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Effects of Pruning Frequency on Biomass Productivity, Non-structural Carbohydrates and Nitrogen Fixation Rates of *Sesbania Sesban*

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Abstract: Tree pruning is a management tool in agroforestry systems for reducing shade, enhancing nutrient cycling or providing fodder. However, little information is available on the effect of pruning management on plant growth, non-structural carbohydrate (NSC) levels in the roots and N_2 fixation of *Sesbania sesban*. A glasshouse experiment was conducted to assess the effect of pruning frequency on biomass production, NSC levels and N_2 fixation of *Sesbania sesban.* Treatments included pruning at (i) 3 months only, (ii) 3 and 6 months, and (iii) 3, 6 and 9 months, with each pruning removing shoot biomass above 50% of the initial height. Increased pruning frequency decreased above- and belowground dry matter (DM), and root NSC levels. The decrease in aboveground DM correlated with reduced levels of starch, sugar and total non-structural carbohydrates. Pruning more frequently significantly decreased nodulation, the percentage N derived from the atmosphere and N_2 fixed. It could be concluded that more frequent prunings decreases biomass and NSC levels which could reduce the regeneration capacity of trees since they rely on NSCs to regrow. The suppressive effects of increased pruning frequency on N_2 fixation could decrease the desirable benefits of legume trees on soil N fertility improvement.

Keywords: Biomass; Pruning frequency; *Sesbania sesban*; [15]N Natural abundance; N_2 fixation; Ndfa

1. Introduction

Sesbania sesban is an important multipurpose woody legume capable of providing fuelwood, fodder and/or nutrient-rich biomass for nutrient cycling. It has been widely used in agroforestry in Eastern and Southern African regions for replenishing soil fertility [1–2]. It has a deep root system which allows it to capture and recycle nutrients efficiently. Rhizobia that nodulate on the roots of *Sesbania* spp. are fast-growing strains and are found in a wide range of African soils [3]. *S. sesban* trees, through their symbiotic association with soil rhizobia, have been shown to contribute 84 kg N ha^{-1} in Zimbabwe [1] and between 280 – 360 kg of N ha^{-1} in Kenya [2].

In many agroforestry systems, shoots of *S. sesban* are often frequently pruned during the growing season for several production goals such as harvesting N-rich fodder for livestock [4] or providing residues to be applied as soil amendments [5-6]. Following pruning, energy requirements for regrowth of the new shoot is obtained from C assimilation in the residual photosynthetic tissue and mobilization of non-structural carbohydrates (NSC) stored in branches, stems and roots [7–8]. The pool of carbohydrate reserves is replenished once sufficient leaf area is re-established [7,9]. This means that the amount of carbohydrate reserves stored in the plant tissues and their subsequent translocation largely determine the success of new leaf and shoot regrowth [9–10].

Since tree regrowth largely depends on the reserve carbohydrates [10], pruning at short time intervals (more frequently) may result in depletion of these energy resources [8], reduced the regenerative capacity or even death of trees [11]. A study by Latt et al. (2000) reported differences in post-cutting biomass production due to various cutting frequencies in *Leucaena leucocephala* and *Gliricidia sepium* that were linearly correlated with carbohydrate reserves. If shoot regrowth and carbohydrate levels of *S. sesban* plants are affected by pruning more frequently, this will not only have implications on future biomass production but also soil N fertility contributions in agricultural landscapes.

S. sesban can derive between 5 and 90% of its N requirements from atmospheric N_2 [1–2]. Because leaves are the main source of carbon assimilates required for nodule functioning, tree pruning may negatively affect photosynthate supply to nodules [13]. Earlier studies show that, after shoots are removed, nodule biomass and nodule functioning are significantly reduced legume species [13– 15], thus leading to suppression of symbiotic N_2 fixation. Because growth and N_2-fixing capacity of woody legumes may vary depending on the species, they are most likely to react differently to frequent pruning. Therefore, the objectives of this study were to (i) evaluate the effects of pruning frequency on biomass productivity and non-structural carbohydrate reserves, (ii) assess if there is a relationship between biomass productivity and nonstructural carbohydrate root reserves, and (iii) assess the effects of pruning frequency on nodulation and N_2 fixation of *S. sesban*.

