night, due to air temperatures being higher during the day. It has been suggested that the degree of inhibition of ecosystem respiration is larger towards the start of the growing season than in later months because of leaf respiration likely being higher in the first half of the growing season than the second, due to leaves having higher energy demands for growth and expansion (Brooks et al., 1991; Keenan et al., 2019; Wehr et al., 2016). Here, dark leaf respiration (c) was isolated from ecosystem respiration, and quantified at the ecosystem scale. In agreement with prior suggestions, it was found to be higher in the early growing season than the late.

There being no significant difference between b (sensitivity of leaf respiration to PAR) in the early and late growing season, suggests that the degree to which the Kok effect impacts the various chemical processes involved in leaf respiration remains steady throughout the growing season. Combining this with higher c during the early growing season than the late implies that inhibition is greater in the early growing season simply because there is more leaf respiration to inhibit than later in the season. Considering the impact of this on seasonal ecosystem respiration; reduced leaf and increased soil respiration results in DER being higher in the second half of the growing season than the first, while night-time ecosystem respiration is higher in the first half than the second. This is presumably because the magnitude of uninhibited leaf respiration is greater than that of soil respiration, even as soil respiration increases over the growing season due to priming by root exudates (Keenan et al., 2019; Wehr et al., 2016). The PAR value at which leaf respiration plus photorespiration equals carboxylation (I_{p0}) being higher in the early growing season than the late is an expected consequence of differing c and constant b; there is higher leaf respiration in the early growing season than the late, but the potency of the Kok effect remains the same, so the PAR level required for the rate of carbon fixation through photosynthesis to outstrip the rate of carbon release through respiration is higher.

The finding that light saturated net photosynthesis ($a + c$) is constant between the early and late growing season is similar to that of Wehr et al. (2016), who found that the response of GEP to APAR remained stable between the completion of leaf expansion and the onset of autumnal senescence. As c is smaller in the late growing season than the early, this implies that the size of the reduction in leaf respiration caused by light inhibition is approximately equal to the size of the reduction in leaf respiration between the early and late growing season at this site. This is supported by leaf level measurements taken at Harvard Forest which show little or no decline in photosynthetic capacity before senescence in site dominant tree species (Wehr et al., 2016).

It is unclear why a negative correlation between dark leaf respiration (c) and air temperature was found (Figure 4). Temperature sensitivity of leaf respiration has been reported to be lower during the day than during the night (Atkin et al., 2000). As all data used here were obtained during the day, this could explain a relatively low correlation, but not a negative sign. Atkin et al. (2000) also reported an increasing degree of inhibition with increasing leaf temperature in *Eucalyptus pauciflora* (snow gum), which helps to explain the positive correlation between b and air temperature, but not c. After analysing a global data set, Atkin et al. (2017) reported a decrease in temperature sensitivity of baseline leaf respiration as leaves warm, which could help explain the large temperature range at which the highest rates of dark leaf respiration, around 5 μmol CO_2 m^{-2} s^{-1}, occur, but does not explain why the lowest rates of leaf respiration occur only at higher temperatures (Figure 4). However, Heskel et al. (2018) found no seasonal variation in the temperature sensitivity of daytime leaf respiration for Red Oak, Crous et al. (2017) reported differing responses to temperature from dark and light leaf respiration, and Zha et al. (2001) found that growth respiration in Scots pines was insensitive to an elevated temperature treatment. Temperature dependence of leaf respiration can also be impacted by drought conditions, with well-watered plants being more temperature sensitive than those experiencing drought (Crous et al., 2012). Therefore, varying soil water content over the growing season may have altered how leaf respiration responded to temperature, lessening the correlation, but still not explaining the negative sign. One example of respiration potentially decreasing with temperature was found at Harvard Forest, where increasing temperature coincided with net ecosystem exchange (NEE) becoming increasingly negative, which could indicate a reduction in ecosystem respiration, but is more likely to indicate that the rate of increase in photosynthesis outstripped that of respiration (Yi et al., 2004). However, the same study also showed NEE becoming less negative with increasing temperature at a temperate deciduous forest, similar to that studied here, in Tennessee. Overall, it is unclear how temperature effects daytime leaf respiration at this site and should be investigated further.

Soil water content has also been found to impact leaf respiration in ways other than temperature sensitivity. In *Eucalyptus saligna* (blue gum), the degree of light inhibition of leaf respiration was greater in trees that experienced a growing season under drought conditions versus those that were well-watered (Crous et al., 2012), and leaf respiration in both the light and the dark was reduced after a month under drought conditions, with the greatest impact on respiration in the light (Ayub et al. 2011). However, in *Querus ilex* (holm oak), day respiration was higher during a sustained multi-year drought than in normal conditions (Sperlich et al., 2016). Different tree species respond to drought differently. For example, whether a species is isohydric or anisohydric can determine if it will survive a sustained or severe drought, and species with varying hydraulic traits are often present in the same ecosystem (Roman et al., 2015). The varied impacts of drought on leaf respiration in different species could explain the lack of correlation between soil water content and the light response parameters seen at this mixed forest site (Figure 4). Another possibility is that the soil water content never got low enough, or did not remain low for a sufficiently long time, for it to have an impact on the studied ecosystem. Further research on the impact of environmental variables on leaf respiration could be done at this site using data from multiple growing seasons, ensuring a larger data set, and therefore allowing data to be binned based on temperature or drought conditions, so the impacts of each variable could be seen independently.

This work confirms previous suggestions that leaf respiration is higher during the first half of the growing season than the second, and provides a possible explanation of why percentage inhibition has been found to be higher in the early growing season than the late in deciduous broadleaf forests. These findings emphasise the importance of leaf respiration on seasonal respiration cycles, with the magnitude possibly being higher than that of soil respiration. It also proposes that the reduction in leaf respiration over the course of the growing season is of a similar size to the reduction in daytime leaf respiration caused by the Kok effect in the early growing season. The relationship between the Kok effect and environmental variables such as air temperature and soil water content remains unclear. Future research should aim to ascertain whether leaf respiration varies with these environmental variables consistently within ecosystem types, which would allow the response to be incorporated into Earth-system models.

Figures

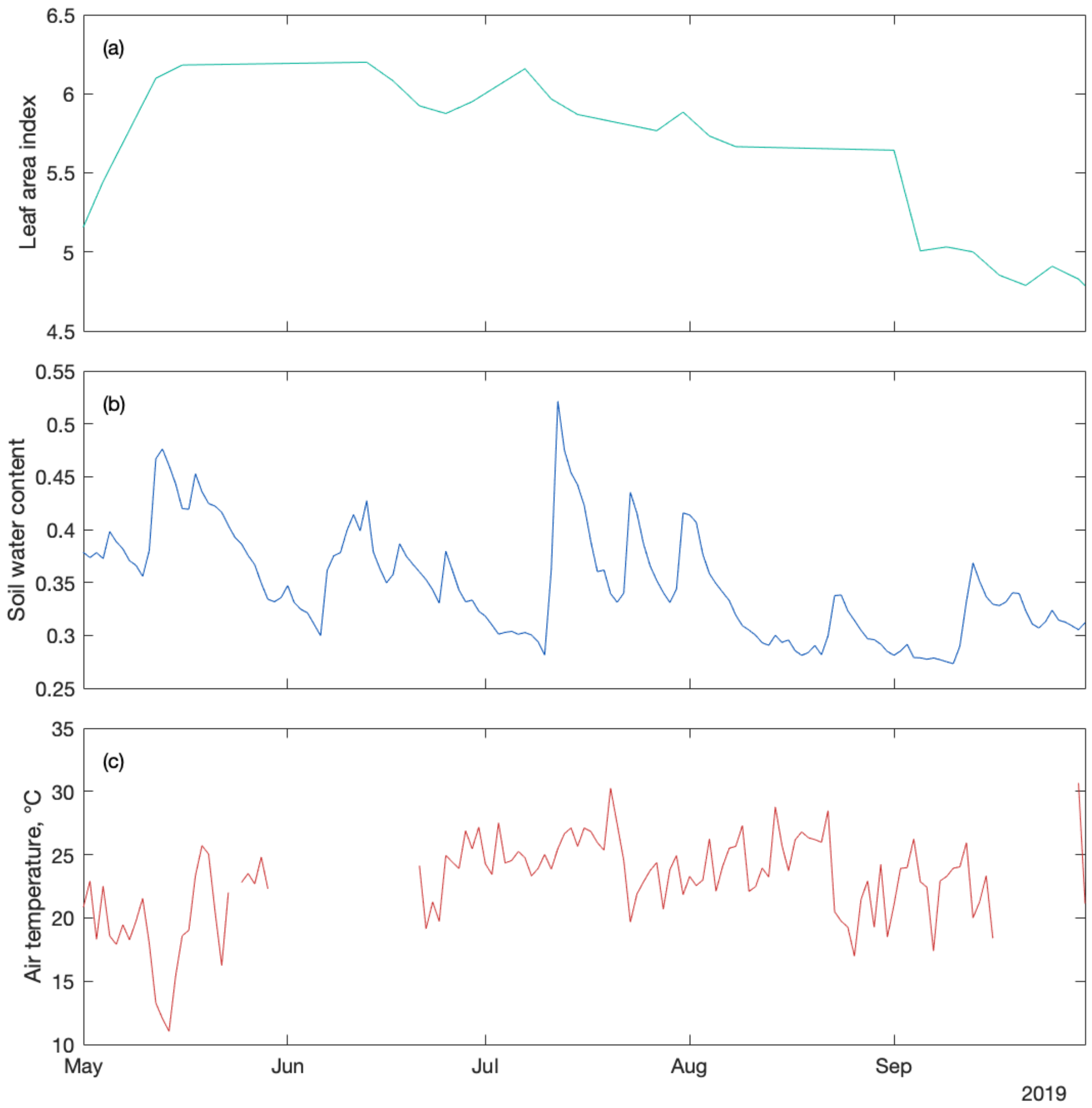

Figure 1 | Time series of environmental variables over the course of the 2019 growing season. (a) Leaf area index was taken from the LAI MODIS Land Product, which has a frequency of 8 days. Points corresponding to cloudy days were removed and the remaining data were linearly interpolated to a frequency of 30 minutes. (b) Soil water content values from two 30 cm soil moisture probes were averaged. (c) Displayed air temperature was mean daytime (incoming shortwave radiation >10 Wm^{-2}) temperature, measured with a temperature and humidity sensor mounted above the forest canopy.

