Nitrogen fixation in *Vigna unguiculata* (L.) Walp. in Senegal is affected by organic input and variety.

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Abstract:

Legumes such as cowpea (*Vigna unguiculata*), significantly contribute to sustainable agriculture by offering economic, ecological, and nutritional advantages. A key mechanism behind these advantages is their capacity to fix atmospheric nitrogen through symbiosis with soil bacteria. This study quantified the nitrogen fixation of a local and a research-derived cowpea variety intercropped with millet across six farmers' fields. These fields presented contrasting histories of farmyard manure application. N fixation was assessed using the natural abundance of 15N method. Interestingly, while the local cowpea variety obtained a smaller proportion of its nitrogen from the atmosphere (61%), it fixed a considerably larger amount of nitrogen (40 kg ha^{-1}) and assimilated more total nitrogen (72 kg ha^{-1}) particularly in amended fields. The substantial increase in nitrogen uptake per unit area, driven by significantly higher biomass production of the local variety in amended fields, was associated with a trade-off: the cowpea competed strongly with the intercropped millet, likely for the limited soil water resources. We concluded that organic input and cowpea variety influenced cowpea nitrogen fixation in these agroecosystems. Farmers typically sow the local cowpea variety at lower density than tested here, probably to minimize its competition with millet. The selection of cowpea varieties should therefore balance the goal of limiting cowpea-millet competition against maximizing cowpea grain and fodder yields.

Keywords: Biological nitrogen fixation, legume, ^{15}N natural abundance, sub-Saharan Africa, homefields, outfields, variety, millet-cowpea intercropping

1. Introduction

Legumes are the main cereal diversification crops in sub-Saharan Africa. They have both economic and ecological advantages. Harvests are used for human diet, livestock feed and farmers' income. The symbiotic nitrogen-fixing capacity of a legume such as cowpea in sub-Saharan Africa can account for up to 96% of its N requirements (Namatsheve et al., 2020). By rapidly covering the soil, creeping varieties reduce the risk of soil erosion and weed development (Kocira et al., 2020; Zougmore et al., 2000). Legumes may enhance the soil's fungal flora, particularly mycorrhizae, and their services in terms of phosphorus and soil water use efficiency (Tang et al., 2022; Alvey et al., 2001). Legumes may also play a role in the natural regulation of pests on cereals grown in rotation or in intercropping with them (Franke et al., 2018).

However, just cultivating legumes is insufficient to ensure the implementation of ecological services. The symbiotic nitrogen fixation of legumes and their contribution to nitrogen recycling in agrosystems could be significantly influenced by various factors and practices, as minor element content of soils (Bonilla & Bolaños 2009), nitrogen and phosphate fertilization (Franke et al, 2018), liming (Costa et al., 2023), intercropping (Namatsheve et al., 2020; Binacchi et al., 2022), disease management (Rathjen et al., 2020), ploughing and crop residue management (Franke et al., 2018; Binacchi et al., 2022), soil moisture conditions (Munjonji et al., 2018), legume species and variety (Fall et al., 2003; Bado et al., 2018). Therefore, understanding the specific factors that limit nitrogen fixation in legumes within each field is crucial for maximizing this process.

In Senegal, cowpea (*Vigna unguiculata*) is a fast-growing crop (FAOSTAT, 2023)), often grown in intercropping with millet, under rainfed conditions and using few inputs (Mbengue et al., 2024). Available results show that this legume is more resistant to drought (Halilou et al., 2015) and has greater nitrogen-fixing activity than groundnuts (Bado et al., 2007; Hamidou et al., 2018). Of the factors likely to affect symbiotic nitrogen fixation of cowpea in the Senegalese groundnut basin, soil fertility is probably one of the most important. Differences in soil fertility levels are largely linked to the heterogeneity of organic fertilization practices (Tounkara et al., 2020). While homefields receive manure almost every year or two, outfields generally do not. Another major factor influencing the symbiotic nitrogen fixation of cowpea could be the varieties (Fall et al., 2003; Bado et al., 2018). Breeding programs have created shorter-cycle varieties, but research has yet to fully assess their symbiotic nitrogen fixation under farmers' fields conditions and against local varieties. This lack of data hinders the ability to provide farmers with relevant varietal advice.