2. Materials and method

2.1. Experimental site and plant culture

The experiment was carried out under controlled greenhouse conditions at the University of KwaZulu-Natal, South Africa. Seeds of *S. sesban* (var. *nubica*) and *Senna siamea* used in this study were obtained from natural populations near Empangeni (28°39'S, 31°57'E), KwaZulu-Natal Province, South Africa. The seeds of *S. sesban* and *S. siamea* were surface scarified by immersing in boiled water for 10 – 15 minutes and cooled with five rinses of cool tap water. The seeds were planted into seedling trays containing a mixture of 'Umgeni River' sand and seedling growth mix (Farmyard Organics). The sand was purchased from commercial suppliers in Pietermaritzburg. The seedling trays were placed in a glasshouse (temperature 21 – 32 °C and relative humidity of 60 – 90%) and watered with fresh tap water once or twice a week, depending on the water requirements.

At 60 days after planting, healthy seedlings of both woody legumes were transplanted into 5 L free draining pots containing a 7:1 kg mixture of local unsterilized Umgeni River sand and sterile vermiculite. One seedling was transplanted per pot. During the course of the experiment, plants were fed with 1 L of modified Hoagland N-free nutrient solution once a week, and in some cases, particularly on hot days, 1 L of water was used to supplement irrigation. Whenever aphids or red spidermites were observed on plants, a solution of Aphicide Plus (Chloro-nicotinyl) and Red Spidermicide (Tetradifon) were sprayed on plants at a rate of 62.5 mL per pot.

2.2. Nodulation

To ensure the presence of compatible rhizobia essential for symbiotic N_2 fixation, rhizosphere soils were also collected from *S. sesban* populations during seed collection. The soils were collected using shovels to prepare soil inoculum. About 700 – 800 g of soil from rhizospheres of *S. sesban* was collected in the top 0.5 – 20 cm of the soil profile and stored in a freezer (10 °C) prior to preparation of soil inoculum. The inoculum was prepared by adding 1200 mL of sterile distilled water to 350 g of soil in a 2000 mL container. The contents were stirred for 20 to 30 minutes and left to settle before applying the soil suspension to seedlings. To guarantee effective nodulation, the seedlings were inoculated immediately after transplanting and 14 days after transplanting at a rate of 15 mL per pot per event.

2.3. Pruning treatments

At approximately 100 days after transplanting when mean plant height (measured from root collar to the terminal bud) reached 91.4 cm and the mean number of leaves per plants was 87, the pruning treatments were applied to the pots. The treatments included three cutting frequencies, i.e. removal of shoot biomass at 50% height at (i) 3 months only, (ii) 3 and 6 months, and (iii) 3, 6 and 9 months, subsequently referred to as PF1, PF2 and PF3, respectively. The experiment was laid out in a complete randomized design (CRD) with four replications.

2.4. Plant sampling and processing

At 4 weeks after the final pruning, plants were destructively harvested by decapitating at 2 cm above the soil line, and the aboveground biomass was partitioned into leaves, twigs, branches and main stem. The roots were carefully recovered from pots, washed free of soil over a sieve and root length was measured using a ruler. For determination of non-structural carbohydrates, a 5 cm sample was taken from the uppermost portion of the tap root and put into an envelope. The envelopes were kept in a cooler box containing crushed ice and later oven-dried. The nodules were detached from roots and counted.

Non N_2-fixing *Senna* plants were also harvested, and the biomass was partitioned into leaves, twigs, branches and main stem. In this study, the non-nodulating *S. siamea* was used as a reference plant for the estimation of N_2 fixation as its N uptake pattern and rooting phenology is very similar to that of *S. sesban* [16]. All collected samples were oven-dried separately at 60 °C for 72 hours (h) and weighed for determination of dry matter yield. All the leaves and petioles of *S. sesban* and *S. siamea* were ground into fine powder for the analysis of total N (%N) and ^{15}N natural abundance (δ^{15}N). Root samples for the determination of starch and soluble sugars were ground into fine powder using a hammer mill, and later homogenized (Precellys evolution, Bertin technologies).