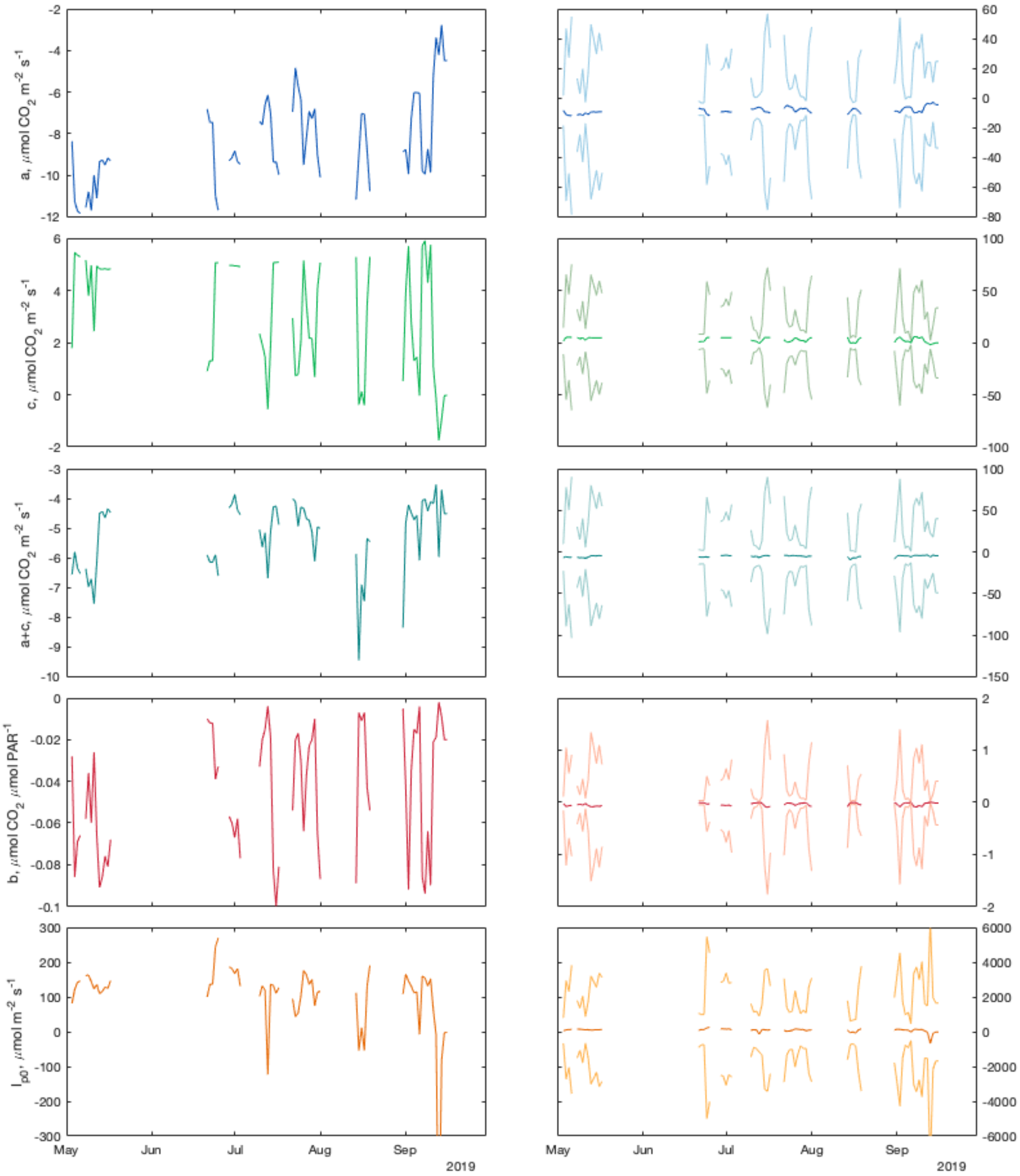

Figure 2 | Time series of LAI normalised parameters of light response curves for the 2019 growing season. *c* represents dark leaf respiration, *a+c* represents the photosynthesis saturation value, *b* represents the sensitivity of leaf respiration to PAR, and *Ip0* represents the sensitivity of leaf respiration to PAR. Left column shows mean values of light response curve parameters fitted over 5 days of data. Right column shows mean values (dark lines) with 95% confidence intervals (pale lines).

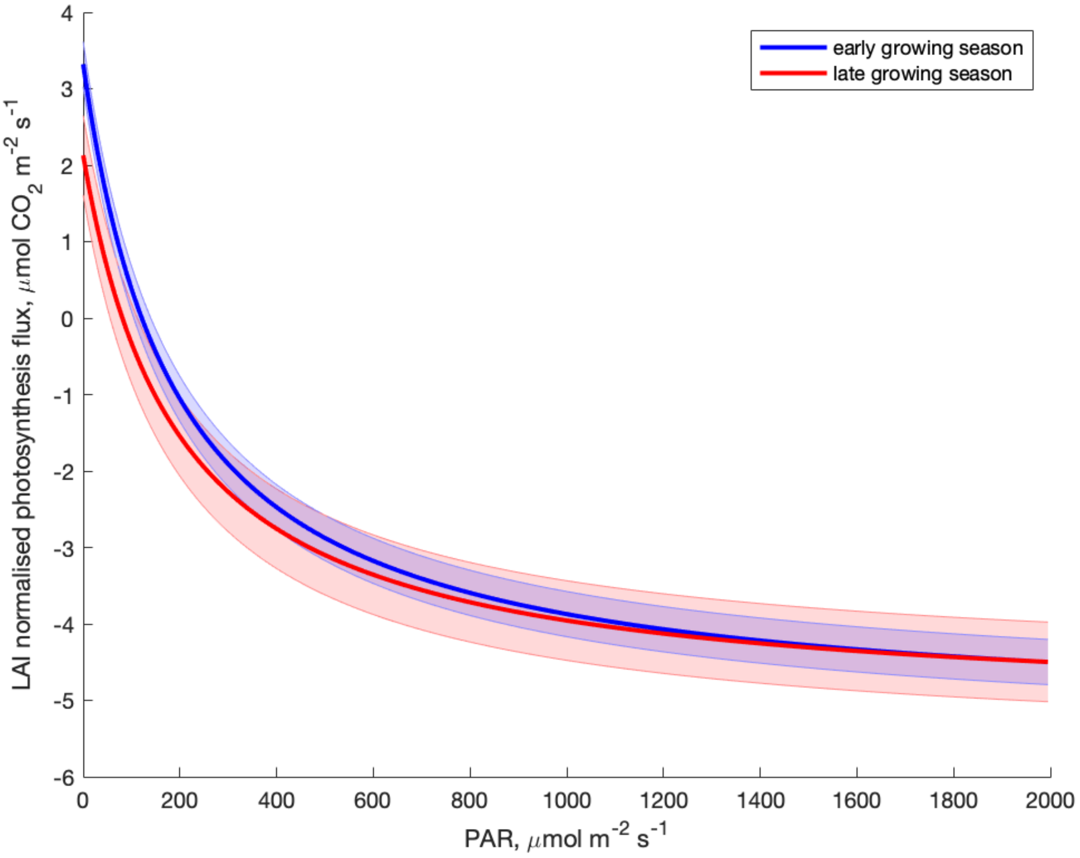

Figure 3 | Net photosynthesis light response curves for the 2019 growing season. Lines represent LAI normalised photosynthesis values calculated using equation (1) where values for a, b, and c are the mean of the daily values found by fitting light response curves in a 5-day moving window over the course of the growing season. Shaded areas represent one standard error about the mean. Early growing season refers to May, June, and July, while late growing season refers to August and September.

