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Computing for Sustainable and Smart Agriculture

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Research Article

Understanding the Impact of the Intercropping System on Carbon Dioxide (CO_2) Emissions and Soil Carbon Stocks in Limpopo Province, South Africa

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Understanding the carbon dioxide emission rates under different agricultural practices is a critical step in determining the role of agriculture in greenhouse gas emissions. One of the challenges in advocating for an intercropping system as a sustainable practice in the face of climate change is the lack of information on how much CO_2 is emitted by the system. A factorial randomized complete block design study was set up at two distinct agroecological locations (Syferkuil and Ofcolaco) in the Limpopo Province of South Africa to investigate carbon dynamics in sorghum-cowpea intercropping and sole cropping system over two seasons. Intercropping system emitted less CO_2 compared to sole cropping system. In 2018/19 at Syferkuil and 2020/21 at Ofcolaco, intercropping systems emitted 11% and 19% less CO_2, respectively, than sole cropping systems. In both agroecological regions, low cowpea density consistently resulted in higher CO_2 emissions than high density. During the 2018/19 cropping season, sorghum emitted more CO_2 of 5.87 t·ha^{-1} than cowpea with 5.14 t·ha^{-1} in a sole cropping system at Syferkuil. Cowpea, on the other hand, emitted more CO_2 of 6.5 t·ha^{-1} and 10.18 t·ha^{-1} than sorghum during the 2020/21 cropping season at Syferkuil and Ofcolaco, respectively. Furthermore, intercropping improved the carbon emission efficiency (CEE) of the individual crops in the system. The treatments used in the intercropping and sole cropping systems had a significant impact on the strength of the relationship between carbon stocks and CEE. Our results revealed that sorghum-cowpea intercropping system at a relatively higher cowpea density in a no-till system reduces the amount of CO_2 lost to the atmosphere. The system can thus, be promoted as one of the sustainable farming practices to reduce emissions and improve carbon storage in the soil.

1. Introduction

Agricultural activities, such as crop production, are major contributors to global CO_2 emissions. Agriculture accounts for more than 21% of global greenhouse gas emissions[1]. CO_2 emissions increased by 13% in the agricultural sector between 2007 and 2016 [2]. These emissions are a result of the need to increase food production in order to feed the world's growing population. Sustainable crop intensification is the key to producing food on less land while protecting the natural ecosystem [3] Preferred crop production practices such as sole cropping system coupled with conventional tillage do not enhance the retention of organic matter [4]. According to Paustian et al. [5], crop production practices emit more greenhouse gases that contribute to climate change from planting to harvesting compared to other agricultural practices. Climate plays an important role in determining the potential of agricultural activities such as crop production in South Africa, with a particular emphasis on smallholder farmers [6]. As a result, climate change variability has a significant impact on smallholder farmers, particularly in Limpopo Province's semiarid regions, where most farmers produce under rainfed conditions. As a climate-smart practice, intercropping has the potential to increase crop production while lowering greenhouse gas emissions and increasing resilience to climate change [1].

Sustainable crop production practices such as minimum tillage and intercropping systems are required for farmers to continue producing in a way that is environmentally friendly. Adoption of these practices necessitates a thorough understanding of the effects of farming practices on the soil and the environment [7]. Most of the research on intercropping systems in Limpopo Province has focused on productivity as well as nitrogen dynamics in the soil [8, 9]. However, there is little to no information available on the system's impact on carbon dynamics in the soil, with a focus on CO_2 emissions in intercropping. Such information is critical in understanding the role of conservation practices in reducing greenhouse gases such as CO_2.

The study conducted in China revealed that when combined with other sustainable crop production practices such as mulching and conservation tillage, intercropping could reduce CO_2 emissions by more than 15% [10]. According to research, intercropping systems combined with conservation tillage can help reduce CO_2 emissions while increasing soil organic carbon [11]. However, carbon emissions are highly influenced by the growing conditions such as temperature, soil moisture, and precipitation. Hence, the specific cropping system, as well as the agroecological condition, must be studied to determine the extent to which an intercropping system reduces CO_2 emissions. The study aimed at investigating soil carbon emissions and carbon stocks in intercropping versus sole cropping systems under distinct environmental conditions in Limpopo Province. The study also focused on determining whether increased plant density of the companion legume crop in the intercropping system can reduce the CO_2 emissions while increasing accumulation of dry matter. The research hypothesised that grain sorghum-cowpea intercrop under high and low density of cowpea would reduce soil CO_2 emission rates and increase soil carbon stocks.

2. Materials and Methods

2.1. Experimental Sites. The study was conducted at two distinct agroecological zones in Limpopo Province, South Africa (Figure 1). The first location was the University of Limpopo Experimental Farm, Syferkuil, situated at geographical coordinates of 23° 50′ 02.7″S and 29° 41″ 25.5″E. The area receives an annual rainfall of about 350 to 500 mm with an average maximum and minimum temperatures of 30°C and 15°C,' respectively. The second location was the Itemeleng Ba-Makhutjwa Primary cooperative at the farmers' field at Ofcolaco, located at 24° 06″ 38.3″S and 30° 23″ 11.8″E near Tzaneen town. Ofcolaco receives an annual rainfall of approximately 650 to 700 mm with an average maximum and a minimum temperature of 35°C and 18°C, respectively. The two locations also have different soil types: sandy-clay at Syferkuil and clay-loam at Ofcolaco. Both soils were classified as Hutton according to the Soil Classification System for South Africa. Syferkuil soils were also classified as chromic Luvisols while those at Ofcolaco were classified as rhodic Luvisols according to the World Reference Base of 2014.

2.2. Weather Data. Two automatic weather stations were used to provide daily weather data. At the University of Limpopo Experimental Farm (Syferkuil), the weather station was located at the farm, whereas at Ofcoalco, an automatic weather station, situated approximately 25.7 km from the experimental site were used to access daily weather data during the period of experimentation. The variables measured daily minimum and maximum temperatures, daily rainfall during the growing seasons. The variables were used to plot Figures 2 and 3 as presented under results.

2.3. Experimental Design. The experiment was laid out in a randomized complete block design (RCBD) in a $2 \times 4 \times 2$ factorial arrangement, replicated four times. The treatment factors studied were two cropping systems (intercrop and sole), four sorghum cultivars (Avenger, Enforcer, Titan, and NS5511), and two cowpea densities of 37037 $p \cdot ha^{-1}$ (low) and 74074 $p \cdot ha^{-1}$ (high) under no-till dryland conditions. Each experimental unit was 3.0 m × 3.6 m, consisting of four rows of sorghum and four rows of cowpea in the intercropped treatment (Figure 4). For grain sorghum, seeds were planted at inter- and intrarow spacings of 0.9 m and 0.3 m, respectively. The cowpea was planted at an inter-row spacing of 0.9 m and an intrarow spacing of 0.3 and 0.15 m to obtain the treatment densities of 37037 and 74074 plants ha^{-1}, respectively. The spacing between sorghum and cowpea in the intercropped treatment was thus 0.45 m, and the size of the experimental unit was 10.8 m^2. The details of the experimental design and management are also outlined by Mogale et al. [12].

2.4. Installation of Collars and Measurement of Soil CO_2. For this research, CO_2 emission measurements were taken between 09h00 and 15h00 throughout the experiment from each gas chamber. The CO_2 measurements were taken using GMP343 CO_2 probe along with MI70 data logger. The gas chambers were installed at each experimental unit from the onset of the experiment during the 2018/19 and 2020/21 cropping seasons. The chambers were installed in the middle rows of each plot and between sorghum and cowpea in intercropping (Figure 5(a)). The gas chambers consisted of two separate PVC collars. One PVC ring (0.20 m diameter and 0.15 in height) was inserted to the ground using hammer to about 0.05 m. The other PVC collar (0.20 m diameter and 0.10 m height) was used as a lid, fitted CO_2 probe on it, and had a small gas valve on it to discourage pressure build up in the chambers during measurements (Figure 5(b)). Modification of chambers, the size of PVC ring/collar, the information on the chamber lid, and measurements and calculations of CO_2 were done following the procedure described by Munjonji et al. [13].

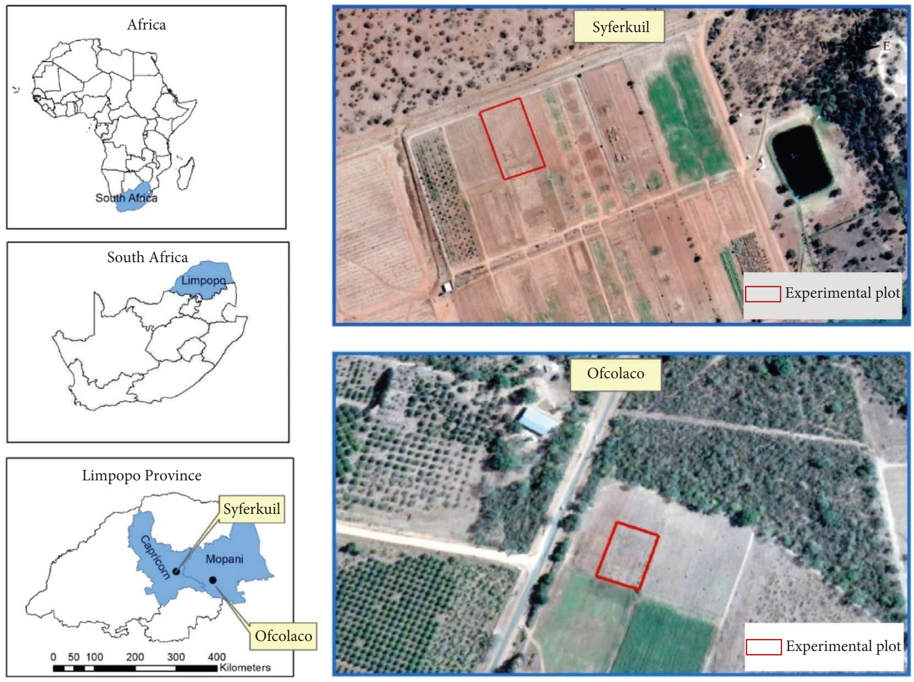

Figure 1: The map of agroecological regions where the field experiments were conducted during the 2018/19 and 2020/21 cropping seasons (Risk and Vulnerability Science Centre, University of Limpopo).

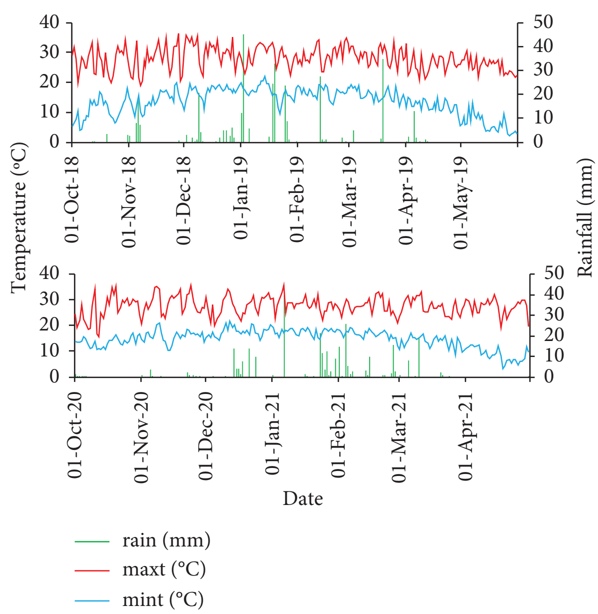

Figure 2: Rainfall, maximum and minimum temperature from Syferkuil during the 2018/19 and 2020/21 cropping seasons.

