

## Article

# Cowpea Growth and Nitrogen Fixation Performance under Different Mulch Treatments

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**Abstract:** Mulching is regarded as the most important of the three conservation agriculture principles in increasing crop yield in the short term. Thus, the main objective of this study was to investigate the impact of mulch type and mulch application rate on biological nitrogen fixation (BNF), physiological and yield responses of cowpea. A multi-locational (two locations) and multi-seasonal (two seasons) study was carried out under rainfed conditions in the Limpopo Province of South Africa. Three mulch types (*Moringa oleifera* stems; *Moringa oleifera* leaves and twigs; and *Vachellia karroo* leaves and stems) were uniformly spread on the surface at four rates (0, 3, 6, 9 t/ha). The application of mulches, regardless of the rate and type, improved cowpea chlorophyll content and agronomic parameters, such as stem diameter and plant height. Grain yield at Syferkuil responded to the mulching effect in both seasons, while at Ofcolaco, differences were only observed in one of the seasons. Cowpea under control discriminated against <sup>15</sup>N more than under mulched treatments, resulting in more than 70% of the nitrogen being derived from air compared to 50% in mulched plots. This study demonstrated that organic surface mulches improved the physiological responses of cowpea and that organic surface mulches with a lower C:N ratio significantly reduced BNF.

**Keywords:** biological nitrogen fixation; chlorophyll content; grain yield



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## 1. Introduction

Mulching has been reported as the most important of the three conservation agriculture (CA) principles in increasing crop yield in the short term [1]. The three principles of conservation agriculture are minimum mechanical soil disturbance (<25% of the cropped area), maintenance of permanent organic soil cover (at least 30%) and diversification of crop species (crop rotation and intercropping), which include legumes [2]. While it has been proved that, when simultaneously combined, the three principles sustainably improve crop yields [3,4], many of the farmers, particularly smallholder farmers, have failed to combine these three practices [1] due to various reasons that range from the shortage of land to practise crop rotation to weed pressure under minimum tillage.

The application of mulch is known to reduce soil evaporation and thus conserve soil moisture, suppress weed growth, control soil structure and temperature, as well as influencing soil micro-organisms [5]. The conservation of soil moisture is of particular importance in arid and semi-arid regions where rainfall is erratic, and droughts occur more frequently. According to Ref [5], the application of mulches, such as wheat straw, grass clippings and leaf debris, increased soil moisture by 10% compared to bare soils. Soil microbiology is influenced by mulching due to its influence on soil moisture and temperature. Organic mulch materials also add nutrients to the soil when decomposed, enhancing microbial activity. Due to this reported impact of mulches on soil microbes, this current study sought to investigate how biological nitrogen fixation (BNF) is impacted by mulching. Biological nitrogen fixation does not only depend on the genetic makeup

of the *Rhizobium* bacteria and the legume crop but also on environmental factors, such as moisture and temperature [6]. In soybean, soil moisture stress has been shown to reduce nodule weight and numbers, while suboptimal temperatures are also known to impede nodulation [6]. Nodule numbers and dry weight of individual nodules were found to have an average increase of more than 40% in mulched plots compared to unmulched plots. However, to our knowledge, there is limited information reported on cowpea.

In agriculture, different kinds of mulches have been used. Studies have reported the use of plastics, stones, sand, organic materials, among others [5]. Plastic mulches are traditionally common in vegetable production [7] and less common in field crops, such as maize and cowpea. Organic mulches, such as straw, sawdust and manures, are more common in field crops. Even though mulching has been reported as the most important of the three CA principles, its impact depends on the type, the rate of mulching applied and the climatic conditions [5].

Cowpea (*Vigna unguiculata* (L.) Walp) is an important protein-rich food consumed by rural and urban communities of Africa and other countries [8]. Most of the cowpea is produced by smallholder farmers who are resource poor and farm on marginal soils [9]. Thus, it is critical for these farmers who cannot afford inorganic fertilisers or manures to maximise on biologically fixed nitrogen and the retention of high-quality organic residues in their soils. In sub-Saharan Africa, many resource-poor farmers are located in arid and semi-arid areas, and they rely heavily on rainfall, which has become increasingly erratic. Climate change and variability are further exacerbating the challenges faced by the farmers due to the increased severity and frequency of drought [10]. Even though cowpea is generally regarded as drought tolerant, it has been shown that its capacity to fix nitrogen and its yield performance can be hindered by drought [11]. The susceptibility of smallholder farmers to yield losses due to drought calls for research into methods that can conserve soil moisture. In addition, most smallholder farmers are cultivating on inherently infertile soils lacking nitrogen and phosphorus [1,12].

To find sustainable ways to improve cowpea productivity in dry and semi-arid regions, this study investigated the impact of different organic surface mulch materials and different application rates on cowpea yield performance and biological nitrogen fixation. Firstly, it was hypothesised that mulching improves yield and biological nitrogen fixation (BNF) in cowpea grown in a semi-arid region of Limpopo, South Africa; secondly, we hypothesised that the yield performance and BNF of cowpea strongly depend on the type of organic surface mulch applied; and thirdly, it was hypothesised that the amount of mulch applied also has an impact on the performance of cowpea.

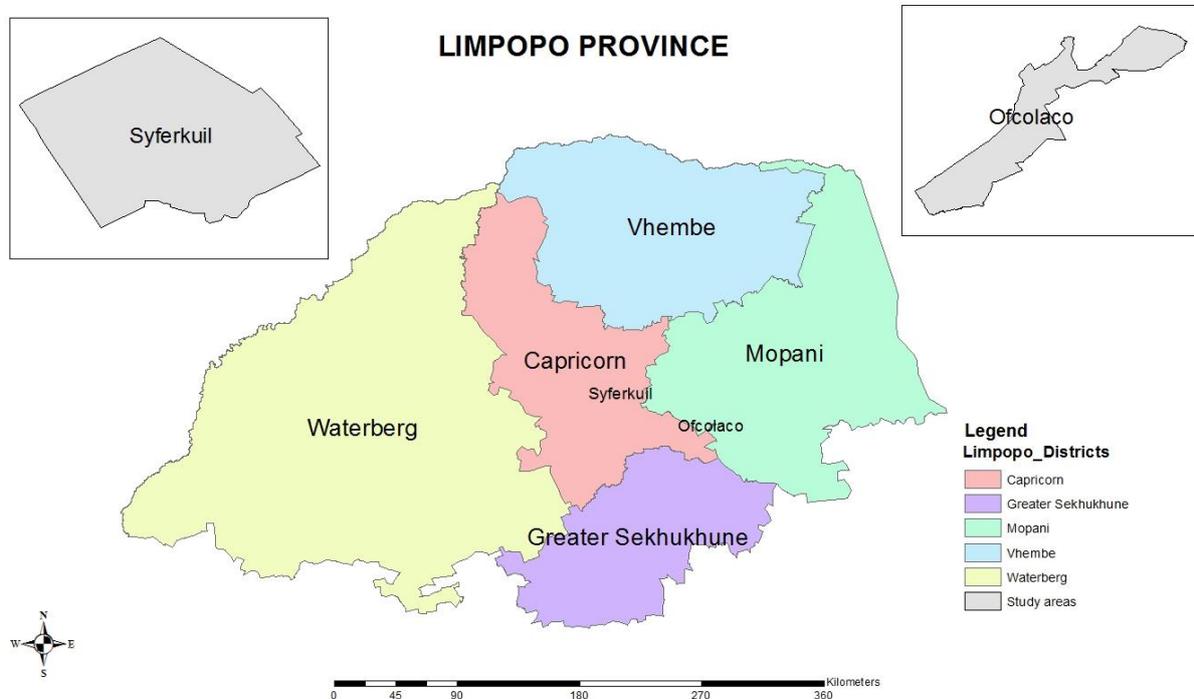
## 2. Methodology

### 2.1. Study Site

The study was conducted under dryland conditions during the 2016/17 and 2017/18 growing seasons under two climatically different locations in the Limpopo Province of South Africa. The locations were the University of Limpopo's experimental farm, commonly known as Syferkuil (23°49' S, 29°41' E), and the farmers' field at Ofcolaco (24°6' S, 30°23' E). Using pegging and GPS, the 2017/18 plantings occurred at the same sites as the previous season at both locations. The study sites were located approximately 120 km apart (Figure 1).

