Seasonal variation in soil CO₂ emission and leaf gas exchange of well-managed commercial *Citrus sinensis* (L.) orchards

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2 ***Citrus sinensis* (L.) orchards**

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11

12 ABSTRACT

13 Purpose

14 To investigate whether soil clay content, cultivar and seasonal variation have any effect on soil
15 CO₂ emission rates and leaf CO₂ assimilation rates in a drip-irrigated commercial *Citrus sinensis*
16 orchard.

17 Methods

18 The study was carried out in the field as a randomised complete block design in 2x2 factorial
19 consisting of two soil types and two citrus cultivars on a drip-irrigated commercial *Citrus sinensis*
20 orchards with 2-week interval measurements of soil CO₂ emission and leaf gas exchanges over
21 period of a year.

22 Results

23 Soil clay content did not influence plant CO₂ assimilation rates and soil CO₂ emission rates in
24 irrigated citrus. However, seasonal variation significantly influenced both processes. Soil CO₂
25 emission rates were highest in summer and were more than double the rates observed in winter
26 while leaf CO₂ assimilation rates were highest in autumn and four times higher than the winter
27 season rates. Mean seasonal soil CO₂ emission rates were strongly influenced by mean minimum
28 seasonal temperatures while leaf CO₂ assimilation rates only showed a relatively weak relationship
29 with mean maximum seasonal temperatures.

30 Conclusions

31 Soil clay content did not influence soil CO₂ emission and assimilation rates in drip irrigated
32 irrigated citrus. Citrus CO₂ assimilation rate peaks in the autumn while soil CO₂ emission rates
33 peak in summer. Snapshot analysis of CO₂ sequestration rates suggests that irrigated citrus orchards

34 are net sinks of CO₂. Empirically measured CO₂ flux rates in a commercial drip-irrigated citrus
35 orchard are presented.

36 **Keywords:** CO₂ assimilation, CO₂ emission, citrus, seasonal variation, leaf gas exchanges

INTRODUCTION

Soil CO₂ fluxes play a vital role in the terrestrial carbon cycle as they integrate the decomposition of organic matter and root respiration (Paudel et al. 2018; Wu 2020). It is believed that 25 to 60 % of CO₂ assimilated through photosynthesis is lost through respiration and that soil respiration alone contributes 88% (Paudel et al. 2018). Due to climate change, soil respiration is anticipated to contribute even more CO₂ to the atmosphere due to its sensitivity to global warming (Davidson and Janssens 2006). However, the dynamics of soil respiration are still not well understood due to the high measured variability in terrestrial ecosystems (Bond-Lamberty and Thomson 2010). The high measured variability in soil respiration and the need to accurately predict the response of soil respiration to climate change as well as its feedback to global warming, have promoted the urgent need to determine soil respiration at higher temporal and spatial scales.

In agricultural systems, it is even more crucial to understand this spatiotemporal variation in soil respiration in combination with CO₂ assimilation to better understand if systems are net sinks or net emitters of CO₂. Soil CO₂ emission rates and leaf CO₂ assimilation rates respond strongly to environmental conditions such as temperature and water availability (Avola et al. 2008; Bond-Lamberty and Thomson 2010; Davidson and Janssens 2006; Qu et al. 2020; Tankari et al. 2019; Wu 2020). Some studies have found soil water content and temperature to have a confounding effect on soil respiration (Wu 2020; Yuste et al. 2003) while others such as Davidson et al. (1998), found the two to either act independently or together in controlling soil respiration. Nonetheless, there are still several studies that dispute the temperature dependence of organic matter decomposition (Fang et al. 2005; Giardina and Ryan 2000). Thus, there is no consensus on how global warming affects CO₂ fluxes. Soil texture is also another factor that can influence soil CO₂

fluxes as it influences gas movement in soils (Yang et al. 2018). Soils with high clay content present restrictions to gas movements due to tortuosity of the micro pores (Neira et al. 2015).

Similar to soil respiration, leaf CO₂ assimilation is also controlled by both ambient temperature and soil moisture. However, unlike soil respiration which is predominantly affected by soil moisture and temperature, leaf CO₂ assimilation is also strongly influenced by other factors such as light, nutrient availability and atmospheric CO₂ concentration (Davidson and Janssens 2006). Several studies have shown that soil moisture controls CO₂ assimilation through its impact on stomatal conductance (Carvalho et al. 2019; Munjonji et al. 2017; Rivas et al. 2016; Tankari et al. 2019; Zhang et al. 2013). Under unlimited soil moisture, leaf CO₂ assimilation is high due to a higher stomatal conductance with the reverse being true for limited water supply. Likewise, temperature also controls CO₂ assimilation by increasing vapour pressure deficit and the subsequent effect on stomatal conductance. High vapour pressure deficit leads to lower leaf CO₂ assimilation as the plant closes its stomata to limit water loss (Aliniaiefard et al. 2014).