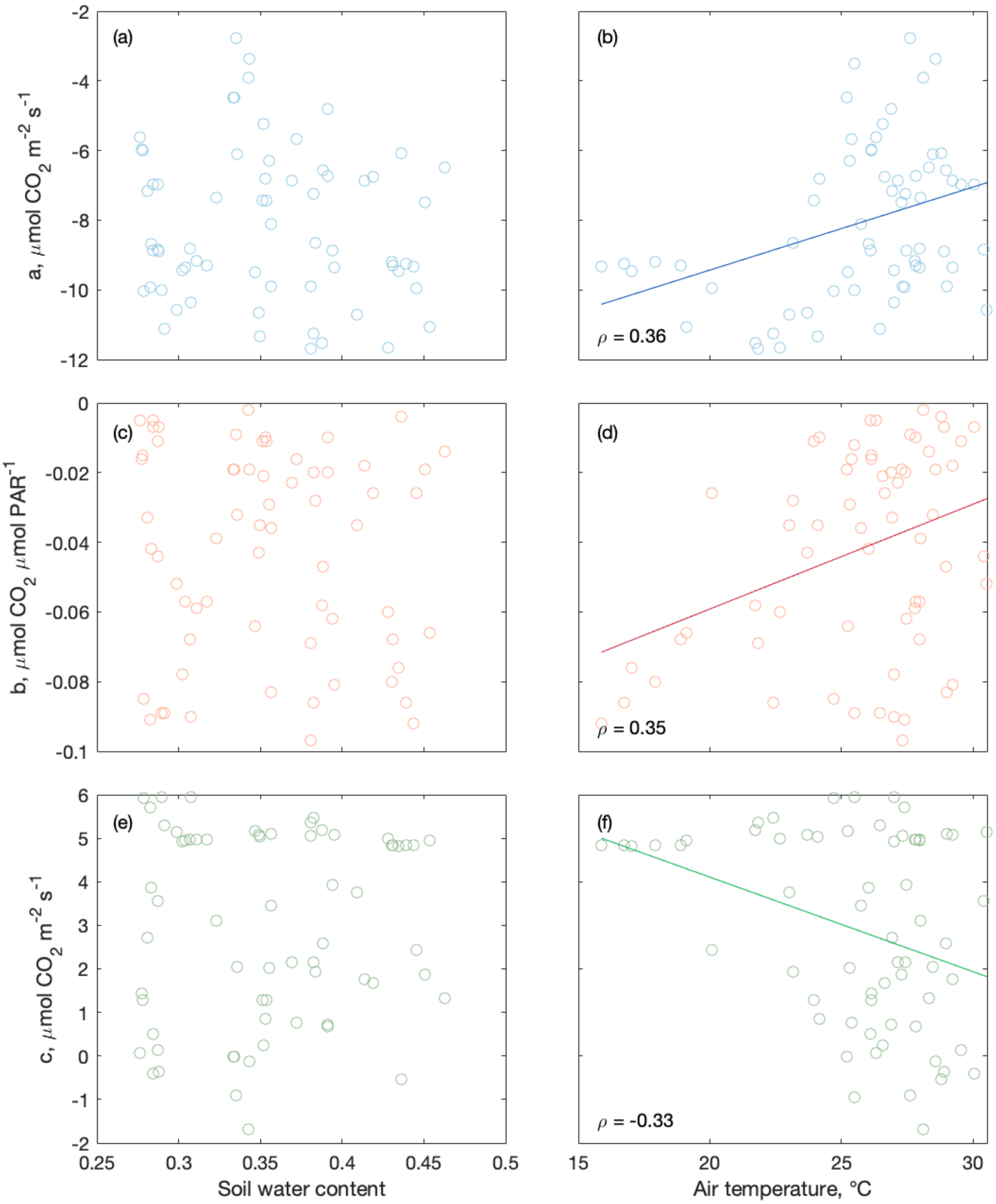

Figure 4 | Scatter plots of daily values of parameters of light response curves against soil water content and air temperature. *a*, *b*, and *c* values are daily LAI normalised values found by fitting light response curves in a 5-day moving window over the course of the 2019 growing season. ρ represents correlation coefficient. Soil water content values, shown in (a), (c) and (e), are 5-day averages. Temperature values, shown in (b), (d) and (f), are daily means, and the solid lines show linear least squares fits ($p < 0.05$).

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Chapter 3: Comparison of Methods of Measuring Water Use Efficiency and its Response to Water Availability

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Abstract

Water use efficiency (WUE) is defined as the ratio of photosynthesis and transpiration fluxes, therefore linking carbon and water cycles. As climate change produces rising air temperatures and causes precipitation patterns to differ from the past, the water availability of many ecosystems is likely to alter. In order to understand how ecosystems will be impacted by these future changes there must first be an understanding of how ecosystems respond to current variations in water availability. The majority of studies to date have used partitioned eddy covariance data for photosynthesis or transpiration flux estimates, or both, but there has been little work validating the results of eddy covariance partitioning with other techniques, even though they are often used to validated climate models. Here, the WUE of a temperate broadleaf deciduous forest is measured using two independent methods, and its response to canopy vapour deficit (*D*) and soil water content (SWC) is investigated. The first method obtains values of photosynthesis and transpiration fluxes by using flux variance similarity (FVS) to partition water vapour and carbon dioxide fluxes measured using the eddy covariance technique, while the second method uses solar induced chlorophyll fluorescence (SIF) as a proxy for photosynthesis, and transpiration is measured using sap flow sensors. Results using the SIF and sap flow method show that the magnitude of WUE decreases as SWC increases, while no correlation was found using FVS. Results using the FVS method show that the magnitude of WUE decreases as *D* increases, while SIF and sap flow data produces the opposite trend. Inherent water use efficiency, the product of WUE and *D*, is calculated and found to observe trends similar to those of WUE with SWC for both methods. Multiple linear regressions of WUE with SWC and *D* are also performed, the results of which

agree with the single variable linear regressions when an interaction term is not included. The unexpected relationship between D and WUE calculated using the SIF and sap flow method, which makes very few assumptions about the underlying functioning of the forest ecosystem, calls previous understanding of the response of forest carbon and water exchanges to water availability into question. The lack of agreement between the two methods of calculating WUE also poses challenges for scaling up the results of SIF and sap flow based studies to the ecosystem scale for validation purposes.

1. Introduction

Water use efficiency (WUE) is defined as the ratio of photosynthesis flux (F_p) to transpiration flux (F_t) and is an important factor in regulating global carbon and water cycles (Beer et al., 2009). Over the last couple of decades, WUE has increased in Northern Hemisphere boreal and temperate forests due to rising atmospheric CO_2 concentrations (c_a) (i.e. CO_2 fertilization effect), meaning there has been a decline in the stomatal conductance required for a given rate of photosynthesis (Babst et al., 2014; Keenan et al., 2013; Rezaie et al., 2018). In addition, multiple general circulation models (GCMs) predict a future increase in atmospheric water demand, i.e. vapour pressure deficit, due to rising air temperatures, further reducing plants' stomatal conductance in order to limit water loss (Williams et al., 2013). It is unclear how the combination of these changing conditions will impact future WUE and carbon uptake (Novick et al., 2016; Sulman et al., 2016). In the southeastern United States, this uncertainty is further confounded by GCMs forecasting increasing drought frequency and severity despite increased precipitation (which increases soil moisture), due to the rate of evaporation increase outstripping that of precipitation, though there is significant uncertainty surrounding these projections (Mearns et al., 2003; Mitra et al., 2018; Seager et al., 2009; Strzepek et al., 2010). Understanding the response of ecosystem WUE to changing water availability, both in terms of canopy vapour deficit (*D)* and soil moisture, will become increasingly important as water limitation increases in many areas of the globe (Williams et al., 2013).

In multiple ecosystem types, including broadleaf deciduous forests, WUE has been found to increase under drought stress. A peak is then reached, and the trend reverses as drought levels become increasingly severe (Huang et al., 2017; Lu & Zhuang, 2010). Like many Earth-system models, these studies used only soil water content (SWC) as an indicator of drought. However, it has been shown that *D* can impact carbon and water fluxes just as severely as low SWC (Novick et al., 2016; Sulman et al., 2016). At monthly and seasonal timescales *D* correlates strongly with SWC, whereas at shorter timescales the correlation is significantly smaller (Novick et al., 2016). Therefore, to understand the response of ecosystems to changing SWC, the effect of *D* must be taken into account. Inherent water use efficiency (IWUE) is the product of WUE and *D*. IWUE can therefore be used to investigate WUE response to changing SWC, even as *D* varies (Beer et al., 2009; Keenan et al., 2013). Beer et al. (2009) observed that, similarly to WUE, IWUE increases during periods of moderate drought in broadleaf deciduous forests. This conclusion was based upon values of IWUE derived from traditional CO_2 flux partitioning methods, and the assumption that F_t is equal to evapotranspiration flux after a few days without rainfall, the drawbacks of which are discussed below.

The photosynthesis flux used to calculate WUE is generally found by partitioning of net CO_2 fluxes measured using the eddy covariance technique (e.g. Lasslop et al., 2010), as was done by Beer et al. (2009). Commonly used flux partitioning methods may overestimate gross primary productivity (GPP) because the light inhibition of leaf respiration is disregarded (Wehr et al. 2016; Keenan et al. 2019). A widely used method to calculate F_t is to assume the contribution of evaporation to evapotranspiration is negligible after a few days without rainfall. Data from days where this assumption is deemed invalid are discarded. However, whether this assumption is met or not varies between ecosystem types (Stoy et al., 2019). Methods of estimating WUE that do not rely on often unfulfilled assumptions are therefore needed.

Flux variance similarity (FVS) is an alternative method of partitioning eddy covariance derived fluxes. The most recent iteration of FVS calculates WUE based on a modified version of the optimisation theory of Cowan and Farquhar (1977), stating that stomatal behaviour operates such that canopy carbon gain is maximised while water loss is minimised (Scanlon et al., 2019). Values of WUE produced using this method were similar to those from more commonly used partitioning methods, implying that the overestimations of GPP and transpiration are of comparable size. While FVS avoids making the same assumptions as other partitioning techniques, it still relies exclusively on eddy covariance measurements. Independent measurements are required to verify the results of partitioning methods.

Forest transpiration can be quantified independently of eddy covariance measured water vapour fluxes by taking sap flow measurements of trees (Burgess et al., 2001). Sap flow may provide a particularly useful measure of transpiration for this investigation, as it is controlled by both atmospheric evaporative demand and soil water availability (Bovard et al., 2005). GPP calculated through partitioning of flux tower data has been found to correlate well with solar induced chlorophyll fluorescence (SIF; Yang et al., 2015), which is a by-product of photosynthesis (Van der Tol et al., 2014). SIF can therefore be used as a proxy for GPP, independent of net ecosystem CO_2 flux measurements (Yang et al., 2018). Taking the ratio of these measures of transpiration and photosynthesis would provide estimates of WUE independent of eddy covariance estimates, and could serve as verification for other methods of WUE calculation. Combining them with canopy vapour deficit data derived from a thermal camera, would provide a method of calculating IWUE entirely independently of standard eddy covariance methods.