The Syferkuil experimental farm is characterised by hot dry summers (November to January) and cool winters (June to August), with an annual rainfall range of 400 to 500 mm. The 5-year average calculated from the weather data recorded at the Syferkuil weather station showed that the temperature generally ranges from an average minimum of 10 °C in the winter to an average maximum of 28 °C in the summer. Ofcolaco is characterised by hot rainy summers and warm winters, with an annual rainfall of about 700 mm, falling predominantly in summer. The average maximum and minimum temperatures are 31 °C and 18 °C, respectively. These weather data were obtained from a nearby weather station (Metz weather station. According to the Köppen–Geiger climate classification, the climate

at both locations falls under BSh (arid, steppe, hot) [13]. The soils at both locations are characterised by reddish brown soils. Sandy clay loams dominate the surface soils at Ofcolaco, while sandy clays dominate the surface soils at Syferkuil. The soils fall under the Hutton form according to the South African Soil Taxonomy System [14].



**Figure 1.** Locality map of the study sites.

## 2.2. Research Design, Layout and Treatments

The experiment was established as a randomised complete block design under a no-till system with four replications at each location. The treatments consisted of tree and grass (*Hyparrhenia hirta*) mulch materials applied to the surface of the soil. Tree mulches were first spread uniformly on the surface, followed by grass. The tree mulches used were (i) *Vachellia karoo* (*V. karoo*) leaves and twigs; (ii) Chipped *Moringa oleifera* stem; (iii) *M. oleifera* leaves and twigs, each applied at rates of 0, 3, 6 and 9 t/ha in the 2016/17 and 2017/18 growing seasons. *Vachellia karoo* leaves and twigs were obtained from the bush nearby, while *M. oleifera* mulching materials and the grass were acquired from local farmers. Mulching materials were air dried under a shade first before being applied. All the tree mulch treatments were applied in combination with a 3 t/ha grass as a base material (Table 1). Cowpea (*Vigna unguiculata*), var. Bechuana white, was inoculated with a commercial *Bradyrhizobium* sp. prior to planting. Each experimental unit was 5 m × 4 m, consisting of 4 rows at an inter-row spacing of 90 cm and intra-row of 15 cm at both locations.

## 2.3. Soil Sampling and Analysis of Mulch Material

Prior to the establishment of the trials, soil samples were randomly collected at each location at a depth of 0–30 cm using a soil auger to document the pre-plant fertility status of the soil. The soil was analysed to determine the texture and chemical properties, such as pH (KCl), nitrogen (N), phosphorus (P), organic carbon, calcium (Ca), magnesium (Mg). The mulch materials were analysed for total N, total P, Ca, Mg, Mn and Fe (mg kg<sup>-1</sup>).

**Table 1.** Experimental treatments applied during 2016/17 and 2018/19 growing seasons.

Mulch Material	Rate (t/ha)		Code
	2016/17	2017/18	
<i>Valechia karroo</i> leaves and twigs	3 + grass	3 + grass	VK3
	6 + grass	6 + grass	VK6
	9 + grass	9 + grass	VK9
<i>Moringa oleifera</i> stem	3 + grass	3 + grass	MS3
	6 + grass	6 + grass	MS6
	9 + grass	9 + grass	MS9
<i>Moringa oleifera</i> leaves and twigs	3 + grass	3 + grass	MLT3
	6 + grass	6 + grass	MLT6
	9 + grass	9 + grass	MLT9
Control	0	0	C

#### 2.4. Land Preparation

Glyphosate herbicide was applied three weeks before planting to control the weeds present at the sites. The dead vegetation was then slashed and raked from the field two days before planting. This was performed to remove weeds that would add to the applied mulch and ultimately affect the mulching rates. The field was demarcated according to the experimental design of RCBD. Rows were created using a hand hoe and a string to achieve the desired 90 cm inter-row spacing. Two seeds of cowpea var. Bechuana white were sown manually per planting station on 31 January 2017 and 2 February 2018 at Syferkuil and 9 February 2017 and 7 February 2018 at Ofcolaco. Seedlings were thinned out to one plant per station 14 days after planting (DAP) to reach the desired plant population. Mulching materials were applied 15 days after planting when the seedlings had fully emerged.

#### 2.5. Crop Management

Single superphosphate (10.5%) was applied uniformly to all experimental units at a rate of 30 kg P/ha before planting using the row banding method at Syferkuil. No P fertiliser was applied at Syferkuil, as it had enough P of 49 mg kg<sup>-1</sup>. Weeding was performed by hand hoe to remove miniature weeds that were present before the application of mulch. No weeding was performed after applying mulch materials until harvest. The pesticide Lambda-cyhalothrin (Karate zeon) was applied to control aphids whenever observed at both locations. No irrigation was applied, as the trial was conducted during the rainy season.

#### 2.6. Agronomic Measurements

Plant height was determined at 50% flowering at both locations. Measurements were taken from the ground surface to the tip of the youngest fully expanded leaf on five plants from each experimental unit using a measuring tape. The chlorophyll content of the fully expanded flag leaves was measured using a Chlorophyll Content Meter (CCM-200 plus Opti-Sciences, Inc., Hudson, NY, USA), while stem diameter was measured at flowering using a vernier calliper.

Plant biomass was determined at the flowering stage from five randomly selected plants from each experimental unit. The samples were oven dried at the temperature of 60 °C to constant weight. Pod length was measured from five randomly selected pods using a measuring ruler. The number of pods per plant and number of seeds per pod were counted from five randomly selected plant samples per harvest area, and the weight of 100 seeds was taken. The grain yield was determined at harvest maturity from a 2 m × 2 m area from each experimental unit.

### 2.7. Soil Water Content

Gravimetric soil moisture content was determined at the vegetative, flowering and physiological maturity growth stages. Sampling was performed to a depth of 30 cm using a soil auger at both locations. The samples were then placed in labelled zip log plastic bags to conserve moisture, weighed and then oven dried at a temperature of 105 °C until a constant weight was attained. Gravimetric moisture content was determined using the following formula:

$$\text{Gravimetric Water Content (\%)} = \frac{\text{Weight of wet soil} - \text{Weight of dry soil}}{\text{Weight of dry soil}} \times 100 \quad (1)$$

### 2.8. Nodulation and Biological Nitrogen Fixation

Five randomly selected plant samples were uprooted at flowering to determine the number of nodules per plant during the 2016/17 growing season. The nodules were then oven dried at 60 °C to constant weight. The measurement of biological nitrogen fixation (BNF) was performed using the  $\delta^{15}\text{N}$  natural abundance technique. Biological nitrogen fixation was only determined at the Ofcolaco sites. The isotope composition was reported as  $\delta^{15}\text{N}$  in parts per thousand (‰) using air as an international standard ( $R_{\text{Standard}} = 0.3663$ ) and calculated using the formula:

$$\delta^{15}\text{N}_{\text{Sample}} = 1000 \times \left[ \frac{R_{\text{Legume}} - R_{\text{Standard}}}{R_{\text{Standard}}} \right] \quad (2)$$

where  $R_{\text{Legume}} = {}^{15}\text{N}/{}^{14}\text{N}$  of the legume.

The percentage of nitrogen derived from air (%Ndfa) was determined using the formula:

$$\% \text{Ndfa} = 100 \times \left[ \frac{\delta^{15}\text{N}_{\text{Refplant}} - \delta^{15}\text{N}_{\text{Legume}}}{\delta^{15}\text{N}_{\text{Refplant}} - B} \right] \quad (3)$$

*Refplant* was the non-nodulating grass found in the field of experimentation. The B value was determined by growing inoculated cowpea seed in a pure sand culture, so that the crop solely depended on atmospherically fixed nitrogen.

