High rates of nitrogen fixation and transfer by Cajanus cajan to associated crop in a semi-arid agroforestry system

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Abstract

In semi-arid regions where nitrogen deficiency limits crop productivity, the integration of leguminous tree species into agroforestry systems (AFS) is likely to enhance nitrogen availability for associated crops. We tested this hypothesis in southern Madagascar, a region characterized by erratic and low rainfall, and nutrient-poor soils where the pigeon pea grown as a pure crop or mixed with cassava. Over a 3-month period, we estimated the atmospheric nitrogen fixation rate (%Ndfa) by two-year-old pigeon pea (*Cajanus cajan*) grown as a pure crop and in association with cassava (*Manihot esculenta*) using N dilution method, and the transfer of N from pigeon pea to cassava (%Ndft) in the mixed pigeon pea-cassava plots. Six-month-old pure cassava plots were used as reference. Each system was replicated three times. ^{15}N soil labeling (98 atom% ^{15}N) was used to estimate %Ndfa and %Ndft. %Ndfa was 100% in both pigeon pea systems. Considering %Ndfa of 100% from planting date, the quantity of N fixed was two times higher in pure pigeon pea plot than in mixed pigeon pea-cassava plots, with values of 17.1 kg N ha^{-1} and 8.5 kg N ha^{-1}, respectively. This finding was consistent with the 2.8 times higher planting density in pure pigeon pea plots compared to mixed pigeon pea-cassava plots. Nitrogen transfer from pigeon pea to cassava was greater (72.9%) when the cassava plant was positioned farther from pigeon pea rows (in the middle) compared to when it was planted next to pigeon pea row (42.4%). Our results suggest that integrating pigeon pea into semiarid agroforestry systems could enhance nitrogen status of the associated crop, particularly in unfertilized AFS.

1. Introduction

Agroforestry systems (AFS) propose a sustainable and productive ecosystem by combining trees and/or shrubs with crops. These perennial cropping systems require less tillage and provide more organic matter to the soil than annual cropping system thereby improving crops nutrition (Bekele-Tesemma 2007; Mafongoya et al. 2007; Sileshi et al. 2020). In tropical areas, the majority of trees in these systems are nitrogen fixing species (NFS)to improve soil fertility and crop nutrition (Crews 1999; Menge et al. 2014), whereas in semi-arid climates, NFS not only improve the availability of nitrogen where it is frequently deficient, but also help to create favorable micro-environments for crop development (Nair et al., 2021). The loss of nitrogen in cropping systems through export at harvest (Kim and Isaac 2022), ammonia (NH_3) volatilization, and nitrate (NO_3) and ammonium (NH_4^+) leaching (Cameron et al. 2013; Kim and Isaac 2022) is a frequent problem in AFS cropping systems. On the one hand, NFS are likely to reduce nitrogen loss within AFS and on the other hand, they can supply nitrogen to the system through atmospheric fixation (Kim & Isaac, 2022; Nygren et al., 2012).

While fixation of atmospheric N_2 in NH_4^+ form, through symbiosis with Rhizobium bacteria, is a major source for nitrogen input in agroecosystems (Nygren et al. 2012), environmental factors can reduce the N_2 fixation (Bordeleau and Prévost 1994; Paula et al. 2015). Several studies on soybean indicated that drought stress (Freitas et al. 2022; Serraj et al. 1999),soil nutrient deficiency such as low P availability (Yao et al. 2022) can decrease N_2 fixation as a consequence of photosynthesis reduction and decrease in photosynthates supply to the nodules (Nandwal et al. 1991; Sheoran et al. 1988).

Crops associated with NFS can benefit by direct transfer through root exudates and common mycorrhizal networks (CMN), or indirectly through litter mineralization, and fine root and nodule turnover (Munroe and Isaac 2014; Nygren et al. 2012; Paula et al. 2015; Thilakarathna et al. 2016). Previous studies in tropical environments have suggested that N-transfer may be stronger when the associated crop is grown close to the NFS (Paula et al., 2015). Zayed et al. (2023) reported that under nutrient deficiency, especially N deficiency, plants may generally activate physiological and molecular mechanisms to improve their nitrogen uptake efficiency. Eucalyptus trees grown in association with acacia have been shown to develop more extensive root systems and increase root exudation (Oliveira et al. 2021). These responses contribute to improve N-transfer within mixed species where the associated crop increase their demand for available N and the NFS respond (Oliveira et al. 2021).

