

Effect of retaining versus removing green prunings on N₂ fixation of *Cajanus cajan* and *Sesbania sesban*

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Research Article

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Abstract

Aim

To compare the effects of retention versus removal of green prunings on symbiotic N₂ fixation of *Sesbania sesban* and pigeon pea (*Cajanus cajan*).

Methods

A factorial experiment was conducted to evaluate the effects of retention versus removal of prunings on N₂ fixation of pigeon pea and *S. sesban*. The plots had three rows of woody legumes, and *Zea mays* (maize) was planted in between the legume rows. Three prunings were conducted between April 2017 and February 2018 by cutting the legumes back to 75 cm height. Pruned leaves and twigs were either spread evenly (retained) on the soil surface or completely removed (removed). N₂ fixation was measured using the ¹⁵N natural abundance method.

Results

Whether fresh prunings were retained or removed, pigeon pea derived a significant proportion of its N nutrition from N₂ fixation as compared with *S. sesban*. Retaining prunings on the soil surface significantly depressed symbiotic N₂ fixation by 18% in pigeon pea and 20.6% in *S. sesban* compared to when prunings were removed. The results also showed that retaining prunings increased the reliance of pigeon pea and *S. sesban* on soil N uptake.

Conclusions

Retaining both leaves and twigs in agroforestry systems reduces N₂ fixation by pigeon pea and *S. sesban*. The observed reduction in N₂ fixation following retention of prunings could diminish the beneficial effects of N₂-fixing species on soil N fertility in agroforestry systems.

Introduction

Smallholder farming systems in southern Africa are continuously losing their capacity to support crop growth and productivity (Barnard and Du Preez 2004; Sosibo et al. 2017). This issue emanates from nutrient mining associated with continuous cultivation of staple food crops without adequate nutrient input (Mafongoya et al. 2006). The decline in soil fertility is a root cause of persistently lower crop yields on small household farms and contributes considerably to food insecurity in rural poor communities (Sanchez 2002). Nitrogen (N) deficiency, in particular, is the major factor limiting productivity of staple food crops such as maize, sorghum and millet in southern Africa (Mafongoya et al. 2006).

The use of locally derived nutrient sources, such as crop residues, can provide reasonable amounts of N, phosphorus (P), potassium (K) and other mineral nutrients required for crop growth and productivity (Chen et al. 2014). However, smallholder farmers in southern Africa often let their livestock feed on crop residues in winter after harvest, use them as fuel, or burn the remaining residues just before the cropping season commences (Tongwane et al. 2016). In these situations, alternative sources of N are required to help improve soil fertility and crop productivity in smallholder farming systems.

The integration of fast-growing woody species, especially N₂-fixing legumes, represent an alternative strategy needed for addressing soil N fertility challenges confronted by many resource-poor farmers. In most agroforestry systems, N₂-fixing trees are regularly pruned to minimize competition and shading of companion crops (Youkhana and Idol 2009). The tree prunings can be either retained in the soil to enhance nutrient cycling or removed to provide fodder for livestock (Franzel et al. 2014; Henriksen et al. 2002; Youkhana and Idol 2009). Retention of tree prunings has been shown to increase soil organic matter and N availability, crop yields and N uptake, and also improve soil physical, chemical and biological properties (Isaac et al. 2003, 2004; Youkhana and Idol 2009).

Fresh prunings and litter of pigeon pea (*Cajanus cajan*) and *S. sesban* are capable to recycle 115 and 152 kg N ha⁻¹, respectively (Chikowo et al. 2004), thus indicating that prunings from woody species could be valuable sources of N for resource-poor farmers. Pigeon pea and *S. sesban* prunings are high-quality organic inputs (high N but low lignin and polyphenol contents) capable of releasing nutrients into the soil rapidly (Mafongoya et al. 1998). While legumes have the ability to derive majority of their N requirements from atmospheric N₂, they are also capable of taking up soil mineral N like non-N₂-fixing plants (Unkovich 2012). Mapfumo et al (1999) showed that the percentage of N derived from atmospheric N₂ by pigeon pea decreased with increasing soil N availability. In the context of intercropping systems, this means that the addition of N through fresh prunings and litter can gradually increase the soil N availability leading to the reduction or cessation of N₂ fixation if most of the added mineral N is not taken up by companion crops. Therefore, the beneficial effects of N₂ fixing legumes to soil N fertility in intercropping systems may not be fully realized.

The objective of this study was to compare the effects of retention versus removal of green prunings on symbiotic N₂ fixation of *S. sesban* and pigeon pea. It was hypothesized that the retention of fresh prunings on the soil surface will decrease %Ndfa (percentage of N derived from atmospheric N₂) of *S. sesban* and pigeon pea as compared to their removal.