The B value for cowpea is in the range of  $-0.50\%$  and  $-1.84\%$  [15]. Total N yield was calculated from the total above-ground biomass as follows:

$$\text{Total N yield (kg ha}^{-1}\text{)} = \text{Shoot biomass (kg ha}^{-1}\text{)} \times \% \text{N} \quad (4)$$

Biologically fixed N was then calculated as

$$\text{BNF (kg ha}^{-1}\text{)} = \text{Total N yield (kg ha}^{-1}\text{)} \times \% \text{Ndfa} \quad (5)$$

### 2.9. Data Analysis

All data were analysed using the Statistical Analysis System (SAS), Enterprise Version 9.4. Analysis of variance (ANOVA) was carried out to determine differences among the treatments. Where differences were significant, means were separated using the Tukey (HSD) method at a probability level of  $p \leq 0.05$ .

## 3. Results

### 3.1. Soil and Mulch Material Analysis Results

#### 3.1.1. Soil Analyses Results

The analyses results show that the soils at Syferkuil were sandy clay with 35% clay, while the Ofcolaco one was sandy clay loam with 24% clay (Table 2). Soil pH was slightly acidic at both sites, whereas organic carbon was relatively higher at Ofcolaco compared to Syferkuil. Total nitrogen was low in both locations. Phosphorus was marginally deficient

(18 mg kg<sup>-1</sup>) for cowpea production at Syferkuil but was relatively high (49 mg kg<sup>-1</sup>) at Ofcolaco.

**Table 2.** Soil chemical and physical properties at Syferkuil and Ofcolaco.

Soil pH and Nutrients	Location	
	Syferkuil	Ofcolaco
pH (KCl)	6.9	5.5
N (%)	0.05	0.05
P (mg kg <sup>-1</sup> )	18	49
K (mg kg <sup>-1</sup> )	335	238
Ca (mg kg <sup>-1</sup> )	1166	716
Mg (mg kg <sup>-1</sup> )	756	185
Carbon (%)	0.56	1.02
C:N	11.2	20.4
Physical properties		
Silt (%)	14	19
Clay (%)	35	24
Sand (%)	52	57
Textural class	Sandy clay	Sandy clay loam

N = Nitrogen, P = Phosphorus, K = Potassium, Ca = Calcium, Mg = Magnesium.

### 3.1.2. Quality of the Mulching Materials

The results of the organic mulch material analysis showed that they did not have significant amounts of P, Ca, Mg and Mn in them (Table 3). However, the mulching materials had significant amounts of nitrogen. Moringa leaves had 5.5% of N in the mulch material. Table 4 shows the total amount of N in the applied mulch at different rates. The results show that at 3 t ha<sup>-1</sup> of mulch, moringa leaves could potentially supply 165 kg ha<sup>-1</sup> of N, moringa stems 84 kg ha<sup>-1</sup> and the grass only 39 kg ha<sup>-1</sup>. Mulch rates of 9 t ha<sup>-1</sup> could supply significantly high amounts of N, with moringa leaves having a potential N supply of almost 500 kg ha<sup>-1</sup> (assuming all the N is mineralised).

**Table 3.** Organic mulch material nutrient status.

Mulch Type	Total N (%)	Total P %	Ca %	Mg %	K %	Mn (mg/kg <sup>-1</sup> )	Fe (mg/kg <sup>-1</sup> )
<i>V. karroo</i>	2.12	0.23	1.94	0.50	0.82	40	433
Moringa leaves	5.51	0.34	1.79	0.70	1.56	74	323
Moringa stem	1.30	0.32	0.55	0.50	1.42	19	181
Grass	2.80	0.02	0.16	0.05	0.04	103	1429

N = Nitrogen, P = Phosphorus, Ca = Calcium, Mg = Magnesium, Mn = Manganese, Fe = Iron, Al = Aluminium.

**Table 4.** Total nitrogen supplied in mulch at the three rates of mulching.

Mulch Type	Total N (%)	Mulching Rate (t ha <sup>-1</sup> )		
		3 t ha <sup>-1</sup>	6 t ha <sup>-1</sup>	9 t ha <sup>-1</sup>
		Total Nitrogen in Mulch (kg ha <sup>-1</sup> )		
<i>V. karroo</i>	2.12	64	127	190
Moringa leaves	5.51	165	331	496
Moringa stem	1.30	84	168	252
Grass	2.80	39	-	-

### 3.2. Growth and Yield Determining Parameters Response to Mulching

At Syferkuil, the growth and yield parameters measured at the flowering stage showed that chlorophyll content differed among the mulch types and rates (Table 5, Supplementary

Table S1). In the 2017 season, differences were observed mainly between the control and mulching rates of more than 6 t/ha, regardless of the mulch type. Moringa stems at 9 t/ha had 34% higher chlorophyll content compared to the control. In the 2018 season, the control treatment also had lower chlorophyll content compared to the mulched treatments. There were also variations observed between the mulch types in the two seasons. For instance, MLT6 showed higher chlorophyll content compared to VK3. Differences in the effect of the mulch type were also observed for plant height and pods per plant. In the 2017 season, the pod length and the number of seeds per pod did not differ, while for 100 seed weight, the control had the lowest weight of 12.97 g compared to the other treatments. In the 2018 season, both the pod length and number of seeds per pod differed between the treatments. Higher pod lengths were observed in *Vachelia karroo* mulches and moringa leaves and twigs and lower pod lengths in the control and moringa stems.

**Table 5.** Yield determining parameters as influenced by the combined effect of mulch type and rates at Syferkuil.

Syferkuil 2017							
Treatment	Chlorophyll	Stem Diameter (mm)	Plant Height (cm)	Pods/Plant	Pod Length (cm)	Seed/Pod	100 Seed Weight (g)
Control	22.85c	6.75b	23.98c	6.45c	15.7	12.60	12.97b
MLT3	26.09bc	7.75ab	25.11bc	8.10bc	16.6	11.85	15.60a
MLT6	31.35a	7.86ab	27.86	11.50a	15.3	12.00	15.45a
MLT9	29.90ab	7.74ab	25.65bc	8.13bc	16.2	11.85	16.07a
MS3	26.17bc	7.80ab	26.90bc	10.23ab	15.0	12.45	15.74a
MS6	29.43ab	7.95ab	26.88bc	10.08ab	16.2	12.75	15.53a
MS9	30.64a	8.66a	28.51a	12.15a	15.8	12.40	15.83a
VK3	25.72bc	7.59ab	27.39	7.60c	16.2	12.40	16.00a
VK6	29.47ab	8.43ab	28.17	10.05ab	16.3	12.80	15.38a
VK9	29.88ab	8.05ab	26.81bc	10.14ab	16.3	13.35	15.51a
	***	ns	**	***	ns	ns	**
Syferkuil 2018							
Control	23.25d	7.93c	23.22f	4.98f	14.64cd	10.50d	12.20e
MLT3	29.34a	8.78bc	23.83ef	5.40f	16.49a	12.78a	15.53cd
MLT6	23.05d	9.52ab	24.55bcde	9.59b	15.74abc	12.55ab	15.56cd
MLT9	25.74c	8.50	24.92bcd	11.03a	16.27ab	11.34bcd	17.06a
MS3	26.21c	8.42bc	23.42e	6.72de	14.34d	10.81cd	14.70d
MS6	30.04a	8.54bc	25.53ab	5.50f	16.12b	11.98abc	17.36a
MS9	26.15c	9.93a	26.40a	8.19c	15.16bcd	12.83a	16.43abc
VK3	25.84c	8.44bc	23.73ef	5.58ef	16.33ab	13.22a	15.67bcd
VK6	25.82c	7.99c	25.08bc	7.91cd	16.78a	12.86a	16.81ab
VK9	27.44b	9.05abc	26.42a	8.08c	16.59a	12.77ab	16.35abc
	***	***	***	***	***	***	***

MLT is Moringa Oleifera leaves and twigs applied at rates of 3 tons/ha (MLT3), 6 tons/ha (MLT6) and 9 tons/ha (MLT9); MS is M. Oleifera stems applied at rates of 3 tons/ha (MS3), 6 tons/ha (MS6) and 9 tons/ha (MS9); VK is Vachelia Karoo leaves and twigs applied at rates of 3 tons/ha (VK3), 6 tons/ha (VK6) and 9 tons/ha (VK9). Significance levels: \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , ns means not significant. Different letters in the same column refer to significant differences.