The main challenge of using leaf CO₂ assimilation rates to estimate carbon capture is that they are mostly instantaneous and represent individual leaves. Over the years models have been developed and used to upscale leaf measurements to canopy level using leaf area index (LAI) (Friend 2001; Gara et al. 2019; Luo et al. 2018; Sprintsin et al. 2012). The most common methods of upscaling are the Big-Leaf model, Two-Big-Leaf model and Two-leaf model which are all well described by Luo et al. (2018). The Big-Leaf model assumes that canopy carbon fluxes have the same relative responses to the environment as any single unshaded leaf on a canopy top (Sprintsin et al. 2012) while the other two stratify the canopy into sunlit and shaded leaves (Luo et al. 2018). The Big-Leaf model is believed to under-estimate CO₂ assimilation by approximately 20% (Friend 2001) thus the Two-Big-Leaf model and the Two-leaf model are more accurate.

In many agroecosystems in semi arid environments, soil water content appears to be the most studied due to it being the most limiting as well as the seasonality of crop production. Many annual crops are seasonal and hence are produced when ambient temperatures are relatively similar. However, the dynamics may differ in perennial fruit trees. Deciduous fruit trees such as apples and peaches lose their leaves in winter while evergreen fruit trees such as citrus maintain them. A perennial evergreen crop such as citrus is subjected to large seasonal variation of environmental conditions throughout the annual cycle (Ribeiro and Machado 2007) hence their ecophysiology is unique and probably less understood. As leaves are the main photosynthetic organs in citrus trees (Iglesias et al. 2013), their annual CO₂ assimilation would be expected to be higher than for deciduous as reported in similar studies by Iglesias et al. (2013) and Luyssaert et al. (2007). However, soil respiration was found to vary similarly with the season between deciduous and evergreen forest trees (Sun et al. 2020).

According to Paudel et al. (2018), carbon balance i.e. the net result of CO₂ assimilation (sequestration) and emission may vary with season, management practices, irrigation method and water quality. Thus, it is important to understand the variation and the factors that control CO₂ efflux and assimilation to accurately predict the impact of climate change and global warming on CO₂ assimilation and loss from agroecosystems. Currently, not much work has been done on the variation of CO₂ assimilation rates and emission rates in irrigated citrus orchards. The objectives of this current study were to investigate 1) whether soil clay content has any effect on soil CO₂ emission rates and leaf CO₂ assimilation rates in a drip-irrigated commercial *Citrus sinensis* orchard and 2) how soil CO₂ emission rates and leaf CO₂ assimilation rates vary with seasons in a drip-irrigated commercial *Citrus sinensis* orchard.

METHODOLOGY

Study Site

The study was conducted at Mahela Group, a commercial citrus orchard, in Letsitele area, located about 100km east of Polokwane in the Limpopo province of South Africa (Fig 1) from May 2018 to May 2019. The coordinates of the location are 23°52'5.10" S and 30°23'28.65" E. The general climate of the area is warm-temperate, dry winter and hot summer (Cwa) (Kottek et al. 2006). The area receives a unimodal rainfall, most of which falls between December and April. A total of 485 mm of rainfall were received during the study period with the highest amount of 193 mm being received in February alone. The mean minimum temperatures ranged from 8 to 21°C while mean maximum temperatures ranged from 24 to 32°C. The weather data shown in Fig 1 was obtained from an automatic weather station installed at the experimental site.

[Insert Fig 1]

Experimental design and management

This experiment was conducted on a commercial farm and thus the orchards were already established. The designing of the experiment was done after a survey on the soil types and cultivars grown on the farm. The experiment was then laid out in a randomised complete block design in a 2 x 2 factorial arrangement consisting of two soil types and two citrus cultivars. The treatments were replicated three times. The two soil types were rich clay content soil (RC) with more than 20% clay and a moderate clay content soil (MC) with 10 – 20 % clay. The two citrus cultivars studied were Alpha Valencia and Valencia late. All cultivars were at the fruit-bearing stage and more than 10 years old. The trees were spaced at 7 x 3 m resulting in 476 trees per hectare. The irrigation was by drip and the schedule differed with the season, weather and soil type. Generally, irrigation was applied for two hours a day and three times a week in autumn and winter (March to

August) and 3 hours a day, three days a week in spring and summer (September to February). Fertilisation is done following soil and folier analysis. Foliar and soil samples are normally collected in March and send for analysis. Depending on the deficient nutrient fertilizers are then applied through fertigation, foliar sprays or as granules.