Materials And Methods

Description of study site

The agroforestry experiment was conducted between November 2016 and February 2018 at Fountainhill Research Station (29°27'S, 30°32'E) situated 2 km outside Wartburg and approximately 30 km northeast of Pietermaritzburg in KwaZulu-Natal, South Africa. The site has an average elevation of 853 m above

sea level and receives mean annual rainfall of 805 mm. The rainfall pattern is unimodal and 85% of the rains fall between October and April, followed by a dry season from May to September (Fig. 1). During the winter months, the area experiences occasional frost.

Experimental design and methodology

The experiment commenced in November 2016. The study was set up in a 2 x 2 x 2 factorial experiment and the treatments were arranged in a randomized complete block design (RCBD) with 3 replicates. The combinations of treatment factors were:

Factor A: Two woody legume species: *S. sesban* and pigeon pea.

Factor B: Two methods of pruning management: retain (as with a green manure) and remove (as with harvesting of fodder).

Factor C: Two dates of sampling i.e. November 2017 and February 2018, referred to as November and February, respectively.

Seeds of *S. sesban* (var. *nubica*) used in this study were obtained from natural populations near Empangeni (28°39'S, 31°57'E), KwaZulu-Natal Province, South Africa. The seeds were surface scarified by immersing in boiled water for 10–15 minutes and cooled with five rinses of cool tap water. *S. sesban* trees were established from seedlings raised in poly bags for 60 d in a glasshouse (24°C). The most homogeneous seedlings of *S. sesban*, according to leaf surface area, biomass and height, were selected for transplanting in the field to minimize non-treatment variation among the trees. Long duration pigeon pea plants were established by direct seeding using seeds obtained from a 2-year fallow experiment at Owen Sithole College of Agriculture, Empangeni. Two seeds were sown per hole and, at 90 d after establishment, the seedlings were thinned to one per hole.

The plots were 10 m x 7 m in size and included three hedgerows with 13 woody legume plants each. The woody legume species were spaced at 3 m between rows and 0.75 m within rows, giving a population density of 3 900 plants ha⁻¹. Maize seeds (OPV Border king) were obtained from a local seed supplier (McDonald seeds Pty Ltd.) in Pietermaritzburg, KwaZulu-Natal. Four rows of maize were planted in the interspaces formed by rows of woody legumes, at 0.3 m within and 0.5 m between rows giving a population of 38, 095 plants ha⁻¹. The distance between maize rows and hedgerows was 0.75 m. Two maize seeds were planted per hole, but later thinned to one at four weeks after planting.

No synthetic fertilizers or inoculants were applied to plants throughout the duration of this experiment to simulate conditions of smallholder farmers. Plots were weeded using hand hoes during the two cropping seasons. During early plant growth stages, the presence of the rhizobia–legume symbiosis was visually evaluated following a few root excavations. The presence of pink root nodules distributed on roots of test legume species was indicative that the soils in the study site contained sufficient populations of native rhizobia for establishing an effective N₂-fixing symbiosis.

Pruning and sampling for ^{15}N isotopes

The first pruning was conducted in April 2017; subsequent prunings were conducted in November 2017 (end of the dry season) and February 2018 (middle of the rainy season). At pruning, the legume species were cut back to 75 cm height using secateurs. Sampling for ^{15}N isotopic measurements was conducted at each pruning date from four randomly selected plants in the middle rows. Collected samples were oven-drying for 96 h at 60°C and finely ground into powder using a cross beater mill (Retsch KG, West Germany) for the determination of total N (%N) and $\delta^{15}\text{N}$ natural abundance ($\delta^{15}\text{N}$). *Zea mays* L. (maize) from unfertilized plots and *Panicum maximum* from adjacent paths were used as reference plants. For comparing the effects of retaining (for soil fertility improvement) versus removing (for livestock fodder), prunings (twigs and leaves) were evenly spread in the 'retain' plots as mulch whereas in the 'remove' plots these biomass components were completely removed.

$^{15}\text{N}/^{14}\text{N}$ isotopic analysis

Symbiotic N_2 fixation of pigeon pea and *S. sesban* was assessed using the ^{15}N natural abundance technique (Unkovich et al. 2008). The measurements of $\delta^{15}\text{N}$ (‰) and additionally the %N were performed from aliquots of 1.1 to 1.2 mg subsamples of finely ground plant material. Isotopic analysis was conducted 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.

The ^{15}N abundance, expressed as $\delta^{15}\text{N}$, i.e. parts per million (‰) ^{15}N excess over atmospheric N_2 , was determined using the following equation (Mariotti et al. 1981):

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

where $^{15}\text{N}/^{14}\text{N}$ sample and $^{15}\text{N}/^{14}\text{N}$ standard are respectively ratios of the sample and the standard (atmospheric N_2). The international standard for atmospheric N = 0.0036765.