At Ofcolaco, in the 2017 season, differences in the chlorophyll content were observed only between the control and the other treatments (Table 6; Supplementary Table S2). Similarly, in 2018, the chlorophyll content was lowest in the control and MLT9 treatments and highest in MS9 and MLT6. In both seasons at Ofcolaco, stem diameter, plant height and number of pods per plant differed among the treatments. It was observed that applying mulch, regardless of the type and quantity, increased stem diameter. The pod length did not vary between the treatments in both seasons, and it averaged 17.08 cm in 2017 and 15.79 cm in 2018. The number of seeds per pod was also not significant in 2017 but differed in 2018. In the 2017 season, 100 seed weight was lowest in the control treatment and highest

in the MS9. In the following year (2018), 100 seed weight was also lower in the control treatment, though not significantly different to MS6, VK6 and VK9.

**Table 6.** Yield determining parameters as influenced by the combined effect of mulch type and rates at Ofcolaco.

Ofcolaco 2017							
Treatment	Chlorophyll	Stem Diameter (mm)	Plant Height (cm)	Pods/Plant	Pod Length (cm)	Seed/Pod	100 Seed Weight (g)
Control	12.26b	8.41c	31.56ab	12.15d	16.36	11.70	13.19c
MLT3	23.46a	10.77ab	32.10ab	15.00cd	17.10	13.35	16.26ab
MLT6	22.96a	11.30b	31.50ab	21.31ab	17.23	13.35	16.33ab
MLT9	21.10a	11.75a	30.65b	23.85a	17.38	13.50	17.01ab
MS3	22.74a	10.79ab	32.30ab	20.61abc	17.31	12.67	16.68ab
MS6	21.98a	10.41ab	30.65b	20.70abc	16.74	12.00	16.05ab
MS9	21.92a	11.51b	31.37b	18.05abc	17.40	13.10	17.37a
VK3	22.95a	10.72ab	30.40b	21.89ab	17.01	13.05	16.38ab
VK6	21.21a	9.32b	34.90a	16.20bcd	16.81	11.85	15.51b
VK9	21.27a	10.80b	31.15b	17.63bcd	17.45	13.25	16.69ab
	***	***	**	***	ns	ns	***
Ofcolaco 2018							
Control	16.31d	6.33g	33.36d	13.23c	15.01	8.56c	12.92b
MLT3	20.47b	8.55f	39.82b	16.25bc	15.39	10.16ab	15.80a
MLT6	22.97a	11.68cd	37.24c	15.53bc	16.06	10.41ab	16.14a
MLT9	16.28d	15.56a	43.76a	21.09ab	16.31	10.78a	15.52a
MS3	20.70b	12.81bc	40.70b	22.13a	16.08	10.31ab	15.61a
MS6	20.31b	10.42e	40.78b	15.84bc	15.55	9.45bc	15.25ab
MS9	22.05a	13.99b	40.54b	22.00a	15.89	10.25ab	15.68a
VK3	19.77b	11.10de	37.09c	15.44bc	16.29	10.59ab	16.27a
VK6	17.75c	10.96de	37.59c	15.58bc	15.60	10.11ab	15.27ab
VK9	19.69b	11.60d	40.80b	18.07abc	15.73	9.80ab	14.39ab
	***	***	***	***	ns	***	***

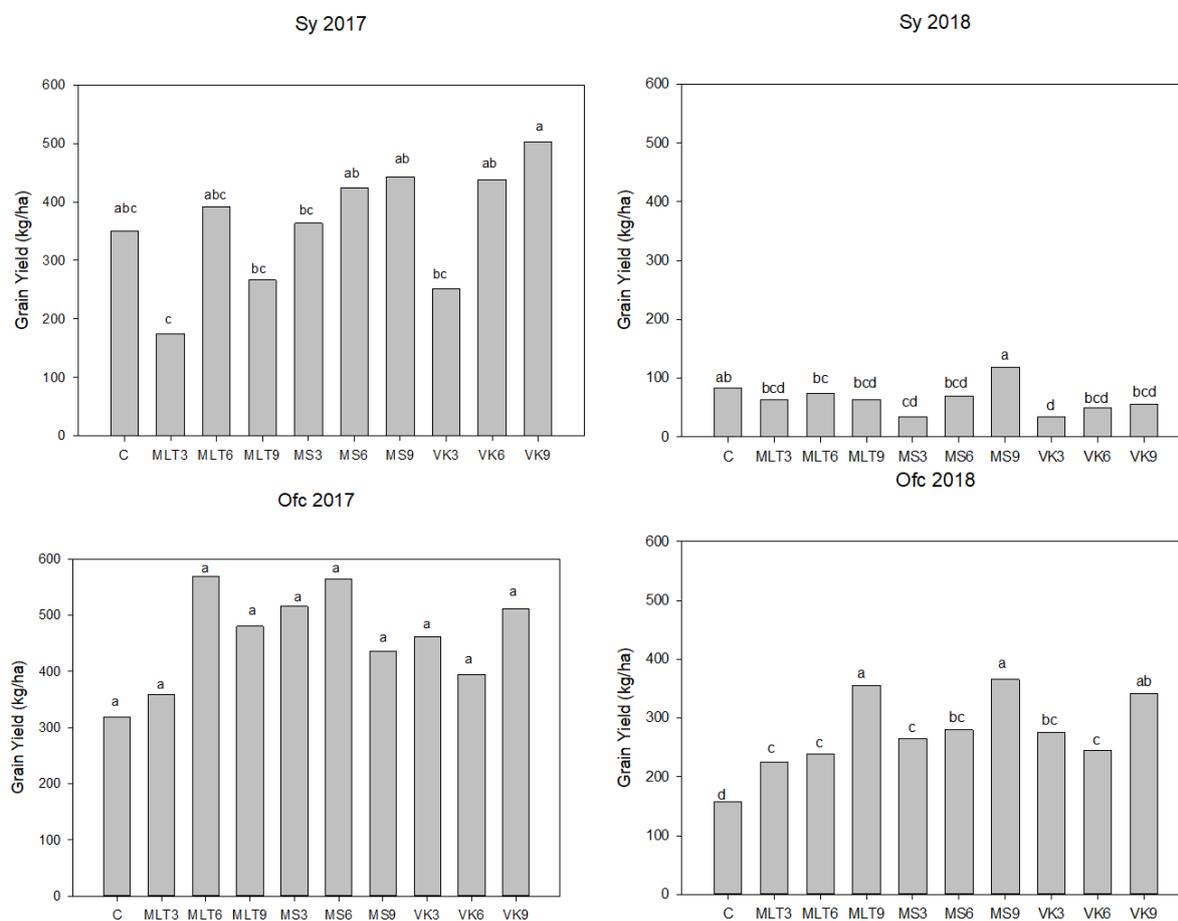
MLT is *Moringa Oleifera* leaves and twigs applied at rates of 3 tons/ha (MLT3), 6 tons/ha (MLT6) and 9 tons/ha (MLT9); MS is *M. Oleifera* stems applied at rates of 3 tons/ha (MS3), 6 tons/ha (MS6) and 9 tons/ha (MS9); VK is *Vachelia Karoo* leaves and twigs applied at rates of 3 tons/ha (VK3), 6 tons/ha (VK6) and 9 tons/ha (VK9). Significance levels: \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , ns means not significant. Different letters in the same column refer to significant differences.

MLT is *Moringa Oleifera* leaves and twigs applied at rates of 3 tons/ha (MLT3), 6 tons/ha (MLT6) and 9 tons/ha (MLT9); MS is *M. Oleifera* stems applied at rates of 3 tons/ha (MS3), 6 tons/ha (MS6) and 9 tons/ha (MS9); VK is *Vachelia Karoo* leaves and twigs applied at rates of 3 tons/ha (VK3), 6 tons/ha (VK6) and 9 tons/ha (VK9). Significance levels: \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , ns means not significant. Different letters in the same column refer to significant differences.

