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Research article

Nitrogen fertilization increases the growth and nutritional quality of the forage legume, *Calobota sericea* – A preliminary investigation

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A B S T R A C T

Calobota sericea is being evaluated as a forage for drought stressed areas. The nutritional quality of *C. sericea* from native populations are poor, and this is believed to be due to poor soil fertility. Therefore, a greenhouse trial was established to determine the impact of N-fertilization level (0, 25, 50, 75 and 100 kg/ha) on the growth and nutritional quality of *C. sericea* plants. Three-month-old plants were harvested and the root and shoot length, as well as branching intensity on each plant determined. Thereafter, the shoots were separated into leaves and stems and all plant parts were oven dried for dry mass determination. After weighing, the leaves and stems for each plant were combined and the dried shoots used for nutrient determination. Results indicated that increased N application levels is positively correlated with improved *C. sericea* growth. Similarly, mineral nutrient uptake increased significantly under all the N-fertilization treatments and crude protein content increased from 9.6% to 18.6%. Plant growth was only statistically significantly ($p < 0.05$) improved when N was applied at rates of 50 kg/ha and more, but crude protein content increased from the lowest N application rates (25 kg/ha). The improved growth and nutrient uptake could primarily be explained by improved resource allocation under N-fertilization. Therefore, appropriately fertilized *C. sericea* can result in improved forage production and improved quality forages and when N is applied at high enough rates.

1. Introduction

Calobota sericea (Thunb.) Boatwr. and B-E van Wyk is a deciduous, indigenous, perennial legume species native to the winter rainfall regions of South Africa [1]. The plant has been identified as a potential cut-and-carry forage resource for water-limited agro-ecological areas due to its current contribution to extensive livestock production systems within the rangelands of the

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Mediterranean-like region of South Africa [2,3]. To date, studies into its distribution [1], climatic and edaphic niche [3], drought tolerance [4], germination requirements [5–7] and nutritional quality [8,9] has been done to characterise the species for its agronomic and forage potential. From these, it has been proposed that this species could potentially be developed further for use as a supplementary feed during the dry season within water-limited Mediterranean type agro-ecological areas.

Recently, it was shown that when *C. sericea* forages are harvested at different phenological stages, crude protein from forages collected from native stands ranges between 5.9% in dry plant litter collected in the dry summer season to 10.3% when harvested at the flower bud stage in spring [9]. It was therefore suggested that collection from native stands for supplementary fodder should be done at either non- or early reproductive stages where the highest crude protein and energy content was found, and digestibility was also sufficient for proper utilization by ruminants [9]. However, at 10%, the level of crude protein is only sufficiently high to meet the minimum requirements (7–8%) of ruminants, but for highly productive livestock herds, a crude protein content of approximately 13–14% is required [10]. Thus, forages collected from native stands will not be able to sustain highly productive livestock herds and further intervention is needed if the forage species is to be used as supplementary feed for highly productive livestock herds.

Although legumes can acquire N from symbiotic N_2-fixation, due to the distribution of *C. sericea* being primarily in areas that are prone to droughts [1,11], the establishment of infection and subsequent symbiotic N_2-fixation could be severely compromised or delayed due to the sensitivity of this relationship to drought stress [12–15]. This, in turn, could explain the relatively low N and crude protein content found in the plant materials collected from native *C. sericea* populations [8,9]. Under these conditions and other limiting environments [15–20], the application of N-fertilizer could result in improved crop productivity, even if this is at the expense of biological nitrogen fixation [15,21–24].

Nitrogen fertilization is known to improve plant growth and biomass production [25–27], however, it has also been shown to result in the reduction of certain mineral nutrients, while certain other mineral nutrients are increased in the biomass produced [27,28]. The inverse relationship between plant growth and mineral concentration, termed the dilution effect, occurs when dry-weight accumulation increases at a faster rate than mineral-nutrient accumulation [29]. Conversely, the synergism effect will result when increased mineral-nutrient accumulation increases at a faster rate than dry-weight accumulation [29]. Riedel [28] showed that both dilution and synergism under N-fertilization can occur at the same time. For example, in maize plants fertilized with N, Ca and Mn concentrations increased, while P and K concentrations were significantly lower compared to plants that did not receive N-fertilizers [28]. Furthermore, N-application has also been shown to increase crude protein content of forages [30], and therefore has a potential to improve livestock production in areas where natural N sources are limiting. This study therefore assessed whether biomass production, mineral nutrient and crude protein content could be improved by nitrogen (N) fertilization in planted *C. sericea* pastures.

