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Research Article

The Development of Analytical Methodologies and Advances in Plant Physiology, Measurable Amounts of Elements in Nutrients Contribution and Carbon Assimilation by Five Grain Legumes, Bambara Groundnut; Groundnut, Cowpeas, Blackgram and Mungbeans Planted in Mpumalanga province, South Africa during 2011/2012 Season

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Abstract

A field experiment was carried out in the 2011/2012 summer cropping season at the Lowveld Research Station, Nelspruit (Bombela), in Mpumalanga province, where five grain legumes planted under field conditions during the planting season 2011 replicated four times as randomized complete block design (RCBD). The grain legume species cultivars for discussion were Bambara-groundnut (*Vigna subterranea* L. Verdc) (cv. Brianbeck MB 51), Groundnut (*Arachis hypogaea* L.) (cv. JL 24), Cowpea (*Vigna unguiculata*) (cv. Pan 311), Blackgram (*Vigna mungo*) (Local market seed), and Mungbean (*Vigna radiata*) (cv. VC 1973A).

Recent consideration of essential and other (non-essential and toxic) nutrient elements in crops, both macro- and micro-elements - can cause nutrient imbalances, reduction in growth and yield losses. Beneficial "elements", they are not required by all plants but can promote plant growth and may be essential for several plant species. Recently, nutrient content is expressed as element content in dry matter (DM) percentages in DM for macro-elements, in DM for micro-elements (also known as ppm= pars pro million, 1 mg per 106 kg). Average concentration ranges of essential nutrient elements in crops, Oxygen (45), Carbon (45), Hydrogen (6), Sulphur (0.1 - 0.4), Chlorine (0.2 - 2.0), Na (Sodium) (0.01 - 10), Silicon (0.2 - 2.0), Manganese (0.1 - 0.4), Calcium (0.2 - 1.0), Potassium (0.2 - 6.0), Phosphorus (0.01 - 0.7), Nitrogen (0.1 - 6.0) and Concentration range percentages in DM Macro-elements Cobalt (0.02).

Carbon (C) is ranked along N and P as mineral nutrients that are needed the most by plants for their growth and development. For C_3 plants such as grain legumes, C is acquired through the process of photosynthesis and these C compounds play a significant role in the growth and development of C_3 plants. However, the disproportionate alteration of the microbial C and N contents led to a distinct decrease of the C:N ratio for mung-bean residues at flowering but no change in the groundnut and Bambara-groundnut treatments. This suggests that the changes in microbial C:N ratio are mainly caused by the 'recoverable' residue inputs.

Basically, the composition of the C isotopes is compared to that in the air to reveal water-use efficiency of the test plant species during its growth period. Calculated as $\delta^{13}C$, the water-use efficiency provides information on the ability of C_3 plants to balance the process of acquiring carbon through the process of photosynthesis in relation to the water that get lost in the process. With regards to grain legumes such as cowpea, Bambara-groundnut, groundnut, mung-bean and black gram, quality residues include that which have a C/N ratio that is < 24 g g-1. There is a need to select crops particular that cultivated by smallholder farmers which can tolerate drought because South Africa has had high temperatures, and evapotranspiration coupled with reduced and scanty rainfall.

The implications of future increases in atmospheric CO_2 for the productivity of indeterminate C_3 crops grown in rain-fed subsistence agricultural systems in the semi-arid tropics are stress resistance, therefore JL 24 cultivar in the study at Nelspruit, under field conditions manage to have shoot C content of 45% as best performer legume species planted. Plants also affect the residue turnover by excreting easily available organic C and N compounds which can interact directly with the microbial biomass and affect its size, activity, turnover rate or physiological status. However, following this concept, N will be set free from the microbial biomass as microbial residues, the relation between the parameters is difficult to prove because data concerning the residue quality are only available for the 'recoverable' residues and not for the rhizo-deposits.