### 3.3. Grain Yield and Shoot Biomass

The grain yield at Syferkuil responded to the treatment effect in both 2017 and 2018 seasons, ranging from 170 to 500 kg ha<sup>-1</sup> in 2017 and 34 to 120 kg ha<sup>-1</sup> in 2018 (Figure 2). In 2017, a relatively higher grain yield was recorded under *Vachelia karoo* at the mulch rate of 9 t ha<sup>-1</sup> (VK9) compared to VK3, MS3 and MLT9 but was similar to that recorded under moringa leaf and twig at 6 t ha<sup>-1</sup>, moringa stem at 6 and 9 t ha<sup>-1</sup>, and control. Grain yield under VK9 was almost three times higher than that observed under the control. In 2018 at Syferkuil, the grain yield under moringa stem at 9 t ha<sup>-1</sup> was higher than all the other treatments, except the control treatment. The average grain yield obtained in 2017 was more than five times higher than that observed in the 2018 season. At Ofcolaco, the grain yield did not show any differences across all treatments; however, differences were observed in the 2018 season. In the 2018 season at Ofcolaco, higher grain yields were observed under

MS9 and MLT9, and the lowest grain yield was observed under control. As observed at Syferkuil, the average grain yield in the 2017 season (461 kg/ha) was higher compared to the average yield obtained in the 2018 season (275 kg/ha).



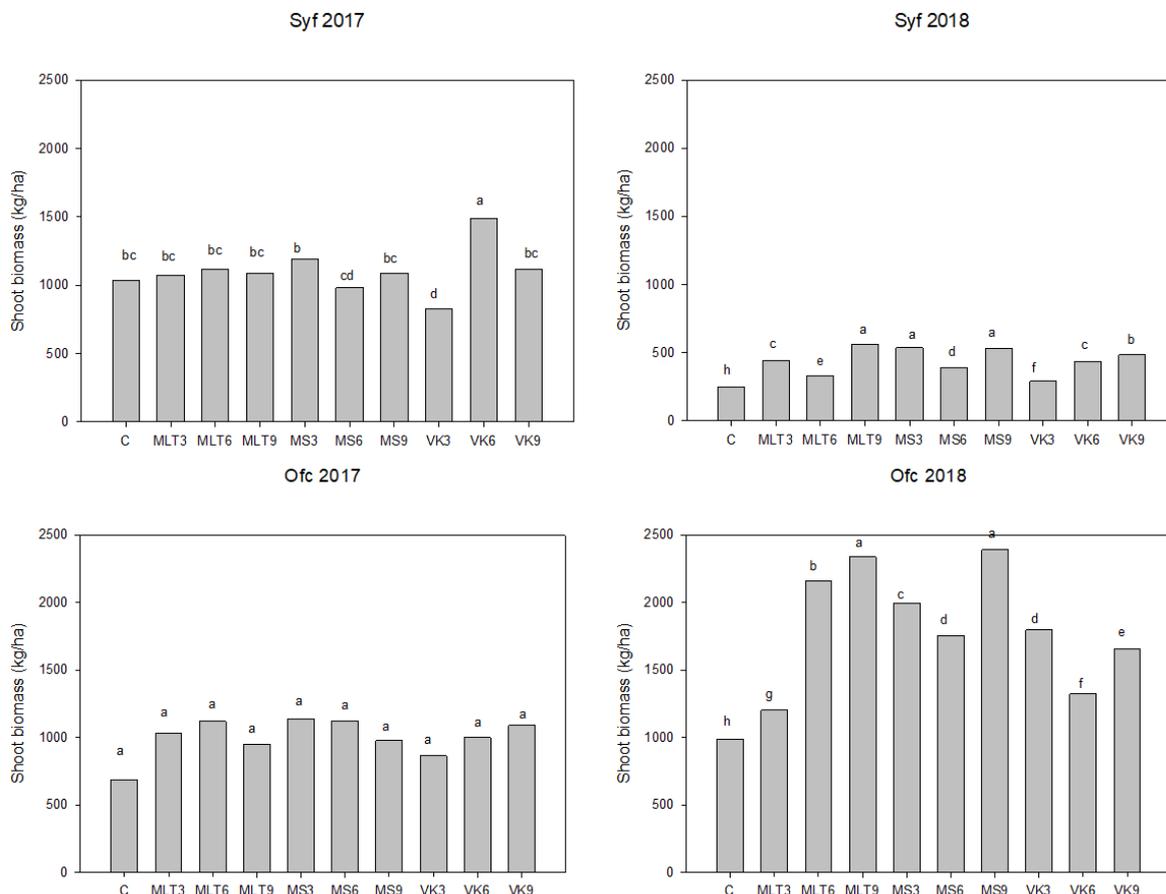
**Figure 2.** Grain yield at Syferkuil and Ofcolaco in the 2017 and 2018 seasons. MLT is *Moringa Oleifera* leaves and twigs applied at rates of 3 tons/ha (MLT3), 6 tons/ha (MLT6) and 9 tons/ha (MLT9); MS is *M. Oleifera* stems applied at rates of 3 tons/ha (MS3), 6 tons/ha (MS6) and 9 tons/ha (MS9); VK is *Vachelia Karoo* leaves and twigs applied at rates of 3 tons/ha (VK3), 6 tons/ha (VK6) and 9 tons/ha (VK9). Different letters show significant differences.

Similar to grain yield, the biomass yield at Syferkuil showed variations in response to the treatments in both years (Figure 3). In the 2017 season, the highest biomass yield was observed under VK6 (1486 kg/ha) and lowest under VK3 (825 kg/ha), while in 2018, higher yields were observed under MLT9, MS3 and MS9, and lowest under control. At Ofcolaco, no differences in biomass were observed in the 2017 season, while in 2018, higher biomass yields were observed under MLT9 and MS9, and the lowest under control. The average biomass yield, contrary to the observation made with grain yield and with biomass at Syferkuil, was higher in 2018 (1761 kg/ha) compared to 997 kg/ha in 2017.

### 3.4. Biological Nitrogen Fixation under Mulching

The impact of mulch type and mulch rate on %NDFa and BNF is shown in Figures 4 and 5. It was observed that the control had the highest %NDFa of more than 70% compared to the mulched plots, and this was observed in both years at Ofcolaco (Figure 4). It was also observed that the control, which was equivalent to zero application of mulch, had higher %NDFa compared to the other rates. In 2017, the highest rate of 9 tons/ha had the lowest %NDFa. However, in 2018, no differences were observed among the rates of 3 to 9 tons/ha (Figure 5). However, the actual amount of N derived from air (BNF) did not vary among

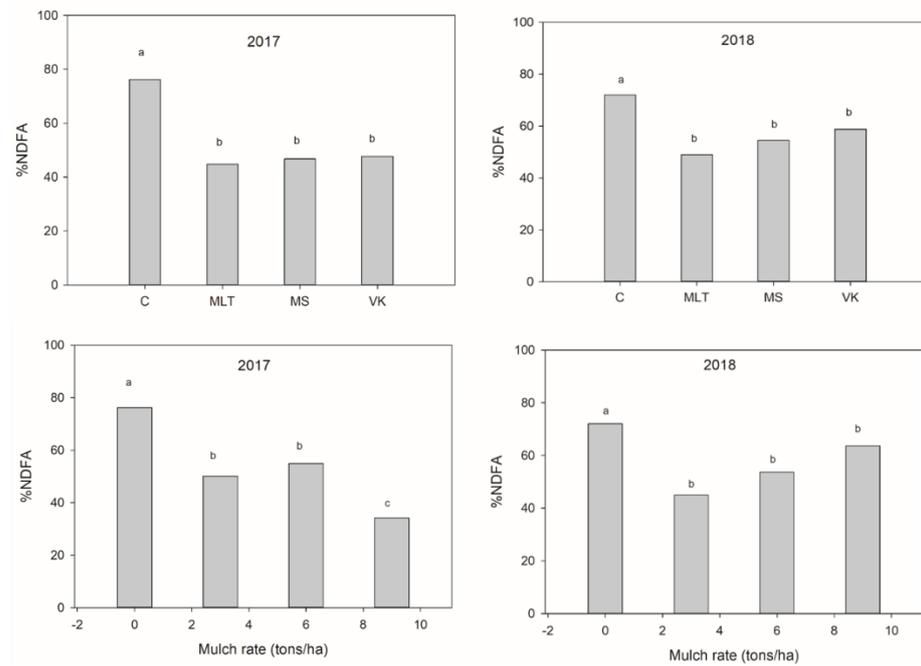
the mulch types in 2017, while in 2018, significant differences were observed between the control and MS. The mulching rates also differed significantly in BNF in both seasons. In 2017, 6 tons/ha had the highest BNF compared to the other rates, while in 2018, 9 tons/ha had the highest, followed by 6 tons/ha. The control and 3 tons/ha had the lowest BNF.