Installation of chambers and measurement of soil CO₂

The CO₂ chambers were installed in May 2018 at a distance of 1m from the main stem. Three chambers were installed in three adjacent rows at the centre of each orchard. A total of 12 chambers were installed i.e. 3 chambers x 4 treatments (n=12) The CO₂ chambers were made and installed in the field according to the USDA-ARS GRACEnet Project Protocols (Parkin and Venterea 2010). The gas chambers were however modified and interfaced with an infrared CO₂ sensor, GMP343 CO₂ probe along with MI70 data logger (Viasala, Vantaa, Finland) to allow for in-situ CO₂ measurements. The chambers consisted of two separate PVC rings: 1) a PVC ring/collar (0.20 m diameter and 0.15 m in height and 2) another PVC ring (0.20 m diameter and 0.10 m height) sealed on one side with a PVC circle to make a chamber lid. The chamber lid had a small gas ball valve on it to discourage pressure build up in the chambers during measurements. The chamber lid was also perforated on top to fix the mounting flange that holds the GMP343 CO₂ probe. The collars were hammered into the soil to a depth of 0.05 m leaving 0.10 m above the soil. These collars were left in the soil for a few days to settle before the measurements were taken (Fig 2).

[Insert Fig 2]

When taking measurements, the chamber lid with the fixed CO₂ probe on it was attached to the collar and secured with a tube strip to make it airtight (Fig 2). The CO₂ probe GMP343 was set to record measurements every 30 seconds for 5 minutes. All measurements were taken between

10h00 and 14h00. CO₂ fluxes, which is the emission rate of CO₂, were measured for 12 months starting from June 2018 to May 2019 at 2 weeks intervals.

The emission rates were then calculated as described in Munjonji et al. (2020).

Calculation of CO₂ fluxes

The CO₂ probe GMP343 gives measurements of CO₂ in parts per million (ppm). The measurements were first converted to mg m⁻³ using the ideal gas law (equation 1):

$$PV = nRT \quad (1)$$

Where P = pressure, V = volume, n = moles of gas, R = gas law constant (8.3145 J mol⁻¹ K⁻¹), and T = temperature in Kelvin. The molar volume of an ideal gas at 1atm pressure (101.325 kPa) and 25°C is 22.4 L mol⁻¹. Thus, at different pressures, the molar volume of the gas can be calculated as follows:

$$\text{Molar Volume} = \frac{RT}{P} \quad (2)$$

At different temperature and pressure the concentration of CO₂ in mg m⁻³ was calculated as follows:

$$CO_2 \text{ (mg m}^{-3}\text{)} = \left(\frac{CO_2 \text{ ppm} \times \text{Molar weight } (CO_2)}{22.4 \text{ L mol}^{-1}} \right) \times \left(\frac{273.15 \text{ K}}{T \text{ (K)}} \right) \times \left(\frac{P \text{ (kPa)}}{101 \text{ kPa}} \right) \quad (3)$$

Where CO₂ ppm is the measured concentration of CO₂ at any given time, T is the chamber temperature (Temperature in °C + 273.15 K) and P is the ambient pressure.

CO₂ concentration in mg m⁻³ was then plotted against time (min) giving a slope in mg m⁻³ min⁻¹.

The slope of the resulting regression lines was then determined for each installed chamber. The

slope was then multiplied by the volume of the chamber and divided by the area covered by the chamber giving the resultant flux in $\text{mg m}^{-2} \text{min}^{-1}$, which describes the CO_2 flux out of the soil. Cumulative CO_2 was calculated by assuming that the rate of CO_2 release was constant between two measurement points.

Leaf gas exchange

Leaf gas exchanges were measured on the trees that were next to the CO_2 chambers. Leaf gas exchanges were measured using LCi-SD Ultra-Compact Photosynthesis System (BioScientific, UK). The measurements were taken between 10h00 and 14h00 on clear sunny days on fully grown illuminated leaves at a height of about 1.5m. The leaves were located outside the canopy. When conditions allowed, measurements were taken every 2 weeks. The measured parameters included CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E) and intercellular CO_2 concentration (C_i). Leaf gas exchanges were upscale to canopy level using the Big Leaf Model (Luo et al. 2018). The upscaling was done using the formula:

$$A_c = A_0 \times \frac{1 - \exp(-k \times \text{LAI}_{\text{tot}})}{k} \quad (4)$$

Where A_c is the total canopy photosynthesis rate, A_0 is the photosynthetic rate of the fully illuminated leaves on the canopy, k is the extinction coefficient and LAI_{tot} is the total leaf area index of the canopy.