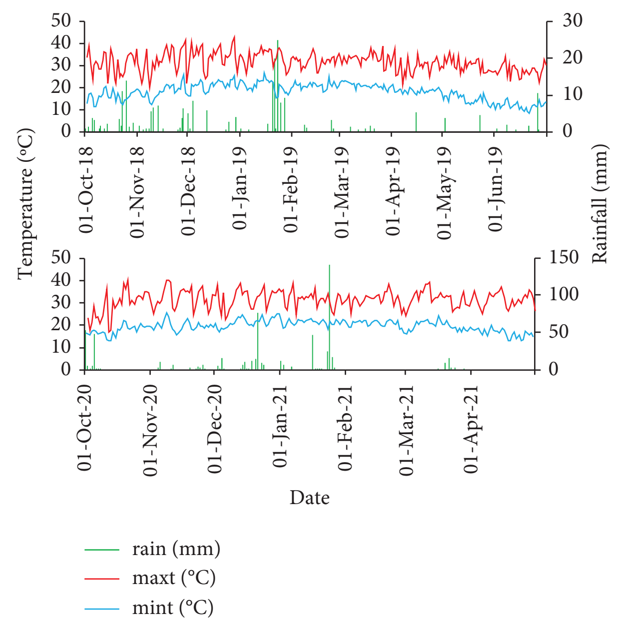

Figure 3: Rainfall, maximum and minimum temperature at Ofcolaco during the 2018/19 and 2020/21 cropping season.

2.4.1. CO_2 Flux Calculations. The CO_2 collected from the field was in part per million, therefore was converted to $mg{\cdot}m^{-3}$ using the following equation:

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

where P is the pressure, V is the volume, n is the moles of gas, R is the constant value of gas law, and T is the temperature.

The molar volume was calculated at different pressures using the following formula:

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

The CO_2 in $mg{\cdot}m^{-3}$ was calculated at different temperatures and pressure as follows:

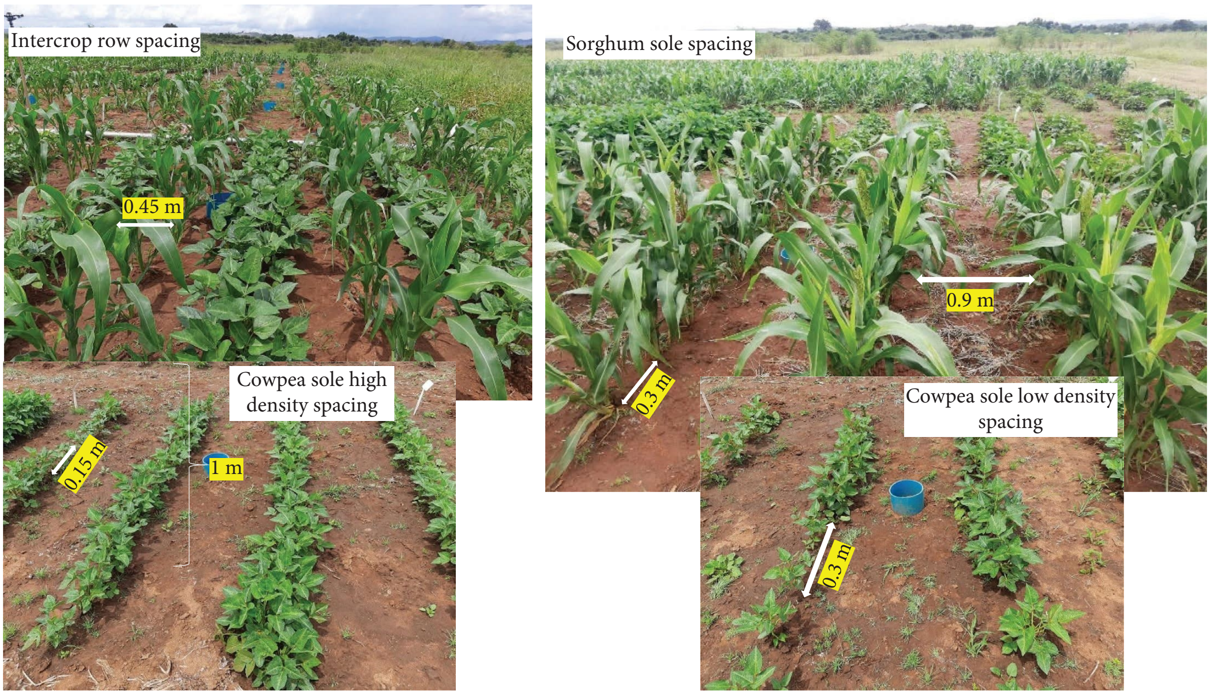

Figure 4: Binary and sole row arrangements.

(a) (b)

Figure 5: (a) Installed pvc chamber/collar and (b) soil CO_2 chamber to measure CO_2 emission rates.

$$CO2\left(\frac{mgm}{3}\right) = \left(\frac{CO2ppm x Molarweight(CO2)}{22.4Lmol}\right) x \left(\frac{273.15K}{T(K)}\right) x \left(\frac{P(kPa)}{101kPa}\right), \quad (3)$$

where CO_2 in ppm is measured every 0.5 second for 5 minutes, T represent the temperature of the chamber, and P is the ambient pressure.

The CO_2 in mgm^{-3} was plotted against time to get the slope in $mgm^{-3}\cdot min^{-1}$. The CO_2 flux was calculated using the following formula:

$$\text{CO2 Flux}\left(\frac{\text{mgM}}{2}\quad\frac{\text{min}}{1}\right)=\frac{\text{Slope } x \text{ voleme of the chamber}}{\text{area covered by the chamber}}. \tag{4}$$

The cumulative CO_2 emission was calculated by assuming the CO_2 emission rate was constant from one data point to another.

2.5. Determination of Carbon Dioxide Emission Efficiency (CEE). Dry biomass was collected at harvest maturity of sorghum and cowpea at an area of 2.7 m^2 for each crop. At each harvesting area, a total of 10 plants were sampled. The samples were dried in the laboratory in an oven at 65°C to a constant weight to determine biomass weight. The correlation between dry biomass (DB) and the rate of carbon dioxide emission (CO_2E) of each crop was measured using carbon dioxide emission efficiency (CEE) as described by Hu et al. [14]. The authors used the following formula to calculate carbon emission efficiency:

$$\text{CEE}=\frac{\text{DB}}{\text{CO2}E}, \tag{5}$$

where CEE is the carbon emission efficiency, DB is the weight of dry biomass (kg·ha^{-1}), and CE (kg·ha^{-1}) is the rate of CO_2 emission.

2.6. Determination of Soil Bulk Density and Soil Carbon Stocks. Bulk density was measured at two soil depth, i.e., 0–10 cm and 10–20 cm, sampled four times each level per plot using the core ring method. Cores with a diameter of 5 cm and a height of 5 cm were used. Sampled soils were then oven dried at a temperature of 105°C for 24 hours. The bulk density was collected close to where the chambers for CO_2 emission rates were installed. Initial and final soil samples were collected per plot at two different depth, i.e., 0–15 cm and 15–30 cm for two cropping seasons of 2018/19 and 2020/21. The drying of samples was done using the oven dry method at a temperature of 105°C for 24 hours before weighing them. The soil carbon stock was determined using soil organic carbon (SOC), bulk density (BD), and depth (D), from which soil samples were collected as described by Mbanjwa et al. [15]. The following formula was used:

$$\text{CS}=\text{SOC}\, x\, \text{BD}\, x\, D, \tag{6}$$

where CS is carbon stocks (kg·m^{-2}), SOC is soil organic carbon (%), BD is soil bulk density (kg·m^{-3}), and D is the soil depth (m).

2.7. Gravimetric Water Content and Soil Chemical Analysis. Pre- and postplanting soil samples were collected on each experimental unit at the depth of 0–30 cm using an auger at the two experimental sites. Each sample was stored in a zip bag and sealed after being collected to avoid moisture loss. The samples were taken to the laboratory where the fresh weight of each sample was determined using a weighing balance. The samples were air-dried for seven days in the laboratory and were weighed again to obtain dry weight. Gravimetric water content was calculated using the following formula:

$$\text{GWC (\%)}=\frac{\text{Fresh wegiht}-\text{dry weight}}{\text{dry weight}}\, x\, 100. \tag{7}$$

The samples were sieved to pass through a 2 mm sieve and analysed for chemical properties. Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), and copper (Cu) were analysed following the procedure of Mehlich-III multinutrient extraction method. Soil organic carbon was determined using Walkley and Black method.

2.8. Statistical Analysis. The relevant model assumptions, including normality, independence, and constant variance, were checked before data analysis. The Statistical Analysis System (SAS) version 9.4 was used to fit CO_2 emission and other soil data collected using a multivariate multifactor analysis of variance (ANOVA) model. Mean separation was done where the means were different, using the least significant difference (LSD) at probability levels of $p \leq 0.05$. A regression analysis was done to test the relationship between the CO_2 emission rate and carbon stocks.

3. Results

3.1. Weather Conditions during Growing Seasons. Rainfall occurred in most months during the trials at Syferkuil. In the 2018/19 cropping season, it rained from late October throughout the cropping season until mid-April, as shown in Figure 2. The highest rainfall of more than 40 mm occurred in January 2019, with the lowest rain of less than 5 mm at the beginning of the season and towards the end of data collection. During the 2020/21 cropping season, the rain fell from November throughout the growing season until April, when the experiment was terminated (Figure 2). The highest rainfall of more than 40 mm occurred in January, with the lowest at the beginning and towards the end of the cropping season. Temperatures fluctuated during the two cropping seasons. The maximum temperature reached more than 35°C while the minimum temperature dropped to less than 10°C.

Rainfall from Ofcolaco occurred mainly from October to February during the 2018/19 cropping season. The highest rainfall of more than 20 mm was observed in February 2019 (Figure 3). During the 2020/21 cropping season, the highest rainfall of about 140 mm occurred in February 2021. The fluctuations in temperatures were also observed at Ofcolaco. In 2018/19, the highest maximum temperature reached 40°C, whereas the highest minimum temperature was about 10°C. The highest maximum temperature occurred a month before the highest rainfall occurred (Figure 3). In the 2020/21 cropping season, the highest maximum temperature reached about 40°C and the minimum temperature was more than 10°C throughout the growing season.

3.2. The Effect of Cropping System on Soil Physical and Chemical Properties. Bulk density (BD) was higher in sole compared to the binary culture at Syferkuil during the

2018/19 cropping season, with a mean of 1270.01 kg·m^{-3} and 1260.41 kg·m^{-3}, respectively (Table 1). The results indicated that binary cultures had more gravimetric water content (GWC) of 11% compared to sole cultures, which had 10%. Phosphorus (P), potassium (K), calcium (Ca), and zinc (Zn) concentrations were higher in the sole compared to the binary cultures, with means of 28.49 mg·kg^{-1}, 301.84 mg·kg^{-1}, 1061.30 mg·kg^{-1}, and 3.05 mg·kg^{-1}, respectively. The results revealed that organic carbon (Org.C), the carbon and nitrogen ratio (C:N ratio), and carbon stocks (CS) were 8%, 18%, and 8% higher in binary compared to sole cultures, respectively. Phosphorus was higher, with a mean of 45.2 mg·kg^{-1} in binary compared to sole culture, which had 29.21 mg·kg^{-1} P. The soil had 8% more K in the sole compared to binary cultures. In 2020/21 season, the BD was 1463.10 kg·m^{-3} in binary and 1448.70 kg·m^{-3} in sole cultures. Organic carbon and carbon stocks were 12% and 11% more in sole compared to binary cultures during the 2020/21 cropping season, respectively.