Keywords: Nutrients Contribution; Carbon Assimilation; Grain Legumes; Bambara Groundnut; Groundnut; Cowpeas; Blackgram; Mungbeans

Abbreviation

Pg: Picogram= 10 - 12g: F: Fluorine; As: Arsenic; Cr: Chromium; Li: Lithium; Pb: Lead; Al: Aluminum; Co: Cobalt; Na: Sodium; Se: Selenium; Si: Silicon; S: Sulfur; Mg: Magnesium; Ca: Calcium; K: Potassium; P: Phosphorus; N: Nitrogen; Ni: Nickel; Na: Sodium*; Cl: Chlorine*; V: Vanadium; Co: Cobalt; Si: Silicon**; Mo: Molybdenum; Cu: Copper; Mn: Manganese; Zn: Zinc; Fe: Iron; DM: Dry Matter; C: Carbon; O: Oxygen; SOM: Soil Organic Matter; RCBD: Randomized Complete Block Design; cv.: Cultivar/Genotypes; TOC: Total C Analyser; TNb: Total N Analyser

Background

Background of plant macro and micro-nutrients concentration

Recent consideration of essential and other (non- essential and toxic) nutrient elements in crops

Lowest measurable amount: pg (picogram = 10 - 12g). (a) Excessive concentration of a mineral element - both macro- and micro-elements - can cause nutrient imbalances, reduction in growth and yield losses. The element has been considered as "toxic". (b) Plants may contain small amounts of elements with no evidence of essentiality: Fluorine (F), Arsenic (As), Chromium, (Cr), Lithium (Li), Lead (Pb).

New terms were introduced by Epstein (1999), Epstein and Bloom (2005). Instead of the term "non-essential", it is suggested to use the term "apparently non-essential" or not known to be essential. The element is classified as "quasi-essential" when essentiality and plant responses are different among plant species, it is suggested to use the term "toxic concentration" rather than "toxic element".

Other terms used by several authors

"Beneficial" elements (Pilon-Smits., *et al*. 2009) Aluminum (Al), cobalt (Co), sodium (Na), selenium (Se) and silicon (Si) are considered "beneficial" elements for plants. They are not required by all plants but can promote plant growth and may be essential for several plant species. Silicon is considered a "quasi-essential" element for plants because its deficiency can cause various abnormalities with respect to plant growth and development. This term was introduced by Epstein (1999), Epstein and Bloom (2005).

Principal form (s) taken up by roots Chemical symbol Element Sulfur (S), Sachs J, Knop, Magnesium (Mg), Willstatter Salm-Horstmar F, Calcium (Ca) Potassium (K), Sachs J, Knop, Phosphorus (P), Ville, Nitrogen (N), Rutherford, G.K., Essential nutrients [1], Ni, 1983 Brown, Welsh and Cary^{2+}, Nickel Ni, Na 1980's + Sodium* Na and Cl, 1954 Broyer, Stout - Chlorine* Cl, Co^{2+}, Si $(OH)_4$0, Mo (O), B (OH), Cu^{2+}, Mn^{2+}, Zn^{2+}, Zn (OH) and Fe^{2+}, Fe^{3+}, Micro-nutrients, Vanadium (V), Cobalt (Co), Silicon* * (Si), Molybdenum (Mo), Arnon and Stout, Boron (B), Warington, Lipman and MacKinney, Copper (Cu), Manganese (Mn), McHargue, Zinc (Zn) Sommer and Lipman, Iron (Fe) Sachs, J., Knop, *Macro-nutrients for several crops, **"Quasi-Essential Element".

Expressing plant nutrient content

Recently, nutrient content is expressed as element content in dry matter (DM) percentages in DM for macro-elements: N %, P %, K %, Ca %, Mg %, S %, mg per kg (mg/kg^{-1}) in DM for micro-elements (also known as ppm= pars pro million, 1 mg per 106 kg) Previously, element contents were commonly expressed as oxides (e.g. P_5, K_2O etc.) Conversion factors: 5% x 0.436 = P % or P % x 2.29 = P_2O % x 0.83 = K% or K % x 1.2 = K.

Average concentration ranges of essential nutrient elements in crops

O (45), C (45), H (6), S (0.1 - 0.4), Cl (0.2 - 2.0), Na (0.01 - 10), Si (0.2 - 2.0), Mg (0.1 - 0.4), Ca (0.2 - 1.0), K (0.2 - 6.0), P (0.01 - 0.7), N (0.1 - 6.0) and Concentration range percentages in DM Macro-elements (Co (0.02).