2.5. Carbohydrate analyses

Starch and soluble sugar concentrations were analysed using a method described in Poorter and Kitajima (2007). However, in this study, hydrochloric acid rather than amyloglucosidase was used to hydrolyse the starch and sugars to glucose [18]. The root sugar and starch concentrations were used to calculate total non-structural carbohydrates and the ratio of sugar to starch concentrations.

2.6. Analysis of isotopic composition

To determine the ^{15}N/^{14}N ratio of *S. sesban* and *S. siamea*, aliquots of 1.1 to 1.2 mg subsample of finely ground plant material were weighed into aluminium tin capsules that have been pre-cleaned in toluene. The isotopic analysis was done on a Flash EA 1112 Series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (all equipment supplied by Thermo Fischer, Bremen, Germany), housed at the Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria. During analysis, two laboratory running standards, Merck Gel (δ^{15}N=7.89‰; N%=15.29) and DL-Valine (δ^{15}N=-6.15‰; N%=11.86), and a blank sample were run after every 11 unknown samples.

The ^{15}N abundance, expressed as δ^{15}N, i.e. the per million (‰) ^{15}N excess over atmospheric N_2, was determined as [19]:

$$\delta^{15}N = \frac{(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{standard}}{(^{15}N/^{14}N)_{standard}} \times 1000$$

where ^{15}N/^{14}N sample and ^{15}N/^{14}N standard are respectively ratios of the sample and the standard (atmospheric N_2). The international standard for atmospheric N is = 0.0036765 [20].

The proportion of N derived from the atmosphere (%Ndfa) was obtained by comparing the ^{15}N natural abundance of N_2-fixing *S. sesban* with that of non N_2-fixing *S. siamea*,

which in this case, is assumed to represent a measure of the isotopic signature of plant available soil mineral N for *S. sesban* [21]:

$$\%Ndfa = \frac{(\delta^{15}N)_{non-fixing\ leg} - (\delta^{15}N)_{fixing\ leg}}{(\delta^{15}N)_{non-fixing\ leg} - B\ value}$$

where B value is the ^{15}N natural abundance of the nodulated test legume when grown with N_2 fixation as the sole source of N for its N nutrition. The B value replaces the value of atmospheric N as it incorporates the isotopic fractionation associated with N_2 fixation. The B value for *S. sesban* used in this study was -1.76 and was obtained from literature [22].

The amount of N_2 fixed in dry matter was determined from the %Ndfa and the amount of N accumulated in the dry biomass [23]:

$$N_2\ fixed\ (g\ plant^{-1}) = \frac{\%Ndfa}{100}\ x\ N\ content$$

where N content is the product of %N and dry matter yield:

$$N\ content\ (g\ plant^{-1}) = \frac{\%N}{100}\ x\ DM\ yield$$

2.7. Statistical analysis

A 1-Way ANOVA was carried out to compare treatment means and where significant differences were found, the Duncan Multiple Range Test (DMRT) was used to separate treatment means at $p \leq 0.05$ and significance level at *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ indicated; NS= not significant. Correlation analysis was done using Pearson's simple correlation coefficients to test the relationships between DM yield and non-structural carbohydrate reserves.

3. Results

3.1. Above- and belowground biomass productivity

Aboveground dry matter (DM) yield of *S. sesban* plants was significantly altered by the frequency of pruning (Table 1). Increased pruning frequency significantly reduced branch, leaf and therefore total aboveground DM yield. However, the most frequently pruned plants (PF3) had significantly more twig DM relative to PF1 plants.

Table 1. Aboveground dry matter (DM) productivity of *S. sesban* as affected by pruning frequency.