This study therefore aimed to determine the impacts of N-fertilization on the growth, resource allocation, nutrient uptake and crude protein content of *C. sericea* forages. It was hypothesized that (1) increased N-fertilization will improve biomass production as well as the mineral nutrient content of in *C. sericea* forages, and (2) that the crude protein content will increase to levels of approximately 13–14% which are sufficient to sustain highly productive livestock herds.

2. Materials and methods

A complete randomized block trail was conducted to determine the impact of N-fertilization in the growth, nutrient uptake, and nutritional quality of *C. sericea* plants. Nitrogen was applied to 10 m^2 plots as a single application of 0, 25, 50, 75 and 100 kg/Ha N as a LAN fertilizer. These levels did not correspond to any known N-requirements, but it was done so that baseline data could be collected for more detailed further studies based on N-fertilizer applications for *C. sericea*. Prior to fertilization, the plots were cleaned/weeded manually, and soil cores collected to a depth of 15 cm for soil characteristics determination (Table S1), after which the LAN was applied and rotavated to a depth of 10 cm. The plots watered to 75 mm (measured using a portable rainfall meter) and were left unplanted for two months. After two months, soils were collected (to a depth of 15 cm) from the 10 m^2 nursery plots and added to 10 cm wide and 15 cm deep pots, creating six replicates per N-treatment. Within each pot, two seedlings were established resulting in a total of 12 plants per treatment. The pots were arranged in a complete randomized block design in the tunnels at the Agricultural Research Councils Roodeplaat experimental farm (longitude of 25°34′11.27″S, and latitude of 28°22′05.36″E). The pots were irrigated ones a week to pot capacity (water draining from the pot) and the *C. sericea* plants were allowed to grow for three months before the materials were harvested and measurements taken. During this time, no additional N-fertilization was done. At harvesting, the plants were carefully removed from the pots and the soil was washed from the roots, taking care not to break the roots. Thereafter, the plants were separated into roots and shoots. Measurements taken included shoot branching intensity, root and shoot length, as well as roots, leaves, and stems mass (fresh mass) on each plant. Thereafter the plant material were oven dried at 65 °C until a constant mass was achieved and the dry mass taken for each plant part. With this information, the root:shoot length and dry mass ratios were calculated. After drying and weighing, the stems and leaves for each plant were grouped again and milled to pass through a 2 mm mesh sieve. The dry, milled samples were thereafter send for mineral nutrient (N, Ca, Mg, P, K, Na, Mn, Fe, Zn and Cu) analyses.

A sample of the dry, milled plant material were ignited at 950 °C in oxygen from where total N concentration in the plant samples were analyzed using a dry oxidation method using a Flash 2000 CHNS-O Analyzer (Thero Scientific, United States) which was calibrated against a known standard (Phenylalanine) containing 8.48% N. For the other elements, a different sample of the dry, milled *C. sericea* plant materials were digested in HNO_3 and $HClO_4$ in a heating block and the digestate was brought to volume (100 ml) using dH_2O. Thereafter the digested solution was used for the determination of Ca, Mg, P, K, Na, Mn, Fe, Zn and Cu using an ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer - Agilent 725 (700 Series), Agilent Technologies, USA). All analyses were done using analytical grade chemicals and reagents. Deionized water (dH_2O) was used for reagent, standard and sample preparation, and dilution. Calibration curves were carefully developed through dilution of 1000 mg/l single element standards for the

Table 1
Pearsons correlation coefficients between the different plant growth, nutritional quality parameters and N-fertilizer application level in *C. sericea* forages.