The objectives of this work are to (1) determine the relationship between IWUE and SWC at a broadleaf deciduous forest site, (2) determine the relationship between WUE and water availability at a broadleaf deciduous forest site, (3) calculate IWUE using a novel combination of techniques, entirely independent of standard eddy covariance measurements, and (4) compare the results of this method to those from using the FVS partitioning technique.

2. Methods

2.1. Site Description

The Virginia Forest Research facility (37.9229 N, 78.2739 W) is located in a secondary growth mixed deciduous forest in Fluvanna County, central Virginia. The facility includes a 40m tower, around which the forest extends >1 km in all directions. The forest is dominated by oaks (*Quercus* spp.), Virginia pine (*Pinus virginiana*), red maple (*Acer rubrum*), hickory (*Carya* spp.), American beech (*Fagus grandifolia*) and tulip tree (*Liriodendron tulipifera*) (Chan, 2011).

The 30-year average temperature of the growing season (May to September) in Fluvanna County is 20.5 °C, with an average rainfall of 59.1 cm. The average annual precipitation is 114.2 cm. The 2019 growing season, which was studied here, experienced an average temperature of 22.1 °C and 55.5 cm of rainfall, though reliable sap flow data for this study was only available for the late growing season (July-September), so this was the period studied. During this time, the average temperature was 24.7 °C and there was 20.5 cm of rainfall. Total annual precipitation for 2019 was 107.1 cm (NOAA National Centers for Environmental Information, Climate at a Glance: County Time Series, https://www.ncdc.noaa.gov/cag/). Volumetric soil water content varied between 0.26 and 0.50 over the growing season.

2.2. Data Collection and Processing

2.2.1. Calculating Inherent Water Use Efficiency

Inherent water use efficiency (IWUE) is a useful measure that accounts for the strong control vapour deficit exerts on WUE, and is defined as

$$IWUE = D\frac{F_p}{F_t} = \frac{c_a - c_i}{1.6} \tag{1}$$

(adapted from Beer et al., 2009) where D is canopy vapour deficit, F_p is photosynthesis flux, F_t is transpiration flux, c_a is ambient CO_2 concentration, c_i is internal leaf CO_2 concentration, and 1.6 is a factor that arises from the ratio of the molecular diffusivities of CO_2 and water vapour. When using flux values derived from the eddy covariance technique, both F_p and F_t have units of mmol m^{-2} s^{-1}, leaving IWUE with the same units as D, which, for the partitioning method that was used in this study, are mmol mol dry air^{-1}, which would also be the units for c_a and c_i. At other instances in this study, F_p was represented by SIF measurements (mw m^{-2} sr^{-1} nm^{-1}) and F_t by transpiration measured using sap flow (cm^3 hr^{-1}), so IWUE did not have the same units as D (kPa) in this case. Though equation (1) doesn't strictly hold for this second combination of measurements, the concept of IWUE that underlies equation (1) still stands. It should be noted that the first equality in equation (1) assumes aerodynamic resistance through the boundary layer can be neglected (Beer et al., 2009; Keenan et al., 2013).

IWUE was calculated using two different methods. The first used standard eddy covariance data and the WUE optimization theory of Scanlon et al. (2019), which assumes that canopy carbon gain is maximised while water loss is minimised. Calculated WUE was then used to generate values of F_p and F_t. The second method used measurements of solar induced chlorophyll fluorescence (SIF) as a proxy for F_p, and sap flow measurements were used to find F_t. Both methods used values of D calculated using measurements of air temperature and relative humidity made using a sensor (HMP45C, Vaisala, Vantaa, Finland), which were used to calculate actual air vapour pressure, and canopy temperature measurements were taken using a thermal camera (FLIR A655sc with 45-degree field of view lens; FLIR Systems, Wilsonville, OR, USA), and used to calculate saturation vapour pressure at the canopy level. The difference between these values yielded *D*. Further details can be found in Yi et al. (2020). The camera has three trees within its field of view (FoV), Quercus alba, Quercus falcata, and Pinus virginiana, which represent a relative dominance of 23.6%, 11.9%, and 20.1%, respectively, within a 500m radius of the tower (Chan, 2011). The following sections describe these methods in more detail.

2.2.2. Optimisation Theory and Eddy Covariance

CO_2 and water vapour concentrations were measured with an open-path infrared gas analyser (IRGA; LI-7500, LI-COR, Lincoln, NE, USA), and wind velocities measured with a sonic anemometer (CSAT3, Campbell Scientific, Logan, UT, USA). Measurements were taken at a frequency of 10 Hz at a height of 32 m, above the 24 m canopy. Shortwave and longwave radiation was measured using a net radiometer (CNR4; Kipp & Zonen, Delft, Netherlands).

The optimisation theory method of calculating IWUE requires values of CO_2 concentration and vapour deficit at the canopy level (c_a and D respectively), and photosynthesis and transpiration fluxes (F_p and F_t respectively). D was calculated as described in section 2.2.1., while c_a was found using a logarithmic flux profile to project CO_2 concentration down from the measurement height to the canopy. In order to calculate F_p and F_t, five input parameters were required: net carbon dioxide and water vapour fluxes (F_c and F_q respectively), standard deviations in CO_2 and water vapour concentrations, and the correlation coefficient between those concentrations. Half-hourly F_c and F_q values were calculated using EddyPro software (LI-COR, Lincoln, NE, USA). The correlation coefficient is not included in the output of EddyPro, so was calculated through analysis of the 10 Hz eddy covariance data using MATLAB (version 9.8.0.1323502 (R2020a), The MathWorks, Inc., Natick, MA, USA). Before calculation of the required variables, the following standard eddy covariance pre-processing steps were performed: Only half-hour sections where at least 75% of the possible 18000 time points were present were used. Any half-hour sections of the 10 Hz data that occurred during night-time, when average incoming shortwave radiation is < 10 Wm^{-2}, were discarded. Those that remained were despiked using a modified version of the method described by Vickers and Mahrt (1997), a double axis rotation was performed (Wilczak et al., 2001; Cheynet, 2018), and the Webb-Pearman-Leuning (WPL) correction (Detto and Katul, 2007) applied. Further data quality assessment was performed using the flagging policy of Foken (2003) generated by EddyPro for F_c and F_q.

2.2.3. Solar Induced Fluorescence, Sap Flow, and Canopy Temperature

Continuous automated measurements of SIF made using FluoSpec 2 (Yang et al., 2018), mounted on the same tower as the eddy covariance instruments, were used. The sensor has the same three trees within its FoV as the thermal camera. SIF values (mw m^{-2} sr^{-1} nm^{-1}) were used directly as a proxy for F_p in equation (1), providing a measure of photosynthesis independent of flux tower measurements.

Sap flow sensors (HRM30 Sap Flow Sensors and SL5 Smart Logger, ICT International, Armidale, NSW, Australia) installed in the three trees in the FoV of FluoSpec2 were used to find their transpiration rates. Sapwood depths were found by taking tree cores, allowing the sensors to be positioned to measure sap flux densities (J_s, cm hr^{-1}) of the sapwood area. Measurements were taken every ten minutes using the heat ratio method (Burgess et al., 2001). A detailed description of sensor installation and J_s calculation can be found in Yi et al. (2020). J_s was found for each of the three trees and multiplied by their sapwood area at breast height to find their transpiration rate, F_t (cm^3 hr^{-1}) (Bosch, 2014). The transpiration rate of the three trees was then scaled according to their relative dominance within the forest, and averaged.

2.2.4 Soil Moisture

Volumetric soil moisture content was measured using two 30 cm water content reflectometers (CS616, Campbell Scientific, Logan, UT, USA). Measurements were taken every 30 minutes and an average of the two measurements was taken.

2.3. Analysis

The same analysis was applied to each method of calculating IWUE, only the data sources were different. As the various data sources had varying measurement frequencies, all data was first standardised to a frequency of 30 minutes. Sapflux data had an original frequency of 10 minutes, so was temporally averaged, and linear interpolation was used for canopy vapour pressure deficit and temperature data as they had an original frequency of 60 minutes. All other datasets had an original frequency of 30 minutes.

To ensure IWUE values would not be heavily influenced by measurements taken at times when the underlying assumptions about stomatal gas exchange may not be fulfilled, for example, early in the morning when dew may be present on leaves, it was required that each day should have at least 3 measurements during the middle of the day, defined here as between 10:00 and 14:30 local time. Days that did not meet this requirement were discarded. Equation (2) was then applied to each remaining day

$$IWUE = \frac{\sum_{i=1}^{n} D_i F_{p_i}}{\sum_{i=1}^{n} F_{t_i}} \tag{2}$$

where n is the number of available 30- minute periods on a given day. For the FVS method, D represents vapour deficit (mmol H_2O mol dry air^{-1}), calculated using vapour pressure deficit and air pressure, and F_p (mmol CO_2 m^{-2} s^{-1}) and F_t (mmol H_2O m^{-2} s^{-1}) represent photosynthesis and transpiration fluxes respectively, partitioned using the FVS method of

Scanlon et al. (2019). The resulting units for IWUE and WUE are mmol CO_2 mol dry air $^{-1}$ and mmol CO_2 mmol H_2O^{-1} respectively. For the SIF and sap flow method, D represents vapour pressure deficit (kPa), F_p represents measurements of SIF (mw m^{-2} sr^{-1} nm^{-1}), and F_t (cm^3 hr^{-1}) represents the average transpiration from the three trees studied. The resulting units for IWUE and WUE are kPa hr mw^{-2} sr^{-1} nm^{-1} cm^{-3} and hr mw^{-2} sr^{-1} nm^{-1} cm^{-3} respectively. Once calculated, possible WUE outliers were identified using residual intervals. These were inspected and 1–2 high leverage points were removed from the data sets of each method. F_p values have a negative sign, denoting the downward direction of photosynthesis flux, while F_t values have a positive sign, denoting its upwards flux direction. This sign convention results in WUE and IWUE values being more negative (of greater magnitude) when the forest's water use is more efficient, and less negative (of smaller magnitude) when the forest's water use is less efficient.