Treatment[1]	Aboveground DM yield (g plant^{-1})				
	Stem	**Branch**	**Twig**	**Leaf**	**Total**
PF1	16.56ab	49.50a	0.70b	9.33a	76.10a
PF2	13.20b	15.17b	1.83a	6.50b	36.70b
PF3	17.88a	8.00b	1.67a	5.83b	33.38b
LSD (0.05)	3.65	9.17	1.29	1.67	12.31
F-statistics	4.48*	59.91***	10.20**	12.64**	38.13***

[1] PF1 = pruned once; PF2 = pruned twice; PF3 = pruned 3 times.

As with total aboveground DM yield, root DM yield was significantly reduced by increased pruning frequency (Table 2). In comparison with PF1 plants, pruning frequency reduced root DM yield by 27 and 35% in PF2 and PF3 plants, respectively. the root length of PF2 and PF3 treated plants were significantly reduced by 19 and 25%, respectively, when compared with that of PF1 plants (Table 2).

Table 2. Root DM yield and root length of *S. sesban* as affected by pruning frequency.

Treatment[1]	DM yield g plant^{-1}	Length cm
PF1	65.45a	58.75a
PF2	47.02b	47.85b
PF3	42.78b	44.20b

| LSD (0.05) | 9.13 | 7.19 |
| F-statistics | 17.86*** | 11.36** |

[1] PF1 = pruned once; PF2 = pruned twice; PF3 = pruned 3 times.

3.2. Reserve carbohydrate concentrations

There was a significant effect of pruning frequency on root starch, sugar and total non-structural carbohydrates (TNC) concentrations in roots of *S. sesban* (Table 3). The two most frequently pruned plants (PF3) had lower starch concentrations than the less frequently pruned ones. Relative to PF1 plants, starch concentration of PF2 and PF3 plants was decreased by 11 and 13%, respectively. Similarly, the concentration of sugar in roots of PF2 and PF3 treated plants decreased by 17 and 25%, respectively, as compared with PF1 plants. Relative to PF1 plants, the TNC of PF2 and PF3 plants was decreased by 12 and 16%, respectively (Table 3).
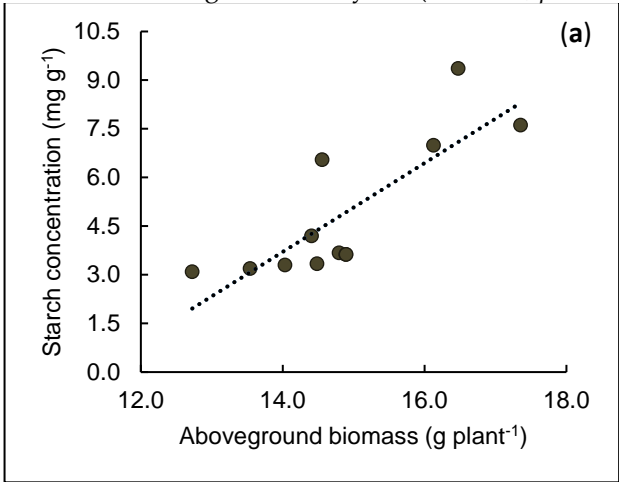
Table 3. Concentration of starch, sugar and total non-structural carbohydrates (TNC) in roots of *S. sesban* plants subjected to different pruning frequencies.

Treatment[1]	Non-structural carbohydrates (mg g^{-1})		
	Starch	Sugar	TNC
PF1	161.27a	58.82a	220.09a
PF2	144.09b	48.91b	193.01b
PF3	140.34b	43.89b	184.23b
LSD (0.05)	14.97	6.84	19.23
F-value	5.69*	12.62**	9.67**

[1] PF1 = pruned once; PF2 = pruned twice; PF3 = pruned 3 times.