Intrinsic and Instantaneous water use efficiencies were calculated from the gas exchange measurements using the following formulas:

$$\text{Intrinsic WUE} = \frac{A}{g_s} \quad (5)$$

189 Instantaneous WUE = $\frac{A}{E}$ (6)

190 Determination of bulk density, infiltration rate and particle size

191 Bulk density was measured using the core ring method. Cores with a diameter of 5 cm and a height
192 of 5 cm were used to collect undisturbed soils cores in the top 5 cm of the soils. A total of 12 soil
193 cores were collected following the design of 2 x 2 x 3 replications. The soils were then oven-dried
194 at 105°C for 24 hours before being weighed. Infiltration rate was measured using mini-disk
195 infiltrometers (Decagon Devices, USA). The infiltration measurements were taken once in each
196 experimental plot (n =12). Soil samples for particle size were also collected from each
197 experimental plot (n =12) and determined using the pipette method (Day 1965). All the three
198 parameters (bulk density, infiltration rate and particle size) were measured about 1m from the tree
199 trunk and close to the installed chambers.

200 Soil Chemical Analyses

201 Soil samples were collected from two depths i.e. 0 -15 cm and 15 – 30 cm using augers. Similar
202 to the other parameters 12 soil auger samples were collected per depth (one sample per
203 experimental plot). The samples were then air-dried and sieved to pass through a 2 mm sieve. Soil
204 pH was determined in both water and KCl. De-ionised water and 1M KCl solution were used in a
205 ratio of 1:2.5 and measured using a glass electrode. Calcium and Mg were determined by atomic
206 absorption after being extracted using 1M KCl, filtered and diluted with 20 ml of 0.0356M SrCl₂.
207 Phosphorus, K, Zn, Cu, and Mn were extracted using Ambic-2 solution and determined using
208 atomic absorption. Total C and N were analyzed by the Automated Dumas dry combustion method
209 using a LECO CNS 2000 (Leco Corporation, Michigan).

210 Determination of soil carbon stocks

211 Soil carbon stocks refer to the amount of carbon in a soil layer of known bulk density. Soil carbon
212 stocks are normally expressed as weight/mass per unit area and is commonly limited to the soil
213 fraction of less 2mm in size. Soil C stocks were determined by the following formula (Batjes
214 1996):

$$215 \text{ Soil C stock} = C_{\text{tot}} \times BD \times d \quad (7)$$

216 Where Soil C stock is soil carbon stock (kg C m^{-2}), C_{tot} is the total carbon content (g C g^{-1} soil),
217 BD is bulk density (kg m^{-3}); d is soil depth (m). Carbon stocks were calculated from the top 5 cm
218 since bulk density was only determined in that depth.

219 Data analysis

220 A two-way ANOVA was run using SPSS 25 (SPSS, USA) to determine the effect of soil type,
221 cultivar and their interaction on the measured parameters. A further ANOVA was carried out to
222 determine the impact of season on the measured parameters. Where differences were significant,
223 Tukey HSD was used to separate means. Paired T tests were also carried out to compare leaf level
224 gas exchanges and canopy level gas exchanges (up scaled values). Regression analyses were run
225 to compare minimum, maximum and mean seasonal temperatures with mean seasonal CO_2 flux
226 rates and mean seasonal photosynthesis rates. Where cultivar by soil type interaction was non-
227 significant, the impact of soil type was compared across cultivars, whereas that of cultivar was
228 made across soil types. When running ANOVA for seasonal variation, data of the two cultivars
229 and the two soil types were combined.

RESULTS

Physical and chemical characteristics of the soils

The average clay content in the rich clay content (RC) soil (22%) was twice that of the moderate clay content (MC) soil (11%) (Table 1). A two-sample T-test, showed that the potassium (K) was significantly higher in the RC soil compared to the MC soil while the other macronutrients (Ca and Mg), did not differ between the two soils but were also relatively higher in the RC soil compared to the MC soil. Phosphorus (P) on the other hand did not differ between the two soils but was relatively higher in the MC soil compared to the RC soil. The concentrations of micronutrients Mn and Zn in the RC soil were significantly higher compared to in the MC soil. The soil pH was 1 unit higher in MC soil but both soils were slightly acidic with pH values of 6.9 and 5.9 for MC and RC soil, respectively. The total carbon content was not different between the two soils only with only a 0.2 % difference between MC soils and the RC soil. Due to the 0.2% difference observed between the two soils, carbon stocks were relatively higher in MC soil compared to the RC soil (Fig. 3A,). Figure 3B also shows that bulk density was higher in MC soils when compared to a RC soil. Moderate clay content soils had an average bulk density of about 1450 kg m⁻³ while the RC soil had an average bulk density of 1310 kg m⁻³.