	SL	RL	BI	LM	SM	SHM	RM	R : SM	R : SL	N	K	Ca	Mg	P	Na	Fe	Mn	Zn	Cu	CP
N F	0.73**	0.50**	0.68**	0.59**	0.63**	0.67**	0.61**	0.35**	−0.36**	0.93**	0.84**	0.70**	0.77**	0.02^{ns}	0.86**	0.57**	0.38^{ns}	−0.08^{ns}	0.81**	0.93**
SL		0.6	0.47**	0.36**	0.51**	0.49**	0.58**	0.27*	−0.52**	0.68**	0.62**	0.65**	0.46*	−0.11^{ns}	0.59**	0.48*	0.41^{ns}	−0.04^{ns}	0.50*	0.68**
RL			0.47**	0.42**	0.45**	0.48**	0.51**	0.21^{ns}	0.35**	0.44*	0.43*	0.32*	0.19^{ns}	−0.22^{ns}	0.35*	−0.02^{ns}	0.10^{ns}	−0.18^{ns}	0.38*	0.44*
BI				0.57**	0.61**	0.65**	0.63**	0.41**	−0.09^{ns}	0.51*	0.52*	0.15^{ns}	0.45*	−0.02^{ns}	0.49*	0.35^{ns}	0.30^{ns}	0.06^{ns}	0.55*	0.51*
LM					0.67**	0.86**	0.46**	0.11^{ns}	0.01^{ns}	0.48*	0.62**	0.18^{ns}	0.22^{ns}	0.08^{ns}	0.42^{ns}	0.13^{ns}	−0.01^{ns}	−0.32^{ns}	0.62**	0.48*
SM						0.96**	0.55**	0.08^{ns}	−0.13^{ns}	0.48*	0.51*	0.14^{ns}	0.40^{ns}	−0.03^{ns}	0.48*	0.20^{ns}	0.52*	0.18^{ns}	0.56*	0.48*
SHM							0.56**	0.10^{ns}	−0.09^{ns}	0.53*	0.60**	0.17^{ns}	0.38^{ns}	0.01^{ns}	0.51*	0.19^{ns}	0.38^{ns}	0.01^{ns}	0.64**	0.53*
RM								0.64**	−0.13^{ns}	0.55*	0.58**	0.57**	0.35*	0.14^{ns}	0.45*	0.17^{ns}	0.38*	−0.15^{ns}	0.52*	0.55*
R:S M									−0.08^{ns}	0.29^{ns}	0.39	0.56*	0.19^{ns}	0.28^{ns}	0.24^{ns}	0.12^{ns}	0.24^{ns}	−0.14^{ns}	0.05^{ns}	0.29^{ns}
R:S L										−0.35^{ns}	−0.28^{ns}	−0.40^{ns}	−0.41^{ns}	−0.05^{ns}	−0.36^{ns}	−0.58**	−0.35^{ns}	−0.19^{ns}	−0.22^{ns}	−0.35^{ns}
N											0.76**	0.74**	0.84**	−0.09^{ns}	0.93**	0.66**	0.44^{ns}	−0.15^{ns}	0.79**	1.00**
K												0.57**	0.65**	0.23^{ns}	0.80**	0.44^{ns}	0.34^{ns}	−0.18^{ns}	0.70**	0.76**
Ca													0.53*	−0.08^{ns}	0.61**	0.44^{ns}	0.30^{ns}	−0.20^{ns}	0.52*	0.74**
Mg														0.02^{ns}	0.91**	0.62**	0.44^{ns}	0.09^{ns}	0.71**	0.84**
P															−0.07^{ns}	−0.03^{ns}	0.04^{ns}	−0.19^{ns}	0.09^{ns}	−0.09^{ns}
Na																0.64**	0.50*	−0.04^{ns}	0.79**	0.93**
Fe																	0.38^{ns}	−0.03^{ns}	0.43^{ns}	0.66**
Mn																		0.13^{ns}	0.39^{ns}	0.44^{ns}
Zn																			−0.11^{ns}	−0.15^{ns}
Cu																				0.79**