Introduction

Carbon (C) is ranked along N and P as mineral nutrients that are needed the most by plants for their growth and development. For C_3 plants such as grain legumes, C is acquired through the process of photosynthesis and these C compounds play a significant role in the growth and development of C_3 plants. It is therefore on this background that the C content in organs of C_3 plants is used as an indicator of their growth. Basically, during photosynthesis, the CO_2 fixation process in plants involves discriminates of the heavier isotope of C, that is, $^{13}CO_2$, in favour of the lighter isotope of carbon, namely $^{12}CO_2$. The ratio of $^{12}CO_2/^{13}CO_2$ or $^{12}C/^{13}C$, calculated as $\delta^{13}C$ or ^{13}C in organs of C_3 plants is used to indicate water-use efficiency. Among other techniques, the water-use efficiency of C_3 plants is determined through the use of the isotopes of carbon ($^{13}C/^{12}C$) in dried shoots among other techniques.

As reported by several authors above-ground residue incorporation causes an increase in microbial C and N contents followed by a subsequent decrease [2,3]. However, the disproportionate alteration of the microbial C and N contents led to a distinct decrease of the C:N ratio for mung-bean residues at flowering but no change in the groundnut and Bambara-groundnut treatments. Under N-limiting conditions (Table 1) and sufficient C supply by the roots combined with an increasing competitiveness of the plants for N, the microbial biomass was severely N limited and this reduced the microbial growth [4]. It is not clear how this N becomes plant available.

The significant differences in the microbial C:N ratio might be caused by the differences in residue N inputs and quality. At the start of the experiment the distribution in microbial C:N ratio varied from that observed at maturity (Table 1). This suggests that the changes in microbial C:N ratio are mainly caused by the 'recoverable' residue inputs. However, the rhizo-deposition quality might also contribute to differences in microbial C and N immobilization due to an alteration of the microbial population. Smith and Kirkegaard (2002) found that bacteria were generally more tolerant against isothiocyanates than the eukaryotic group, although both groups showed considerable variability in response.

C. Assimilation	Bambara	Groundnut	Cowpea	Blackgram	Mungbean
Shoot biomass	45 ($g.plant^{-1}$)	35 ($g.plant^{-1}$)	25 ($g.plant^{-1}$)	20 ($g.plant^{-1}$)	10 ($g.plant^{-1}$)
Shoot C/N ratio	15 ($g.g^{-1}$)	12$g.g^{-1}$)	18 ($g.g^{-1}$)	15 ($g.g^{-1}$)	20 ($g.g^{-1}$)
Shoot $\delta^{13}C$	-26 (%)	-25 (%)	-28 (%)	27 (%)	-27 (%)
Shoot C content/Concentration	44%	45%	41.5%	42%	41%
Shoot C content	20 ($g.plant^{-1}$)	15 ($g.plant^{-1}$)	10 ($g.plant^{-1}$)	7.5 ($g.plant^{-1}$)	5 ($g.plant^{-1}$)

Table 1: Carbon assimilation by five legumes species grown under field conditions at Nelspruit during the 2011 planting season.

These interactions can have direct effects on the C and N mineralization of the native soil organic matter (SOM) and the decomposition of plant residues. Beside well known adaptation mechanisms for nutrient acquisition such as root morphology, mycorrhiza or release of organic acids and phytosiderophores (Jones, 2004; Marschner, 2018), plants are able to influence the mobilisation-immobilisation turnover in soils [5,6] and on top of that might be differences between plant species (Hodge., *et al*. 2004).

Basically, the composition of the C isotopes is compared to that in the air to reveal water-use efficiency of the test plant species during its growth period. Calculated as $\delta^{13}C$, the water-use efficiency provides information on the ability of C_3 plants to balance the process of acquiring carbon through the process of photosynthesis in relation to the water that get lost in the process (Condon., *et al.* 2012). It therefore is an important measure of plant and environmental parameters that influence photosynthetic gas exchange processes over time that CO_2 is fixed (Roussel., *et al*. 2009).

Furthermore, it provides useful knowledge on the ability of crops to withstand periodic drought during the growth season, making the technique useful in selecting agricultural crops for drought tolerance [7]. In general, C concentration determined in legume plants typically ranged up to 30% (Sprent., *et al*. 2014), however, there are instances where the C concentration in legume tissue organs is greater than 40% [8]. For example, high %C values (40 - 49) have been reported in Bambara-groundnut collected from farmers' fields in the Mpumalanga Province [9]. According to Post., *et al.* [8] such high values indicate high lipid distribution. There is no literature available on the %C of cowpea, groundnut and black gram yet knowledge on such data could improve our knowledge on these plants' growth and C nutrition.