3.3. Relationship between aboveground DM yield and carbohydrate concentrations

Starch concentration correlated positively with aboveground DM yield ($R^2 = 0.66$, $p<0.001$), just as sugar concentration correlated positively with aboveground DM yield ($R^2 = 0.75$, $p<0.001$) (Figure 1a and b). Similarly, total non-structural carbohydrates positively correlated with aboveground DM yield ($R^2 = 0.76$, $p<0.001$) (Figure 1c).
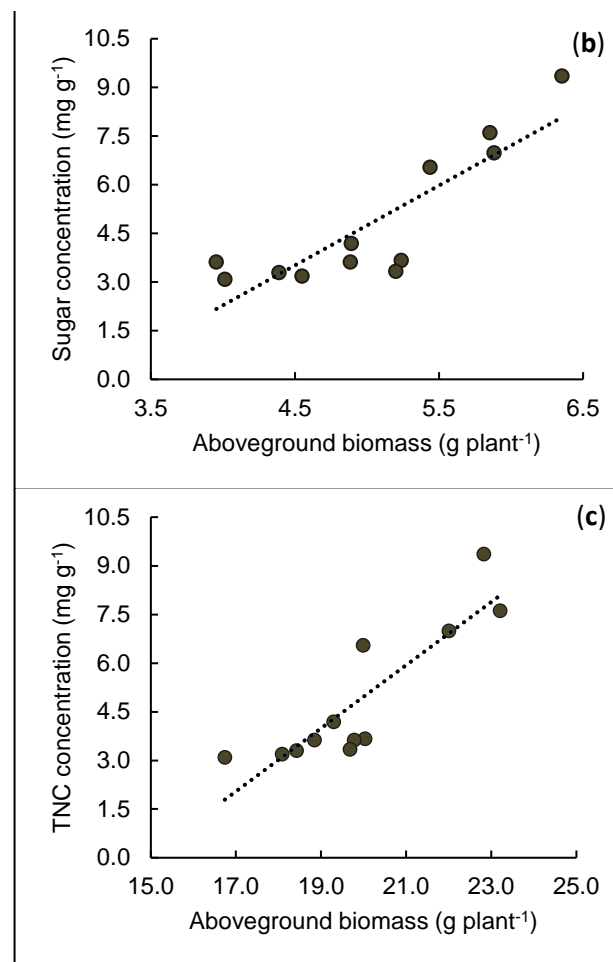
Figure 1. Relationship between aboveground biomass and **a**) starch, **b**) sugar and **c**) total non-structural carbohydrate concentration of *S. sesban* roots subjected to three pruning frequencies.

3.4. Nodulation and symbiotic performance

Nodulation, measured as nodule dry weight (DW) and nodule number per plant, was significantly affected by pruning frequency, but this effect was more pronounced in PF3 plants (Table 4). When compared with nodulation recorded in PF1 plants, nodule DM of PF3 plants declined by 30% whereas the number of nodules declined by 32%.

Table 4. Nodule dry weight (DW) and number of *S. sesban* as affected by pruning frequency.

Treatment[1]	Nodule DW g plant^{-1}	Nodule number no. plant^{-1}
PF1	2.82a	163a
PF2	2.71a	140a
PF3	1.97b	110b
LSD $_{(0.05)}$	0.44	27.59
F-statistics	11.15**	9.94**

[1] PF1 = pruned once; PF2 = pruned twice; PF3 = pruned 3 times.

Isotopic analysis revealed that pruning frequency significantly affected N content, ^{15}N natural abundance (δ^{15}N), percent N derived from the atmosphere (%Ndfa) and amount of N$_2$ fixed by *S. sesban* but not foliar %N (Table 5). The less frequently pruned plants (PF1) recorded the highest N content in biomass as compared with the most frequently pruned plants (PF3). The foliar δ^{15}N signatures of plants significantly increased with increasing frequency of pruning. As a result, %Ndfa estimates of PF2 and PF3 plants decreased by 3 and 15%, respectively, as compared with PF1 plants. The %Ndfa estimates

varied from 82 – 87, 75 – 90 and 73 – 85% for PF1, PF2 and PF3 plants, respectively. The amount of N_2 fixed significantly decreased with increased pruning frequency (Table 5).

Table 5. Symbiotic performance (measured as %N, N content, $\delta^{15}N$, %Ndfa and N_2 fixed) of *S. sesban* as affected by pruning frequency.