Among the NFS used in agroforestry systems, pigeon pea (*Cajanus cajan* (L.) Millsp) is one of the species recommended for semi-arid areas (Daniel and Ong 1990; Mula and Saxena 2010; Saxena et al. 2019). This leguminous shrub is adapted to harsh climatic and environmental conditions such as low rainfall and nutrient poor soil (Mula and Saxena 2010; Odeny 2007; Saxena et al. 2019). In addition, pigeon pea is efficient to fix atmospheric N_2 (Bopape et al. 2022; Kumar Rao and Dart 1987; Sameer Kumar et al. 2017). In semi-arid Kenya, the percentage of nitrogen derived from fixation (%Ndfa) of five pigeon pea varieties were estimated over 80% in roots, leaves, stover, grains (Kwena et al. 2019).

The southern Androy region of Madagascar, is characterized by a semi-arid climate, frequent drought, and severe aeolian erosion. These environmental constraints, combined with widespread poverty, have led to deforestation, poor soil fertility and low agricultural productivity (Violas 2020). To address this issue, a land-use strategy design described as agroecological blocks has been implemented since 2014. These blocks combine physical and biological components over a minimum area of 10 ha (Violas 2020). AFS play a central role in these blocks, with the aim of improving environmental protection and soil fertility by integrating ground cover species and nitrogen-fixing trees, thereby reducing erosion, strengthening the resilience of agro-ecosystems and supporting food production (Fahad et al. 2022; Pancholi et al. 2023; Octavia et al. 2023; Violas 2020). Pigeon pea is one of the key species used in these AFS, either as sole crop or in associated crop. These systems are mainly adopted by resource-limited farmers, consequently, no fertilizers are added, which likely lead to progressive soil nutrient depletion (Vanlauwe et al. 2023; Violas 2020). The integration of pigeon pea into these AFS is therefore expected to promote nitrogen inputs and improve the N status of associated crops. To optimize the management of AFS in this semi-arid area, this study aims to estimate the amount of atmospheric nitrogen fixed and transferred to the associated crops. The results are intended to guide spatial configurations of this agroforestry system to enhance biological nitrogen inputs and improve soil fertility without external inputs.

2. Materials and methods

2.1 Study site

The study was carried out in the Karoke Mahandrorano agroecological block (25°15'15"S, 46°1'21"E), located in the Androy region of South Madagascar near the city of Ambovombe, at 200 m asl. Established in 2013, this is one of the oldest agroecological blocks in the region, and serves as a reference site for the other blocks. The site is located on a coastal area, on limestone rock. The relief was characterized by gently undulating topography with slope < 5%. The soil texture was uniform below a depth of 30 cm with sand content around 80%. The soil pH_{H2O} ranged from 7.2 to 9.1 depending on the plots. Soil chemical analyses down to a depth of 0.30 m are given in Table 1.

The climate of the region is classified as BSh (semi-arid hot) according to Köppen-Geiger classification (Beck et al. 2018), with wet season from December to April and dry season from May to November (Fig. 1). The average annual temperature is 24.4°C, with temperature varying from 19°C (minimum in June) to 40°C (maximum in January). Annual rainfall ranges from less than 300 and 600 mm, occurring mainly during wet season. Sporadic and limited rainfall may also occur in May and June. However, precipitation is highly erratic, with frequent droughts and irregular rainfall patterns. For instance, in 2020, no rainfall was recorded for 18 consecutive months, and in other years, only 3 to 4 rainfall events per season (UNICEF 2024). Potential evapotranspiration is 518 mm. $year^{-1}$, which generally exceeds rainfall throughout the year (JICA 2006). From August to October, violent soil-eroding winds occur (Morlat and Castellanet 2012; Violas 2020).

The study was conducted during 90 days from January 2024 to April 2024. During this period, the average air temperature was 22.5° C and the cumulative rainfall was 224 mm (https://www.historique-meteo.net).

Table 1
Main chemical soil attributes of the top soil without pigeon pea at the experimental area (n = 4)

Soil depth (cm)	0–10	10–20	20–30
pH	8.92	8.62	8.70
Organic C ($g.kg^{-1}$)	2.83 ± 0.69	2.46 ± 0.58	1.95 ± 0.61
Total soil N ($g.kg^{-1}$)	0.29 ± 0.08	0.21 ± 0.06	0.20 ± 0.05
Available P ($mg.kg^{-1}$)	5.07 ± 2.24	2.58 ± 1.32	2.88 ± 1.45
K ($cmol+.kg^{-1}$)	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.02
Ca ($cmol + kg^{-1}$)	1.65 ± 1.04	1.68 ± 1.03	1.70 ± 1.24
Na ($cmol + kg^{-1}$)	0.01 ± 0	0.01 ± 0	0.02 ± 0

2.2 Experimental layout

The ^{15}N isotope dilution method was used to estimate the %Ndfa. The method was applied to pure pigeon pea, mixed pigeon pea-cassava (Manihot esculenta Crantz), and pure cassava plots.