The results from Ofcolaco revealed that BD was higher in binary compared to sole cultures during the 2018/19 cropping season (Table 2). Sole cropping had a higher GWC of 26% compared to binary culture, which had 21%. The soil had 34%, 15%, and 10% higher P, Zn, and Mn in binary culture compared to sole culture, respectively. The results further revealed that soil under a sole cropping system had higher K and Ca compared to the soil under binary culture of 10% and 1%, respectively. The CN ratio was higher in binary cultures compared to sole cultures. The soil from Ofcolaco had a BD of 1277.48 kg·m^{-3} compared to binary culture which had 1201.91 kg·m^{-3} (Table 2) during the 2020/21 cropping season. P, Zn, and Mn were higher, whereas K was lower in binary compared to sole cultures. The soil had 3% more CN ratio in the sole compared to binary culture.

3.3. The Effect of Cropping System and Temperature on CO_2 Emission Rate. During the 2018/19 growing season, the grain sorghum-cowpea intercropping system significantly ($p < 0.01$) influenced CO_2 emissions at 42, 28, and 56 days after planting at Syferkuil (Figure 6). The cropping system did not affect CO_2 emissions at 11, 78, 88, 98, and 112 days after planting. Sole CO_2 emissions were higher in sole cultures, ranging from 0.05 t·ha^{-1}·day^{-1} to 0.09 t·ha^{-1}·day^{-1}, compared to binary cultures, which were between 0.04 t·ha^{-1}·day^{-1} and 0.06 t·ha^{-1}·day^{-1} from 11 to 56 days after planting (Figure 6) with the average temperature ranging from 21 to 23°C. The CO_2 emission rate decreased from 76 to 112 days after planting in both cases as the average temperature dropped from 22 to 18°C.

In the 2020/21 cropping season, the CO_2 emission rate was higher at Syferkuil in binary cultures compared to sole cultures between 39 and 67 days after planting, which ranged from 0.1 t·ha^{-1}·day^{-1} to 0.07 t·ha^{-1}·day^{-1} and 0.09 t·ha^{-1}·day^{-1} to 0.04 t·ha^{-1} day^{-1}, respectively. From 91 to 117 days after planting, the CO_2 flux dropped in binary and increased in sole cultures (Figure 7). The average temperature did not influence the fluctuations in CO_2 emission rates in the 2020/21 cropping season. However, at 117 days after planting when CO_2 emission was lower compared to other sampling days when the average temperature dropped to 15°C.

At Ofcolaco, the CO_2 emission rate was higher in sole compared to the binary culture at 39 days after planting, as shown in Figure 8 with the means of 0.1 t·ha^{-1}·day^{-1} and 0.07 t·ha^{-1}·day^{-1}, respectively. However, at 49 and 63 days after planting, the CO_2 emission rate was similar in sole and binary cultures. The CO_2 emission rate continued to increase in sole culture from 83 to 101 days after planting.

Plant density had a significant effect ($p \leq 0.05$) on CO_2 emission at Syferkuil from 28 to 56 days after planting during the 2018/19 cropping season. In the 2020/21 cropping season, CO_2 emission was significantly different ($p \leq 0.05$) between low and high density between 104 and 117 days after planting. During the 2018/19 cropping season, low density cowpeas emitted more CO_2 between 11 and 56 days after planting (DAP) than high density cowpeas (Figure 9(a)). CO_2 emissions ranged between 0.05 t·ha^{-1}·day^{-1} and 0.87 t·ha^{-1}·day^{-1} at low density, and between 0.05 t·ha^{-1}·day^{-1} and 0.058 t·ha^{-1}·day^{-1} at a high density from 11 DAP to 56 DAP. CO_2 emissions did not differ between binary and sole cultures, as well as low and high cowpea density between 76 and 112 days after planting, according to the findings. CO_2 emission rates in low and high density were comparable from 39 to 91 days after planting in the 2020/21 cropping season. Low density, on the other hand, emitted more CO_2 than high density from 104 to 117 days after planting (Figure 9(b)).

The results further revealed that the low density of the companion crop emitted more CO_2 compared to the high density from 39 to 63 days after planting with the means of 0.087 t·ha^{-1}·day^{-1}, 0.133 t·ha^{-1}·day^{-1}, and 0.072 t·ha^{-1}·day^{-1}. However, between 83 and 101 days after planting, CO_2 emission rates were similar in low and high density (Figure 10). On average, low density emitted about 0.098 t·ha^{-1}·day^{-1} from 39 DAP to 63 DAP, while under high density, 0.086 t·ha^{-1}·day^{-1} was emitted between the same days after planting.

3.4. The CO_2 Emission Rate for Each Crop and the Combination of the Two Crops. Sorghum had higher emissions of CO_2 0.065 t·ha^{-1}·day^{-1} in monocropping between 28 and 76 days after planting compared to cowpea in monocropping and the combination of sorghum and cowpea which had 0.052 t·ha^{-1}·day^{-1} and 0.054 t·ha^{-1}·day^{-1}, respectively, during the 2018/19 cropping season at Syferkuil (Figure 11(a)). The sorghum-cowpea combination emitted less CO_2 compared to when the two crops are planted in sole cultures between 28 and 76 days after planting. CO_2 emissions were similar in binary and sole cropping between 88 and 112 days after planting. When compared to other sampling dates during the 2020/21 cropping season, sorghum-cowpea combination and cowpea had high CO_2 emissions 39 days after planting. Sorghum emitted less CO_2 in the sole at 39 days after planting. At 91 to 117 days after planting, CO_2 emissions were higher in cowpea soles compared to sorghum soles and the combination of sorghum and cowpea

Table 1: Bulk density (BD), gravimetirc water content (GWC), and soil chemical properties from Syferkuil collected at the end of 2018/19 and 2020/21 cropping seasons.

Chemical properties	2018/19		2020/21	
	Binary	Sole	Binary	Sole
BD (kg·m^{-3})	1260.41 ± 192.30	1270.01 ± 210.62	1463.10 ± 412.10	1448.70 ± 335.41
GWC (%)	11.08 ± 5.99	9.61 ± 4.46	10.74 ± 2.06	10.80 ± 2.49
P (mg·kg^{-1})	25.29 ± 14.19	28.49 ± 20.71	45.20 ± 18.65	29.21 ± 11.74
K (mg·kg^{-1})	250.87 ± 81.60	301.84 ± 82.55	255.60 ± 56.18	325.09 ± 53.90
Ca (mg·kg^{-1})	1057.92 ± 93.26	1061.30 ± 88.68	992.79 ± 72.97	1001.84 ± 57.71
Mg (mg·kg^{-1})	595.90 ± 98.18	589.01 ± 83.06	658.39 ± 95.30	712.20 ± 109.24
Zn (mg·kg^{-1})	2.48 ± 1.69	3.05 ± 2.49	6.25 ± 3.65	2.92 ± 1.45
Mn (mg·kg^{-1})	13.43 ± 3.86	13.85 ± 2.20	15.58 ± 2.13	15.08 ± 2.87
Cu (mg·kg^{-1})	2.83 ± 0.45	2.94 ± 0.33	3.19 ± 0.40	3.27 ± 0.35
Org.C (%)	0.65 ± 0.22	0.60 ± 0.23	0.75 ± 0.18	0.84 ± 0.14
C : N ratio	13.93 ± 8.10	11.83 ± 7.97	12.68 ± 2.87	12.68 ± 3.60
CS (kg·m^{-2})	1.46 ± 0.60	1.40 ± 0.63	2.88 ± 0.87	3.19 ± 0.66

Table 2: Soil chemical properties from Ofcolaco collected at the end of 2018/19 and 2020/21 cropping seasons.

Chemical properties	2018/19		2020/21	
	Binary	Sole	Binary	Sole
BD (kg·m^{-3})	1555.25 ± 404.03	1440.97 ± 269.56	1201.91 ± 289.70	1277.48 ± 368.98
GWC (%)	21.27 ± 5.97	25.58 ± 7.86	15.71 ± 4.10	15.10 ± 4.85
P (mg·kg^{-1})	71.73 ± 35.66	53.43 ± 21.09	50.66 ± 26.89	43.63 ± 19.44
K (mg·kg^{-1})	151.50 ± 37.40	166.47 ± 43.74	116.78 ± 44.30	141.95 ± 46.80
Ca (mg·kg^{-1})	748.18 ± 98.77	756.69 ± 94.54	744.38 ± 98.69	741.08 ± 76.38
Mg (mg·kg^{-1})	141.87 ± 18.44	163.41 ± 24.79	149.84±	163.24 ± 21.37
Zn (mg·kg^{-1})	8.29 ± 3.21	7.21 ± 2.73	9.00 ± 4.79	5.75 ± 1.96
Mn (mg·kg^{-1})	39.75 ± 12.44	36.21 ± 11.98	30.91 ± 5.04	28.57 ± 4.21
Cu (mg·kg^{-1})	4.64 ± 0.39	4.51 ± 0.35	4.37 ± 0.61	4.45 ± 0.53
Org.C (%)	1.51 ± 0.14	1.58 ± 0.13	1.38 ± 0.13	1.41 ± 0.14
C : N ratio	70.50 ± 30.05	50.96 ± 26.67	69.68 ± 57.48	71.88 ± 36.03
CS (kg·m^{-2})	6.99 ± 1.82	6.84 ± 1.49	2.49 ± 0.56	2.67 ± 0.84

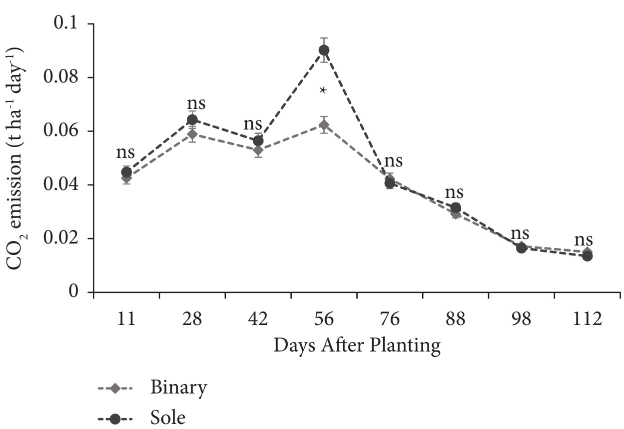

Figure 6: CO_2 emission rates in intercropping (binary) and sole cropping system during the 2018/19 cropping season at Syferkuil. ns = not significant; * = significant at $p \leq 0.05$.

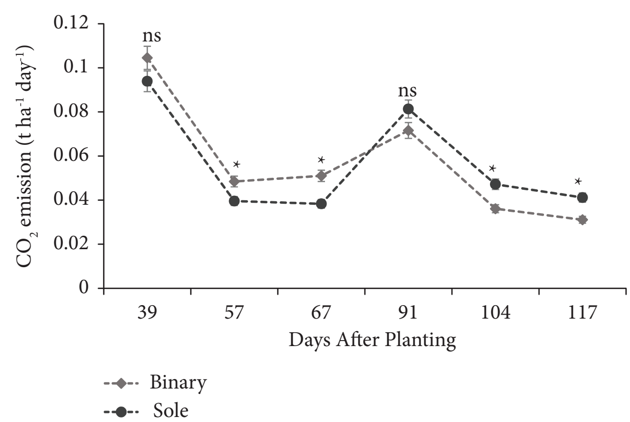

Figure 7: CO_2 emission rates in intercropping (binary) and sole cropping system during the 2020/21 cropping season at Syferkuil. ns = not significant; * = significant at $p \leq 0.05$.

(Figure 11(b)). On average, cowpea sole emitted 0.060 t·ha^{-1}·day^{-1} of CO_2, while a combination of sorghum and cowpea and sorghum sole emitted 0.057 and 0.054 t·ha^{-1}·day^{-1}, respectively.