N-F = N-fertilizer, SL = Shoot length, RL = Root length, BI = Branching intensity, LM = Leaf mass, SM = Stem mass, SHM = Shoot mass, RM = Root Mass, R:S M = Root:Shoot mass ratio, R:S L = Root: Shoot length ratio, N = Nitrogen, K = Potassium, Ca = Calcium, Mg = Magnesium, P = Phosphorus, Na = Sodium, Fe = Iron, Mn = Manganese, Zn = Zinc, Cu = Copper, CP = Crude protein, ns = not significant ($p \geq 0.05$), ** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level.

elements of interest (Fluka TraceCERT® or Merck CretiPUR®). The machine was regularly re-calibrated using these certified standards for each element and washed with dH_2O to avoid any analyte being deposited in the instrument. Additionally, with each batch of samples digested, reagent blanks were prepared which was subtracted from the concentrations obtained in the samples.

2.1. Statistical analyses

SPSS v. 22 (IBM SPSS Inc., Chicago, IL) was used to test the data for normality using a Shapiro-Willks test. Thereafter, a one-way ANOVA was performed on all variables to determine whether significant differences ($p \leq 0.05$) were found between the different nitrogen treatments. Where significant differences were observed, an LSD post hoc test was performed to separate the means.

3. Results

3.1. Plant growth and resource allocation

N-fertilization generally improved plant growth and the uptake of certain mineral nutrients (K, Ca, Mg, Na, Fe, Cu, and N). This was seen in the significant correlations between N-fertilizer application levels and several of the growth parameters evaluated and mineral nutrient contents in *C. sericea* shoots (Table 1). A N-fertilizer application level of at least 50 kg/ha is needed to significantly increase shoot length, and 75 kg/ha for increased root length, better branch formation, as well as increased root, stem, leaf and total shoot mass in *C. sericea* plants (Fig. 1). Only at 75 kg/ha N were resource allocation between roots and shoots (in terms of mass) significantly increased, while root to shoot mass ratio was generally reduced by N-fertilization. This suggest that root mass increased more than shoot mass under N-fertilization but shoot and root length increased proportionally under N-fertilization (Fig. 1).