In total, nine farmer plots were selected within the AFS agroecological block for the experiment, corresponding to three cropping system with three replicates each: pure pigeon pea, mixed pigeon pea-cassava, and pure cassava plots. All pigeon pea plants were two years old, while the cassava plants were six months old at the time of the experiment. Cassava, a non- N fixing species, was chosen as the reference plant for %Ndfa estimation (Boddey et al. 1990).

2.3 ^{15}N soil labeling

An area of 100 m^2 was delimited in each plot. Ammonium chloride ($Cl-^{15}NH_4$) with 98 atom% ^{15}N was applied in this area at a rate of 0.8 kg N ha^{-1} on 15th, 16th, and 17th January 2024. The fertilizer was diluted in water and applied uniformly to the soil using a watering can. We assumed that the low amount of applied N did not affect the N_2 fixation of pigeon pea (Bouillet et al. 2008).

2.4 Plant sampling and analyzes

Aboveground leaf biomass of pigeon pea plants

In each pure pigeon pea and mixed plots, a pigeon pea inventory was carried out by counting individual plants and measuring their height. To estimate aboveground leaf biomass, all leaves were harvested randomly from 10 shrubs per plot, replicated four times, resulting in a total of 80 shrubs sampled (Table 2). Both fresh and dry masses were recorded.

Leaf sampling for N fixation measurements

For nitrogen analysis, leaf samples were separately collected in each system and for each species at two time points: before soil labeling in January 2024 and 90 days after soil labeling in April 2024.

In the pure pigeon pea plots, leaf sampling was carried out on five shrubs arranged diagonally in a cross pattern (Fig. 2.A). For each shrub, twenty leaves from the upper part of the crown on two pairs of opposite branches (north-south and east-west) were collected and pooled to make a composite sample representative of the plot (Table 2).

In the pure cassava plots, this sampling was carried out on 10 plants arranged diagonally and in a cross pattern (Fig. 2.A): four leaves were sampled per plant, one in each direction (Table 2).

In the mixed pigeon pea - cassava plots, pigeon pea leaves were collected from five shrubs in a row, with 20 leaves per shrub (Table 2), while the cassava, leaves were collected at three different distances from the pigeon pea row: the first plant close to the shrub (near), the second at a medium distance (middle), and the last between two pigeon pea rows (far) (Fig. 2.B).

The sampled leaves were then dried at 60°C and ground, and sent to SILVATECH Laboratory (https://www6.nancy.inrae.fr/silva/Plateformes/SilvaTech) for analysis. For this purpose, 10 mg of leaf powder dry matter were placed in tin capsules for isotopic analyzes. N concentrations and $\delta^{15}N$ values of the leaves were determined using an elemental analyzer (vario ISOTOPE cube, Elementar, Langenselbold, Germany) coupled, via a gas box interface, to a continuous flow isotope ratio mass spectrometer (Isoprime100, IRMS, Elementar UK, Cheadle, United Kingdom). The precision of $\delta^{15}N$ measurement was 0.5‰.

Table 2
Number of pigeon pea sampled for leaf biomass and leaf samples for nitrogen analysis in pure and mixed plots

		Pigeon pea sample for leaf biomass	**Leaf sample for N analyzes**
Pure crop	Pigeon pea	10	100
	Cassava	-	40
Mixed crop	Pigeon pea	10	100
	Cassava	-	30
Replicates		4	3
Total		80	810

2.5 N derived from atmospheric fixation (%Ndfa)

The percentage of N derived from N_2 fixation (%Ndfa) for each plot during the experiment is estimated by examining ^{15}N Atom Excess (AE) using the following equation (Bouillet et al. 2008):

$$\%Ndfa = 100\left(1 - \frac{\frac{\left(N_{Ffinal}\,AE_{F\ldots}\right.}{\left(N_{\ldots}\right.}}{\frac{\left(N_{REFfinal}\,AE_{REF\ldots}\right.}{\left(N_{REF\ldots}\right.}}\right.$$

1

where: AE = $^{15}N \times 100 / (^{15}N + ^{14}N) - 0.003663$, AE_{Ffinal}, $AE_{Finitial}$, $AE_{REFfinal}$, and $AE_{REFinitial}$ are the percentage atom excess of pigeon pea 90 days after soil labeling, the percentage atom excess of pigeon pea at soil labeling, the percentage atom excess of cassava 90 days after soil labeling, and the percentage atom excess of cassava at soil labeling, respectively. N_{Ffinal}, $N_{Finitial}$, $N_{REFfinal}$, and $N_{REFinitial}$ are the N content of pigeon pea 90 days after soil labeling, the N content of pigeon pea at soil labeling, the N content of cassava 90 days after soil labeling, and the N content of cassava at soil labeling, respectively.