Cowpea sole had a higher CO_2 emission of 0.11 t·ha^{-1}·day^{-1} at 39 days after planting compared to sorghum sole (0.09 t·ha^{-1}·day^{-1}) and the combination of sorghum and cowpea (0.07 t·ha^{-1}·day^{-1}) at Ofcolaco (Figure 12). However, CO_2 emissions were similar for cowpea and sorghum in sole and binary at 49 days after planting. From 63 to 101 days after planting, cowpea sole had a higher emission of CO_2 compared to sorghum sole as well as the combination of the two crops.

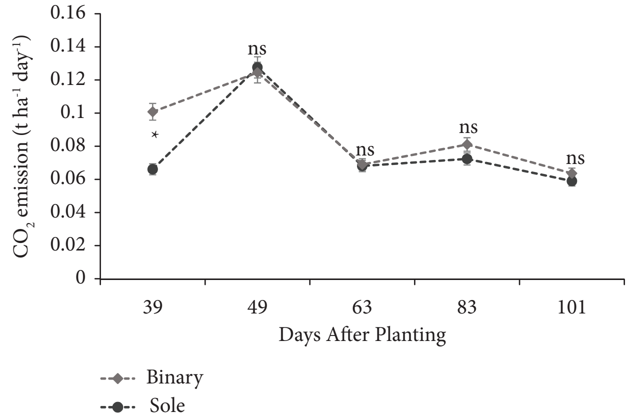

Figure 8: CO_2 emission rates in intercropping (binary) and sole cropping system during the 2020/21 cropping season at Ofcolaco. ns = not significant; * = significant at $p \leq 0.05$.

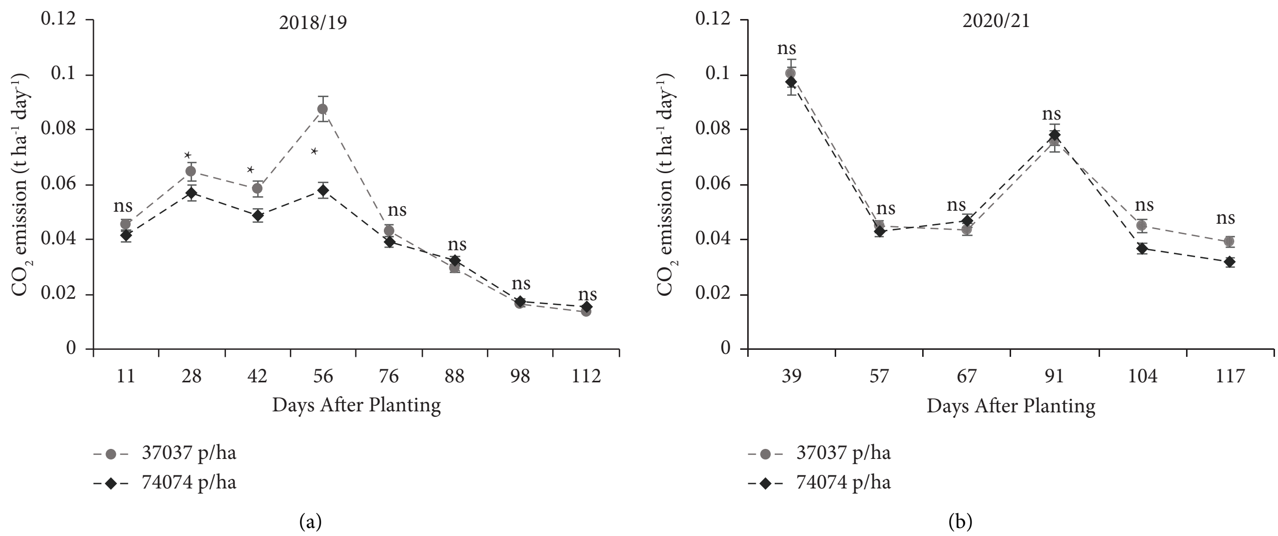

Figure 9: CO_2 emission rate in low and high density of cowpea at Syferkuil during the 2018/19 and 2020/21 cropping seasons. ns = not significant; * = significant at $p \leq 0.05$.

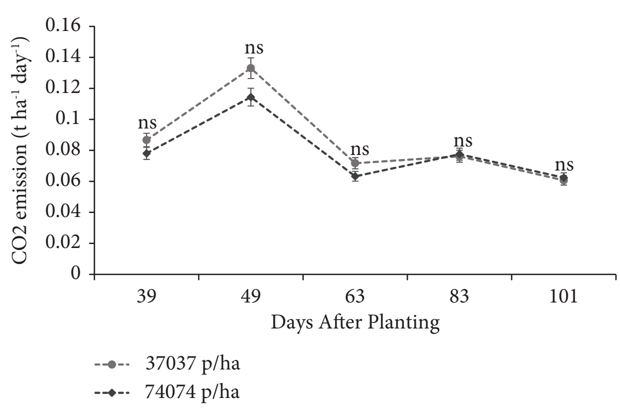

Figure 10: CO_2 emission rate in low and high density of cowpea at Ofcolaco during the 2020/21 cropping season. ns = not significant.

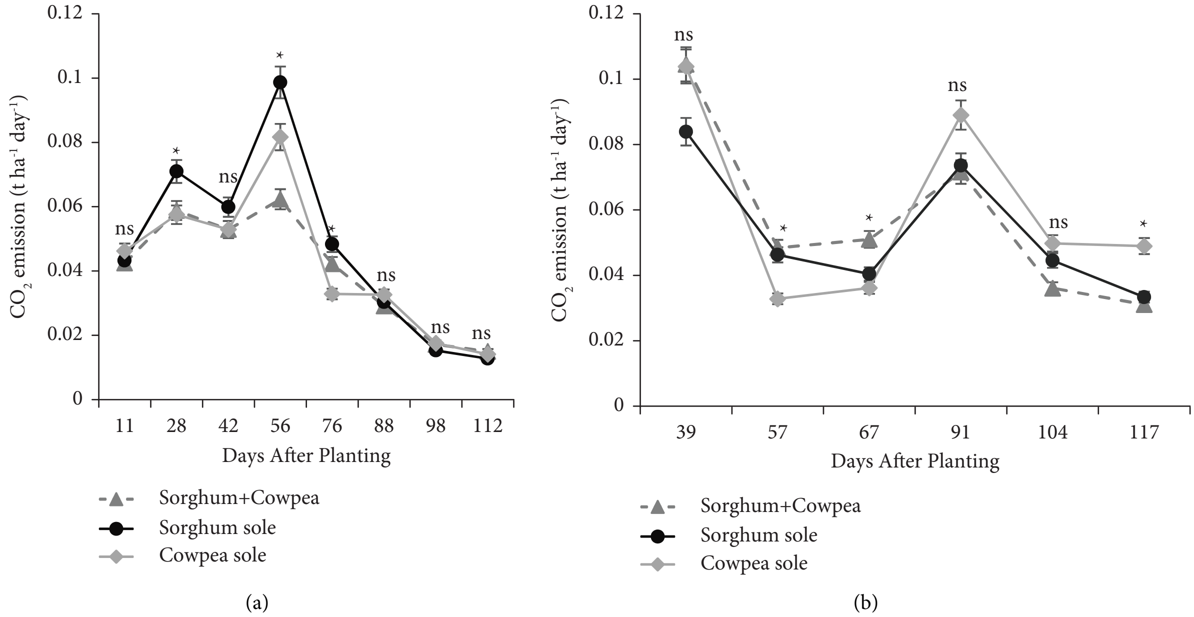

Figure 11: CO_2 emission rates of sorghum intercropped with cowpea, sorghum in sole and cowpea in sole cultures collected at Syferkuil during the 2018/19 (a) and 2020/21 (b) cropping seasons. ns = not significant; * = significant at $p \leq 0.05$.

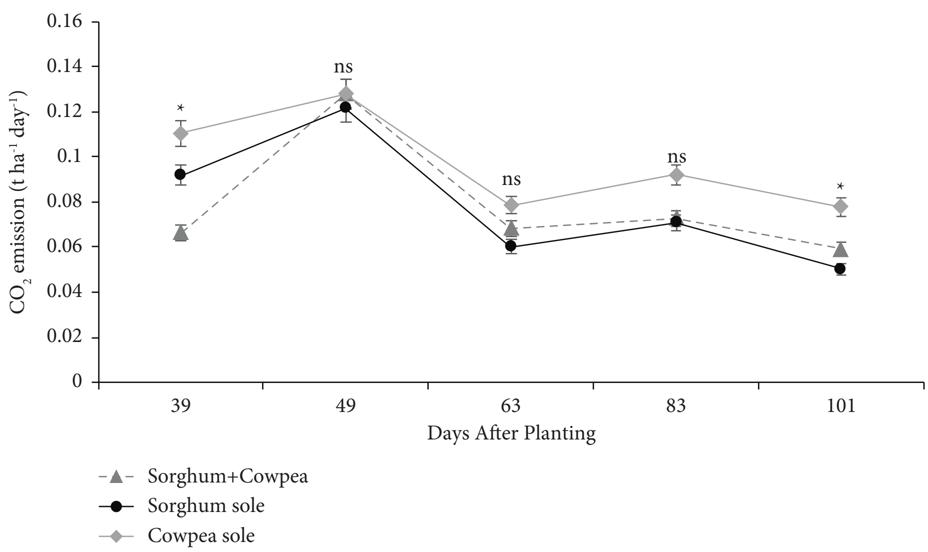

Figure 12: CO_2 emission rates of sorghum intercropped with cowpea, sorghum in sole and cowpea in sole cultures collected at Ofcolaco during the 2020/21 cropping season. ns = not significant; * = significant at $p \leq 0.05$.

3.5. The Cumulative CO_2 Emission during the Growing Seasons. The cumulative CO_2 emissions emitted during the 2018/19 cropping season were significantly different ($p \leq 0.05$) in binary and sole cultures (Figure 13(a)). In the 2020/21 cropping season, there was no variation in the cumulative CO_2 emitted in binary and sole cultures at Syferkuil. Ofcolaco showed a significant variation in cumulative CO_2 flux in sole and binary cultures during the 2020-21 cropping season. The cumulative CO_2 emissions were 13% and 26% more in sole compared to binary cultures at Syferkuil and Ofcolaco during the 2018/19 and 2020/21 cropping seasons, respectively (Figure 13(a)). The density of companion crops showed a significant variation in CO_2 emission rates at Syferkuil during the 2018/19 cropping season. During the 2018/19 cropping season, there was a high emission of cumulative CO_2 at low density compared to high density. However, there was no significant difference in cumulative CO_2 flux at Syferkuil and Ofcolaco during the 2020/21 cropping season (Figure 13(b)). Although there was no statistical difference between low and high density during the 2020/21 cropping season at Syferkuil and Ofcolaco, more CO_2 was emitted under low density compared to high density (Figure 13(b)).