In addition to single independent variable linear regressions, multiple linear regression (MLR) models were run using data from both the FVS and SIF and sap flow methods, and both without (equation 3), and with (equation 4), an interaction term,

$$WUE = a_1 + a_2D + a_3SWC \tag{3}$$

$$WUE = a_1 + a_2D + a_3SWC + a_4D \times SWC \tag{4}$$

where WUE represents water use efficiency, a_i represents regression coefficients, D represents vapour deficit, and SWC represents soil water content. When examining the relationships between variables, a significance threshold of $p < 0.05$ was used.

3. Results

No significant correlation was found between SWC and IWUE (ρ = -0.11, p = 0.53), or SWC and WUE (ρ = -0.21, p = 0.25), calculated using FVS (Figure 1a and 1b). However, weak, significant correlations between SWC and IWUE (ρ = 0.26, p = 0.025), and SWC and WUE (ρ = 0.30, p = 9.5×10^{-3}) calculated using measurements of SIF and sap flow were found (Figure 1c and 1d), indicating that the magnitudes of IWUE and WUE decrease as SWC increases. There was no significant correlation between the two methods of calculating IWUE (ρ = 0.17, p = 0.30) or WUE (ρ = 0.30, p = 0.097) (Figure 2).

The response of WUE to *D* was also investigated. It was found that WUE calculated using the partitioning method of Scanlon et al. (2019) displayed the relationship of increasing in magnitude as vapour deficit reduced (ρ = 0.72, p = 3.3×10^{-6}), i.e. more CO_2 is fixed per unit of water lost as the air around the leaf becomes more humid, while the relationship of WUE calculated using SIF and sap flow measurements displayed a relationship with the opposite sign (ρ = -0.25, p = 0.031) (Figure 3).

Table 1 shows the results of the MLR models of WUE with *D* and SWC for the FVS and SIF and sap flow methods, both with and without an interaction term. SWC and *D* were not well correlated (ρ = -0.23, p = 0.052, VIF = 1.1). Including an interaction term in the MLRs made little difference to the predictive power of the models for both the FVS and SIF and sap flow methods, demonstrated by the R^2 values of the models with interaction terms being only slightly higher than those of the models without an interaction term. Without an interaction term, the coefficients a_2 and a_3 are in agreement with the signs of the corresponding single variable regressions. When an interaction term is included in the MLR models, the signs of a_2 and a_3 are reversed in all cases, except SIF and sap flow with *D*, which remains negative. Agreement between observed and modelled values was greater for FVS than SIF and sap flow values, both with and without an interaction term (Figure 4).

Table 1 | Water use efficiency MLR coefficients and statistics. Coefficients correspond to those in equations (3) (no interaction term) and (4) (with an interaction term). Strength and significance of regressions given by R^2 and p values, respectively. Values of $p < 0.05$ were deemed significant.

	Flux Variance Similarity		SIF and Sap Flow	
	Without Interaction Term	With Interaction Term	Without Interaction Term	With Interaction Term
a_1	-9.6×10^{-3}	6.7×10^{-4}	-1.4×10^{-4}	-6.7×10^{-5}
a_2	2.7×10^{-4}	-2.9×10^{-4}	-1.1×10^{-5}	-7.7×10^{-5}
a_3	8.8×10^{-5}	-3.2×10^{-2}	1.6×10^{-4}	-6.5×10^{-5}
a_4	-	1.7×10^{-3}	-	2.0×10^{-4}
R^2	0.66	0.69	0.13	0.15
p	1.6×10^{-7}	3.0×10^{-7}	6.3×10^{-3}	0.012

4. Discussion

With climate change, air temperatures are expected to rise and seasonal precipitation patterns are forecast to change in many areas, including the southeastern United States. These changes will cause an increase in average *D*, and will lead to periods of increased SWC, as well as times of severe and long-term drought stress (Mearns, 2003; Mitra, 2018; Novick et al., 2016; Williams et al., 2013). As WUE plays an important role in determining the carbon and water cycling of terrestrial ecosystems, it is important to understand the response of WUE to these variables in order to predict how ecosystems will respond in turn. Many WUE studies use traditional partitioning techniques to obtain values of GPP from CO_2 fluxes and transpiration from H_2O fluxes measured using the eddy covariance technique, but only a few studies have attempted to validate these findings using other methods (Beer et al., 2009; Keenan et al., 2013; Rezaie, 2018; Ruehr et al., 2012; Stoy et al., 2019; Sulman et al., 2016). In addition, several evapotranspiration partitioning techniques use values of WUE as their starting point, compounding the need for verification (Stoy et al., 2019, Zhou et al., 2016). Here, the WUE of a southeastern US forest was measured using two methods. The first used an eddy covariance flux partitioning method based on the assumption that plants work to optimise WUE, while the second used a novel combination of SIF and sap flow measurements entirely independent of eddy covariance data. Measurements of *D* derived from thermal camera canopy temperature data were also incorporated in calculating IWUE, which takes into account the decoupling of *D* and SWC at short timescales. The response of WUE to SWC and *D* was investigated, as well as the response of IWUE to SWC.

The correlations between SWC and both IWUE and WUE calculated using SIF and sap flow measurements showing that they decrease as SWC increases (Figure 1) agree with previous findings for moderate droughts, similar to the one experienced at this site (Beer et al., 2009; Huang et al., 2017; Lu & Zhuang, 2010; Ruehr et al., 2012). The lack of correlation between SWC and IWUE and WUE calculated using FVS could be due to a number of factors. Firstly, the range of soil moisture values experienced over the study period was relatively limited, with very few instances of low SWC. It has been shown that ecosystem fluxes are quite insensitive to changes in SWC when SWC is not limiting (Ruehr et al., 2012; Sulman et al., 2016). Secondly, the number of points in the FVS data set is considerably smaller than that of SIF and sap flow due to the strict quality controls imposed on the eddy covariance data. Thirdly, the assumptions that the FVS partitioning technique are based upon are focused on stomatal processes and their almost instantaneous response to *D*, with *D* playing a key role in the calculation of WUE (Scanlon et al., 2019). This may be at the cost of homogenizing the diversity of response to environmental conditions that different tree species display, such as the differences in response to environmental conditions between isohydric and anisohydric species. Isohydric species strongly regulate stomatal function during dry conditions (soil moisture and atmospheric dryness), while anisohydric species' stomata remain open during dry periods, prioritising carbon gain over water loss (Roman et al., 2015; Sulman et al., 2016; Yi et al., 2020). FVS may characterise the behaviour of isohydric species well, but may not correctly model the response of anisohydric species. However, Stoy et al. (2019) tested the assumption of optimal response to *D* and found it to be a reasonable assumption across many ecosystem types, though the conditions under which this assumption holds require further investigation.

It is possible that the measurements of SWC in the top 30 cm of soil may not reflect SWC deeper into the rooting zone, though this is unlikely to have had a large impact here as the SWC remained relatively high throughout the study period, so the top soil layer did not dry out. The two probes whose results were averaged to obtain the SWC data displayed very different time series (data not shown), demonstrating the heterogeneity of soil texture at this site. It is therefore possible that the locations at which SWC was measured did not represent what the three studied trees, and perhaps other trees within the flux tower footprint, experienced. Improvements in SWC data could be obtained by emulating the methods of Roman et al. (2015). In their study, four 30 cm soil moisture probes were installed, the data was scaled using gravimetric samples, and was used to develop an empirical soil water potential model. SWC was also monitored to a depth of 50 cm by one probe, and this was used to scale the 30 cm measurements to a larger portion of the rooting zone. In addition, Beer et al. (2009) notes that SWC alone may not be a good measure of leaf level water demand, and that root distribution and the hydraulic conductivity within the soil matrix should be considered.

The lack of agreement between the two methods of calculating both IWUE and WUE (Figure 2) displays possible challenges in scaling up the SIF and sap flow derived data to the size of the flux tower footprint, or even ecosystem scale. A potential problem may be that the three trees studied are not representative of the forest as a whole. White oak (*Quercus alba*), southern red oak (*Quercus falcata*) and Virginia pine (*Pinus virginiana*) represent a relative dominance of 23.6%, 11.9%, and 20.1%, respectively, within a 500m radius of the tower (Chan, 2011), while when only the three observed trees are considered, they represent 30.1%, 20.9% and 49.1%. Although the contributions of the three studied trees were scaled to reflect their relative dominances, they are still not representative of the forest as a whole as many other species present in the forest were not accounted for. Oaks are known to be strongly anisohydric, while southern pines are much further towards the isohydric end of the spectrum. The other dominant species at the study site fall between these two extremes (Roman et al., 2015). The combination of the assumptions behind the FVS partitioning technique potentially not representing some of the dominant species at this site, and the three SIF and sap flow trees not being representative of the wider forest, likely played a role in the lack of agreement between the techniques.

The question of whether the three trees measured by SIF and sap flow measurements are representative of the wider ecosystem arises again when considering the unexpected negative correlation between WUE and *D* for this method (Figure 3b). The negative correlation describes the magnitude of WUE increasing as the vapour deficit becomes larger, or in other words, the amount of carbon fixed per unit of water vapour lost to transpiration decreases even as the air gets closer to saturation. This is contrary to previous findings (Beer et al., 2009; Huang et al., 2017; Lu & Zhuang, 2010; Ruehr et al., 2012), and the trend found using results from the FVS method (Figure 3a). This trend appears to be the result of the unexpected relationship between SIF and *D* (ρ = -0.49, p = 8.4×10^{-6}) (Figure 5a), which shows the magnitude of SIF increasing even as the air gets drier, while transpiration shows the expected trend of increasing with increasing *D* (ρ = 0.40, p = 4.3×10^{-4}) (Figure 5b). The data shown in Figure 5 are daily averages calculated using only midday values, when incoming solar radiation is at its highest, to control for possible light intensity effects on the optical SIF measurement. As described in the methods section, data displayed in other figures includes all daytime values as long the day had at least 3 midday measurements available.