2.6 N derived from transfer

The proportion of cassava N derived from transfer (%Ndft) from pigeon pea during the experiment period was estimated using Eq. 2 (Snoeck et al. 2000) :

$$\%Ndft = 100 \times \frac{\delta^{15}N_{REF} - \delta^{15}N_{R\ldots}}{\delta^{15}N_{REF} - \delta^{15}N_{fi\ldots}}$$

2

where: $\delta^{15}N_{REF}$ is the $\delta^{15}N$ value of cassava leaves in pure crop, $\delta^{15}N_{REFass}$ is the $\delta^{15}N$ of cassava leaves in mixed crop, and $\delta^{15}N_{fixation}$ is the $\delta^{15}N$ of pigeon pea leaves in pure crop.

2.7 Statistical analyses

One-way analysis of variance (ANOVA) was used to compare the means between the N content, $\delta^{15}N$ and AE of the different system as fixed factor and, %Ndft values of cassava in mixed crop with distance as fixed factor. Tukey's post-hoc tests were used to examine pairwise differences. Assumptions of normality and homoscedasticity of residuals were tested using Shapiro-Wilk and Bartlett tests, respectively. Statistical analyses were performed using R 4.0.3 et RStudio 4.1.1, with a significance level α of 0.05.

3. Results

The experimental season was characterized by low rainfall (224 mm) and low topsoil fertility. According to Boyer. (1982), the critical deficiency levels for tropical plants grown on ferralitic soils are 0.5 $g\text{-}kg^{-1}$ for total N and 3 $mg\text{-}kg^{-1}$ for available P, while the deficiency thresholds are 1.5 $g\text{-}kg^{-1}$ and 10 $mg\text{-}kg^{-1}$ respectively. Our soil analysis (depth 0–30 cm) showed total N levels below both the critical level and the deficiency threshold, while available P levels were comparable to the critical level but still above the deficiency threshold. This following section presents data from shrub inventory, pigeon pea biomass assessments, and leaf N analysis, with a focus on N derived from atmospheric fixation (%Ndfa), their transfer in mixed plots (%Ndft), and the overall contribution of fixed N to the system.

3.1 ^{15}N values and percentage of N derived from atmospheric fixation (%Ndfa)

No significant difference in AE mean values between the different crop systems was observed at the beginning of the experiment (*p-value* = 0.23), with $AE_{initial}$ ranging from 8.42–9.39% (Table 3). After 90 days, the AE of cassava leaves in the pure crop was significantly higher than that of pigeon pea leaves in the pure and mixed crops (*p-value* = 0.008). No significant difference in N content of the cassava and pigeon pea leaves in pure and mixed crops was found at the beginning of the experiment (*p-value* = 0.18) or after 90 days (*p-value* = 0.78). $N_{initial}$ ranged from 3.56–4.09%, while N_{final} ranged from 4.08–4.25%. The %Ndfa was estimated to be 100% during the 90 days experiment both in the pure pigeon pea and in mixed pigeon pea-cassava plots.

Table 3

N content, ^{15}N atom excess of leaf samples (AE) in pigeon pea and cassava, and %Ndfa of pigeon pea (Mean values, n = 3)

Systems	Leaf samples	Observed parameters				
		$N_{initial}$	$AE_{initial}$	N_{final}	AE_{final}	%Ndfa
Pure cassava	Cassava	4.09a	8.42a	4.08a	9.95a	
Pure pigeon pea	Pigeon pea	3.56a	9.39a	4.14a	8.58b	100
Mixed pigeon pea - cassava	Pigeon pea	3.78a	8.92a	4.25a	8.18b	100

*$N_{initial}$ and N_{final} are the N content of leaf samples on soil labeling and after 90 days respectively, expressed in %.

$AE_{initial}$ and AE_{final} are the values of atom excess at soil labeling and after 90 days respectively, expressed in %.

%Ndfa is the percentage of nitrogen derived from fixation atmospheric.

Letters (a–b) indicate significant differences ($p < 0.05$) between systems.

3.2 Nitrogen input from N_2 fixation

The planting density of pigeon pea was higher in pure system (3464 individuals ha^{-1}) compared to the mixed pigeon pea-cassava system (1216 individuals ha^{-1}), due to inter-row spacing constraints in the mixed plots. Despite this, the average height of pigeon pea shrubs was around 180–190 cm in both systems, suggesting comparable vertical growth regardless of planting arrangement. (Table 4).