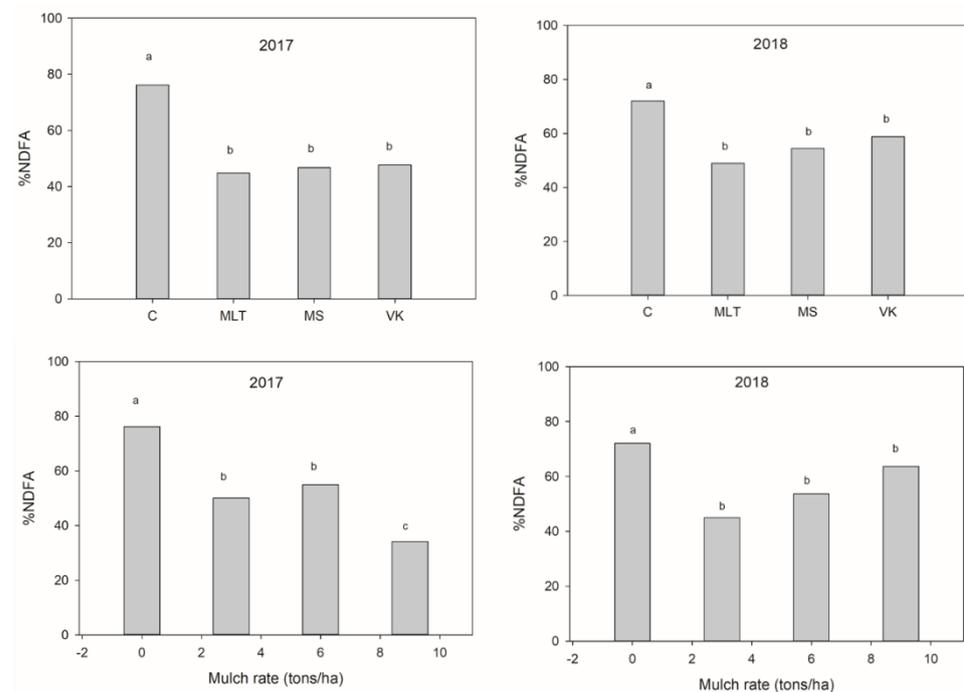
**Figure 3.** Shoot biomass at Syferkuil and Ofcolaco in the 2017 and 2018 seasons. MLT is *Moringa Oleifera* leaves and twigs applied at rates of 3 tons/ha (MLT3), 6 tons/ha (MLT6) and 9 tons/ha (MLT9); MS is *M. Oleifera* stems applied at rates of 3 tons/ha (MS3), 6 tons/ha (MS6) and 9 tons/ha (MS9); VK is *Vachelia Karoo* leaves and twigs applied at rates of 3 tons/ha (VK3), 6 tons/ha (VK6) and 9 tons/ha (VK9). Different letters show significant differences.

At Ofcolaco,  $\delta^{15}\text{N}$  responded to the combined effect of mulch type and mulching rate (Figure 6). Generally, cowpea discriminated more against  $^{15}\text{N}$  under the control treatment compared to mulched plots, except under moringa stem at 6 tons  $\text{ha}^{-1}$  and *V. karoo* under 3 tons  $\text{ha}^{-1}$ , which were similar to that of the control treatment. In 2018, only moringa leaves and twigs at 3 and 6 tons  $\text{ha}^{-1}$  and moringa stem at 3 tons  $\text{ha}^{-1}$  were superior to the control. In the 2017 season, the biggest difference was observed between MS9 and the control where  $\delta^{15}\text{N}$  under MS9 was more than twice as high as the  $\delta^{15}\text{N}$  under control. A similar margin of difference was observed in 2018 between MLT6 and the control. Due to the differences observed in  $\delta^{15}\text{N}$ , variations were also observed in the percentage of nitrogen that was derived from air. It was observed that the treatments with high amounts of N derived from air came from the control (76.2%) treatment, *M. oleifera* stem at 6 tons  $\text{ha}^{-1}$  and *V. karoo* at 3 tons  $\text{ha}^{-1}$  in the 2017 season. In the 2018 season, the control was also among the highest, with 72%, but did not differ with *M. oleifera* leaves and twigs at 9 tons/ha, *M. oleifera* stem at 6 and 9 tons  $\text{ha}^{-1}$ , as well as *V. karoo* at 6 and 9 tons  $\text{ha}^{-1}$ . There were no huge differences in the biologically fixed nitrogen (BNF) in both

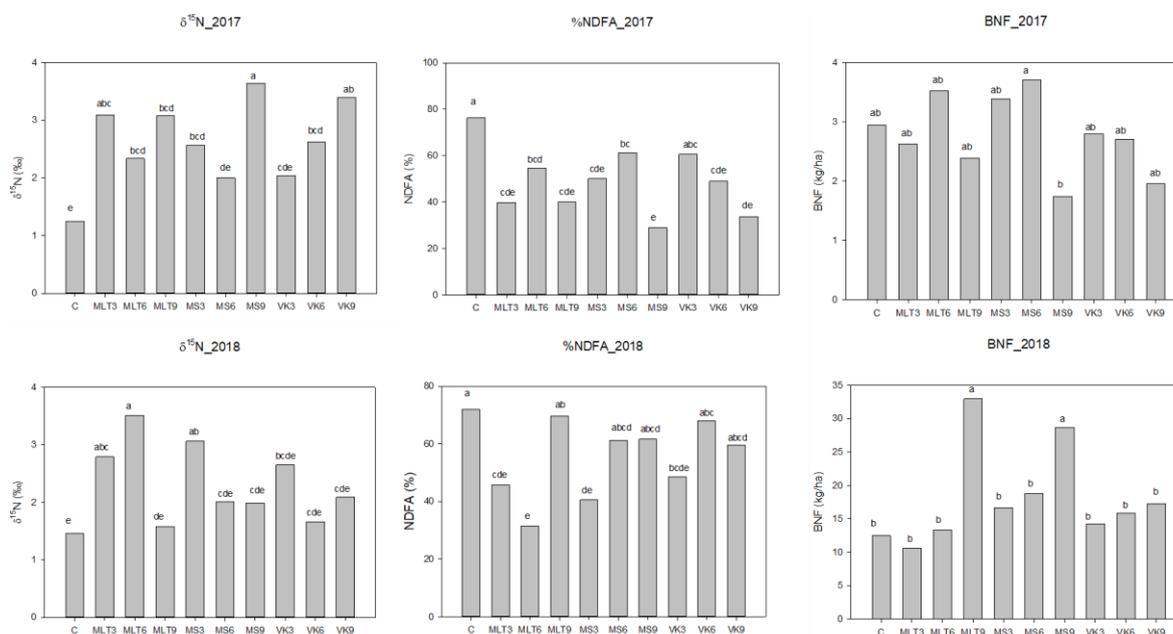
seasons. In 2017, significant differences were only observed between MS6 and MS9, while in 2018, MLT9 and MS9 had significantly higher BNF compared to the other treatments.