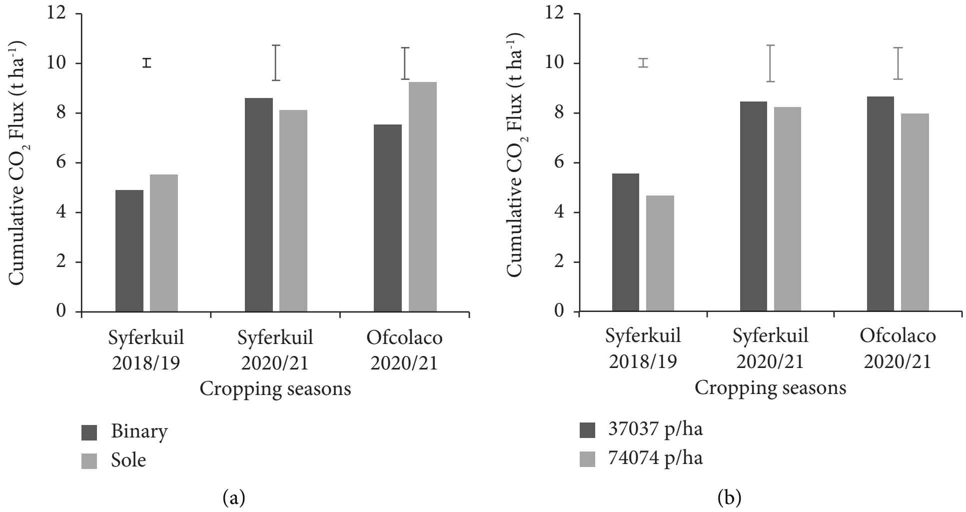

Figure 13: Cumulative CO_2 emission rates in binary and sole cultures (a) as well as low (37037 p/ha) and high (74074 p/ha) population density (b) at Syferkuil and Ofcolaco during the 2018/19 and 2020/21 cropping seasons.

Sorghum sole cumulatively emitted higher CO_2 of 5.87 t·ha^{-1} in 2018/19 compared to cowpea sole and the intercrop of the two crops which had 5.14 t·ha^{-1} and 4.88 t·ha^{-1} respectively. However, in the 2020/21 cropping season, sorghum sole had the lowest cumulative CO_2 compared to cowpea when the two crops were intercropped together. At Ofcolaco, cowpea sole had the highest cumulative CO_2 emitted followed by sorghum sole while the two crops emitted less when grown in the intercropping system (Figure 14).

The linear regressions of cumulative CO_2 at Syferkuil and Ofcolaco during the two cropping seasons are represented in Figure 15. The coefficient of determination (R^2) for all treatments in the sole cropping and intercropping systems was more than 0.9445 during the 2018/19 and 2020/21 cropping seasons. Sorghum intercropped with cowpea; sorghum and cowpea in sole cropping showed a strong linear relationship at the two locations. From 42DAP to 112DAP, a day increase resulted in cumulative CO_2 of sorghum sole increasing by 0.83 t·ha^{-1} followed by cowpea sole with 0.70 ta·ha^{-1}. Sorghum and cowpea intercrop emitted 0.66 t·ha^{-1} CO_2 for an everyday increase during the 2018/19 cropping season (Figure 15(a)). At Syferkuil, the cumulative CO_2 was similar in sorghum sole cropping, cowpea sole, and a combination of sorghum and cowpea between 11DAP and 28DAP in the 2018/19 cropping season. In the 2020/21 cropping season, sorghum sole had 0.87 increase in CO_2 for every increase in days which was the lowest compared to cowpea sole and sorghum + cowpea at Syferkuil which had 0.92 and 0.94. The results from Ofcolaco indicated that cowpea sole had the highest cumulative CO_2 followed by sorghum sole during the 2020-21 cropping season (Figure 15(b)). At Ofcolaco, sorghum + cowpea had the lowest cumulative CO_2 flux compared to sole cultures. Cowpea sole had 1.5 t·ha^{-1} followed by sorghum sole with 1.3 t·ha^{-1} of CO_2 emitted with an increase in each day, whereas intercrop of the two crops had 1.2 t·ha^{-1} of cumulative CO_2 emission (Figure 15(c)).

3.6. Carbon Dioxide (CO_2) Emission Efficiency of Sorghum and Cowpea in Sole Cropping and Intercropping System. The cropping system had a significant effect ($p \leq 0.05$) on the CO_2 emission efficiency (CEE) of sorghum and cowpea at Syferkuil in the 2018/19 cropping season. Cultivar NS5511 had a higher CEE when intercropped with cowpea, followed by cultivars Enforcer intercropped with cowpea and Enforcer sole, with means of 1.15, 1.10, and 1.00, respectively (Table 3). The treatments Avenger + Cowpea, Titan sole, and Avenger sole had lower CEE of 0.84, 0.82, and 0.74 compared to all other treatments. At Ofcolaco, the CEE of sorghum and cowpea was significantly affected by the cropping system in the 2020/21 cropping season. The treatment Avenger + Cowpea had a higher CEE of 0.75 compared to all other treatments in intercrop and sole systems (Table 3). The cultivar Enforcer utilized CO_2 emitted less efficiently compared to all other treatments.

Cowpea sole had the highest CEE of 0.83 compared to all other cowpea treatments in the intercropping system. In the 2020/21 cropping season, the cropping system did not affect sorghum; only cowpea showed significant variation in terms of CEE (Table 4). In terms of cowpea, all cowpea treatments in the intercropping system utilized CO_2 emitted more efficiently at Ofcolaco compared to the sole system, as shown in Table 4.

3.7. The Relationship between Carbon Stocks and CO_2 Emission Rate of Intercropped and Sole Treatments. Carbon stocks and CO_2 flux were regressed for each treatment in binary and sole cultures for the two cropping seasons at the test locations (Figures 16–18). The results presented are of the treatments that showed either a strong negative or strong positive relationship between carbon stock and CO_2 flux. At Syferkuil, Avenger + cowpea, Enforcer + cowpea, and cowpea sole showed negative regression, whereas Titan + cowpea had a strong positive linear regression between carbon stocks and CO_2 flux during the 2018/19 cropping season (Figure 16).

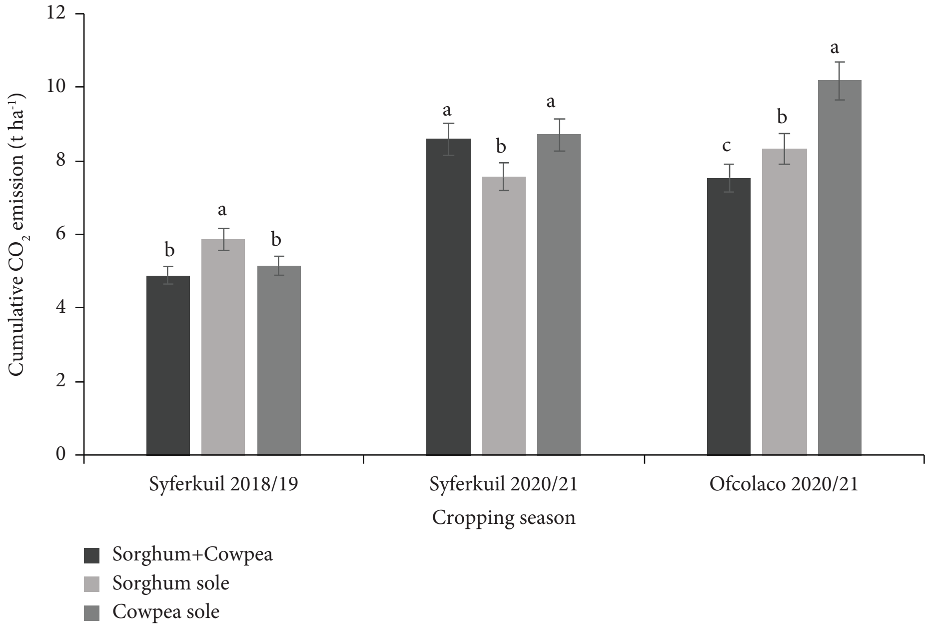

Figure 14: Cumulative CO_2 emission rates of sorghum and cowpea in binary and sole cultures at Syferkuil and Ofcolaco during the 2018/19 and 2020/21 cropping seasons.

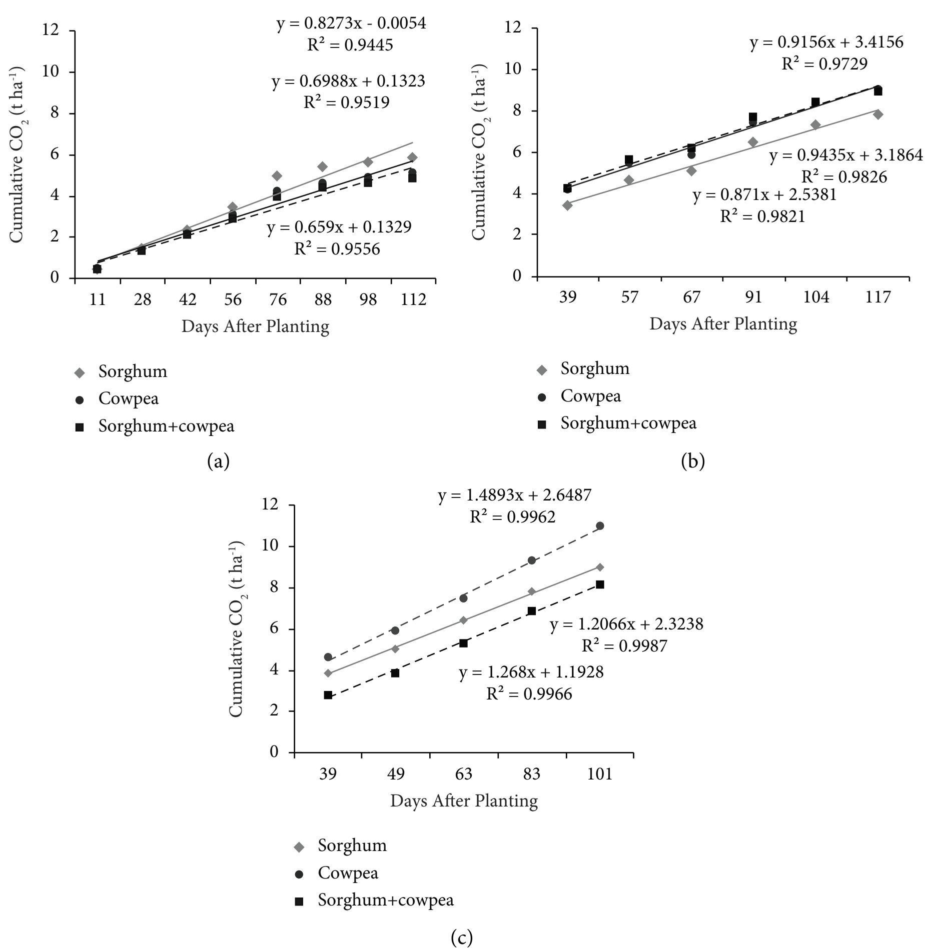

Figure 15: Cumulative CO^2 emissions of sorghum and cowpea in sole, sorghum, and cowpea in intercrop at Syferkuil during 2018/19 (a) and 2020/21 (b) as well as Ofcolaco in 2020/21 (c).

Table 3: Carbon dioxide emission efficiency (CEE) of sorghum in intercrop and sole systems at Syferkuil and Ofcolaco during the 2018/19 and 2020/21 cropping seasons.

Treatments	Syferkuil 2018/19	Syferkuil 2020/21	Ofcolaco 2020/21
NSS551-intercrop	1.15[a]	0.44	0.51[b]
Enforcer-intercrop	1.10[ab]	0.40	0.47[b]
Enforcer sole	1.00[abc]	0.41	0.27[c]
NSS5511 sole	0.97[abc]	0.51	0.45[b]
Titan-intercrop	0.90[bcd]	0.45	0.52[b]
Avenger-intercrop	0.84[cd]	0.44	0.75[a]
Titan sole	0.82[cd]	0.57	0.55[b]
Avenger sole	0.74[d]	0.49	0.58[b]
Grand mean	0.94	0.46	0.51
$P \leq 00.5$	**	ns	**

Table 4: Carbon dioxide emission efficiency (CEE) of cowpea in intercrop and sole systems at Syferkuil and Ofcolaco during the 2018/19 and 2020/21 cropping seasons.