Considering individual plants for each system, there was more N fixed in the mixed pigeon pea-cassava plots (6.97 g N $plant^{-1}$) than in the pure pigeon pea plots (4.94 g N $plant^{-1}$), which is expected given the higher leaf biomass per plant in the mixed plot. However, taking planting density into account, the total N input over two years was higher in the pure pigeon pea plots, reaching 17.11 kg N ha^{-1}, compared with 8.48 kg N ha^{-1} in the mixed pigeon pea -cassava plots (Table 4).

Table 4

Aboveground pigeon pea growth parameters and nitrogen supply from %Ndfa in 2-year-old pigeon pea plants, in both pure and mixed pigeon pea plots plots (Mean values ± SE, n = 4)

Systems	Pure pigeon pea	Mixed pigeon pea - cassava
Pigeon pea planting density (individuals ha^{-1})	3464	1216
Height (cm)	196.5 ± 44.9	188.3 ± 49.7
Leaf biomass (kg)	0.13 ± 0.11	0.17 ± 0.08
%Ndfa	100	100
g N $plant^{-1}$ from %Ndfa	4.94	6.97
kg N ha^{-1} from %Ndfa	17.11	8.48

3.3 N transferred from pigeon pea to cassava

After 90 days, the mean values of $^{15}N_{final}$ varies slightly but significantly from 0.37–0.44% between the three systems (Table 5). The highest value of $^{15}N_{final}$ was recorded in leaves of the pure cassava plots (0.44%), while the lowest value was observed in the pigeon pea leaves of the mixed pigeon pea-cassava plots (0.38%).

Cassava leaves located at different distances from pigeon pea (near, at ¼ inter-row and ½ inter-row) showed similar and non-significant $^{15}N_{final}$ values (0.41%, 0.42% and 0.40% respectively), (Table 5). However, the percentage of N transferred from pigeon pea to cassava (%Ndft) varied significantly with increasing distance from the pigeon pea plants (*p-value* = 0.0009). Cassava plants located in the ½ inter-row received the highest proportion of nitrogen transferred (72.95%), while those positioned near to the pigeon pea (51.73%) or slightly further in the ¼ inter-row (42.45%) received less N. These results suggest that spatial arrangement plays a key role in promoting N transfer. No significant difference in %Ndft was found between the last two positions.

Table 5

Mean leaf samples ^{15}N values in the three cropping system and %Ndft in the mixed plots in relation to the distance between the non-leguminous crop (cassava) and the leguminous crop (pigeon pea)

System	Leaf samples	Distance from pigeon pea	$^{15}N_{final}$	%Ndft
Pure pigeon pea	Pigeon pea	-	0.39d	-
Pure cassava	Cassava	-	0.45a	-
Mixed pigeon pea - cassava	Pigeon pea	-	0.38d	-
Mixed pigeon pea- cassava	Cassava	Near (S1)	0.41bc	51.73b
Mixed pigeon pea - cassava	Cassava	¼ inter-row (S2)	0.42b	42.45b
Mixed pigeon pea - cassava	Cassava	½ inter-row (S3)	0.40cd	72.95a

$^{15}N_{final}$ is the value of $\delta^{15}N$ expressed in % of leaf samples after 90 days. %Ndft is the proportion of N derived from transfer. Letters (a–d) indicate significant differences ($p < 0.05$) between systems.

4. Discussion

4.1 High efficiency of pigeon pea to fix atmospheric N_2

The nitrogen fixation rate of pigeon pea was estimated at 100% in both, pure pigeon pea and mixed pigeon pea-cassava plots over the 90-day experimental period. These results indicate that, over this period, all the N in pigeon pea leaves was derived from N_2 fixation. This also suggests that, at least during the second vegetative season, pigeon pea relied entirely on biological nitrogen fixation, regardless of the cropping system. According to Dovrat and Sheffer (2019), biological N fixation is generally optimal during wet season. Soil N deficiency (Dovrat et al. 2018) and high P availability (Alon et al. 2021) has been shown to increase nitrogen fixation rates by NFS. In our study, although P availability was close to the critical level but likely sufficient to support the energy costs for the N_2 fixation. In addition, the low levels of N in the soil and the fact that the experiment was conducted during the rainy season probably contributed to maximizing symbiotic nitrogen fixation. It is consistent with previous studies showing that biological N fixation is more efficient under unfertilized conditions (Figueiredo et al., 2025). Similar high levels of %Ndfa have been reported in other semi-arid regions: Rao et al. (1987) recorded 88% in sole pigeon pea and 96% in mixed pigeon pea–sorghum systems in India, while Kwena et al (2019) found values exceeding 80% in pigeon pea–maize intercropping in Kenya without rhizobia inoculation. Mhango et al (2017) reported %Ndfa about 76% and no significant difference between pure pigeon pea and mixed pigeon pea-maize crop system.