[Insert Table 1]

[Insert Fig 3]

CO₂ flux rates over the year and seasons

CO₂ flux rates fluctuated throughout the year as shown in Fig 4A. Figure 4A shows averaged values for the two cultivars under each soil type. The CO₂ flux rates did not significantly differ between the RC soil and the MC soil. However, the average CO₂ flux rate for the MC soil was slightly higher (0.109 ± 0.037 tons ha⁻¹ day⁻¹) as compared to 0.107 ± 0.036 tons ha⁻¹ day⁻¹ of the

RC soil. Cumulative CO₂ over the whole experimental period did not differ between the two soils (Fig 4B). However, at the end of the experimental period, a MC soil had cumulatively emitted 36.29 tons ha⁻¹ which was slightly higher (7.56%) than that of the RC soil with 33.74 tons ha⁻¹. CO₂ flux rates differed in response to the season. The flux rates were lowest in the late autumn and winter with an average of 0.065 tons ha⁻¹ day⁻¹ and highest in summer 0.136 tons ha⁻¹ day⁻¹ (Fig 5A). Spring and autumn seasons did not differ in their CO₂ flux rates. The results showed that CO₂ flux rates in summer were more than double the rates observed in winter. Similarly, the cumulative CO₂ emitted per season was also different (Fig 5B). As observed with the flux rates, the winter season emitted the least amount of CO₂ with an average cumulative amount of 3.16 tons ha⁻¹ which was more than three times lower than emitted in summer of 9.982 tons ha⁻¹.

[Insert Fig 4]

[Insert Fig 5]

Annual and seasonal leaf gas exchanges

Leaf gas exchanges of the two citrus cultivars were measured throughout the year. Stomatal conductance (*g_s*), photosynthesis rate (*A*), transpiration rate (*E*) and intercellular CO₂ concentration (*C_i*) did not differ between the two cultivars (Fig 6A-D). All cultivars peaked in *g_s*, *A* and *E* in March. On the other hand, *C_i* remained relatively constant throughout the year with values ranging from 200 to 400 ppm. A similar trend was also observed with the two soils (results not shown) and no differences were also observed. Similar to CO₂ flux rates, only season had a significant influence on the leaf gas exchanges (Fig 7). Seasonal transpiration rate was highest in autumn (3.77 mmol m⁻² s⁻¹) followed by the summer season (2.68 mmol m⁻² s⁻¹) and then winter and spring (Fig 7B). No differences in seasonal transpiration rate were observed between winter and the spring season. Seasonal CO₂ assimilation rates responded similarly to transpiration rates

with the rates following the order of autumn ($9.75 \mu\text{mol m}^{-2} \text{s}^{-1}$) > summer ($4.64 \mu\text{mol m}^{-2} \text{s}^{-1}$) > winter ($2.13 \mu\text{mol m}^{-2} \text{s}^{-1}$) = spring ($2.20 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Fig 7D). Seasonal stomatal conductance was not different between summer and autumn seasons but was higher than the winter and the spring seasons. As expected intercellular CO_2 concentration did not vary over the seasons (Fig 7A).

[Insert Fig 6]

[Insert Fig 7]

Leaf area index and canopy leaf gas exchanges

Leaf area index varied with the season (Fig 8A). Leaf area index was higher in autumn compared to summer and winter. The LAI in spring did not differ with the other seasons. The average LAI in spring was 6.81, summer 5.27, winter 4.91 and spring 5.89. Leaf area index was used to upscale leaf gas exchange parameters such as g_s , E and A to canopy level using equation 1. The results of the upscaling are presented in Fig 8. Differences between seasons at canopy level gas exchanges were the same as those observed with leaf-level gas exchanges. The only difference was that canopy values were relatively higher than leaf-level gas exchanges. Paired samples tests showed significant differences between canopy and leaf level for transpiration rate, stomatal conductivity and photosynthesis rate. The canopy level transpiration rate was 1.05 to $1.52 \text{ mmol m}^{-2} \text{s}^{-1}$ higher than the leaf-level transpiration rate. Stomatal conductance at canopy level was 0.41 to $0.62 \text{ mol m}^{-2} \text{s}^{-1}$ higher than leaf-level g_s . Likewise, canopy photosynthesis rate was 2.06 to $3.26 \mu\text{mol m}^{-2} \text{s}^{-1}$ higher than leaf-level A . There were also differences in the upscaling factors for the different seasons. The highest percentage increase from leaf to canopy level of 79% occurred in spring followed by summer 61%, autumn 56% and winter 26%.

Intrinsic (IntrWUE) did not differ between autumn, winter and spring seasons. No differences in IntrWUE were also observed between summer, winter and spring. IntrWUE was however significantly higher in autumn compared to summer (Fig 9A). On the other hand, instantaneous (InstWUE) water use efficiency were higher in autumn compared to the other seasons (Fig 9B). No variation in both IntrWUE and InstWUE were observed between summer, winter and the spring seasons. Also, due to similar scaling up factors, no differences were observed between the leaf level and canopy level. The values shown in Fig 9 are therefore for both leaf level and canopy level.