Treatments	Syferkuil 2018/19	Syferkuil 2020/21	Ofcolaco 2020/21
Cowpea sole	0.83[a]	0.65[a]	0.49[b]
Cowpea-intercrop with Titan	0.69[ab]	0.53[ab]	0.84[a]
Cowpea-intercrop with NS551	0.61[b]	0.51[b]	0.74[a]
Cowpea-intercrop with Avenger	0.50[b]	0.41[b]	0.66[ab]
Cowpea-intercrop with Enforcer	0.50[b]	0.41[b]	0.63[ab]
Grand mean	0.63	0.50	0.67
$P \leq 00.5$	**	**	**

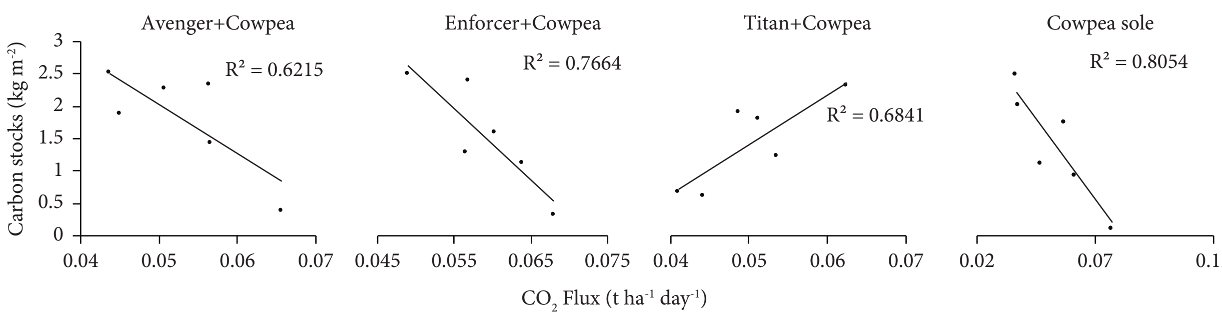

Figure 16: Carbon stocks (y-axis) versus CO_2 emission rate (x-axis) of sorghum and cowpea in binary and sole cultures at Syferkuil during the 2018/19 cropping season.

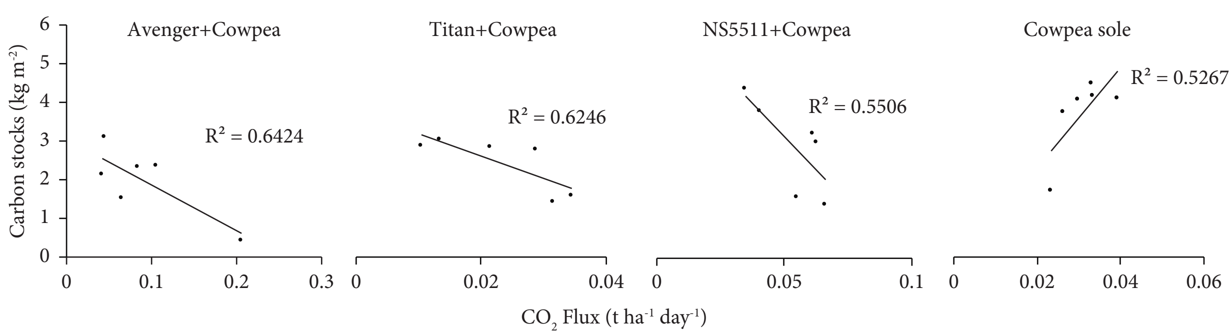

Figure 17: Carbon stocks (y-axis) versus CO_2 emission rates (x-axis) of sorghum and cowpea in binary and sole cultures at Syferkuil during the 2020/21 cropping season.

During the 2020/21 season, the intercropping systems, Avenger + cowpea, Titan + cowpea, and NS5511 + cowpea resulted in a negative linear relationship between carbon stocks and CO_2 flow at Syferkuil (Figure 17). Cowpea sole showed a positive relationship between carbon stock and CO_2 flux.

The results from Ofcolaco revealed that the relationship between carbon stock and CO_2 flux in Avenger + cowpea

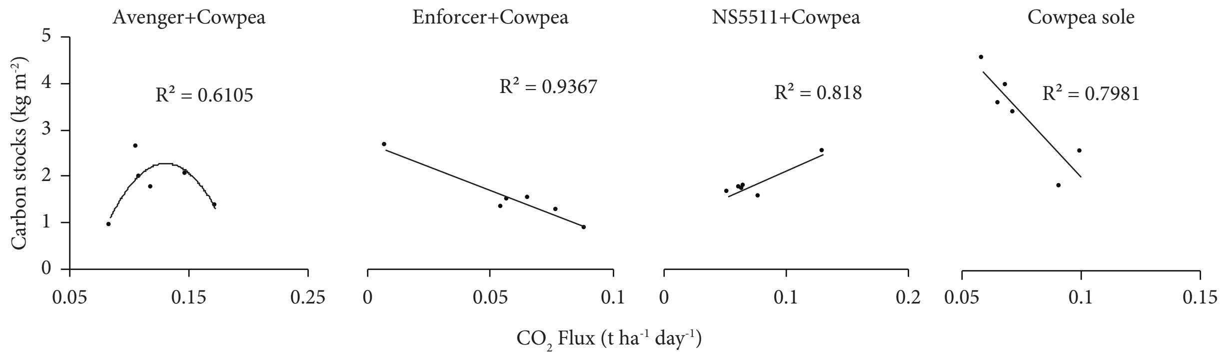

Figure 18: Carbon stocks (y-axis) versus CO_2 emission rates (x-axis) of sorghum and cowpea in binary and sole cultures at Ofcolaco during the 2020/21 cropping season.

intercrop was best described using a polynomial (Figure 18). The treatments Enforcer + cowpea and cowpea sole indicated a strong negative linear regression between carbon stocks and CO_2 flux at Ofcolaco during the 2020/21 cropping season. Of all the treatments, NS5511 + cowpea was the only treatment to show a strong linear regression between carbon stocks and CO_2 flux at Ofcolaco in the 2020/21 cropping season.

4. Discussion

4.1. Weather Conditions during the Growing Seasons and the Effect on Carbon Emissions. The amount of carbon stored in the soil is calculated by balancing the carbon inputs from crop residues with the carbon loss from emissions into the atmosphere [14]. These carbon dynamics in crop production are influenced by cropping systems, management practices, soil conditions such as soil moisture and bulk density, as well as climatic variability. The amount of CO_2 emitted in this study was influenced by the cropping system, the number of plants per unit area (plant density), and the environmental conditions such as temperatures and precipitation of each growing season. Weather variables such as temperature and precipitation were found to play a significant role in the variation in CO_2 emissions from one cropping season to the next in this study. The rainfall and temperature in this study were different from one season to another and across locations. High rainfall in 2020/21 and the minimum temperature of more than 10°C resulted in higher CO_2 emission rates. Warmer summer temperatures, according to Munjonji et al. [16], are the driving factors in the soil releasing more cumulatively CO_2. These findings suggest that seasonal environmental conditions especially temperature and precipitations had an impact on CO_2 emissions. The fluctuations and seasonal variations were also reported by other authors [16, 17].

4.2. CO_2 Emission under Sole and Intercrop Systems. During the 2018/19 season at Syferkuil and 2020/21 seasons at Ofcolaco, intercropping systems emitted 11% and 19% less CO_2, respectively, compared to sole cropping systems. Other authors have also reported relatively low CO_2 emissions in intercropping systems [17–19]. Therefore, planting two crop species on the same plot of land reduces CO_2 emissions compared to planting only one species as a result of the interaction between intercropping populations [20]. Furthermore, Hauggaard-Nielsen et al. [21] reported that the lower CO_2 emissions in cereal-legume intercropping compared to sole cropping are due to the use of fewer amounts of nitrogen fertilizers. Synthetic fertilizers are the primary source of greenhouse gas emissions in cropping systems and thus, planting in a sole cropping system would require more fertilizers to improve productivity. However, in an intercropping system, cereal plants could benefit from the legume thereby reducing the cost of fertilizer. Cereal-legume intercrop may be an appropriate production practice for mitigating high CO_2 emissions as shown by the findings of this study [22].

Our findings also revealed that sorghum sole produced more CO_2 than cowpea sole or the intercrops. Many studies have found that cereal crops emit significantly more CO_2 than legumes or cereal-legume intercrops [23–25]. According to Shao et al. [26], as a coping mechanism for high competition in an intercropping system, cereal crops inhibit growth by reducing their root node. As a result, more CO_2 may be emitted by crops rather than utilized for photosynthetic activities. In this study, sorghum in the intercropping system emitted more CO_2 during the growing period and began to decrease when cowpea was harvested 76 days after planting. The author also stated that the CO_2 peak occurred at the same time in intercrop and sole cropping and decreased significantly as crops matured and harvested. A similar pattern was observed in this study. The CO_2 emission rate decreased after crops have reached flowering and milking stages and were moving towards maturity.

4.3. Different Cowpea Densities and CO_2 Emissions. Plant density is frequently used to gain yield advantage per unit area. The density of the companion crop cowpea had a significant effect on CO_2 emissions in this study where a relatively higher emission of CO_2 was recorded at low density than at high density. The findings contradicted what Yang et al. [25] discovered, as the author reported that high maize density increased CO_2 emissions compared to low density. High density increases plant community components such as dry biomass as a result of efficient utilization of carbon in the soil [27].

4.4. Carbon Dioxide Emission Efficiency of Intercrop and Sole Sorghum and Cowpea. CO_2 emission efficiency is used to calculate how much dry biomass or grain yield is accumulated per unit of carbon emitted under various crop production practices [14]. The study's findings revealed that cropping system had a significant effect on CEE of sorghum and cowpea across various agroecological conditions. Intercropping has a higher CEE than sole cropping, according to Yin et al. [24]. The higher CEE for cultivars NS5511 and Enforcer reported in this study indicates that the cultivars were able to accumulate more biomass per unit of carbon emitted from the soil. CEE by sorghum cultivars, on the other hand, was influenced by cropping season and agroecological regions. When compared to Syferkuil, Avenger was able to use carbon more efficiently in intercrop and sole at Ofcolaco. The results also revealed that cowpea sole cropping had higher CEE than intercropping at Syferkuil due to less competition and an improved root system [28]. Cowpea intercropping had a higher carbon use efficiency than sole cropping at Ofcolaco. According to Mathew et al. [29], carbon allocation is affected by crop species and growing environment temperature.

4.5. Physical and Chemical Properties of the Soil. Regression analysis can be used to determine the relationship between carbon stocks and CO_2 emission rates. Intercrop and sole treatments were used in this study to regress CS and CE. The findings revealed that the strength of the relationship between the two variables varied according to the treatment, which differed from one agroecological region to the next. At Syferkuil, soil carbon stocks increased with an increase in gravimetric water content which also resulted in high organic carbon. Although the cropping system had no significant effect on the physical and chemical properties of the soil, visualization revealed variation from one location to another and across seasons. For example, BD, org.C, and CS were higher in 2020/21 at Syferkuil compared to the 2018/19 cropping season. However, at Ofcolaco, BD, org.C, and CS were higher during the 2018/19 cropping season than during the 2020/21 cropping season. The results were in contrary with what Abbady et al. [30] reported. The author indicated that soil properties such as BD and moisture content were significantly affected by the cropping system. Furthermore, the seasonal variability and treatment effect showed difference in soil properties in intercrop and sole system. The seasonal variability effect on soil physical and chemical properties was also observed in this study. Additional information on the variation across the seasons is outlined by Mogale et al. [31]. Across all cropping seasons of test locations, cropping system did not affect P, K, Ca, Mg, Zn, Mn, and Cu. Munjonji et al. [13] reported no significant difference for P, K, Ca, Mg, Zn, Mn, and Cu under drought conditions.