The %Ndfa was obtained by comparing the ^{15}N natural abundance of N_2 -fixing species with that of reference plants (Shearer and Kohl 1986):

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

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. Due to the unavailability of resources for generating B values for pigeon and *S. sesban*, the B values used in this study were obtained from literature and were - 0.90 for pigeon pea (Peoples 1989) and - 1.76 for *S. sesban* (Gathumbi et al. 2002), respectively.

Statistical analysis

All data collected were tested for normality using the Shapiro-Wilk test prior to analyses. Analysis of variance (ANOVA) was done using STATISTICA version 13.3 (TIBCO software Inc). Mean differences were declared at $P \leq 0.05$.

Results

Soil properties of the study site

Data on the physical and chemical properties of the soil are presented in Table 1. Soil texture is sandy loam (mean; 18.0% clay, 5.0% silt, and 77% sand) with an average pH of 4.3, organic carbon 0.65%, total N 0.05%, available phosphorus 0.02 mg g^{-1} , potassium 0.08 mg g^{-1} , calcium 0.42 mg g^{-1} and magnesium 0.10 mg g^{-1} in the top 40 cm. Soils (0–80 cm depth) of the experimental site are acidic (pH 4.2). The organic carbon ranged from 0.50–0.80%, total organic N varied from <0.05 –0.07% and available phosphorus was in the range of <0.01 –0.02 mg g^{-1} . The amount of potassium, calcium and magnesium were in the range of 0.04 – 0.08 mg g^{-1} , 0.37 – 0.50 mg g^{-1} and 0.10 – 0.15 mg g^{-1} , respectively.

Table 1 Mean values of soil properties in the 0 – 80 cm profile before the commencement of the experiment

Soil depth (cm)	pH	Organic C (%)	Total organic N (%)	Extract P (mg g^{-1})	Exchangeable cations (mg g^{-1})			Soil texture (%)		
					K	Ca	Mg	Clay	Silt	Sand
0-20	4.24	0.80	0.07	0.02	0.08	0.42	0.10	18	5.3	76.2
20-40	4.31	0.50	<0.05	0.01	0.04	0.37	0.11	18	4.6	77.3
40-60	4.48	0.50	<0.05	<0.01	0.04	0.50	0.15	-	-	-
60-80	4.49	0.50	<0.05	<0.01	0.04	0.50	0.15	-	-	-

$\delta^{15}\text{N}$ signatures of reference plants

In all the pruning dates, maize consistently recorded the highest mean $\delta^{15}\text{N}$ in shoots as compared to *P. maximum*. The overall mean $\delta^{15}\text{N}$ value of these reference plants was used to estimate %Nd_{fa} of pigeon pea and *S. sesban*. In April, an overall reference plant $\delta^{15}\text{N}$ value of $+4.56\text{‰}$ was used to estimate the %Nd_{fa} whereas the average mean $\delta^{15}\text{N}$ values of $+3.16\text{‰}$ and $+3.27\text{‰}$ were used for estimating %Nd_{fa} of the test woody species in November and February, respectively (Table 2).

Table 2 Shoot $\delta^{15}\text{N}$ (‰) values of reference plants sampled at different pruning dates

Sampling date	Reference species	Number of plants (<i>n</i>)	$\delta^{15}\text{N}$ (‰)		
			Range	Mean	Overall mean
Pre-treatment					
Apr-17	<i>P. maximum</i>	5	3.58 – 3.93	3.68	4.56
	Maize	5	5.31 – 6.01	5.44	
Post-treatment					
Nov-17	<i>P. maximum</i>	9	2.76 – 3.13	2.91	3.16
	Maize	9	2.50- 4.80	3.42	
Feb-18	<i>P. maximum</i>	9	2.59 – 3.18	2.85	3.27
	Maize	9	2.82 – 5.18	3.68	

Pre-treatment symbiotic performance

A 2-Way ANOVA on the pre-treatment data revealed no significant interaction between woody legume species and pruning management plots for %N, $\delta^{15}\text{N}$, %Nd_fa, N₂ fixed and soil N uptake of test legume species (Table 3). Similarly, the main effect of pruning management plots on measured variables was not significant indicating that soil conditions of the study site were uniform prior to the imposition of experimental treatments. However, the main effect of species was significant for all measured variables. Foliar %N varied considerably among the legume species, with highest values recorded in pigeon pea compared with *S. sesban* (Table 3).

Pigeon pea exhibited significantly lower $\delta^{15}\text{N}$ values relative to *S. sesban*. Because of the low $\delta^{15}\text{N}$ values, %Nd_fa estimates of pigeon pea were considerably higher as compared with those recorded for *S. sesban*. The amounts of N₂ fixed were similarly greater in pigeon pea than *S. sesban*. However, the amounts of soil N uptake were 4.1-fold higher in *S. sesban* as compared with pigeon pea (Table 3).