[Insert Fig 8]

[Insert Fig 9]

Relationships between temperature, CO₂ flux rates and CO₂ assimilation

A strong linear relationship ($P < 0.05$, $r^2 = 0.99$) between mean seasonal minimum temperatures and CO₂ flux rate was observed (Fig 10A). The results showed that 99% of the variation observed in CO₂ flux rates could be explained by mean seasonal minimum temperatures. Mean seasonal maximum temperatures did not show a significant relationship with CO₂ flux rate. CO₂ assimilation rate was also not related to either mean seasonal minimum or maximum temperatures during the measurement period.

[Insert Fig 10]

Comparison between CO₂ assimilation and CO₂ emission rates

Canopy CO₂ assimilation rates and soil CO₂ emission rates were compared per season (Fig 11). Significant differences in the rates were observed for the summer, autumn and winter seasons while the spring season showed no significant variation between the two. In the autumn season,

the citrus trees assimilated CO₂ at rates (578.4 kg ha⁻¹ day⁻¹) that were almost six times higher than the soil CO₂ emission rates (96.8 kg ha⁻¹ day⁻¹). In the summer season, the assimilation rate was almost double the emission rate. Overall, canopy CO₂ assimilation rates were higher than soil CO₂ emission rates. There was a balance between CO₂ assimilation and CO₂ emission rates in the spring season.

[Insert Fig 11]

DISCUSSION

Annual and seasonal soil CO₂ flux rates

Studies, such as that of Neira et al. (2015) and Yang et al. (2018) have shown that soil clay content influences the rate at which gases move in the soil through its influence on soil porosity and gaseous movement. Thus, soils with high clay content would be expected to restrict gas movements due to the tortuosity of the micro pores (Neira et al. 2015). In this study, though not statistically significant, the moderate clay content (MC) soil cumulatively released about 7.56% more CO₂ compared to the rich clay content (RC) soil. This variation could be attributed to the differences in clay content. The MC soil had an average of 11% clay while the RC soil had 22%. The difference might have also resulted from the differences in soil carbon stocks which were observed to be higher in a MC soil (1.47 kg m⁻²) compared to a RC soil (1.16 kg m⁻²).

Seasonal variations of soil CO₂ emission showed that both the rate and the cumulative amount were highest in the summer season and lowest in the winter season (Fig 5). The average soil CO₂ emission rate in the summer season was 0.136 tons ha⁻¹ day⁻¹ which was more than double the rate observed in the winter season (0.065 tons ha⁻¹ day⁻¹). The differences were mainly due to the variation in temperatures between these two seasons as also observed by Yuste et al. (2003). The

influence of temperature on soil respiration has also been reported in other studies (Burton and Pregitzer 2003; Davidson and Janssens 2006). Several studies have shown that soil moisture and temperature are the main drivers of soil CO₂ emission rates (Burton and Pregitzer 2003; Li et al. 2018; Wu 2020). However, in this study moisture was well controlled and kept at near-optimal thus only temperature influenced that variation in the CO₂ release. Due to the differences observed in the rates of soil CO₂ release, the cumulative amounts of CO₂ emitted per season also differed and was congruent to the emission rates. Thus, the summer season also cumulatively released more CO₂ to the atmosphere compared to the other seasons. In the summer season alone, the citrus orchard released 9.98 tons of CO₂ per hectare compared to the winter season where 3.16 tons ha⁻¹ were emitted.

It is interesting to note that Yuste et al. (2003) reported that in the absence of severe drought, soil temperature becomes a more reliable predictor of soil respiration. In this current study, the citrus orchards were well-watered throughout the year. Thus, the differences can therefore be attributed to the differences in mean seasonal temperatures as observed in Fig 10. The results showed that 99% of the variation observed in the soil CO₂ emission rate could be explained by the minimum ambient temperatures while maximum temperatures could only explained 66% of the variation. These results show that minimum ambient temperatures have a stronger influence on soil CO₂ emission rates compared to maximum temperatures. Mean maximum seasonal temperatures were less influential on soil CO₂ emission rates probably because the temperatures are close to optimal. Though variable, depending on substrate and moisture content, the optimal temperature for soil respiration is believed to be around 35°C (Richardson et al. 2012). Temperature sensitivity of respiration is also reported to decreases as temperature increases (Yuste et al. 2003). Even though no actual soil temperature measurements were conducted in this study, Islam et al. (2015) reported

a strong relationship between atmospheric and soil temperature thus the recorded ambient temperatures could be indicative of the soil temperatures. Yilmaz (2012) found both soil and air temperature to significant and positively related to CO₂ emission rates.