There is a need to select crops particular that cultivated by smallholder farmers which can tolerate drought because South Africa has had high temperatures, and evapotranspiration coupled with reduced and scanty rainfall [10,11]. A combination of these events causes poor agricultural production which results in detrimental effects on the livelihoods of largely low-income populations [11]. Unfortunately, low-input smallholder farmers hardly afford irrigation infrastructure and their plight is made worse by the scarcity of water in South Africa with groundwater constrained by underlying hard rock formations, and the 37 to 42% loss of potable water through leaks, wastage and illegal connections (National water security, www.gov.za, 2017).

Generally, with legumes, the level of photosynthetic C accumulation is regulated by N nutrition. The N they contribute becomes available for uptake by the legume, a component crop, microbes or is released during plant growth, at maturity or at decay of legume organs (Genard., *et al.* 2017). Furthermore, the N is incorporated into organic matter, transformed and become available to a succeeding crops [12]. The incorporation of legume residues and the rate of the residue's N transformation into organic matter is crucial especially in rotational systems. In plants, one way to predict N transformation is through the C/N ratio of the above-ground material (Ritchie, 1998). It indicates the extent to which N is released as a plant decomposes (Mulvaney., *et al*. 2017).

With regards to grain legumes such as cowpea, Bambara-groundnut, groundnut, mung-bean and black gram, quality residues include that which have a C/N ratio that is < 24 g g^{-1} (Knops, 1998). A low C/N ratio in plant tissue allows that their mineralization into organic matter by micro-organisms, which typically have a low C/N ratio, happen quite faster [13]. In contrast, the mineralization of plant residues that contain a high C/N ratio can result in immobilisation of N in the microbial biomass [14]. Intriguingly, at times, higher C/N ratio can play a crucial role through protecting soils and maintaining soil water (Luo., *et al*. 2017). Bambara groundnut sampled from farmers' fields in the Mpumalanga Province, South Africa showed shoot C/N ratio that ranged from 10.7 - 26.6 g/g^{-1}, with 21 out of 26 farmers' fields with values ranging from 10.7 - 15.9 g/g^{-1} [9].

Although there are several reports on symbiotic (N) performance of cowpea, Bambara-groundnut, groundnut and mung-bean, studies on their C accumulation, shoot C/N ratio, and shoot-water-use efficiency are few. Therefore, evaluation of these parameters in the test grain legumes widely cultivated in South African smallholder sector is an main objective of the current study.

The interpretation of such data may be further confounded by a carryover of soil nitrate unutilized by the legume crop [15] and possibly a greater degree of immobilization of soil mineral N by the high C:N ratio of the barley stubble with the barley-wheat treatment than with legume residues (e.g. Green and Blackmer, 2009). A quantitative synthesis of intercrop system properties and species trait combinations found that the temporal niche differentiation contributed substantially to high LERs in systems combining C_3 and C_4 species.

Material and Methods

A field experiment was carried out in the 2011/2012 summer cropping season at the Lowveld Research Station, Nelspruit (Bombela), in Mpumalanga province, where five grain legumes planted under field conditions during the planting season 2011 replicated four times as randomized complete block design (RCBD).

Plant sampling and processing

Shoot samples of the five grain legumes were collected from each plot at the flowering stage. The shoots were oven-dried at 70°C for 72h, weighed, and ground to fine powder using Hammer mill, (Wirsam Scientific and Precision Equipment Pty Ltd, Johannesburg, South Africa). The powder (0.85 mm) was used for ^{13}C isotope analysis by mass spectrometry.

Measurement of 13C/12C isotopic ratio

The concentration of C (%C) and $^{13}C/^{12}C$ isotopic ratios of legumes and reference plant species were analyzed using a Carlo Erba NA1500 elemental analyzer (Fisons Instruments SpA, Strada, Rivoltana, Italy) coupled to a Finan MAT252 mass spectrometer (Finnigan, MAT CombH, Bremen, Germany) via a Conflo II open-split device, as described for ^{15}N in introduction section.

Measurement of cellulose and lignin contents

The cellulose and lignin contents were determined by the acid detergent fibre method [16]. The cold water soluble C and N contents of the 'recoverable' residues (leaves + stems + roots) were determined according to Collins., *et al* (2003). A total of 500 mg finely ground residue matter was extracted seven times for 20 minutes with 50 ml deionized water at room temperature (ca. 20°C) on a horizontal shaker. After each extraction the suspension was centrifuged (3000×*g*) for 20 minutes and the clear supernatant was decanted. The five extracts were combined and 545 analysed for total C and N at a TOC/TNb analyser (Dimatoc 100, Dimantec, Germany).