Table 3

A 2-Way ANOVA of symbiotic performance (measured as $\delta^{15}\text{N}$, %Ndfa, %N and N_2 fixed) and soil N uptake by pigeon pea and *S. sesban* prior to imposition of experimental treatments (i.e. retained vs. removed). Data are means $\pm$ standard error. Significant differences ($p < 0.05$) are indicated with dissimilar letters

Treatment	$\delta^{15}\text{N}$	Ndfa	N	N_2 fixed	Soil N uptake
	(‰)	%	%	kg ha^{-1}	kg ha^{-1}
Legume species					
Pigeon pea	$-0.47 \pm 0.18\text{b}$	$89.4 \pm 2.7\text{a}$	$3.23 \pm 0.07\text{a}$	$48.0 \pm 2.4\text{a}$	$5.9 \pm 1.5\text{b}$
<i>S. sesban</i>	$0.59 \pm 0.11\text{a}$	$62.9 \pm 1.7\text{b}$	$2.96 \pm 0.08\text{b}$	$38.2 \pm 1.6\text{b}$	$22.7 \pm 1.3\text{a}$
Pruning management					
Retain plots	$0.08 \pm 0.16\text{a}$	$76.4 \pm 3.3\text{a}$	$3.14 \pm 0.07\text{a}$	$44.2 \pm 2.0\text{a}$	$14.5 \pm 2.1\text{a}$
Remove plots	$0.03 \pm 0.20\text{a}$	$75.9 \pm 3.8\text{a}$	$3.04 \pm 0.09\text{a}$	$42.1 \pm 2.3\text{a}$	$14.1 \pm 2.4\text{a}$
F-statistics					
Species	25.45***	66.9***	6.44*	13.0***	68.2***
Pruning management	0.05NS	0.1NS	0.84NS	0.6NS	0.1NS
Species*pruning management	0.54NS	0.2NS	0.45NS	1.2NS	1.1NS

Effect of retaining vs removing prunings on symbiotic performance

There was no significant interaction between species, pruning management and pruning date for %N, $\delta^{15}\text{N}$, %Ndfa, N_2 fixed and soil N uptake (Table 4). However, there was a significant interaction between species and pruning management for %N, %Ndfa and soil N uptake but not for $\delta^{15}\text{N}$ and N_2 -fixed. Except for %N, there was a significant interaction between species and pruning date for $\delta^{15}\text{N}$, %Ndfa, N_2 -fixed and soil N uptake. There was a significant interaction between pruning management and pruning date for $\delta^{15}\text{N}$, %Ndfa and soil N uptake but not for %N and N_2 -fixed (Table 4).

Table 4

Summary of a 3-Way ANOVA F-statistics on symbiotic performance of pigeon pea and *S. sesban* plants as affected pruning management and pruning date

Source of variation	df	N	$\delta^{15}\text{N}$	Ndfa	N ₂ -fixed	Soil N
		%	(‰)	%	kg ha ⁻¹	
Main effects						
Species	1	33.6***	636.9***	1206.5***	119.0***	457.2***
Pruning management	1	1.78NS	1757.9***	1709.0***	5.1*	67.4***
Pruning date	1	67.1***	140.3***	389.4**	130.1***	42.9***
2-Way interactions						
Species*Pruning management	1	7.9**	2.0NS	6.3*	0.0NS	4.6*
Species*Pruning date	1	0.1NS	3242.4***	3166.4***	113.5***	117.8***
Pruning man. *Pruning date	1	3.6NS	26.5***	29.7***	2.8NS	12.76***
3-Way interactions						
Species*Pruning man. *Pruning date	1	0.4NS	2.3NS	0.9NS	0.2NS	1.0NS

For pigeon pea, removing fresh prunings significantly increased foliar %N by 9% compared to when prunings were not removed (Fig. 2A). In contrast, removing prunings decreased %N of *S. sesban* by almost 3% as compared with when prunings were retained. Retaining fresh prunings on the soil surface significantly reduced %Ndfa of pigeon pea and *S. sesban* by 18 and 29%, respectively (Fig. 2B). As compared with removing prunings, retaining prunings significantly increased soil N uptake by pigeon pea and *S. sesban* (Fig. 2C).

In both pruning dates, pigeon pea exhibited significantly lower $\delta^{15}\text{N}$ values as compared with *S. sesban* (Fig. 3A). As compared with *S. sesban*, %Ndfa estimates of pigeon pea were significantly higher in November and February (Fig. 3B). Although the amount of N₂ fixed was similar for both legume species in November, pigeon pea recorded 2.8-fold higher amount of N₂ fixed as compared to *S. sesban* in February (Fig. 3C). Compared with pigeon pea, *S. sesban* recorded 2.2-fold and 7.7-fold higher amount of soil N uptake in November and February, respectively (Fig. 3D).