The findings of this study suggest that if temperatures get warmer as predicted by the IPCC (2014), more CO₂ would be released in the citrus orchards in winter and spring seasons. The reason being that, increasing global temperatures could potentially cause large increases in root and associated soil respiration (Burton and Pregitzer 2003). The increase in CO₂ emission could result in potentially strong feedback to global warming. However, according to Burton and Pregitzer (2003), if root respiration acclimates to higher temperatures, increases in CO₂ emission could be minimal.

Annual and seasonal leaf gas exchanges

As observed with soil type, citrus cultivars did not vary in the CO₂ assimilation rates and other leaf gas exchange parameters such as *g_s*, *E* and *C_i* but fluctuated throughout the experimental period. Seasonal variation had a strong influence on leaf gas exchanges. The highest rates of CO₂ assimilation were observed in autumn as compared to the other seasons for CO₂ emission rates. These findings are similar to those reported by DAVIS et al. (2014) but are contrary to the findings of Ribeiro and Machado (2007) who found higher CO₂ assimilation rates in spring followed by summer and lowest in winter. However, all studies report the lowest rates of CO₂ assimilation rates in winter.

In this study, CO₂ assimilation rates in the winter season were four times lower than those reported in the autumn season. Ribeiro et al. (2009) who reported similar findings attributed the differences to decreased root permeability and plant hydraulic conductance due to low temperatures. Poor root permeability is thought to result from increased viscosity of water which then affects water supply

to the leaves (Angelocci et al. 2004). The low plant hydraulic conductivity due to poor root permeability results in lower stomatal conductance leading to lower CO₂ assimilation rates (Ribeiro and Machado 2007; Ribeiro et al. 2009). Accordingly, *g_s* in winter was about 75% lower when compared to mean *g_s* in autumn. In a lime orchard, Angelocci et al. (2004) found *g_s* to decrease with lower temperatures and vapour pressure deficit. Lower CO₂ assimilation rates in winter could also be attributed to the reduction in the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) an enzyme that catalyses the fixation of CO₂ in C₃ plants (Hendrickson et al. 2004; Sage 2002). Also, according to Ribeiro et al. (2018), citrus plants have a low temperature threshold of around 13°C. Thus citrus plant metabolism is severely reduced at lower temperatures.

Intercellular CO₂ concentration (*C_i*) is one other parameter that is reported to influence CO₂ assimilation in plants. In this current study, *C_i* did not vary with season and hence could not have effected CO₂ assimilation. In other studies with other evergreen tree crops such as macadamia, CO₂ assimilation rate responded to *C_i* where higher *C_i* resulted in increased CO₂ assimilation (Smit et al. 2020). However, CO₂ assimilation rates were as expected strong related to *E* and *g_s* (results not shown). However, mean minimum seasonal temperatures did not show any significant relationship with CO₂ assimilation rate despite the obvious influence of temperature on CO₂ assimilation rates. Mean maximum seasonal temperatures had a modest positive relationship ($r^2 = 0.41$; Fig 10B) with CO₂ assimilation. This results suggesting that there could be other plant intrinsic factors contributing to the variation.

In many seasonal crops, CO₂ assimilation rate normally peaks just before the flowering and fruiting stages probably due to higher sink activity (Lewis et al. 2002). And indeed some studies have suggested an association between sink demand and photosynthesis rate (Dovis et al. 2014;

Nebauer et al. 2013; Ribeiro et al. 2012). As evergreen species, citrus trees assimilate and consume carbon throughout the year (Dovis et al. 2014). Citrus flowers in the spring but their CO₂ assimilation rates were not highest in spring and this could be because the flowering was driven by stored assimilates. Dovis et al. (2014) showed that reserve assimilate consumption from the roots was high during citrus flowering.

Canopy CO₂ assimilation vs soil CO₂ emission rate

Due to the limitation of studies on the role of orchards in sequestering atmospheric CO₂ this study attempted to provide a snapshot of whether a citrus orchard is a net sink or emitter of CO₂. As such seasonal canopy CO₂ assimilation rates were compared to seasonal soil CO₂ emission rate (Fig 11). The results showed that in most seasons (summer, autumn and winter) CO₂ assimilation rates were significantly higher than CO₂ emission rates suggesting that citrus orchard could be net sinks of CO₂. Even though this snapshot method applied in this study is not as accurate and common as the eddy covariance methods (Aubinet et al. 2012), it is however very applicable and acceptable in that it provides a relatively high spatial and temporal capture of the influence of management practices that are known to strongly influence both plant physiology and soil condition which consequently affect carbon exchange (Nardino et al. 2013). One drawback of this snapshot is that it does not capture CO₂ losses from plant respiration (Huntingford et al. 2017) but it is known that plant assimilates more CO₂ than they emit. Other studies use carbon stocks methods to estimate CO₂ sequestration in tree-based systems (Dube et al. 2018; Ryan et al. 2011)