Prior work has shown that SIF displays a strong correlation with GPP calculated using traditional partitioning methods. However, traditional partitioning methods do not take the light inhibition of leaf respiration into account, which results in GPP being overestimated in the first half of the growing season, when leaf respiration is highest (Brooks et al., 1991; Wehr et al., 2016; Keenan et al., 2019). As the degree of inhibition is not consistent over the course of the growing season, any change in the values of WUE as a cause of this effect are likely unevenly distributed throughout the dataset. This raises the possibility that the trends reported here could change if this phenomenon was incorporated. However, the impact of this on this study cannot be stated with any certainty, as raw SIF values were used (rather than GPP), and the mechanism of SIF and how it relates to GPP and leaf respiration is unknown (Miao et al., 2018). The uncertainty surrounding this is furthered when considering that the data available for this study did not span a whole growing season, and so is not directly comparable to the majority of published works on light inhibition of leaf respiration, which compare early and late growing season behaviour, but not changes on shorter timescales (Keenan et al., 2019; Wehr et al., 2016).

Comparing the MLR models of the two methods of calculating WUE, the signs of the coefficients a_2, a_3, and a_4 are the same for both methods, except for a_2 without an interaction term, which is positive for FVS but negative for SIF and sap flow, in agreement with the single variable regressions (Table 1 and Figure 3b). As this negative correlation was discussed at length above, it is omitted from the following discussion. a_2 and a_3 are both positive in equation (3) (except a_2 for SIF and sap flow), and negative in equation (4) when a_4, the coefficient of the interaction term, is positive. The positive signs of a_2 and a_3 in (3) describe the magnitude of WUE decreasing as *D* and SWC increases i.e. less carbon is assimilated per unit of water lost as the air gets drier and the soil gets wetter, agreeing with previous findings. Interestingly, when an interaction term is introduced, the signs of these coefficients are reversed, which is at least partly compensated for by a_4 being positive for both methods, and there is a very slight improvement in the strength of the regressions, though the significance is reduced. It is unclear why introducing an interaction term would cause *D* and SWC to display the opposite behaviour to when no interaction term is included, but as *D* and SWC are not well correlated (ρ = -0.23, p = 0.052, VIF = 1.1) at the timescale studied here, this result is unlikely to represent a physical process (Novick et al., 2016). Sulman et al. (2016) did similar regression modelling for *D* and SWC with an interaction term for GPP and transpiration, though not WUE. Both fluxes behaved as expected with respect to the studied variables, implying WUE would do the same, contrary to what was observed here. The only discrepancy in sign of the regression coefficients between the two measurement methods is the crossing point of the WUE axis (a_1), which is positive for the FVS method with an interaction term, while a_1 is negative for all other cases. Physically, this positive value represents a reversal in either the CO_2 or H_2O flux through stomata, i.e. CO_2 moving out of the leaf along with H_2O, or H_2O moving into the leaf along with CO_2. This behaviour is unlikely, but not impossible. Shortly after sunrise *D* may be small due to lower air temperatures during the night, while stomata are open in response to photosynthetic demand (Moncrieff et al., 1997). If this coincided with CO_2 concentration outside of a leaf being lower than inside, CO_2 would diffuse out of the leaf. This concentration gradient could be caused by low turbulence during the night resulting in the majority of CO_2 produced by ecosystem respiration remaining below canopy level rather than being mixed into the atmosphere, and night-time leaf respiration causing the CO_2 concentration inside the leaf to rise. However, as a_1 represents the point at which both *D* and SWC are zero, little weight should be given to these extrapolated values, as such extreme conditions would not occur without significant damage to the forest ecosystem, therefore changing its functioning.

This work found that the magnitude of WUE and IWUE decreases as soil water content increases when calculated using photosynthesis and transpiration flux measurements that are entirely independent of each other, and of the more commonly used eddy covariance method. This is in line with findings of previous studies, supporting suggestions that stomatal conductance is less strictly regulated when soil water availability is not limiting. The lack of agreement between the two methods of calculating WUE used here belies the relationship between WUE and canopy vapour deficit. Though substantial conclusions on the correlation between the two methods used here cannot be drawn, this work provides a basis for future studies to build upon. Combining the SIF, sap flow, and thermal camera measurement techniques used here with a more comprehensive picture of soil water availability, and collecting data over a longer time period that includes a greater range of environmental conditions, should allow well supported claims to be made on the validity of using standard eddy covariance measurements and partitioning techniques to characterise the WUE of temperate broadleaf deciduous forest ecosystems. It also provides a proof of concept for using this technique in other forest ecosystem types.

Figures

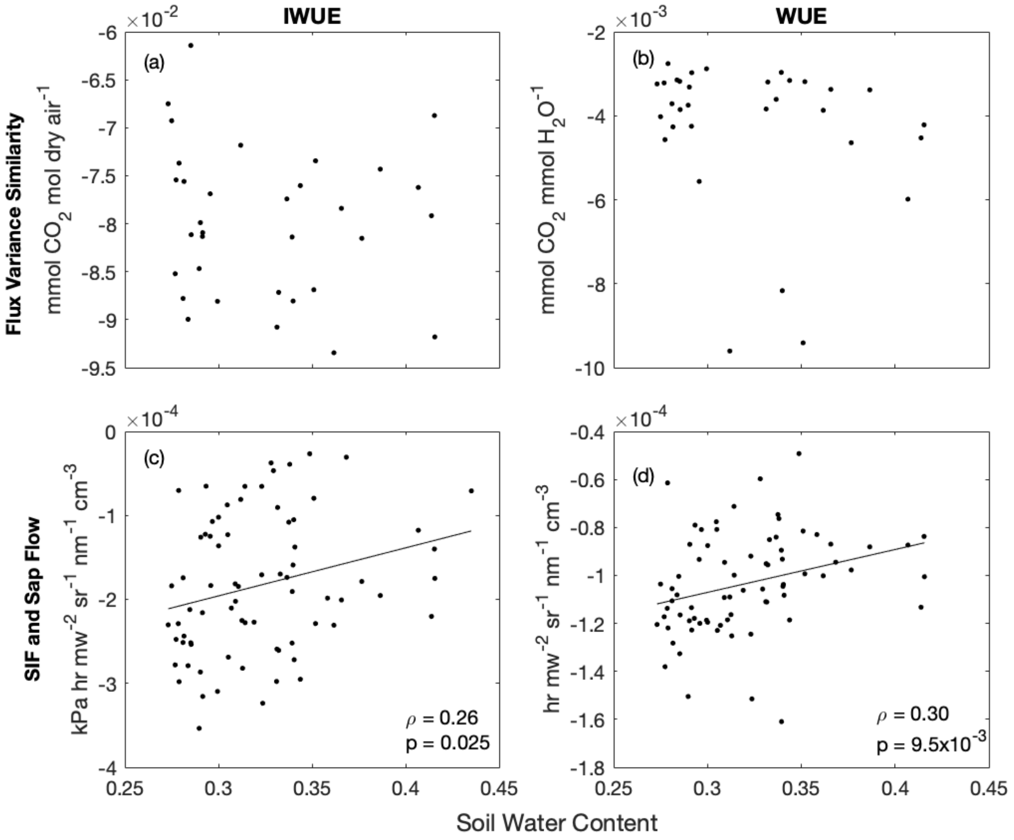

Figure 1 | Correlations between inherent water use efficiency (IWUE), water use efficiency (WUE), and soil water content. IWUE and WUE were calculated using two methods: (a-b) using photosynthesis and transpiration fluxes calculated using the partitioning method on Scanlon et al. (2019) applied to eddy covariance measurements and vapour deficit derived canopy temperature measurements from a thermal camera, and (c-d) using solar induced fluorescence, sap flow, and vapour pressure deficit, also derived from thermal camera measurements. A line of best fit is shown for relationships that are statistically significant ($p < 0.05$).