Whether prunings were removed or retained, the $\delta^{15}\text{N}$ values were significantly higher in November 2017 as compared with February 2018 (Fig. 4A). Surface retention of green prunings increased, on average, $\delta^{15}\text{N}$ values as compared to when prunings were removed. The %Ndfa estimates were higher in February as compared to November (Fig. 4B). %Ndfa values were, on average, decreased by retention of tree prunings as compared to their removal. Whether prunings were removed or retained the amount of soil N

uptake was significantly lower in November as compared with February (Fig. 4C). Soil N uptake values were, on average, increased by retention of tree prunings as compared to their removal.

Discussion

Shoot $\delta^{15}\text{N}$ of reference plants

This study assessed the effect of retention versus removal of green prunings on symbiotic N_2 fixation of *S. sesban* and pigeon pea using the ^{15}N natural abundance technique. With this method, the choice of a suitable reference plant, which is assumed to represent a measure of isotopic signature of plant available soil N for the target N_2 -fixing plant, is of important consideration (Unkovich et al. 2008). The use of more than one non- N_2 -fixing reference species, especially cereal or grass, is also highly recommended for improving the accuracy of N_2 fixation estimates (Unkovich et al. 2008). In this study *P. maximum* and maize were used as reference species, and the latter species consistently exhibited greater shoot $\delta^{15}\text{N}$ values across the pruning dates (Table 2). The variation in shoot $\delta^{15}\text{N}$ of the reference plants used in this study is indicative of inherent differences in isotopic discrimination among the species, or differences in soil N uptake patterns due to varying root phenology and volume of soil explored. Relative to foliar $\delta^{15}\text{N}$ values of woody species, the shoot $\delta^{15}\text{N}$ range of reference plants was sufficiently large to provide reliable estimates of symbiotic N_2 fixation by the ^{15}N natural abundance method.

Pre-treatment symbiotic performance of test legumes

The strong variation in $\delta^{15}\text{N}$ values between the reference plants and test legume species strongly indicate that pigeon pea and *S. sesban* relied mainly on symbiotic N_2 fixation to satisfy their N requirements (Tables 2 and 3), which allowed for reliable assessment of pruning management effects on N_2 fixation of the test species. Similar to what has been reported in a previous study (Chikowo et al. 2004), pigeon pea exhibited significantly lower $\delta^{15}\text{N}$ values and consequently higher %Ndfa values as compared to *S. sesban* (Table 3). The mean %Ndfa estimates were 89 and 63% for pigeon pea and *S. sesban*, respectively, and are within the range of previous reports on pigeon pea symbiotic performance in Zimbabwe (58–100%; Mapfumo et al. 1999) or in South Africa (27–92%; Dakora et al. 2015) and on *S. sesban* N_2 fixation in Zimbabwe (42–73%; Chikowo et al. 2004) assessed using the ^{15}N natural abundance technique. Although the test species were established without assessing the presence of microsymbionts capable of forming N_2 -fixing nodules, the higher %Ndfa values obtained in this study confirmed that pigeon pea and *S. sesban* adapted well to the conditions of the study site, and that both species were able to form a highly functional symbiosis in association with native rhizobia population.

Pigeon pea recorded considerably higher %N and amount of N_2 fixed as compared with *S. sesban* (Table 3). The aerial biomass of pigeon pea and *S. sesban* contained 27–66 kg ha^{-1} and 22–54 kg ha^{-1} of N, respectively, thus indicating that they could be suitable for use as sources of green manure or fodder for livestock. Previous studies in agroforestry systems showed that *Gliricidia sepium* could accumulate

35 kg ha⁻¹ (Hairiah et al. 2000) and 31.4 to 38.0 kg ha⁻¹ (Kaba et al. 2019) of N in pruning biomass whilst species such as *Flemingia conjesta* was shown to fix about 26 kg N ha⁻¹ (Hairiah et al. 2000). The amount of N₂ fixed in prunings obtained in these earlier studies are well within the range of the findings of this current study. *S. sesban* had higher reliance on soil N as compared with pigeon pea suggesting that the vigorous growth nature of *S. sesban* could have induced greater N demand which was unmet through symbiotic N₂ fixation. Therefore, that N deficit was obtained through increased sub-soil N capture. Chikowo et al. (2004) also showed that *S. sesban* took up considerable amount of N from the soil when compared with pigeon pea.