It was also observed that while leaf and canopy CO₂ assimilation rates peaked in autumn, soil CO₂ emission rates peaked in the summer season. These differences could just be attributed to the mechanisms and the optimal operational conditions for each of the process. As discussed earlier, soil respiration rates increase with temperature (Burton and Pregitzer 2003) and since soil CO₂

emission rates were measured under a tree canopy, it is possible that maximum warming of the soil occurred in summer when temperatures were higher (Fig 1). On the other hand, CO₂ assimilation rates might have peaked in autumn due to a combination of factors that include optimal environmental conditions and increase in assimilate demand by the plant. Interestingly, no significant difference in canopy CO₂ assimilation and soil emission rates were observed in the spring season.

CONCLUSIONS

This study is one of the few studies to present empirically measured CO₂ flux rates and CO₂ assimilation rates in a commercial drip-irrigated citrus orchard. The objective was to investigate how soil CO₂ emission rates and leaf CO₂ assimilation rates vary with seasons and the extent to which soil clay content influences these two parameters in a well-managed citrus orchard. The study showed that under near-optimal growth conditions, soil clay content did not influence the rate at which CO₂ was released in the soil neither did it have any effect on the rate at which the citrus assimilated atmospheric CO₂. Despite the near-optimal growth conditions, both soil CO₂ emission rates and leaf CO₂ assimilates rates responded strongly to seasonal variations confirming the influence of environmental conditions particularly of temperature on the release and capture of carbon in agroecosystems. The findings showed that citrus CO₂ assimilation rate peaked in the autumn and was six time higher than the CO₂ emission rates but the two rates balanced up in the spring season. Snapshot analysis of the CO₂ sequestration rates suggests that citrus orchards are net sinks of CO₂.

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461 Conflicts of interest/Competing interests

462 The Authors declare no conflict of interests

463 Availability of data and material

464 Some data can be made available through request

465 Code availability

466 No custom codes were used

467 Author Contributions

468 Conceptualization, LM, KKA and TPM; Data curation, LM; Formal analysis LM; Funding
469 acquisition; Investigation, LM, KEM, TM; Methodology, LM, KKA and TPM ; Project
470 administration; Resources; Visualization, LM, KEM, TM; Writing LM, KKA, TPM, KEM and
471 TM

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648

Figures

Figure 1

The weather data shown in Fig 1 was obtained from an automatic weather station installed at the experimental site.

Figure 2

These collars were left in the soil for a few days to settle before the measurements were taken (Fig 2).

Figure 3

Due to the 0.2% difference observed between the two soils, carbon stocks were relatively higher in MC soil compared to the RC soil (Fig. 3A.). Figure 3B also shows that bulk density was higher in MC soils when compared to a RC soil.

Figure 4

CO₂ flux rates fluctuated throughout the year as shown in Fig 4A. Figure 4A shows averaged values for the two cultivars under each soil type.

Figure 5

The flux rates were lowest in the late autumn and winter with an average of 0.065 tons ha⁻¹ day⁻¹ and highest in summer 0.136 tons ha⁻¹ day⁻¹ (Fig 5A). Spring and autumn seasons did not differ in their CO₂ flux rates. The results showed that CO₂ flux rates in summer were more than double the rates observed in winter. Similarly, the cumulative CO₂ emitted per season was also different (Fig 5B).

Figure 6

Stomatal conductance (gs), photosynthesis rate (A), transpiration rate (E) and intercellular CO₂ concentration (Ci) did not differ between the two cultivars (Fig 6A-D).

Figure 7

A similar trend was also observed with the two soils (results not shown) and no differences were also observed. Similar to CO₂ flux rates, only season had a significant influence on the leaf gas exchanges (Fig 7).

Figure 8

Leaf area index was used to upscale leaf gas exchange parameters such as g_s , E and A to canopy level using equation 1. The results of the upscaling are presented in Fig 8.

Figure 9

No differences in IntrWUE were also observed between summer, winter and spring. IntrWUE was however significantly higher in autumn compared to summer (Fig 9A). On the other hand, instantaneous (InstWUE) water use efficiency were higher in autumn compared to the other seasons (Fig 9B).

Figure 10

A strong linear relationship ($P < 0.05$, $r^2 = 0.99$) between mean seasonal minimum temperatures and CO₂ flux rate was observed (Fig 10A).

Figure 11

Canopy CO₂ assimilation rates and soil CO₂ emission rates were compared per season (Fig 11).