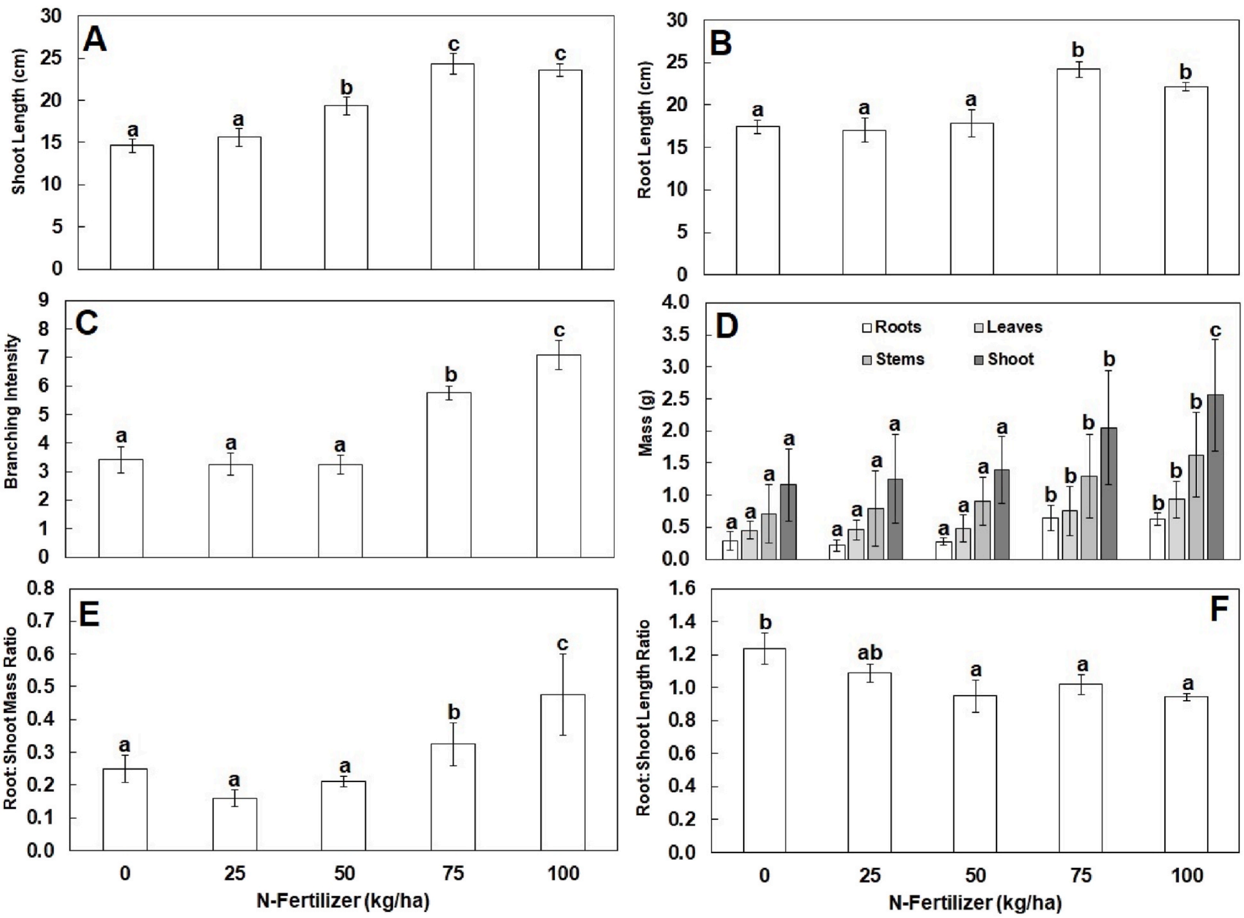

Fig. 1. Shoot length (A), root length (B), branching intensity (C), roots, leaves, stems and total shoot mass (D), root:shoot mass ratio (E) and root: shoot length ratio (F) in *C. sericea* forages at different N-fertilization levels. Statistically significant differences ($p \leq 0.05$) within each variable between the different N-application levels are indicated by different lower case letters.

3.2. Nutritional quality

In terms of mineral nutrient acquisition, although N-fertilization resulted in improved N, Ca, Mg, K, Na, Fe, and Cu concentrations in the *C. sericea* shoots, no significant relationship, were found between N-fertilizer application levels and P, Mn, and Zn concentrations in the shoots of the plants (Tables 1 and 2). Results from the current study showed that an N-fertilizer application level of 25 kg/ha resulted in increased uptake of N, Mg, Na, Fe and Cu concentration, while the uptake of K and Ca only significantly increased with a 50 kg/ha N application (Table 2). The increased N uptake at even the lowest application levels of N-fertilizer in this study also resulted in higher crude protein content, increasing from 9.56% under un-fertilized conditions to 13.51% at an application rate of 25 kg/ha N (Table 2).

4. Discussion

Results from the current study indicates that increased N-fertilization results in improved growth and nutritional quality of *C. sericea* forages. The findings indicate that when planted as pastures and with minimal N-application levels, farmers can improve the quality of *C. sericea* fodders to levels greater than the 13–14% required by highly productive livestock herds [31]. This, in turn, means that farmers are able to plant *C. sericea* as a cut and carry crop under limited N-fertilizer application levels and the fodders created from these pastures can be used as supplementary feed to the basal rangeland diets to maintain highly productive livestock herds in the dry season. This result is important for the initial use of *C. sericea* as a forage because even though the species is a legume, and legumes generally acquire N from symbiotic interactions with N_2-fixing rhizobia bacteria, no information about *C. sericea* N_2-fixation abilities is currently available, apart from the knowledge that the species is nodulated by the α-proteobacteria of the *Bradyrhizobium* and *Mesorhizobium* [32]. Prior work on the nutritional quality of *C. sericea* indicated that N concentrations in the shoots of the plants from native populations are generally low, ranging between 9.6 g/kg and 16.5 g/kg depending on the season and/or phenological stage that the plant material is harvested [8,9]. This suggests that the N_2-fixation abilities of *C. sericea* may be compromised and may not be as efficient within its native ranges. It is well known that in drought prone environments, like the native distribution ranges of *C. sericea*, N_2-fixation can be severely inhibited and/or limited which, in turn, leads to reduced N uptake and consequently reduced plant production and nutritional quality [33,34]. Our results however concurs with several other studies indicating that the addition of N-fertilizers will improve biomass yield and the nutritional quality of legume crops [15,21–24,35,36].