Treatment[1]	N %	N content g plant^{-1}	$\delta^{15}N$ ‰	Ndfa %	N_2 fixed g plant^{-1}
PF1	3.39a	2.59a	-1.24b	84.80a	2.17a
PF2	3.48a	1.29b	-1.16ab	81.99ab	1.05b
PF3	4.00a	1.33b	-1.03a	78.28b	1.04b
LSD $(_{0.05})$	0.85	0.63	0.24	7.47	0.53
F-statistics	1.50NS	27.05***	1.67*	1.67*	15.59***

[1] PF1 = pruned once; PF2 = pruned twice; PF3 = pruned 3 times.

4. Discussion

4.1. Above-and belowground biomass productivity

Pruning more frequently significantly decreased biomass productivity of *S. sesban* (Table 1). Previous studies have shown that tree pruning limits C assimilation by reducing the total leaf area, and when it is done more frequently, it is known to decrease biomass productivity [11] and carbohydrate reserves [8]. The poor recovery of shoot growth in the most frequently pruned plants (PF3) was apparently associated with low levels of carbohydrate reserves in the roots as has also been found for *G. sepium* and *L. leucocephala* [12] or *Terminalia sericea* [8]. This means that the reduced photosynthetic tissue of the most frequently pruned plants led to reduced photosynthetic C supply required to maintain growth of the developing shoots.

In the present study, frequent pruning was found to decrease root DM and root length in *S. sesban* (Table 2), a finding consistent with data of many studies conducted on other important N_2-fixing agroforestry legumes [14, 24, 25]. For example, Kadiata et al. (1997) found that shoot removal reduced root biomass of *Albizia lebbeck* and *L. leucocephala* by 40 and 20%, respectively. The reduction in root DM and length of the most frequently pruned plants (PF3) observed in this study could result from cessation of root growth and their decomposition. Indeed, pruning of trees seems to impact root turnover as suggested by significant reduction in live root abundance observed in the subsoil of pruned trees as compared to unpruned ones [25]. Similar responses were also observed in other agroforestry systems for *Erythrina poeppigiana* [14].

4.2. Reserve carbohydrate concentrations

The results showed that carbohydrate reserves may vary with the frequency of pruning as shown by significantly lower levels of starch, sugar and TNC in PF2 and PF3 plants, which were most affected by the pruning treatment. In agreement with data of Latt et al. (2000), which also showed decreased carbohydrate reserves with increased pruning frequency, these findings might mean that, when the supply of C assimilates was limited by losses of leaves, the demand for C required to rebuild photosynthetic tissue was met from mobilization of nonstructural carbohydrates thus leading to depletion of these reserves in the roots. The results of this study also corroborate findings of previous studies that reported reductions in carbohydrate reserves during periods of tree regrowth [7–8].

It is often stated that carbohydrate reserves are controlling factors of shoot regrowth following pruning, since mobilization of these reserves from roots and remaining branches supply energy for shoot regrowth [8]. Indeed, linear relationships were found between aboveground DM productivity and root starch, sugar and total nonstructural carbohydrates in roots (Figure 1a–c), as already observed in previous studies [12].

4.3. Nodulation and symbiotic performance

In the present study, the most frequent pruning treatment (PF3) was found to decrease nodule DM and nodule numbers of *S. sesban* (Table 4). The finding is consistent with data of [24] which showed that pruning frequency of 3 times in 16 months decreased nodule mass of *A. lebbeck, G. sepium* and *L. leucocephala* by 34.8, 26.8 and 11.6%, respectively, as compared with unpruned controls. In an experiment with *E. poeppigiana,* Chesney and Nygren (2000) found a 71% decline in nodule mass of completely pruned plants as compared with partially pruned plants. The lower nodule biomass and nodule number recorded in PF3 plants in this study could be caused by recurring reductions in C supply to nodules thus leading to decomposition of more nodules. Indeed, removal of aerial parts of nodulated plants has been shown to limit the supply of photosynthates to the nodules thus inducing nodule decay and sloughing off of individual nodules [14].