Statistical analysis

All data collected were subjected to a One-way analysis of variance (ANOVA) using Statistica-10.1 (StatSoft Inc., Tulsa, OK, USA). Analysis were done for shoot N concentration, N content, shoot C/N ratio and shoot $\delta^{13}C$, all compared between the test grain legumes. Where mean values showed differences that were significant, the Duncan's Multiple Range Test was used to separate the means at $p \leq 0.05$. Pearson's correlation was carried out to determine the relationship between the variables evaluated.

Results and Discussion on Data Collected and Analyzed

The grain legume species cultivars for discussion were Bambara-groundnut (Brianbeck cv. MB 51), Groundnut (cv. JL 24), Blackgram (Local market seed), Cowpea (cv. Pan 311) and Mungbean (cv. VC 1973A).

Differences in yield determining processes of groundnut (Arachis hypogaea l.) genotype in non varied drought environment [17]

The shoot biomass, Shoot C content, Shoot C concentration, Shoot $\delta^{13}C$ content and Shoot C/N ratio of five legume species grown under field conditions at Nelspruit during the 2011 planting season were reflected as (Figure 1-5) of these study for caparison and illustration. Assessment of N_2 fixation in groundnut (*Arachis hypogaea* L.) and their relative N contribution to a succeeding maize crop in Northern Ghana [18], Theory and empirical evidence have demonstrated that ^{13}C discrimination (Δ) by leaves of C_3 plants may be associated with intrinsic water-use efficiency or productivity. Plants under dry conditions had lower Δ, which theory predicted could be associated with 62% higher water-use efficiency. The same plants showed a 62% higher ratio of CO_2 assimilation rate to leaf diffusive conductance, but a significant ($P < 0.05$) genotype X drought interaction was observed, which was mainly due to one genotype (This research was partially supported by the Bean/Cowpea CRSP, USAID Grant no. DAN-4048-G-55-2065-00. The opinions and recommendations are those of the authors and not necessarily those of USAID).

This study of five grain legume species undertaken at Nelspruit, during the 2011 planting season revealed that groundnut shoot biomass C was (35 $g.plant^{-1}$) (See figure 1), the C content was (15 $g.plant^{-1}$) (See figure 2) and these differences in comparison of figure 1 and 2 exhibit same level of $^{13}C/^{12}C$ isotopic analysis results. Effects of temperature × photoperiod interaction on vegetative and reproductive growth were in one selected groundnut genotypes (JL 24) by growing it in non-controlled-environment growth conditions with two temperature regimes (26/22 and 30/26°C, day/night) under long (12h, long day), and short (9h, short day) photoperiods.

The effect of photoperiod on the total dry-matter production (TDM) was significant with the genotype producing 35% - 15% greater dry matter under LD than SD (Supportive study conducted at Nelspruit 2011, ICRISAT Journal Article No. 2012). Under normal environmental factors the shoot C/N ration of groundnut contribution is higher than the results reflected by these study of five grain legume species, grown under field conditions at Nelspruit, during the planting season of 2011, where C/N ratio of groundnut was (15 $g.g^{-1}$) (See figure 5). Stomatal conductance decreased almost steadily during the stress period indicating that stomatal conductance was more sensitive than the water loss during the initial stressful period. Relationships between transpiration rate and LWP; stomatal conductance and photosynthesis; and stomatal conductance and transpiration rate are described by many Authors. Results suggested that plants adapt to water stress by slowing down tissue dehydration [19].

The implications of future increases in atmospheric CO_2 for the productivity of indeterminate C_3 crops grown in rain-fed subsistence agricultural systems in the semi-arid tropics are stress resistance, therefore JL 24 cultivar in the study at Nelspruit, under field conditions manage to have shoot C content of 45% as best performer legume species planted [20].