Effects of retaining vs removing prunings on symbiotic performance

Foliar mean %N differed considerably among the test species (Fig. 2A), with greater values in *S. sesban* relative to pigeon pea, thus highlighting inherent variation in rooting phenology and N uptake patterns among the test species (Palm et al. 2001). Because *S. sesban* achieves more rapid stem and leaf growth compared to pigeon pea (Chirwa et al. 2004; Lamage et al. 2021), the greater %N in the leaves could be due to greater root development (Lamage et al. 2021). This probably enabled it to explore greater soil volumes and take up greater soil N to augment N deficit from symbiotically fixed N.

The results of this study show that higher accumulation of soil N due to the decomposition of prunings inhibits symbiotic N₂ fixation of pigeon pea and *S. sesban*. Lower %Nd_{fa} estimates of test species were consistently recorded when fresh prunings were retained on the soil surface compared to when they were removed (Fig. 2B). Pigeon pea and *S. sesban* prunings are high-quality organic inputs (high N but low lignin and polyphenol contents) capable of releasing nutrients into the soil rapidly (Mafongoya et al. 1998). The inhibition of symbiotic N₂ fixation following retention of prunings is most likely due to increased mineralized N levels in the root zone of these species. As tree litters are mineralized, the amount of available N in the soil increases with time, this process leading to the cessation of N₂ fixation in aging plantations (Dommergues 1995). Kadiata et al. (1998) showed that the incorporation of tree prunings into soil significantly reduced the %Nd_{fa} of *Gliricidia sepium* and *Leucaena leucocephala* by 17 and 22%, respectively, as compared with when prunings were removed. The results indicate that the benefits from symbiotic N₂ fixation in agroforestry systems can be offset by the amount of N released from tree prunings or residues.

Soil N uptake by pigeon pea and *S. sesban* was significantly increased by retention of prunings compared to their removal (Fig. 2C), and this could be attributable to the decrease in nodule functioning and N₂ fixation rates associated with elevated levels of N in the root zone of plants. Because N₂ fixation is an energy demanding process, greater reliance on soil N uptake could mean that the N satiety of the species was unmet through decreased N₂ fixation rates. The preferential uptake and assimilation of soil mineral N for meeting N satiety over fixing atmospheric N₂ is common in woody legumes (Dommergues 1995).

Pigeon pea exhibited significantly lower $\delta^{15}\text{N}$ compared with *S. sesban* in both November 2017 and February 2018 (Fig. 3A). Consequently, pigeon derived a greater proportion of its N nutrition from symbiotic N_2 fixation compared with *S. sesban* (Fig. 3B). The reliance on N_2 fixation (%Ndfa) ranged from 54–100% and 27–57% for pigeon pea and *S. sesban*, respectively. The efficiency of N_2 -fixing activity between soil rhizobia and legumes is known to vary among species (Peoples et al. 2009). The superiority of pigeon pea over *S. sesban* in terms of symbiotic performance could be attributable to its high symbiotic promiscuity (Vanlauwe et al. 2019), and most likely, the presence of high population of compatible native soil rhizobia with greater N_2 -fixing efficiency in the study site. Chikowo et al. (2004) also reported considerably higher %Ndfa estimates in pigeon pea as compared with *S. sesban* in improved fallow experiment in Zimbabwe. Due to higher %Ndfa values and most likely biomass, especially in February, pigeon pea accumulated significantly greater amounts of N_2 -fixed compared to *S. sesban* (Fig. 3c). *S. sesban* took up significantly higher soil N compared to pigeon.

Lower $\delta^{15}\text{N}$ and hence higher %Ndfa values were recorded in February 2018 as compared with November 2017 (Fig. 4A and B) could be as a result of moisture stress and lower biomass at the end of the dry season (November). Environmental factors such as drought and extreme temperatures are known to impact growth and symbiotic functioning of nodulated legumes (Hungria and Vargas 2000). Nitrogenase activity has been shown to decrease at low soil moisture levels, probably due to limited supply of C assimilates to the N_2 -fixing nodules (Hungria and Vargas 2000; Chaves et al. 2009) or reduced N demand by plants. Nygren and Leblanc (2009) also found that *G. sepium* trees derived a greater proportion of their N nutrition from symbiotic N_2 fixation in the rainy season as compared with the dry season when water availability in the soil was limiting.

Conclusion

The findings of this study demonstrated that the tested N_2 -fixing legumes adapted well to the conditions of the study site, and both species obtained more than 60% of their N nutrition from N_2 fixation prior to imposition of treatments. Retaining fresh prunings on the soil surface significantly depressed N_2 fixation rates of pigeon pea and *S. sesban*. Additionally, retaining prunings also increased reliance of both species on soil mineral N. The effect of retaining prunings on N_2 fixation can be lessened only if N supply from prunings of N_2 -fixing woody legumes is synchronized with N demand of associated plants. Reasonable benefits in terms of net N additions to an agroforestry system N balance are likely to be achieved by maximising rates of N_2 fixation by removing freshly pruned material from the system. Therefore, in smallholder mixed crop-livestock farming system in Africa it would be more practical to prune woody legumes and retaining in the fields as the source of organic matter and mineral nutrients, especially N for accumulation in the soil. To avoid N accumulation, which will reduce N_2 fixation, it would be practical in mixed crop -livestock farming systems, to only retain prunings during the cropping season when the demand for growing plants is high. Any material pruned during the dry season should be fed to livestock and not retained on the soil surface in the field.