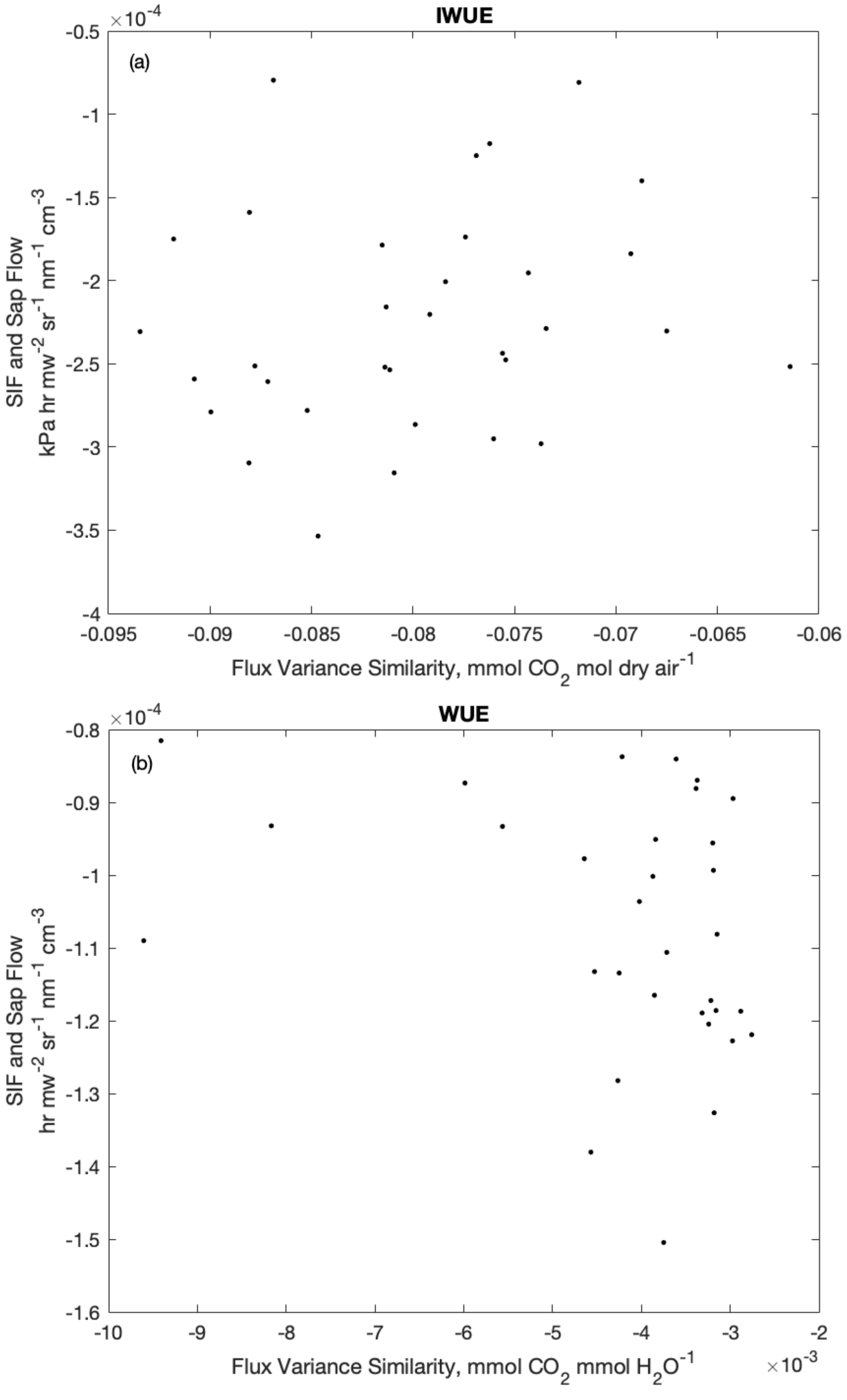

Figure 2 | Comparison of methods of calculating (a) inherent water use efficiency and (b) water use efficiency. X-axis calculated using photosynthesis and transpiration fluxes calculated using the partitioning method of Scanlon et al. (2019) and vapour deficit from thermal camera data, and y-axis calculated using solar induced fluorescence, sap flow, and vapour pressure deficit derived from canopy temperature measured using a thermal camera,

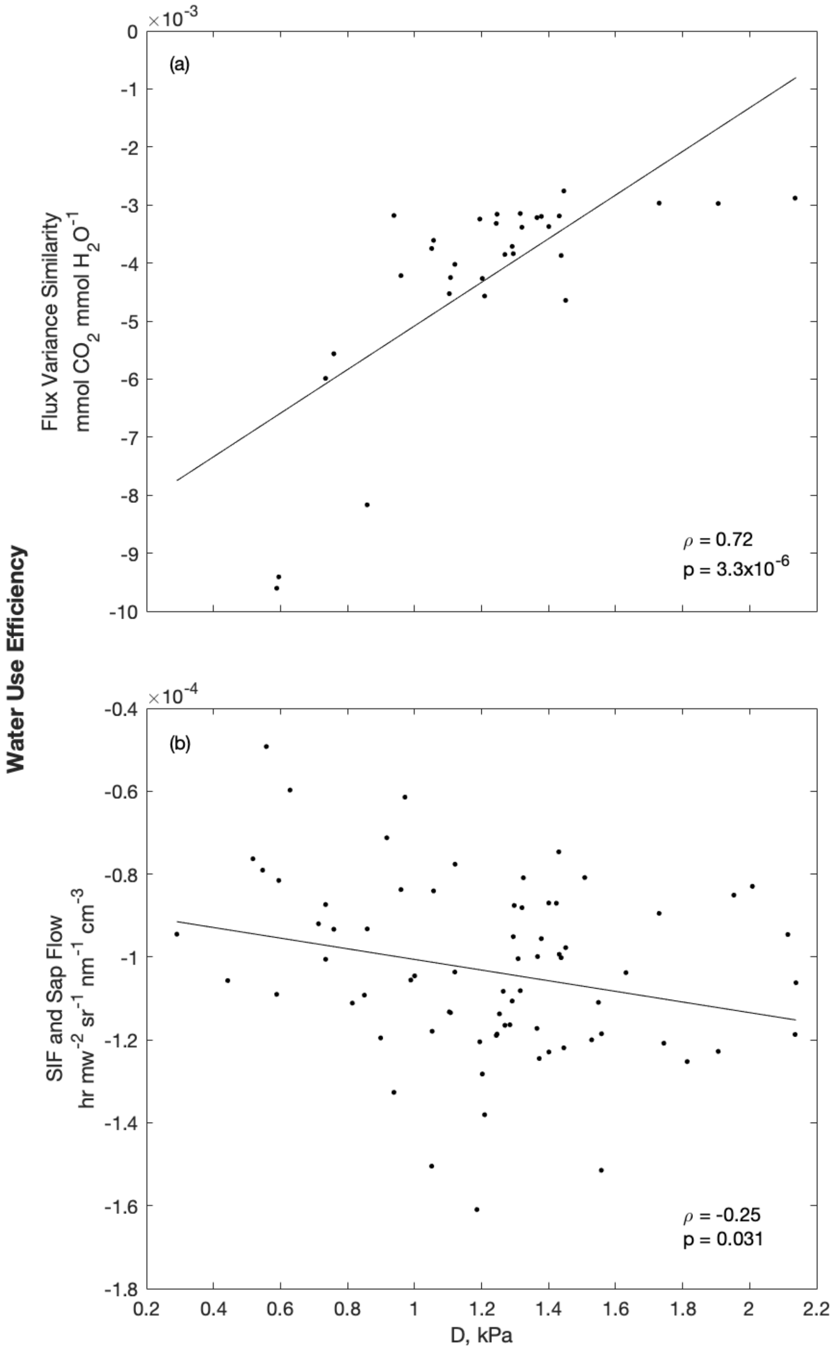

Figure 3 | Water use efficiency (WUE) and vapour deficit. (a) WUE calculated using photosynthesis and transpiration fluxes calculated using the partitioning method of Scanlon et al. (2019), (b) WUE calculated using solar induced fluorescence and sap flow. Vapour deficit in both plots derived from canopy temperature measured using a thermal camera.

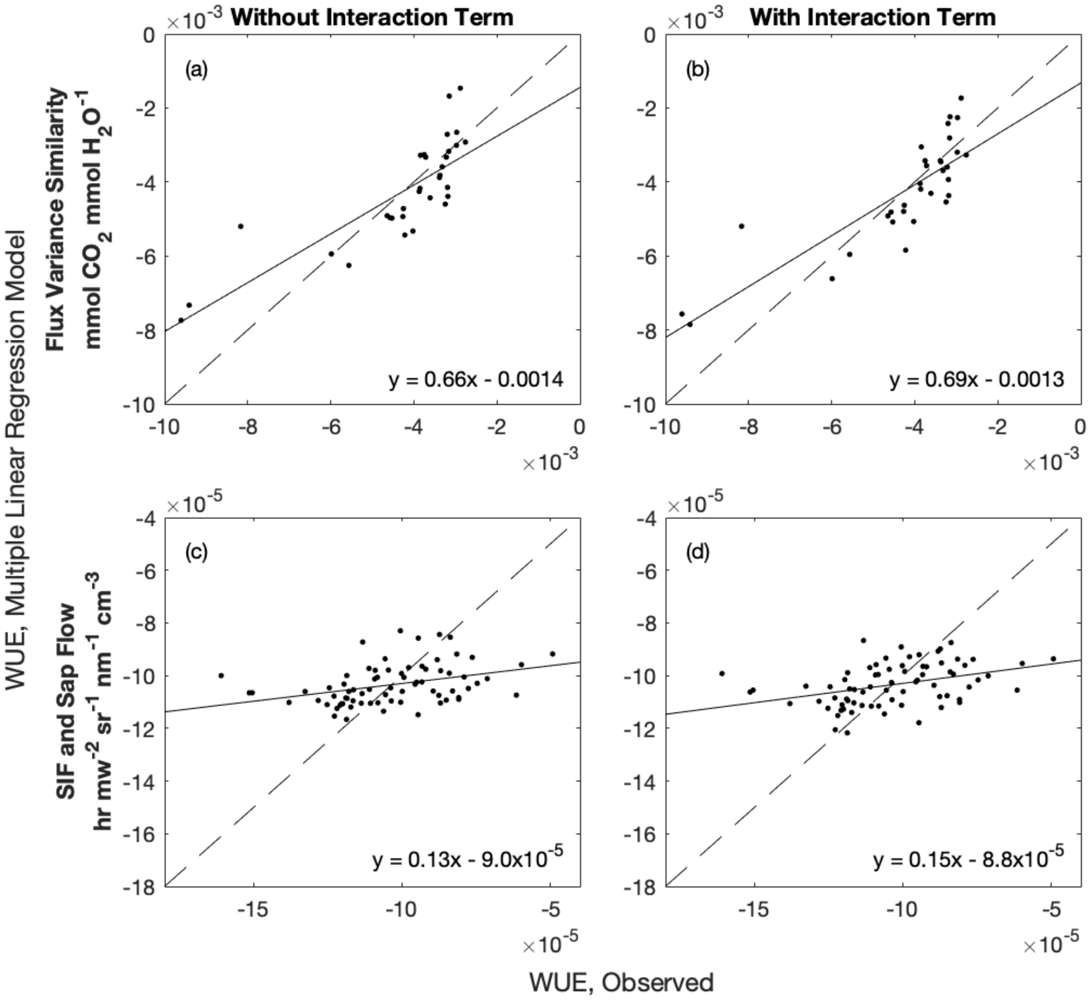

Figure 4 | Comparison of MLR models of water use efficiency with and without an interaction term. X-axis is WUE calculated from observations, while the y-axis is the result of MLR of WUE with SWC and D. The MLRs in (a) and (c) did not include an interaction term, while those in (b) and (d) did. Lines of best fit are shown in solid black, and their equations shown on each plot. 1:1 lines are shown as dashed lines.