Genotype performance of JL 24 in the one environments for which evaluated for the yield and crop growth rates (Carbon), showed that although differences in C existed, differences in the stability of the environment were the dominant attribute of genotypes adapted to the field conditions of Nelspruit region. Data suggested that differences were more attributable to tolerance to temperature and/or humidity than water stress. The grain legume species planted at Nelspruit, under field conditions during the 2011 planting season recorded $\delta^{13}C$ content of JL 24 cultivar being (-25%) as the highest tolerance to stressful conditions. Bambara groundnut (*Vigna subterranea* L. Verdc) (MB 51) is the second most important indigenous food legume in Africa. The aim of this study was to evaluate plant growth, N_2 fixation, N contribution, C accumulation, and plant water relations of Bambara-groundnut grown in One research farm field in Mpumalanga Province of South Africa.

Bambara-groundnut planted at Nelspruit/(Mbombela), under field conditions replicated four times as randomized complete block design (RCBD): The shoot biomass, Carbon content, Shoot carbon concentration, Shoot δ^{13}carbon content and Shoot Carbon/Nitrogen ratio of these study of five legume species grown under field conditions at Nelspruit during the 2011 planting season, represented by figure 1-5 for referral purpose. The cropping system is semi-permanent and the Bambara-groundnut was cultivated as single crop under external field crops with groundnut, blackgram, cowpea and mungbean, Bambara-groundnut (MB 51) was mainly cultivated for the study of nitrogen contribution by five grain legume species at Nelspruit, however the planting density varies between 6 to 29 plants per square meter observation made revealed that more TE (transpiration evaporation) was happening during hot period of the day and stomata conductance was suppressed to enable plant wilting to delay water-use efficiency [21].

Furthermore, Bambara-groundnut (MB 51) has been reported to be a potential crop, antioxidant potential, and a drought resistant crop. Probiotics have been defined as live micro-organisms which when administered in adequate amount confer a health benefit on the host.

Bambara-groundnut (*Vigna subterranea* (L.) Verdc.) MB 51 is an indigenous African legume, where it is the third most important legume in terms of consumption and socio-economic impact in semi-arid Africa behind peanut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*). The crop makes few demands on the soil and is known to be drought tolerant and relatively disease free. It is capable of growing on nutrient poor soils where most crops would not strive. The data revealed marked ($p \leq 0.05$) difference in plant dry matter (DM) (45 g.plant^{-1}) yield, N concentration (44% sample analysis) and N content (20 g.plant^{-1}), $\delta^{13}C$ (-26%), the proportion of shoot C/N ratio (15 g.g^{-1}) and actual the $^{13}C/^{12}C$ was as abovementioned in that one agro-ecology environment and among the replication combination of sample of the research farm surveyed. Plant density correlated positively with %C ($r = 0.44$***), $\delta^{13}C$ (r = -26***), and amount of C content (r = 20*), indicating that the high $\delta^{13}C$ value obtained for Bambara-groundnut in this study and the low C content associated with one research farm were due to low plant density rather than poor C assimilation functioning. The data from this study showed $\delta^{13}C$ value of -26% to which indicates difference in plant water use efficiency on the single field studied.

Furthermore, the positive correlations of $\delta^{13}C$ and N content (r = -26*) and $\delta^{13}C$ and shoot N content (r = 44*) suggest a functional relationship between water use efficiency and N_2 fixation, just as the positively significant correlations between $\delta^{13}C$ and DM yield (r = 45***), C assimilation and DM content (r = 20**), and C content and DM yield (r = 45*), as well as N-fixed and C content (r = 20**) also indicate a functional-relationship between N_2 fixation and photosynthesis. In the same way, the positive correlation between $\delta^{13}C$ and DM biomass (r = 45*), or $\delta^{13}C$ and C content (r = 44*), also implies a functional link between water use efficiency and plant growth. Thus, an increase in water use efficiency in Bambara groundnut, whenever it occurs, seems to functionally enhance plant growth, symbiotic N_2 fixation and photosynthetic activity, just as N_2 fixation in nodules also seems to stimulate leaf photosynthesis (Supportive observation of five grain legume planted, Nelspruit, 2011; Springer Nature Switzerland AG, 2019).