Many methods allow for the quantitative assessment of biological nitrogen fixation in legumes (Herridge et al., 2008). Of all these methods, the natural ^{15}N abundance method attracts particular attention. This method is based on the differences in natural ^{15}N abundance between N in the atmosphere and in the soil. It is therefore easier to implement than the more precise ^{15}N marking method, as it does not require human intervention to supply the marked N. It also has the advantage of being able to incorporate a large part of the crop cycle into the assessment (Herridge et al., 2008; Bado et al., 2018). This method has already been used successfully in the context of cowpea cultivation in the sub-Saharan zone (Namatsheve et al., 2020).

The aim of this article is 1) to quantify nitrogen fixation and cowpea yield in a network of farmers' plots using the natural abundance method for ^{15}N, and 2) to analyse the variability in the quantities of N fixed as a function of organic fertilization management (frequency of farmyard manure application over the previous decade) and cowpea varieties. The study was carried out in the context of cowpea crops grown in intercropping with millet, as practiced more generally in the study region.

2. Materials and methods

2.1. Presentation of the experimental site

The study was conducted during the 2016 rainy season in Diohine, located in west-central Senegal (14°30'4'' N; 16°30'10'' W). The climate is Sahelo-Sudanian, with a long dry season lasting eight months, from November to June, and a short rainy season lasting four months, from July to October. In 2016, rainfall of 593 mm was close to the 20-year average. The soils are arenosols. They are sandy, with a slightly acid pH and low cation exchange capacity (CEC). Tounkara et al. (2020) showed that differences in organic management between plots cultivated with millet and cowpea had significant effects on soil pH, carbon, nitrogen and major mineral element content, and soil bulk density.

2.2. Experimental set-up

In June 2016, 3 homefields and 3 outfields were selected. All the selected homefields had received at least about 10 t ha^{-1} each year over the past 10 years of farmyard manure spread on the soil surface just before sowing. The outfields, on the other hand, have never received organic amendments during this period. Farmyard manure is a mixture of various organic residues available on farms, and is mainly made up of livestock droppings (cattle, sheep, horses) millet and legume residues, and ashes from cooking fires. All the fields selected were less than 500 m apart. They had the same soil type (arenosols) and a similar microclimate. These 6 fields were part of a network of 12, for which Mbengue et al. (2024) provided a detailed map showing their locations within the studied landscape. The differences in soil fertility were not of pedological origin but essentially related to differences in organic fertilization management in recent years. Soil analyses carried out on the 0-10 cm soil horizon in June 2016 (before sowing) revealed significantly higher levels of pH, assimilable phosphorus, total N and total C in the homefields than in the outfields in this network, with values of 6.72, 484 mg kg $^{-1}$, 0.27 % and 0.02 % respectively in the homefields and 5.56, 3.74 mg kg $^{-1}$, 0.12 % and 0.01 % in the outfields (Mbengue et al., 2024).

The experimental set-up was treated as split-plot design: main plots being organic fertilization management (3 homefields and 3 outfields), and the sub-plots being the cowpea varieties grown in association with millet (the local variety: “Baye Ngagne”, and the research-derived variety: “Melakh”). All seeds of each variety originated from a single batch. Each sub-plot (two per field) had a surface area of 36 m^2. The local cowpea variety "Baye Ngagne" is a creeping to semi-creeping variety, with flowering staggered between 40 and 70 days after sowing, and a cycle length varying between 80 and 110 days according to the farmers. Farmers describe it as a dual-purpose variety, with the straw and husk used as feed for livestock on the farm, and the grain contributing to the family diet and farm income. This local variety is also described as having an early flowering compared with the other long-cycle local varieties present in the region. This variety is also said to have good resistance to drought and to the insects and diseases most commonly affecting cowpeas in the region. "Melakh" is a semi-erect variety selected at the Bambey research station and described in the official catalog of released varieties (ECOWAS, 2017), as having its flowering at 45 days, a cycle length of 52-61 days after sowing and a potential yield of 1 t ha^{-1}.