The increased growth and uptake of certain mineral nutrients (K, Ca, Mg, Na, Fe, Cu, and N) in this study could be attributed to improved resource allocation under N-fertilization [26,37–39]. In this study, N-fertilization was found to be strongly positively correlated with root:shoot mass ratio suggesting that N-fertilization increased root mass proportionally more than shoot mass. Furthermore, root:shoot length ratio was strongly negatively correlated with N-fertilization, specifically decreasing from the 0 N treatment to all of the N-fertilizer application levels. This indicates that under N-fertilization, root and shoot length increased proportionally. From these results it was clear that N-fertilization allowed for improved root growth (mass and length) which, in turn could explain the increased shoot growth. Improved root growth generally means that plants are able to access water and nutrients better, which, in turn, increases shoot growth and branching intensity [26,39]. Root length and density are known to influence the acquisition of mineral elements as these increases the volume of soil explored by the root system and the surface area for uptake of mineral nutrients [38,39]. Furthermore, increased root density in the topsoil layers generally improve the uptake of mineral nutrients while increased root length has been shown to be especially important for the acquisition of water and N from subsoil layers [37–39]. In this study, the increased root length found under N-fertilization has been proposed as an important trait which could improve the plants survival during the dry season when water and N in the surface soils has generally been depleted or become unavailable, and N and water harvesting needs to occur from deeper soil depths.

The increase in shoot yield resulting from the application of fertilizers, however, can often be accompanied by decreases in mineral nutrient concentrations in plant shoots [28]. This inverse relationship between plant growth and mineral concentrations is known as a dilution effect [29] which occurs when the dry-weight accumulation increases at a faster rate than mineral-nutrient accumulation. Conversely, higher mineral nutrient content in plant shoots will result when mineral nutrient accumulation increases at a faster rate

Table 2
Mineral nutrient and crude protein content in *C. sericea* forages under different N-fertilizer application levels. Statistically significant differences ($p \leq 0.05$) within each variable between the different N-application levels are indicated by different lower case letters.

N (kg/ha)	K (g/kg)	Ca (g/kg)	Mg (g/kg)	P (g/kg)	Na (g/kg)	Fe (g/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	N (g/kg)	CP (%)
0	11.73 ± 0.08^{a}	13.19 ± 0.13^{a}	3.69 ± 0.09^{a}	2.12 ± 0.02^{a}	2.14 ± 0.04^{a}	0.42 ± 0.01^{a}	41.63 ± 1.63^{a}	44.85 ± 0.95^{a}	9.26 ± 0.26^{a}	15.30 ± 0.12^{a}	9.56 ± 0.08^{a}
25	11.86 ± 0.25^{a}	13.66 ± 0.32^{a}	4.25 ± 0.01^{b}	2.10 ± 0.06^{a}	2.61 ± 0.12^{b}	0.49 ± 0.03^{ab}	44.56 ± 3.05^{a}	44.56 ± 1.38^{a}	11.83 ± 0.69^{b}	21.61 ± 0.19^{b}	13.51 ± 0.13^{b}
50	12.65 ± 0.29^{b}	14.96 ± 0.29^{b}	4.40 ± 0.08^{bc}	2.06 ± 0.06^{a}	3.03 ± 0.05^{c}	0.54 ± 0.04^{b}	46.26 ± 0.82^{a}	43.94 ± 1.62^{a}	11.19 ± 0.23^{b}	27.65 ± 0.58^{c}	17.28 ± 0.36^{c}
75	12.58 ± 0.14^{b}	15.05 ± 0.14^{b}	4.36 ± 0.07^{bc}	2.00 ± 0.11^{a}	3.02 ± 0.05^{c}	0.52 ± 0.02^{b}	46.16 ± 2.41^{a}	43.38 ± 1.61^{a}	12.52 ± 0.41^{c}	28.82 ± 0.25^{d}	18.01 ± 0.16^{d}
100	13.41 ± 0.09^{c}	14.98 ± 0.09^{b}	4.53 ± 0.14^{c}	2.18 ± 0.03^{a}	3.13 ± 0.10^{c}	0.53 ± 0.01^{b}	46.19 ± 1.58^{a}	44.87 ± 1.63^{a}	13.32 ± 0.23^{c}	29.68 ± 0.11^{d}	18.55 ± 0.07^{d}