5. Conclusion and Recommendations

Findings from the study revealed that cowpea-sorghum intercrop released less soil CO_2 compared to the sole of the two crops, and hence could be a more sustainable crop production practice. This assist with provision of data on the intercropping system as a sustainable crop production practice with protection to cultivated land. Furthermore, growing crops in intercrops improved the crop's carbon emission efficiency. More dry matter (biomass) is accumulated with the reduction in CO_2 emission. When the two crops were planted as monocultures, sorghum was found to emit more CO_2 than cowpea. Cowpea density also significantly impacted CO_2 emission rates, with high density (74,074 plants per hectare) emitting less soil CO_2. Furthermore, the study found that agroecological conditions that differ from season to season play an important role in carbon dynamics in the soil. This implies that the long-term seasonal CO_2 emissions in the intercropping system is required to understand the patterns of flux over a magnitude of growing period. The findings from this study may be useful in understanding the importance of intercropping systems on carbon storage and loss. However, more research is needed to fully understand how intercropping systems and conservation practices such as no-till systems affect CO_2 emissions. The study also had limitations of relating the CO_2 emissions results observed in this study to soil microbial activities. Soil microbial activity was not studied in the research but for future research, a serious consideration must be given to it. Furthermore, root activities should be investigated in order to observe the carbon dynamics between plants and soil.

Data Availability

All data and materials used in the write-up of the manuscript were acquired through existing facilities at RVSC, data generated from the research and climatic data from the Agricultural Research Council, South Africa. The data used in this study are available at RVSC of the University of Limpopo which can be accessed through the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Influence of Treated Wastewater on the Percentage of Protein Content during Fodder Intercropping

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This study aims to explore the potential use of treated wastewater in irrigating fodder crops and its effects on protein contents. A comparison of the protein contents in intercropped fodder plants irrigated with fresh water, and rainfall water, against those irrigated with treated grey water was performed under Palestinian climate conditions. Field experiments with different intercropping mixing ratios were carried out in 2017–2019 at the National Agricultural Research Centre in Palestine (NARC). Measurements of the nutritional value of each mixture specifically the protein contents were carried out to get the optimal and best conditions for preparing animal feed crops with three different water sources used. For alfalfa with vetch, the best result for protein percentages was (on average) obtained from the rain-fed experiment (17.1% protein) followed by the freshwater experiment (12.9% protein) and then by the treated grey-water experiment (12.6% protein). It appears that the best result for alfalfa with barley for protein percentages was (on average) obtained from the treated grey-water experiment (13.0% protein) followed by the freshwater experiment (11.1% protein) and then by the rain-fed experiment (10.5% protein). Statistical analysis of the data showed that percent protein for each specific mixing ratio resulted in significant differences in the protein % for the those irrigated with fresh water compared with the other types of water. The highest protein % was found to be for that irrigated with fresh water (31.9 for 10/90 alfalfa/barley ratio) followed by that irrigated with treated grey water (28.4 for 20/80 alfalfa/barley ratio) and then for the 30/70 ratio irrigated with treated wastewater (22.5%), and then for the 100/0 ratio of alfalfa/barley irrigated with rainwater (19.0). Overall, results of this study showed that cereal-legume intercropping irrigated with treated grey water can be used as a suitable management strategy for producing high-quality and high-quantity forage. Furthermore, the use of treated water can reduce the already strained demand on fresh water due to increase in population among other factors.

1. Introduction

Intercropping is widely used by smallholder farmers in developing countries and attracting attention in the context of ecological intensification of agriculture in developed countries [1]. Intercropping becomes particularly important in areas with limited rainfall or semiarid climates [2]. Due to low rain fall and dry areas in certain parts of Palestine [3], diminishing supply of fresh water, and the recognition of using treated water in cropping animal feed crops with significant nutritional values, intercropping was performed by growing of two or more crops simultaneously on the same field to optimize parameters of irrigation and maximize benefits of products.

Integrated intercropping of legumes is an option, therefore aiming at optimizing the agronomic efficiency of applied inputs [24]. Legume integration is an important component of agricultural and animal feed systems [4, 5]. Legume-cereal intercropping, especially corn-beans intercropping, is common throughout many parts of the world [6]. In drier areas, common beans are often replaced by cowpea or groundnut. Farmers commonly intercrop to

Figure 1: Location of the study area.

secure food production by averting risk and to maximize utilization of land and labor [7]. When crops are complimentary in terms of growth pattern, above ground canopy, rooting system, and their water and nutrient demand, intercropping effectively enables a more efficient utilization of available resources (sunlight, moisture, and soil nutrients) and can result in relatively higher yields than when crops are grown separately, as pure stands [8]. Other benefits of intercropping are related to the better soil cover, which has advantages for weed control and leads to reduced erosion and nutrient leaching [9–12]. In addition, regional irrigation with treated grey-water olive orchards and vegetable crops did not show any negative effect on the chemical properties of the fruits and leaves [23].

Because legumes can rely on atmospheric nitrogen (N), they are less likely to compete for N with the cereal. The presence of a cereal, exploiting the soil mineral N, may even stimulate legumes to fix N [13, 14]. Fodders are vital in the world's food resources as plant materials containing high amounts of structured carbohydrates [15–18]. Legumes are a good source of protein and can be used to compensate cereal protein shortage. Thus, growing of crop mixtures with legumes, which is referred to intercropping, can boost the forage protein content of diets [19–22].

Little information is available on the effect of irrigating with treated wastewater in Palestine, especially when dealing with intercropped fodder. This activity come in line with Livestock-Based Livelihood-institutional Component (LBL-i); this is because the FAO works to strengthening the capabilities of and links with applied research to enhance the adoption of innovative approaches in addressing problems and opportunities in the livestock sector and how to use treated wastewater (TWW) for silage crops: quality and safety control in TWW usage, introduction of new fodder crop varieties suitable for TWW irrigation, and growing various barley and vetch species on fresh water, treated grey water, and rainwater; then, use these fodder crops in certain ratios and study the nutritional value of each mix; and then, decide on the optimal and best conditions for preparing animal feed crops regardless of the water source used, inclusively to use treated water as alternative water supply due to scarcity of water in Palestine.

Table 1: Alfalfa/barley and vetch/barley plot ratios.

Plots	Alfalfa/barley	Vetch/barley
1	100/0	100/0
2	90/10	90/10
3	80/20	80/20
4	70/30	70/30
5	60/40	60/40
6	50/50	50/50
7	40/60	40/60
8	30/70	30/70
9	20/80	20/80
10	10/90	10/90
11	0/100	0/100

2. Materials and Methods

2.1. Location. Intercrops of alfalfa with barley and vetch with barley were carried out in the Northern West Bank of Palestine within the Jenin Governorate, as shown in Figure 1. The geographical area is located at latitude *N* 32.40, longitude *E* 35.28, and elevation at 312 m above sea level (m a.s.l.). The rain-fed intercrops receive rainfall of an average of 300 mm/year, which were not fertilized or irrigated throughout growth. The average temperature of the year in the region was 20.3°C (low of 14°C night time and 27°C during daytime), and the average temperature from September to November is about 23.5°C (low of 18°C night time and high of 29°C daytime).

The research team implemented the project by growing various fodder crops from seeds irrigated with rain-fed water, fresh water, and treated grey water. The following crops were intercropped in accordance with Table 1 mixing ratios: barely with vetch and barely with alfalfa. Planting of the crops was done in two areas: outdoor and indoor (inside a greenhouse). For rain-fed, the barley/vetch intercropping and the barely/alfalfa intercropping were performed outdoor because both of these crops are winter crops and are not influenced negatively by the cold weather.

The experiments were carried out in three locations within the NARC facility during the 2017–2019, on terra-rossa brown rendzinas and pale rendzinas soil, which is the type of soil that typically found in northern Palestine. This area of Palestine is dominated by agricultural work. For indoor experiments, barley and alfalfa intercrops were grown inside greenhouse, in which irrigation was performed using fresh water and in another plot using treated wastewater. Another batch of same intercropping was achieved outside, which was dependent upon the rain-fed irrigation method. The same was done to the barley and vetch intercropping fields. Sampling was done in triplicate.

The crops were harvested at physiological maturity. The plots were harvested manually and separated in three fractions, i.e., grain legume, barley, and weeds. The plant samples were dried to constant weight, and total dry matter (DM) production for each plot was determined separately for grain legumes, barley, and weeds After threshing, the grain DM yields were determined.

2.2. Analysis of Samples in the Kjeldahl Apparatus. The analysis essentially involves three steps. First, digestion of 10 grams of each sample that involves oxidative decomposition using concentrated, boiling sulfuric acid for 3 to 5 hours. The bound nitrogen is dissolved out of its bond matrix without any losses and is completely converted into inorganic ammonia nitrogen ($NH_4{}^+$-N). At the end of the digestion reaction, all the sample's nitrogen should be present as ammonia nitrogen as represented in

$$(\mathrm{CHNO})\,(\mathrm{s}) + \mathrm{H_2SO_4}\,(\mathrm{l}) \longrightarrow \mathrm{CO_2}\,(\mathrm{g}) + \mathrm{SO_2}\,(\mathrm{g}) + \mathrm{H_2O}\,(\mathrm{g}) + (\mathrm{NH_4})2\mathrm{SO_4}\,(\mathrm{aq}) + \mathrm{H_2SO_4}\,(\mathrm{l}) \quad (1)$$

Second, the digested solution is distilled by adding concentrated base (33% NaOH solution) and the ammonia is then released and distilled from this solution in accordance with the following equation:

$$(\mathrm{NH_4})2\mathrm{SO}_{4\,(\mathrm{aq})} + 2\mathrm{NaOH}_{(\mathrm{aq})} \longrightarrow \mathrm{Na_2SO}_{4\,(\mathrm{aq})} + 2\mathrm{NH_3}\,(\mathrm{g}) + 2\mathrm{H_2O}\,(\mathrm{l}) \quad (2)$$

Finally, in the third step, applied water steam extracts the volatile component ammonia from the green-colored digestion solution and transports the ammonia through the distribution head and coiled-tube condenser into the collection solution with boric acid, which turns into pink color. The ammonia and boric acid react stoichiometrically to form ammonium borate, which prevents the ammonia from escaping. At the end, the residual boric acid is titrated with base, which provides quantitative conclusions about the nitrogen content in the original sample. The following equation shows collecting the ammonia in boric acid:

$$\mathrm{NH_3}\,(\mathrm{g}) + \mathrm{B}\,(\mathrm{OH})^-_{4\,(\mathrm{aq})} + \mathrm{H}^+ \longrightarrow \mathrm{NH}^+_{4\,(\mathrm{aq})} + \mathrm{B}\,(\mathrm{OH})^-_{4\,(\mathrm{aq})} \quad (3)$$

2.3. Calculating the Nitrogen Content. The consumption of titration solution (H+) during titration of the excess boric acid can be used to simply calculate the percentage nitrogen content in the initial sample. The following formula applies here:

$$\%\mathrm{N} = \frac{(ceq * (\mathrm{V} - \mathrm{VBL}) * M * 100\%)}{\mathrm{E}}, \quad (4)$$

where $\mathbf{c_{eq}}$ is the equivalent concentration of the titration solution (mol/l), **V** is the consumption of titration solution sample [l], $\mathbf{V_{BL}}$ is the consumption of titration solution at blank point (l), **M** is the molar mass of nitrogen (g/mol), and **E** is the weight of the sample (g).

Table 2: Percent protein for alfalfa/barley-intercropped fields.