Each subplot was part of a field cultivated by millet/local cowpea in intercropping. In all the fields, millet was sown in the same geometry as in monocropping, i.e. 12,000 hills ha^{-1}, while the cowpea was intercropped in millet rows, one hill in two. The cowpea density was therefore 6,000 hills ha^{-1}, corresponding to 60% of the cowpea density that would be used in monocropping in the region (Figure 1). Millet was sown before the onset of the rains on 20 June 2016 in all treatments, and just before manure was applied in the homefields. A single millet variety was grown everywhere: Souna III, a variety provided by the Bambey research station by recombining 8 lines, and characterized by its short 90-day cycle, low sensitivity to photoperiod and potential yield of 3 t ha^{-1}. Except for the application of manure at an average rate of 10 t ha^{-1} before sowing, all the homefields and outfields were managed in the same way during the study. None was ploughed before sowing, nor received mineral fertilizers, irrigation or pesticides. The millet sowing density was typical of the association practiced in the region, but the cowpea sowing density was multiplied by three compared with what is more generally done on farmers' plots in this region. A plot consisting of 8 lines of millet without cowpea was set up at the edge of each field. Their natural abundance values in ^{15}N were used as a reference for calculating percentages of nitrogen fixed symbiotically by the legume (% Ndfa).

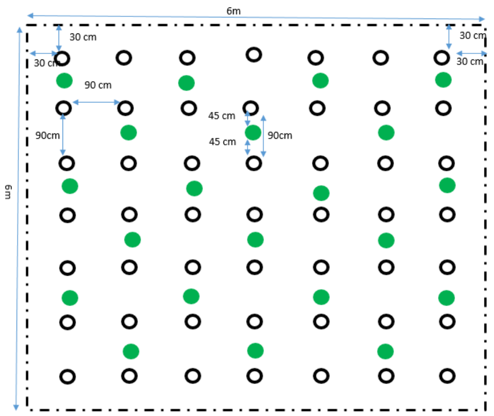

Figure 1: Arrangement of the millet/cowpea intercropping. For millet, the same density as for monocropping is maintained, while cowpea is sown at 60% of the monocrop density in the region.

Cowpea ● Millet ○

Millet/cowpea crop intercropping in farmers' plots in the village of Diohine, Senegal (Photo credit M. Mbengue)

A total of 36 samples of above-ground biomass of cowpea plants (18 in homefields and 18 in outfields) and 36 samples of above-ground biomass of millet plants grown in association with cowpea (18 in homefields and 18 in outfields) were analyzed. Three plants were selected from each subplot as representative of crop growth at subplot level. One sample consisted of the above-ground biomass of a single plant. These samples were taken as soon as the cowpea crop had reached the stage where 50% of the plants in the sub-plot had flowered. The two varieties reached this stage at almost the same time and were therefore sampled on the same day. The samples were oven-dried at 65°C for 72 hours and crushed at the “Laboratoire Mixte International: Intensification Ecologique des sols cultivés en Afrique de l'Ouest” (LMI IESOL) in Dakar. The $^{15}N/^{14}N$ isotope ratio, natural ^{15}N abundance (‰) and %N of cowpea plant and the reference plant (millet) were analyzed at the Laboratoire de Biochimie et Physiologie Moléculaire des Plantes of INRAE in Montpellier, France, using an EA Eurovector autoanalyser and the Isoprime mass spectrometer. Tin capsules were filled with 3 mg of plant material each, then carefully folded and packaged for analysis. The following variables were calculated:

The 15N abundance expressed in delta notation ($\delta^{15}N$), using the formula below:

$$\delta 15\text{N ‰} = \frac{(1000 \text{ x R ech \%} \; - \text{R standard})}{\text{R standard}}$$

where R ech denotes the proportion of ^{15}N in the experimental sample, and R standard the proportion of ($^{15)}N$ in the atmosphere (0.3663%).