However, short rainfall season means low biomass production and lower N fixation. Paula et al (2018) showed variability in N_2 fixation rates between seasons in mixed eucalyptus-acacia cropping system in Brazil. Water availability plays a critical role in improving biological N fixation by reactivating or enhancing biomass nodules of NFS. Pigeon pea is also known for its water use efficiency, allowing it to sustain biological N_2 fixation even under tropical dryland conditions (Berriel and Perdomo 2023). Consequently, it will be important to assess the dynamics of N_2 fixation from the early stages of pigeon pea growth and across different seasons, as seasonal variations in %Ndfa could occur.

4.2 Nitrogen transfer dynamics in mixed cropping systems

Between 42% and 72% of N was transferred from pigeon pea to cassava. Therefore, the association of pigeon pea benefited the crop system by improving the N nutrition of the associated plant. Monroe and Isaac (2014) indicated that N from NFS can be a source of easily available N for the associated crop via fine root decomposition and nodule turnover or N rhizodeposition. Nitrogen could also be transferred from NFS to companion species *via* common mycorrhizal networks (Ingleby et al. 2007; Oliveira et al. 2021).

We found that nitrogen transfer dynamics between pigeon pea and cassava were influenced by cassava localization, with higher %Ndft observed at greater distances from pigeon pea. This invalidates our hypothesis of higher %Ndft at closer distance of cassava from pigeon pea, as observed for N transfer from *Acacia mangium* to *Eucalyptus grandis* in mixed-species plantations (Paula et al. 2015). This could be due to root spatio-temporal dynamics of the fine roots of pigeon pea. Actually, it has been reported that shrubs often have significant lateral root spread, which can favor their interaction with neighboring crops and exploit the surface layers of the soil (Schenk and Jackson 2002). In legumes more generally, an increased root density leads to greater nitrogen release through rhizodeposition (Thilakarathna et al. 2016). A field study revealed, that at two years, pigeon pea roots could reach up to 4 m laterally in the Androy region of Madagascar (Rakotozafy et al. 2024). These observations were not in line with the findings of Ito et al. (1996) who found that the roots of pigeon pea spread more vertically than horizontally in semi-arid conditions. This discrepancy might be due to the soil characteristics in our study area with a rocky layer found at a depth of 150 cm (Rakotozafy et al., personal communication, 2024). However, our results highlight that the distance of 6–8 m between rows of pigeon pea, as adopted by farmers in the Ambovombe region allows effective N transfer from this NFS to cassava.

4.3 Nitrogen input to the system

At two years after planting, N input was at most 17.1 kg N ha^{-1} in pure pigeon pea plots and 8.5 kg N ha^{-1} in mixed pigeon pea-cassava plots, which was consistent with the respective planting density between the systems. Keston et al (2017) also found higher N inputs through biological N fixation in pure crops (85.7 kg N ha^{-1}) than in mixed pigeon pea–cowpea crops (57.4 kg N ha^{-1}) after one growing season in Central Malawi. Similarly, Mhango et al (2017) reported greater nitrogen fixation in pure pigeon pea crop (32 kg N ha^{-1}) than in mixed pigeon pea–groundnut crop (15 kg N ha^{-1}) during a dry year in Northern Malawi. Berriel and Perdomo (2023) also observed high N input through biological nitrogen fixation of pigeon pea used as a cover crop in Uruguay (253 kg N ha^{-1}). In contrast, our study showed relatively lower values of N input, may be due to harsh ecological conditions and soil constraints. Both studies reported higher levels of available P in the topsoil (0–15 cm depth) than those observed at our site, where low P availability may have limited symbiotic fixation capacity. However, the nitrogen contribution is non negligeable considering that there is no fertilizers applied by the farmers in this region (Violas et al. 2018). Mapfumo et al (1999) reported similar findings in pigeon pea, further supporting this observation.

5. Conclusion

This study provides key insights into nitrogen fixation by pigeon pea as well as into nitrogen transfer dynamics to the associated crop. Our results showed that 2-year old pigeon pea largely relied on biological nitrogen fixation, regardless of the cropping system, which is of great importance in soils with high N deficiency. The high %Ndfa observed aligns with previous findings in semi-arid regions, reinforcing the role of pigeon pea as a highly efficient N-fixing species. These findings highlight the potential of agroecological blocks using pigeon pea to maintain nitrogen supply through biological fixation under semi-arid conditions and without external fertilization, thereby contributing to the resilience of low-input farming systems.