Blackgram the alternative drought tolerant crop planted under field conditions at Nelspruit (Mbombela): The Shoot biomass, Shoot C content, the Shoot C concentration, Shoot $\delta^{13}C$ content and Shoot C/N ratio of this study (Figure 1-5) embraces of five legume species grown under field conditions at Nelspruit during the 2011 planting season. Direct transfer of N from the nodulating black gram to the intercropped maize was 18.6 kg/N/ha^{-1}, respectively. However, the transfer of residual N from this legume to the succeeding maize crop was 10.3 kg/N/ha^{-1}, respectively. Blackgram as a newly introduced crop in South Africa research activities shoot biomass (25 g.plant^{-1}), shoot C content (10 g. plant^{-1}) and shoot C concentration (43%) with δ ^{13}C content (-28%) and C/N ration (20 g.g^{-1}) reflected the figures representation during the legume species planted at Nelspruit under field conditions.

Correlation between water-use efficiency and carbon isotope discrimination in pan 311 cowpea genotype and single environmental eco-system: The research result of figure 1-5 are the main focus to the Shoot biomass, Shoot C content, the Shoot C content concentration, Shoot $\delta^{13}C$ content and the C/N ration of five legume species grown under field conditions during the planting season. Cowpea (*Vigna unguiculata*) is an important grain-producing legume that can forego nitrogen fertilization by establishing an efficient symbiosis with nitrogen-fixing bacteria (F.D. Dakora). Several studies that have examined the diversity of the nitrogen-fixing Leguminosae-associated nodulating bacteria have used cowpea [*Vigna unguiculata* (L.) Walp] as the trap plant species. Cowpea is an important agronomic plant; it is also considered promiscuous, capable of establishing symbiotic relationships with a variety of nodulating bacteria at various degrees of efficiency (This article in CS, Vol. 32 No. 1, p. 7-12, Received: Mar 8, 1991, Published (August 2003).

WUE = total biomass production/water use) has been limited by the lack of suitable cultivar screening criteria. Theory has predicted an association between WUE and Δ (leaf discrimination against ^{13}C) that could be used in indirect selection for WUE in C_3 plants. Earlier studies showed genotypic and drought-induced variation in Δ in cowpea (*Vigna unguiculata* (L.) Walp.) and expected associations with leaf gas exchange for drought-induced effects but not for genotypic effects. Pan 311 Carbon isotope composition may be useful for selection in plant breeding. Theory predicts that ^{13}C discrimination (Δ) by leaves can be associated with the ratio of photosynthesis (as indicated by CO_2 assimilation rate) to leaf conductance (g) to diffusion of water vapor or CO_2.

Mungbean (vc 1973A) as green-gram planted for nitrogen contribution with five grain legume at Nelspruit: Shoot biomass, Shoot C content, the Shoot C concentration, Shoot $\delta^{13}C$ and the C/N ration that are recorded in figure 1-5 of five legume species grown under field conditions at Nelspruit during the 2011 planting season represent the discussion of this Chapter. The mung bean is a fast-growing, warm-season legume. It reaches maturity very quickly under tropical and subtropical conditions where optimal temperatures are about 28 - 30°C and always above 15°C. It can be sown during summer and autumn. It does not require large amounts of water (600 - 1000 mm rainfall/year) and is tolerant of drought. It is sensitive to waterlogging. High moisture at maturity tends to spoil the seeds that may sprout before being harvested.

The mung-bean grows on a wide range of soils but prefers well-drained loams or sandy loams, with a pH ranging from 5 to 8 scale. It is somewhat tolerant to saline soils [22]. The mung-bean can be used as a cover crop before or after cereal crops. It makes good green manure. The mung-bean is a N-fixing legume that can provide large amounts of biomass (7.16 t biomass/ha^{-1}) and N to the soil (ranging from 30 to 251 kg/ha^{-1}) [23-25]. The purpose of this study was to determine C-induced N_2-related changes in shoot growth and physiological attributes of (VC 1973A) mung-bean variety.

Reduction in transpiration rate, and stomatal conductance and increase in substomatal CO_2 level, indicated that C reduced net photosynthesis by reducing CO_2 fixation by VC 1973A, albeit these changes were less pronounced in VC 1973A. Positive correlations of shoot biomass (10 g.plant^{-1}) (See figure 1) and $\delta^{13}C$ (-29%) (See figure 4) with C content (5 g.plant^{-1}) (See figure 2) and shoot C content (41%) of shoot dry weight, in VC 1973A suggested that mung-bean sensitivity to C is due to perturbed C and N assimilation (2019, Springer Nature Switzerland AG).