**Figure 4.** The effect of mulch type and mulch rate on %Ndfa (percentage of nitrogen derived from people) at Ofcolaco in 2017 and 2018. MLT is *Moringa Oleifera* leaves and twigs; MS is *M. Oleifera* stems; VK is *Vachelia Karoo* leaves and twigs, and C is the control where no mulch was applied. Different letters (a, b, c) on the bars show significant differences.



**Figure 5.** The effect of mulch type and mulch rate on biological nitrogen fixation (BNF) at Ofcolaco in 2017 and 2018. MLT is *Moringa Oleifera* leaves and twigs; MS is *M. Oleifera* stems; VK is *Vachelia Karoo* leaves and twigs, and C is the control where no mulch was applied. Different letters (a, b, c) on the bars show significant differences.



**Figure 6.**  $\delta^{15}N$ , percentage nitrogen derived from air and biologically fixed nitrogen at Ofcolaco in the 2017 and 2018 seasons. MLT is *Moringa Oleifera* leaves and twigs applied at rates of 3 tons/ha (MLT3), 6 tons/ha (MLT6) and 9 tons/ha (MLT9); MS is *M. Oleifera* stems applied at rates of 3 tons/ha (MS3), 6 tons/ha (MS6) and 9 tons/ha (MS9); VK is *Vachelia Karoo* leaves and twigs applied at rates of 3 tons/ha (VK3), 6 tons/ha (VK6) and 9 tons/ha (VK9). Different letters show significant differences.

The number of nodules per plant in the 2017 season was high in the VK9 treatment, with 6.09, compared to MLT6 and MLT9. In 2018, the highest number of nodules per plant were observed in MS9 and MLT9, which both had just over 13 (Table 7). The average number of nodules per plant was more than double in 2018 (9.11) compared to 2017 (4.21). Nodule weight did not differ between the treatments in 2017 but showed significant differences in 2018. The average nodule weight in 2017 was 0.09 g, while in 2018, it was 1.99 g. The root weight differed significantly among the treatments in 2017, while in 2018, differences were only observed between the control and the rest of the treatments.

**Table 7.** Nodules/plant, nodule weight and root weight at Ofcolaco in 2017 and 2018.

Ofc2017				Ofc2018		
Treatment	Nodules/Plant	Nodule Weight	Root Weight (g)	Nodules/Plant	Nodule Weight (g)	Root Weight (g)
C	4.13ab	0.13	1.71d	5.41d	1.73f	2.38b
MLT3	3.75ab	0.03	4.04abc	8.50b	1.96bcd	4.14a
MLT6	3.02b	0.09	4.25abc	7.29c	2.01b	4.21a
MLT9	2.75b	0.08	3.85abc	13.42a	2.26a	4.93a
MS3	5.38ab	0.07	5.42a	7.31c	1.79ef	4.64a
MS6	3.28ab	0.15	4.64abc	9.21b	1.87de	4.10a
MS9	3.42ab	0.09	3.05cd	13.56a	2.26a	4.70a
VK3	5.42ab	0.12	4.00abc	8.25bc	1.89cd	3.79a
VK6	4.89ab	0.06	3.69bc	9.22b	1.97bc	4.43a
VK9	6.09a	0.13	5.07ab	8.94b	2.18a	4.90a
	**	ns	***	***	***	***

MLT is *Moringa Oleifera* leaves and twigs applied at rates of 3 tons/ha (MLT3), 6 tons/ha (MLT6) and 9 tons/ha (MLT9); MS is *M. Oleifera* stems applied at rates of 3 tons/ha (MS3), 6 tons/ha (MS6) and 9 tons/ha (MS9); VK is *Vachelia Karoo* leaves and twigs applied at rates of 3 tons/ha (VK3), 6 tons/ha (VK6) and 9 tons/ha (VK9). Significance levels: \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , ns means not significant. Different letters in the same column refer to significant differences.

### 3.5. Relationship between BNF and $\delta^{15}\text{N}$ , N%, Shoot Biomass, Total N and NDFA%

A multiple stepwise regression was carried out on the 2018 season data with BNF as the response variable and  $\delta^{15}\text{N}$ , N%, shoot biomass, total N and NDFA% as the predictor variables (Table 8). The output showed that in both seasons, 95% of the variation observed in BNF was explained by that variation in  $\delta^{15}\text{N}$  and total N, as shown in the models below (2017: Equation (6); 2018: Equation (7)). The correlation analyses showed that BNF was negatively related to  $\delta^{15}\text{N}$ , while total N was positively related to BNF.

$$Y = 10.95 + 0.509X_1 - 4.432X_2 \quad (6)$$

$$Y = 13.5 + 0.621X_1 - 7.041X_2 \quad (7)$$

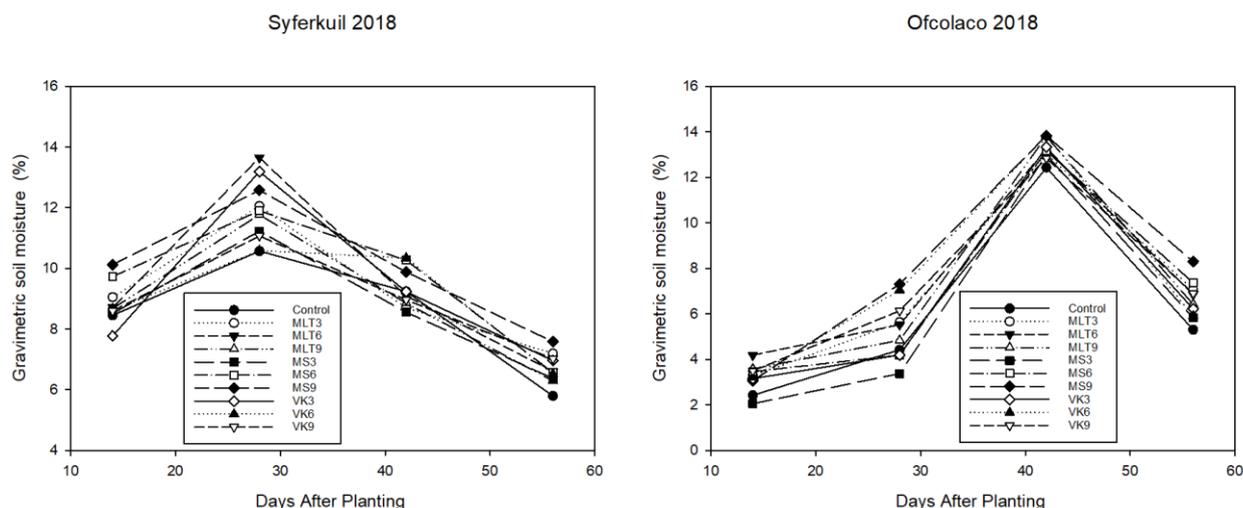
where Y is biologically fixed nitrogen (kg/ha),  $X_1$  is total N (kg/ha), and  $X_2$  is  $\delta^{15}\text{N}$ .