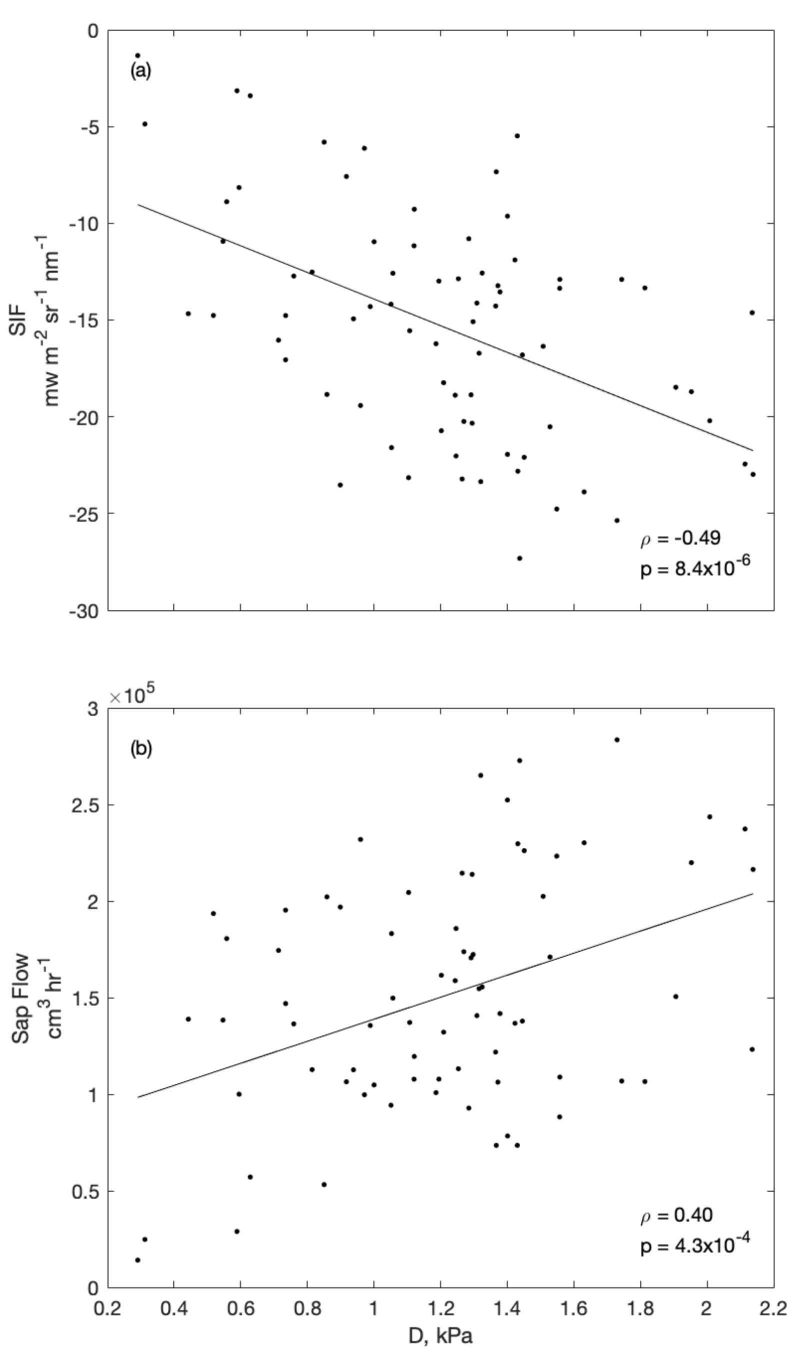

Figure 5 | Solar induced chlorophyll fluorescence (SIF) and sap flow with vapour deficit (*D*). Each point represents a daily average that was calculated using only midday values (10:00 – 14:30 local time), when incoming solar radiation was at its highest. (a) SIF with *D*. Values of SIF are negative as they are used to represent the downwards (negative) flux of carbon uptake by photosynthesis. (b) Sap flow with *D*.

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Chapter 4: Summary and Prospects for Future Research

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1. Seasonality of Leaf Respiration and its Response to Environmental Drivers

I derived net photosynthesis flux from a temperate forest in central Virginia using the flux variance similarity technique of Scanlon et al. (2019). I fitted light response curves to the net photosynthesis values and PAR data in order to obtain daily values of leaf respiration at the ecosystem scale, sensitivity of leaf respiration to PAR, photosynthesis saturation, and the PAR value at which leaves became a net carbon sink rather than a source. I then averaged each of the parameters to give mean values for the early and late growing season.

At this study site, leaf respiration is higher in the first half of the growing season than the second, while the sensitivity of leaf respiration to light remains constant, suggesting that previous findings of daytime ecosystem respiration being inhibited by up to 30% in the early growing season, and not the late, is simply due to larger amounts of leaf respiration in the early growing season, rather than any seasonality of the Kok effect itself. A possible explanation of the seasonal CO_2 flux cycle proposed by Wehr et al. (2016), where both gross ecosystem productivity and daytime ecosystem respiration remain relatively constant throughout the growing season, is that the reduction in leaf respiration over the course of the growing season is of a similar size to the reduction in daytime leaf respiration caused by the Kok effect in the early growing season. Leaf respiration is an important component of seasonal ecosystem respiration cycles, with its magnitude potentially being greater than that of soil respiration.

My findings emphasise the importance of the suggestions of Wehr et al. (2016) and Keenan et al. (2019), that the widely held understanding of seasonal and diel CO_2 flux cycles needs to be revised to include the important role that leaf respiration and its inhibition in the light plays. This would provide a solid base upon which future research into ecosystem functioning could be built. In turn, this would allow Earth-system models to more accurately represent forest functioning, and therefore provide more accurate forecasts on the response of ecosystems to the effects of climate change, such as rising temperatures and changing precipitation patterns.

Future research on this topic should aim to build a comprehensive understanding of leaf respiration and the Kok effect in a wide variety of ecosystem types across the globe, and ascertain whether leaf respiration responds to environmental variables consistently within ecosystem types. To date, studies on the temperature sensitivity of leaf respiration have been performed at a relatively small scale, with many using leaf level measurements. This has resulted in data on individual species with no obvious overarching trends. Studies carried out at the ecosystem scale could produce findings with a greater reach, allowing their incorporation into Earth-system models.

At the site studied in this work, a larger data set spanning multiple growing seasons would allow data to be binned according to SWC and temperature. This would allow the impact of each variable to be seen independently. It would be interesting to know whether the unexpected relationship between leaf respiration and temperature is seen in other growing seasons, as well as at other study sites.

2. Validating Measurements of Ecosystem Scale Water Use Efficiency

I calculated WUE using two methods. In one I used photosynthesis and transpiration fluxes partitioned from net CO_2 and water vapour fluxes measured using the eddy covariance technique. In the other I used SIF values to represent photosynthesis, and transpiration was measured using sap flow sensors. I calculated canopy vapour deficit using a thermal camera, which allowed me to obtain values of IWUE.

The magnitude of WUE decreases as SWC increases when calculated using the SIF and sap flow method. There is a lack of agreement between the two methods of calculating WUE and IWUE, meaning conclusions cannot be drawn on the relationship between WUE and *D*, or on whether the SIF and sap flow method could be used to validate the findings of studies based on eddy covariance derived fluxes. However, as this is the first example of WUE and IWUE being calculated using this combination of measurements, entirely independently of eddy covariance, it provides a starting point from which studies with a wider scope can begin.

At this work's study site, future research would benefit from data collected over a longer period. This would allow the seasonality of WUE to be investigated, and would provide a greater range of environmental conditions. A larger data set would also allow for more extreme incidences to be accounted for, that may be discarded as outliers in smaller data sets due to there only being 1 or 2 data points present at these values. In addition, a more comprehensive characterisation of water availability in terms of root water access would improve some of the shortcomings of this work. As mentioned in Chapter 3, Roman et al. (2015) provides one possible method for doing this. This work would also have benefitted from inclusion of more of the dominant tree species within the SIF and sap flow data set. This could be achieved by either installing a second SIF sensor, or moving the current SIF sensor to include different trees that have sap flow sensors installed in them. From this, the response of the individual tree species to environmental drivers could be described, and scaled to represent their relative dominance within the broader forest. Ideally, this would be accompanied by the thermal imaging camera also being moved to capture the canopies of different tree species, as the temperatures of the canopies of different tree species have been shown to vary (Yi et al., 2020).

If future studies, both at this site and others, showed a good correlation between the SIF and sap flow method used here and eddy-covariance derived fluxes, relationships between the two methods could be developed for a variety of ecosystem types. As eddy covariance data is available across the globe, this would allow the benefits of the SIF and sap flow method (few assumptions required) to be exploited in locations without the equipment to take the required measurements. Additionally, it would be useful to characterise the relationship between SIF and flux partitioning methods that do account for the light inhibition of leaf respiration. This would allow SIF measurements to be used in the process of updating current models of the carbon cycle, and may help in realising the mechanism of SIF and how it relates to photosynthesis at the ecosystem scale.

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