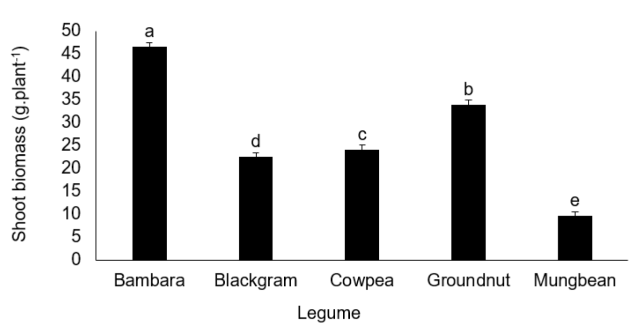

Figure 1: Shoot biomass of five legume species grown under field conditions at Nelspruit during the 2011 planting season.

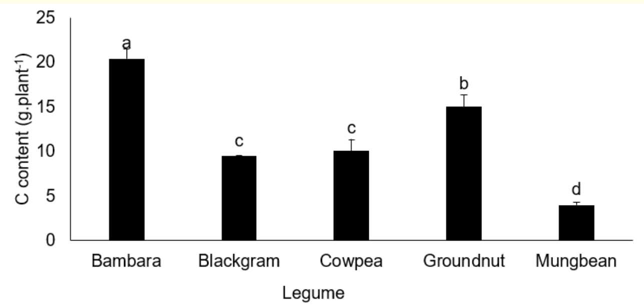

Figure 2: Shoot C content of five legume species grown under field conditions at Nelspruit during the 2011 planting season.

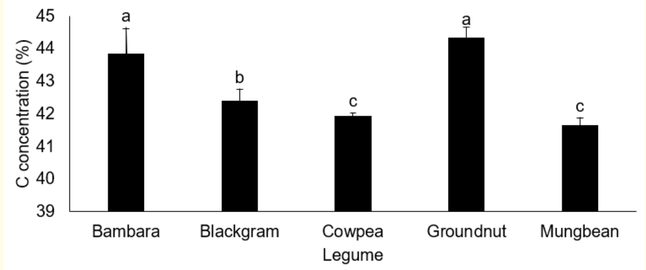

Figure 3: Shoot C content of five legume species grown under field conditions at Nelspruit during the 2011 planting season.

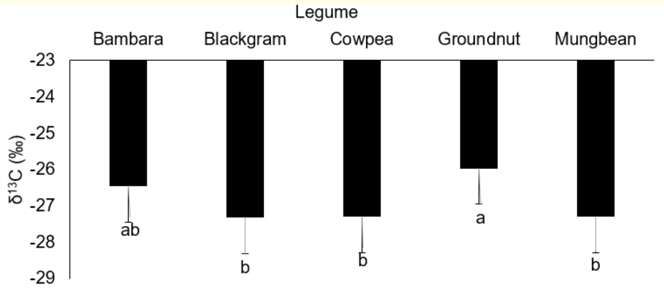

Figure 4: Shoot $\delta^{13}C$ content of five legume species grown under field conditions at Nelspruit during the 2011 planting season.

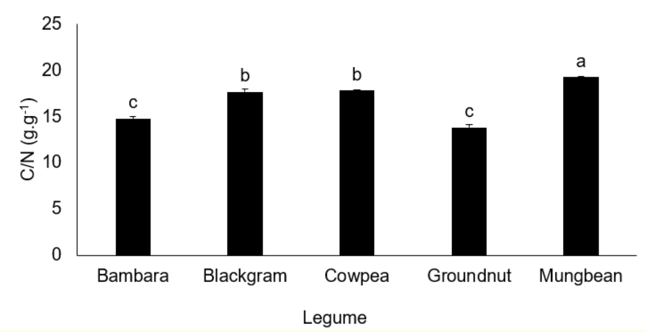

Figure 4: Shoot C/N of five legume species grown under field conditions at Nelspruit during the 2011 planting season.

Conclusion

During the decomposition process the growth of subsequent crops will compete with the microbial biomass for nitrogen and other nutrients (Kuzyakova., *et al.* 2013), leading to a differentiation in the decomposition process compared to a non-cropped soil [26]. Plants also affect the residue turnover by excreting easily available organic C and N compounds which can interact directly with the microbial biomass and affect its size, activity, turnover rate or physiological status [4]. However, following this concept, N will be set free from the microbial biomass as microbial residues, the relation between the parameters is difficult to prove because data concerning the residue quality are only available for the 're-coverable' residues and not for the rhizo-deposits [27-36].

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