**Table 8.** Multiple linear regression on the factors that influence biological nitrogen fixation.

2017											
Model		Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95.0% Confidence Interval for B		Correlations		
		B	Std. Error	Beta			Lower Bound	Upper Bound	Zero-order	Partial	Part
1	(Constant)	20.51	1.66		12.39	0.00	17.16	23.86			
	$^{15}\text{N}$	-3.50	0.61	-0.68	-5.75	0.00	-4.73	-2.27	-0.68	-0.68	-0.68
2	(Constant)	10.95	0.67		16.35	0.00	9.60	12.31			
	$^{15}\text{N}$	-4.43	0.18	-0.86	-24.37	0.00	-4.8	-4.063	-0.682	-0.97	-0.837
	Total N (kg/ha)	0.509	0.025	0.724	20.416	0.00	0.459	0.56	0.507	0.958	0.701
2018											
1	(Constant)	1.965	2.764		0.711	0.482	-3.631	7.56			
	Total N (kg/ha)	0.485	0.079	0.707	6.154	0.00	0.326	0.645	0.707	0.707	0.707
2	(Constant)	13.5	0.963		14.018	0.00	11.548	15.451			
	Total N (kg/ha)	0.621	0.023	0.904	26.551	0.00	0.573	0.668	0.707	0.975	0.868
	N15	-7.041	0.339	-0.707	-20.78	0.00	-7.728	-6.354	-0.455	-0.96	-0.679
Dependent Variable: BNF (kg/ha)											

### 3.6. Gravimetric Soil Moisture Variation in the 2018 Season at Both Locations

Gravimetric soil moisture was measured at four different dates after emergence during the 2018 cowpea growth season. At each measurement date, soil moisture varied between the treatments. Generally, soil moisture was observed to be higher under mulching rates of 6 and 9 t/ha compared to lower mulch levels of 3 t/ha and the control (Figure 7). The moisture level in the control treatment was always among the lowest at all four measurement dates at the two locations. It was observed that MS9 constantly maintained a higher moisture level at all DAEs and the two locations, except at 28 DAE at Ofcolaco. Even though the moisture levels differed among the treatments at each DAE, they followed a similar trend across the season. At Syferkuil, the gravimetric moisture content increased from 14 DAE to 28 DAE and then decreased gradually until the last day of measurement (56 DAE), while at Ofcolaco, the moisture content increased gradually from 14 DAE up until 42 DAE before decreasing on the last day of measurement (56 DAE).



**Figure 7.** Gravimetric soil moisture at Syferkuil and Ofcolaco in 2018. MLT is *Moringa Oleifera* leaves and twigs applied at rates of 3 tons/ha (MLT3), 6 tons/ha (MLT6) and 9 tons/ha (MLT9); MS is *M. Oleifera* stems applied at rates of 3 tons/ha (MS3), 6 tons/ha (MS6) and 9 tons/ha (MS9); VK is *Vachelia Karoo* leaves and twigs applied at rates of 3 tons/ha (VK3), 6 tons/ha (VK6) and 9 tons/ha (VK9).

## 4. Discussion

### 4.1. Agronomic Parameters

The findings of this study indeed confirmed the efficacy of mulching in improving cowpea performance. This multiple site and location study showed that applying mulch improves the physiological aspects of a crop, such as the chlorophyll content. These findings agree with the findings of Zhang, Qian [16], who found chlorophyll content increased with mulching in maize, but is contrary to the findings of Taufiq, Wijanarko [17], who found mulching to have a significant impact on groundnuts. The study also showed differences in chlorophyll content resulting from the mulching treatments. For instance, at Syferkuil in 2018, higher chlorophyll content was observed under MLT3 and MS6 compared to other treatments. At Ofcolaco, in 2018 as well, higher chlorophyll content was observed under MLT6 and MS9 compared to the other treatments. The findings showed that mulching treatments with *Moringa oleifera* tended to perform much better in chlorophyll content compared to those with *Vachellia karroo*.

The results also showed that other agronomic parameters, such as the plant height, pods per plant and stem diameter, were also improved by mulch application. Stem diameters were thicker in mulched plots compared to the control. At Syferkuil in 2018, stem diameter was 25% thicker in the MS9 treatment compared to the control. In the same season at Ofcolaco, the stems were more than two times thicker under MS9 compared to the control. However, the pod length mostly showed no variation among the treatments at both locations and in both seasons, except in the 2018 season at Syferkuil.

The findings of this study showed that both biomass and grain yield were generally lower compared to what has been reported in other studies [11,18], especially in the 2018 season, and this was observed at both locations. The average grain yield was less than 100 kg/ha at Syferkuil in 2018, while in the same season at Ofcolaco, it averaged 275 kg/ha. The biomass averaged less than a ton, except for Ofcolaco in the 2018 season. In the study of Munjonji, Ayisi [11], a biomass yield of more than 2 t/ha was reported for cowpea under water stress conditions. The 2017 grain yield levels were lower but were in the range of what is obtainable and reported under rainfed conditions in many smallholder farming sectors [9]. Despite the low yields, mulching showed that it has a potential to increase the biomass and grain yield of cowpea. Grain yield was significantly higher under MS9 at both locations in the 2018 season. Similarly, biomass was also higher under MS9 in 2018 at both locations. However, contrary to the observations made on yield determining parameters

and chlorophyll content, mulching did not necessarily improve grain yield or biomass compared to the control treatment. Grain yield was only lowest under control at Ofcolaco in the 2018 season. Shoot biomass was also significantly lower under control compared to the other treatments in the 2018 season for both locations. The lower biomass in the control in the 2018 season and the subsequent lower yield can be attributed to the lower moisture content in the control treatment, as shown in Figure 5.

#### 4.2. Biological Nitrogen Fixation (BNF)

Our findings showed that cowpea discriminated more against  $^{15}\text{N}$  when grown without mulch compared to when mulched. In some instances, the mulched plots had more than double the  $\delta^{15}\text{N}$  compared to that of the control, showing that mulched plots had significantly lower discrimination compared to the control. This difference in discrimination can be attributed to the decomposition of the mulching material, providing easily available nitrogen to the crop. It is known that organic surface mulches can decompose, providing nitrogen to the crops [5,19]. The organic mulches used in this study were from tree species that are known to have a relatively low C:N ratio in their leaves [20,21]. Table 4 shows the amount of N that could be supplied by the different mulch materials when decomposed. The table shows that Moringa leaves and twigs (MLT) could supply about 165 kg/ha of N at only 3 tons/ha of mulch. This amount further increases with higher rates. These higher N contents in the mulches could have been mineralised and suppressed BNF. This is because the legume would absorb the easily available N instead of expending energy on fixing N. In addition, studies conducted on soybean have shown that biological nitrogen fixation is reduced when legumes are grown in soils with high mineral N [22,23].

These differences in  $\delta^{15}\text{N}$  resulted in differences in the percentage of nitrogen derived from air (%NDFEA). It was observed that more than 70% of the nitrogen in the cowpea under the control treatment was derived from air. The results also revealed that the main factors (mulch type and rate) all resulted in lower %NDFEA compared to the control. However, despite the high %NDFEA in the control treatment in both seasons, the actual amount of N fixed in the 2018 season was not congruent. This was mainly due to the lower biomass observed in the control treatment at Ofcolaco in 2018. Lower biomass translates to less nitrogen. The lower biomass in the control treatment could have resulted from the lower moisture level, as observed in Figure 5. Figure 5 shows that in the 2018 season at Ofcolaco, the control consistently had relatively lower moisture levels compared to the other treatments and could have resulted in lower biomass accumulation. Soil moisture level is known to significantly influence biomass accumulation in cowpea [11,24–26]. However, in the 2017 season, the biomass did not significantly differ between the treatments, and hence, not many differences were observed in BNF. While the biomass, grain yield and BNF results at Ofcolaco in 2018 show the influence of mulching on the performance of cowpea, the lack of difference in the same parameters at the same location in the 2017 season suggests that cowpea has the ability to perform and yield while depending on atmospheric nitrogen alone, as the other treatments provided N through the mulch degradation.

A regression analysis to identify the factors that influence BNF showed that total nitrogen in biomass and the  $\delta^{15}\text{N}$  are the main factors determining the amount of N fixed. Nitrogen isotope composition was negatively related to BNF, while biomass showed a positive relationship. Thus, in 2018, when lower biomass was observed at Ofcolaco under control, even the corresponding BNF was also reduced.

## 5. Conclusions

The finding of this study showed that the application of mulches, regardless of the rate and type, improved the chlorophyll content and other agronomic properties, such as the stem diameter and plant height of cowpea. However, in this current study, mulching did not necessarily improve the biomass and grain yield. This study also revealed that organic surface mulches with a lower C:N ratio negatively impacted BNF, as very low amounts of nitrogen were derived from air under mulching. Finally, the study showed that BNF in

cowpea grown under mulching is positively associated with the total amount of N in the biomass and negatively related to  $\delta^{15}\text{N}$ .

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/agriculture12081144/s1>, Table S1: Effect of mulch type and mulch rate on selected parameters at Syferkuil; Table S2: Effect of mulch type and mulch rate on selected parameters at Syferkuil.

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