than dry matter accumulation. This relationship has been defined as a synergism effect [29]. Although N-fertilization in this study resulted in increased N, Ca, Mg, K, Na, Fe, and Cu concentrations in the *C. sericea* shoots (Table 2), no significant relationship, were found between N-fertilizer application levels and P, Mn and Zn concentrations in the shoots of the plants (Table 2). In this study, higher concentrations of certain mineral nutrients in the shoots of *C. sericea* were significantly correlated with root length and root mass (Table 1) indicating that vigorous roots enable plants to take up higher amounts of mineral nutrients from the soil and supply higher concentrations of these to the shoots, indicating a synergistic response to N-fertilization [29]. This was evident in the significant positive correlations observed in shoot length and these mineral nutrient concentrations (Table 1). Conversely, although P, Mn and Zn are essential mineral nutrients to plant growth and development[40,41], increased N-fertilization and subsequent increased shoot growth did not result in increases in their concentrations in the shoots of *C. sericea* plants, indicating a dilution effect for these mineral nutrients [29]. This was substantiated by the negative correlations between shoot length and P and Zn concentrations and no relationship between shoot length and Mn concentrations (Table 1). Coblentz et al. [42] and Riedell [28] observed similar results where certain mineral nutrient concentrations in grass forages and maize were not impacted by N-fertilization or, in some instances decreased with increasing N application levels, describing this response as a dilution effect related to increased DM production. Similar to our results, Riedell [28] showed that both dilution and synergism can occur for certain mineral nutrients, where the concentrations of certain mineral nutrients are significantly increased, while others are decreased or remain the same with increased shoot growth under N-fertilization. The results obtained for P concentrations in *C. sericea* shoots however, is a cause for concern. In their native ranges, *C. sericea* plants grow under very low soil P levels, and thus, when harvesting these plants/forages from native populations, they are almost always deficient in P [6,8,9,31]. Plants that grow within these rangelands are well adapted to the low soil P levels and often still thrive under these marginal conditions [31], but the dilution effect observed from the current study, coupled with the already low P-concentrations in the rangeland soils could result in P-deficiencies in livestock if additional P-fertilization is not done.

5. Conclusion

In this study we examined the impact of N-fertilization on the growth, resource allocation and nutritional quality of *C. sericea* forages. Specifically, we asked whether N-fertilization will improve crude protein content to levels that are sufficient to maintain highly productive livestock herds. Our results show that N-fertilization does increase plant growth and the nutritional quality of *C. sericea* forages and the even at the lowest level of N applied to the plants, crude protein content could be increased to levels sufficient to maintain highly productive livestock herds. However, even though N-fertilization can significantly improve the growth and nutritional quality of *C. sericea* fodders, N-fertilizers are often expensive and therefore, not always a viable option for resource poor farmers. Thus, further research into the identification of improved rhizobium strains that is suitable for use under adverse bioclimatic and edaphic conditions is needed. The application of N-fertilizers can however be a good starting point for emerging farmers to already start using this fodder resource in areas where existing commercially available forage species are poorly adapted, and where native stands cannot provide enough material to maintain productive livestock herds.

Author contribution statement

F. Müller, I. Samuels, C. Cupido and L. Cyster - Conceived and designed the experiments; Contributed reagents and materials; Analyzed and interpreted data and Wrote the paper.

E. Britz, N. Ngcobo, L. Masemola, F. Manganyi - Performed the experiments and Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e13535.

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