Overall, our work highlights the potential of pigeon pea as a sustainable nitrogen source in unfertilized agroecosystems. However, further studies are needed to assess seasonal variations in N_2 fixation and long-term effects on soil fertility and crop productivity. In addition, research should focus on identifying pigeon pea varieties best suited to semi-arid region with sandy soil, and on the use of phosphorus fertilizers to improve the N_2 fixation efficiency of this NFS.

Declarations

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Conflicts of interest

The authors declare no competing interests

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HAJASOA Mosa Redida: Investigation

BOUILLET Jean-Pierre : Conceptualization, methodology, formal analysis, writing original draft

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Figures

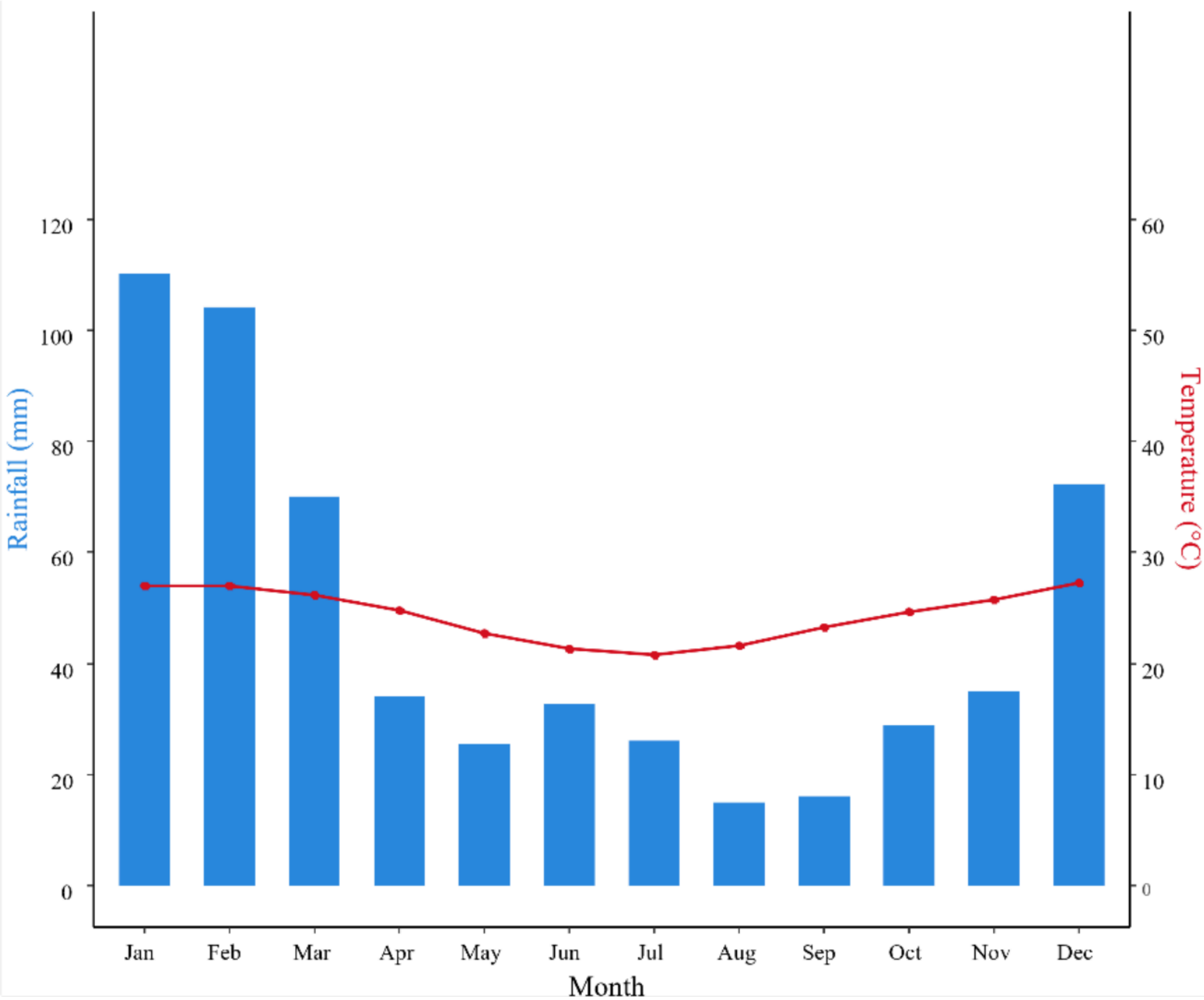

Figure 1

Rainfall (vertical bars) and mean temperature (circle) in Ambovombe from 2014 to 2024 (data source https://www.historique-meteo.net).