	Alfalfa/barley		
Ratio	Fresh water	Rainwater	Wastewater
0/100	5.2 ± 0.12^{Gb}	8.6 ± 0.26^{Fa}	8.0 ± 0.36^{Ha}
10/90	32.0 ± 0.23^{Aa}	8.1 ± 0.22^{Fb}	7.3 ± 0.27^{Ic}
20/80	5.8 ± 0.27^{Gc}	10.7 ± 0.35^{Db}	28.4 ± 0.15^{Aa}
30/70	1.9 ± 0.11^{Hc}	8.0 ± 0.26^{Gb}	22.6 ± 0.24^{Ba}
40/60	24.0 ± 0.35^{Ba}	14.3 ± 0.36^{Bb}	11.4 ± 0.25^{Fc}
50/50	9.4 ± 0.25^{Db}	9.7 ± 0.14^{Eb}	14.5 ± 0.32^{Da}
60/40	6.3 ± 0.33^{Fc}	10.6 ± 0.24^{Db}	12.8 ± 0.23^{Ea}
70/30	8.6 ± 0.25^{Eb}	8.1 ± 0.26^{Fb}	10.0 ± 0.41^{Ga}
80/20	13.4 ± 0.22^{Ca}	4.7 ± 0.33^{Hc}	7.3 ± 0.25^{Ib}
90/10	9.2 ± 0.31^{Dc}	13.3 ± 0.11^{Cb}	15.3 ± 0.25^{Ca}
100/0	6.7 ± 0.21^{Fb}	19.0 ± 0.25^{Aa}	4.9 ± 0.31^{Jc}

2.3.1. Statistical Analysis. Three samples of each treatment were independently analyzed, and all of the determinations were carried out in triplicate. All statistical analyses were carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means were carried out using the GLM procedure, treating main factors separately using one-way analysis of variance (ANOVA). Differences were considered significant if P values were lower than 0.05.

3. Results and Discussion

The protein percentages from the intercropping were determined by measuring the total nitrogen content from 3 to 15 mg subsamples of finely ground material using the Kjeldahl apparatus. The theory is based on determination of the total nitrogen in a sample using the Kjeldahl. This instrument is one of the most accurate and widely used methods for determining nitrogen in substance such as milk, cereal, and flour. The solid is first digested in boiling sulfuric acid, which converts nitrogen to ammonium ion and oxidizes to other elements as in

$$\text{Organic C, H, N} \longrightarrow \mathrm{NH_4^+} + \mathrm{CO_2} + \mathrm{H_2O} \quad (5)$$

Mercury, copper, and selenium compounds catalyze the digestion process. To speed up the rate of reaction, the boiling point of the concentrated sulfuric acid is raised by adding K_2SO_4. After digestion is complete, the solution containing $NH_4{}^+$ is made basic, and the liberated NH_3 is distilled into a receiver containing known amount of HCl. Excess, unreacted HCl is then titrated with standard NaOH to determine how much HCl was consumed by NH_3, as shown in equations (6)–(8). And equation (8) shows treatment of the unreacted acid neutralized with base:

$$\text{neutralization of } \mathrm{NH_4^+}\text{: } \mathrm{NH_4^+} + \mathrm{OH^-} \longrightarrow \mathrm{NH_3} + \mathrm{H_2O}, \quad (6)$$

$$\text{distillation of } \mathrm{NH_3} \text{ into standardized HCl: } \mathrm{NH_3} + \mathrm{H^+} \longrightarrow \mathrm{NH_4^+} \quad (7)$$

$$\text{titration of unreacted HCl with NaOH: } \mathrm{H^+} + \mathrm{OH^-} \longrightarrow \mathrm{H_2O} \quad (8)$$

Table 3: Percent protein from vetch-barley fields.

	Vetch/barley		
Ratio	Fresh water	Rainwater	Wastewater
0/100	5.0 ± 0.12^{Hc}	15.2 ± 0.25^{Da}	10.6 ± 0.21^{Fb}
10/90	13.8 ± 0.25^{Dc}	18.9 ± 0.32^{Cb}	22.5 ± 0.25^{Aa}
20/80	13.6 ± 0.31^{Db}	11.4 ± 0.11^{Gc}	16.9 ± 0.24^{Ba}
30/70	19.3 ± 0.22^{Cb}	23.6 ± 0.26^{Aa}	17.3 ± 0.22^{Bc}
40/60	9.1 ± 0.12^{Ec}	10.1 ± 0.27^{Hb}	15.0 ± 0.36^{Da}
50/50	8.0 ± 0.13^{Fb}	14.3 ± 0.31^{Ea}	14.4 ± 0.28^{Da}
60/40	6.0 ± 0.11^{Gc}	23.2 ± 0.25^{Aa}	6.7 ± 0.11^{Hb}
70/30	1.6 ± 0.12^{Id}	20.3 ± 0.22^{Ba}	11.7 ± 0.13^{Eb}
80/20	24.4 ± 0.25^{Ba}	12.2 ± 0.15^{Fb}	7.8 ± 0.23^{Gc}
90/10	35.1 ± 0.32^{Aa}	21.0 ± 0.15^{Bb}	16.0 ± 0.25^{Cc}
100/0	5.9 ± 0.14^{Gb}	18.1 ± 0.14^{Ca}	$^{*}0.0 \pm 0^{Ic}$

Table 2 shows the percent protein calculated from the total nitrogen under different mixing ratios of alfalfa and barley irrigated with fresh water, rainwater, and treated wastewater.

Results showed that the % protein of the different ratios of alfalfa and barley irrigated with fresh water is significantly different between the different ratios indicated by capital letters (A, B, C, D, E, F, G, and H), except between the ratios 50/50 and 90/10 indicated by capital letter D and between the ratios 60/40 and 100/0 indicated by the capital letter F, which are not significantly different. The highest protein% was found to be 31.9 and the lowest one was found to be 1.9 for the ratios 10/90 and 30/70 of alfalfa/barley, respectively. The same statistical analyses were conducted for the different ratios of alfalfa/barley irrigated with rainwater and treated wastewater. For those irrigated with rainwater, significant differences in the protein% was obtained between the different ratios indicated by the capital letters A, B, C, D, E, F, G, and H. The range of protein % was found to be 4.6–19.0. For those irrigated with treated wastewater, significant differences were also obtained with the range of 4.9–28.4 for % protein.

Comparing the protein % for alfalfa/barley different ratios irrigated with fresh water, rainwater, and grey water: the highest protein % was found to be for that irrigated with fresh water (31.9 for 10/90 alfalfa/barley ratio) followed by that irrigated with treated grey water (28.4 for 20/80 alfalfa/barley ratio) and then for the 30/70 ratio irrigated with treated wastewater (22.5%), and then for the 100/0 ratio of alfalfa/barley irrigated with rainwater (19.0).

Statistical analysis was also conducted to study the effect of irrigation (fresh water, rainwater, and treated wastewater) on the % protein indicated by the small letters (a, b, and c). The results showed significant differences in the protein % of alfalfa/barley ratios when the irrigation varies. As can be seen from Table 2, the protein % was found to be higher for the following mixing ratios when irrigated with wastewater: 0/100, 20/80, 30/70, 50/50, 60/40, 70/30, and 90/10.

Table 3 shows the percent protein calculated from the total nitrogen under different mixing ratios of vetch and barley irrigated with fresh water, rainwater, and treated wastewater.

Results showed that the % protein of the different ratios of vetch/barley irrigated with fresh water is significantly different between the different ratios indicated by capital letters (A, B, C, D, E, F, G, H, and I), except between the ratios 10/90 and 20/80 indicated by capital letter D and between the ratios 60/40 and 100/0 indicated by the capital letter G, which are not significantly different. The highest protein % was found to be 35.0 and the lowest one was found to be 1.6 for the ratio 90/10 and 70/30 of vetch/barley, respectively. The same statistical analyses were conducted for the different ratios of vetch/barley irrigated with rainwater and treated wastewater. For those irrigated with rainwater, significant differences in the protein % was obtained between the different ratios indicated by the capital letters A, B, C, D, E, F, G, and H. The range of protein % was found to be 10.1–23.5. For those irrigated with treated wastewater, significant differences were also obtained with the range of 0.0–22.4 for % protein.

Comparing the protein % for different vetch/barley ratios irrigated with fresh water, rainwater, and wastewater, the highest protein % was found to be for that irrigated with fresh water (35.0 and 24.3 for 90/10 and 80/20 vetch/barley ratio, respectively) followed by that irrigated with rainwater (23.5 for 30/70 vetch/barley ratio) and then for the 10/90 ratio irrigated with treated wastewater (22.4%).

Statistical analysis was also conducted to study the effect of irrigation (fresh water, rainwater, and treated wastewater) on the % protein indicated by the small letters (a, b, and c). The results showed significant differences in the protein % of vetch/barley ratios when the irrigation varies. As can be seen from Table 3, the protein % was found to be higher for the following mixing ratios when irrigated with wastewater: 10/90, 20/80, 40/60, and 50/50. While it was found highest for the mixing ratios 0/100, 30/70, 50/50, 60/40, 70/30, and 100/0 when irrigated with rainwater; on the other hand, it was found highest for the mixing ratios 80/20 and 90/10 when irrigated with fresh water.

The fodder barley/alfalfa and barley/vetch on fresh water, treated grey water, and rainwater have resulted in some interesting positive crop yields that might have some economic benefits. This system of intercropping demonstrated that the fodder barley/alfalfa and barley/vetch arrangement on treated water gave comparable—if not better—outcome and benefits to those on fresh water and rainwater. However, the protein data system fluctuated in the yields, and no obvious trend was deduced as to which is the best ratio would be adopted. This could be attributed to various factors such as temperature and nature of each plant's morphology. Other factors that may influence forage quality are maturity (harvest date), harvest and storage, soil fertility, and variety (cultivar).

Plant morphology for both cereals and legumes has three main plant parts: leaf, stem, and grain. As a structural component of the plant, stems typically contain more fiber for supports. Leaves, on the other hand, provide a means for capture and utilization of energy from sunlight and trend to be lower in fiber content than stems, and thus stems usually are lower in digestibility than leaves, and stem digestibility declines more rapidly with increased plant maturity than that of leaves. Differences between leaf and stem digestibility are normally greater in forage legumes than cereals. Given the large difference between the digestible fiber content of stems and leaves, the proportion of leaf to stem in the given forage plant relates directly to its forage quality. Also, the grain mainly comprises digestible components such as starch and protein. Consequently, the grain-to-stover ratio is considered an indicator for variety selection when high-quality forage is required.

The protein yield data can serve as an indicator of how intercropping on treated wastewater effectively gave better results than that on either fresh or rainwater. This partially (and probably) is due to the fact that treated wastewater is rich with nutrients (N, P, and K) that are necessary for plant growth and yield. For example, at the 10/90 mixing ratio of vetch/barley, the protein content was 22% when cultivated on treated wastewater, 19% when cultivated on rainwater, and only 5% when cultivated on fresh water. Moreover, the alfalfa/barley 20/80 intercropping ratio gave 28% of crude protein when irrigated with treated wastewater, 11% crude protein when grown using rainwater, and 6% when using rainwater. The intercropping irrigated with fresh water was characterized by low-protein crop yields and net benefits.

4. Conclusions

In conclusion, the fodder barley/alfalfa and barley/vetch on treated grey water have resulted in robust comparable results in protein crop yields and net benefits, in comparison with the irrigation of intercropping system on fresh water or rainwater. It appears that the best result for protein percentages was (on average) obtained from the rain-fed experiment (17.1% protein) followed by the freshwater experiment (12.9% protein) and then by the treated wastewater experiment (12.6% protein). Intercropping barley with common vetch improved the forage quality and increased the protein yield of barley without reducing dry matter yield. Overall, results of this study showed that cereal-legume intercropping irrigated with treated wastewater can be used as a suitable management strategy for producing high-quality and high-quantity forage. The yield benefits depend on the correct implementation of the intercropping system, which is recommended for adoption by farmers but will therefore require some investment in workshop training and further research.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

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