There were no significant differences observed between the pruning frequencies in terms of %N in plant biomass. A similar finding was reported for *G. sepium* subjected to varying pruning regimes [26]. Although the foliar $\delta^{15}N$ signatures of plants increased with increasing pruning frequency (Table 5), they remained relatively close to the ^{15}N natural abundance value of -1.76‰ for *S. sesban* grown with N_2 fixation as the sole source of N [22]. The lower foliar $\delta^{15}N$ signatures of *S. sesban* is indicative of higher reliance on N_2 fixation; hence the greater %Ndfa values (Table 5).

Relative to PF1, pruning frequency decreased %Ndfa estimates by 3 and 15% in PF2 and PF3, respectively (Table 5). The progressive decline in %Ndfa estimates with increasing pruning frequency observed in this study is consistent with the results of Ruess et al. (2006) which showed that 15, 25 and 40% weekly defoliation of *Alnus tenuifolia* seedlings reduced %Ndfa by 23, 33 and 67%, respectively, as compared with untreated control. In another greenhouse study of *G. sepium*, a tropical N_2-fixing woody tree, Nygren et al. (2000) found that the nitrogenase activity of partial and complete defoliated plants was decreased by 10 and 60%, respectively, as compared with that of undefoliated plants. In this study, pruning frequency of 3-month intervals led to recurrent fluctuations in leaf area, and under these conditions, photosynthesis from the residual leaf area was in some cases insufficient to supply C assimilates required for nodule functioning and maintenance of high N_2 fixation levels. Therefore, the decline in %Ndfa values with increasing pruning frequency could be due to limited supply of C assimilates to nodules leading reduced nodule functioning [13,27], death and their decomposition [14,24]. Aranjuelo et al. (2014) showed that nodule sucrose, malate and α-ketoglutarate content decreased 7 days following shoot removal thus concluding that shoot removal caused a decline in C availability thus leading to reduced nodule functioning in *Medicago sativa* (alfalfa).

The effects of pruning frequency on amounts of N_2 fixed in biomass followed trends similar to those of DM productivity, i.e. a progressive decline with increasing pruning frequency. Since the amount of N_2 fixed depends mainly on the legume DM yield, the lower amount of N_2 fixed in the most frequently pruned plants was due to lower DM yields [23]. The N_2 fixed values obtained in this study show similar trends to those reported for *G. sepium* but slightly lower than those reported for *A. lebbeck* and *L. leucocephala* subjected to different pruning frequencies [24].

There were several limitations associated with including unpruned control plants and repeating this experiment under field conditions. Firstly, it was very difficult to manage unpruned plants in this experiment because they regularly experienced transpiration induced leaf shedding due to greater aerial biomass. This could be because the irrigation supply from 5 L pots was not sufficient to satisfy the water requirements of plants. Consequently, the unpruned controls were excluded during the analysis. Secondly, under field conditions, it is practically impossible to recover the entire root mass of *S. sesban* for determination of belowground biomass productivity and for measuring root lengths. Additionally, nodules of most woody species (including *S. sesban*) are highly delicate and majority of them can be lost from the root system during digging, with only a minor percentage being recovered. Furthermore, the study used *Senna* species as a reference plant for the estimations of N_2 fixation under controlled conditions. Although it is considered

as a suitable reference plant, establishment of *Senna* species in the field experimental site was prohibited as it is considered an alien plant in South African [28].

5. Conclusion

Results of this study showed that more frequent pruning reduces biomass productivity. The reduction in biomass correlated with concentrations of root starch, sugar and TNC. Additionally, pruning twice or thrice in nine months decreased nodulation and N_2 fixation. It is, therefore, clear that shoot regrowth, and the potential to produce future biomass and to improve soil fertility (particularly N) may decrease drastically if sufficient time is not allowed between pruning events. The findings of this study, however, have implications for designing management of biological nitrogen fixation in agroforestry systems.

6. Acknowledgements

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