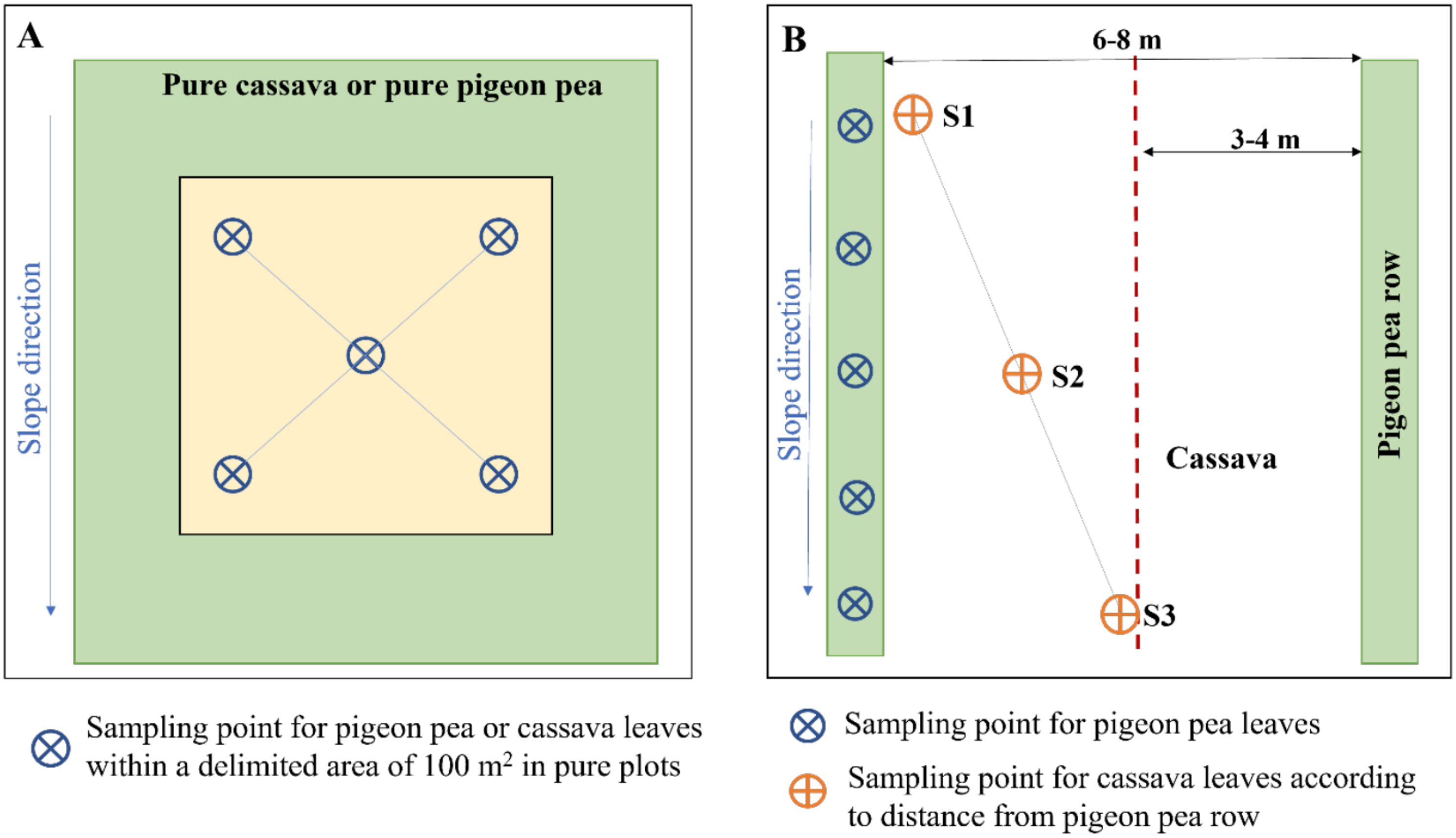

Figure 2

Leaf sampling method in plots for nitrogen analysis. **A)** Sampling point for leaves in the pure cassava or in pure pigeon pea plots. **B)** Sampling point for leaves in the mixed pigeon pea-cassava plots. S1: near pigeon pea, S2: ¼ of the inter-row, and S3: ½ of the inter-row