Declarations

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Competing Interests

Authors declare they have no financial interests.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Makhubedu IT and Letty BA. The first draft of the manuscript was written by Makhubedu IT and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1

Rainfall distribution and average temperature between November 2016 and February 2018 according to the SASA weather website (SASA, 2019)

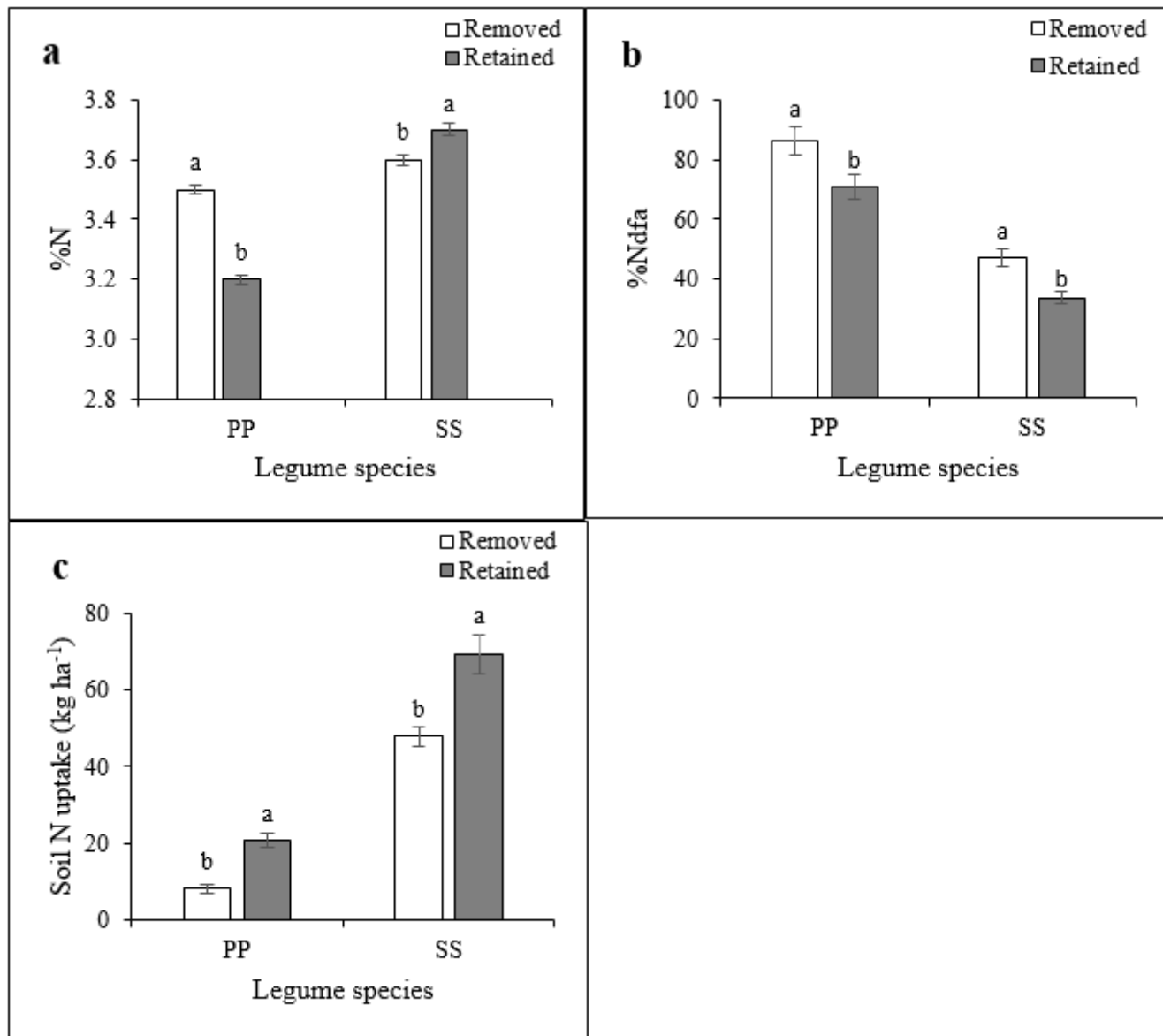


Figure 2

Interaction between woody legume species and pruning management on **a)** %N, **b)** %Ndffa and **c)** soil N uptake. Data are means ± standard error. Significant differences (p < 0.05) are indicated with dissimilar letters

Figure 3

The interaction between woody legume species and pruning date (Nov 2017 versus Feb 2018) on **a)** $\delta^{15}\text{N}$, **b)** %Ndffa, **c)** N_2 -fixed and **d)** soil N uptake. Data are means ± standard error. Significant differences (p < 0.05) are indicated with dissimilar letters

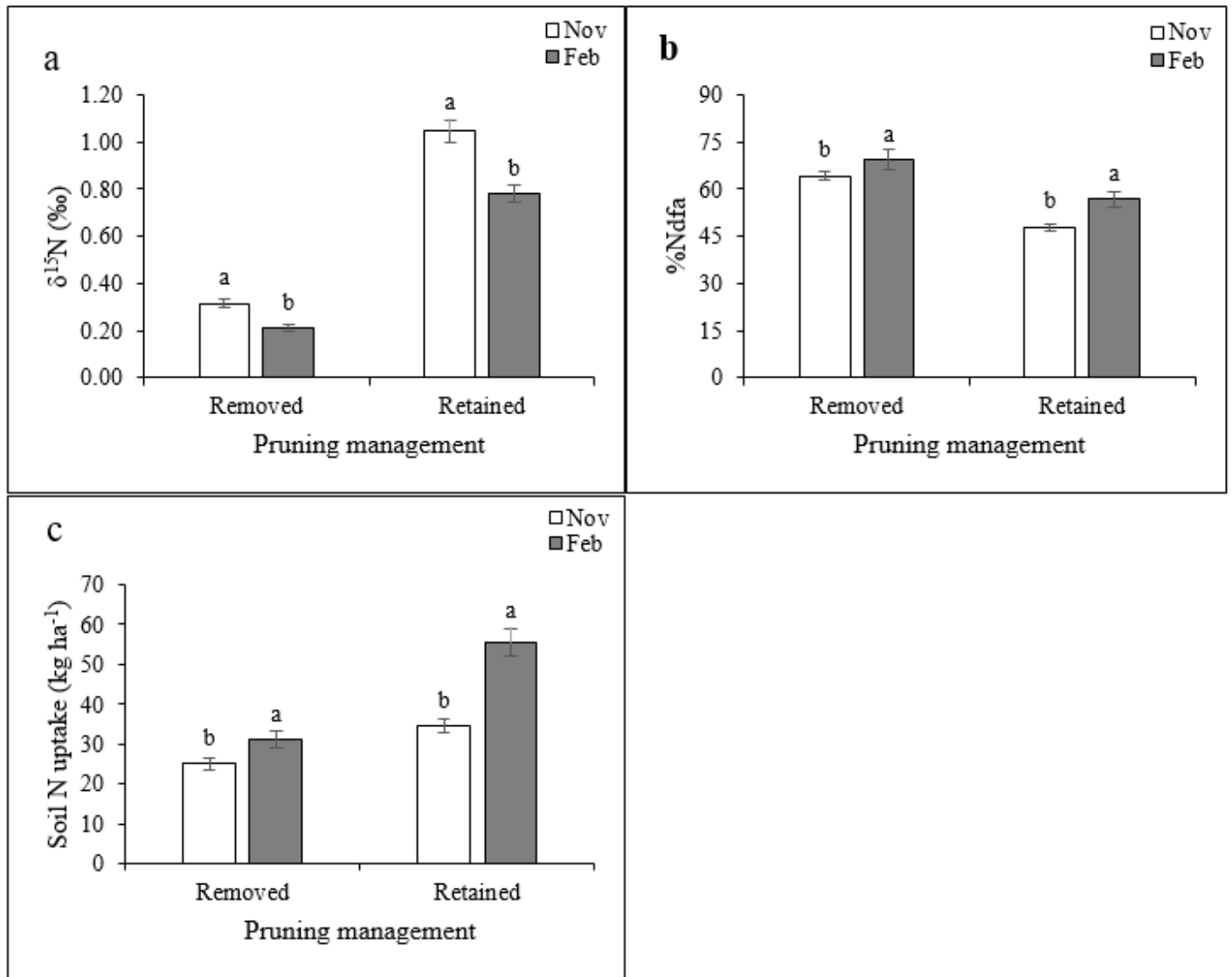


Figure 4

The interaction between pruning management and pruning date (Nov 2017 versus Feb 2018) on **a)** $\delta^{15}\text{N}$, **b)** %Ndffa, and **c)** soil N uptake when combining pigeon pea and *S. sesban* data. Data are means $\pm$ standard error. Significant differences ($p < 0.05$) are indicated with dissimilar letters