The percentage of atmospheric nitrogen fixed by the legume (% Ndfa: N derived from atmosphere). This was calculated following the example of Binacchi et al (2022) and Naab et al (2008), using cereals (millet) as the reference plant:

$$\text{Ndfa \%} = \frac{100 \text{ x } \delta 15\text{N reference plant} \; - \; \delta 15\text{N legume}}{\delta 15\text{N reference plant} \; - \; \beta}$$

where δ15N reference plant and δ15N legume are respectively the natural abundance of non-atmospheric N-fixing plant (millet) and atmospheric N-fixing plant (cowpea), and β the δ15N of the N-fixing plant fixing 100% of air N. The value of -1.7 was chosen for β following the example of Naab et al. (2008).

Total nitrogen accumulated by crops at flowering (kg ha^{-1})

The total nitrogen accumulated by cowpea and millet at flowering (total N) was calculated using the following equation:

$$\text{total N} = \left(\frac{\text{N cap x Ba}}{\text{M cap}}\right) \text{x P hect}$$

where N cap is the percentage of nitrogen in the encapsulated sample, Ba, the above-ground biomass of an average plant at flowering, M cap, the mass of the encapsulated sample, P hect, the number of plants per hectare. We assumed that P hect was equal to the cowpea and millet planting densities that we had initially selected for the subplots (Figure 1).

Amount of nitrogen fixed by cowpeas at flowering stage (kg N ha^{-1})

The quantities of nitrogen fixed by cowpea at flowering were calculated by relating the proportions of ^{15}N from the atmosphere (Ndfa in %) to the total accumulated nitrogen (in kg ha^{-1}).

Fixed N = Ndfa (%) x Total N (kg N ha^{-1})

The number of cowpea nodules at flowering stage

On each subplot at the flowering stage, the 3 cowpea plants taken for ^{15}N concentration measurements were also used to count the nodules present on their roots. Cowpea roots were sampled from a radius of 50 cm and a depth of 50 cm using a spade.

Grain yields of cowpea and millet at the end of the cycle

Cowpea grains were harvested 57 days after sowing for the improved variety, and twice up to 75 days after sowing for the local variety. Millet grain was harvested 85 days after germination. Cowpea grain yields were determined in a yield square measuring 10 m^2 in area at the center of each subplot. In each yield square, we had 6 cowpea and 12 millet hills. The cowpea grain harvests were oven-dried at 65°C for 72 hours to determine their dry matter yields. Grain yields of millet combined with cowpea were measured at the end of the millet cycle after the same oven drying as for cowpea.

2.4 Data analysis

The effects of organic fertilization management (homefields or outfields) and cowpea varieties (local or selected) on nitrogen fixation and yields were analyzed by ANOVA using Statistica software, version 8, and the means were compared using a Tukey test with a threshold of 5%.

3. Results

3.1. Variation in nitrogen fixation by cowpea at flowering as a function of organic management and the variety grown

Cowpea Ndfa values ranged from 61 to 80%, with significantly higher values in fields not amended with manure (outfields) and for the selected variety (Melakh). However, expressed per unit area, the amount of atmospheric nitrogen fixed by cowpea was significantly higher in manure-amended fields (homefields) and for the local variety (Baye Ngagne). For the same variety, the homefields fixed 8 to 10 times more nitrogen than the outfields (Table 1). The local cowpea variety fixed 2 times more nitrogen (expressed in kg ha^{-1}) than the improved variety in the homefield and 4 times more in the outfields (Table 1). A significant interaction was found between organic fertilization and cowpea variety regarding the quantities of N fixed by the cowpea, indicating that the superiority of the local variety increased when the soil was poor. Cowpea nodulation was significantly increased in the homefields, but this difference was not significant (P> 0.05). The variety effect was not significant for the number of nodules.

Figure 2 shows the respective contributions of soil N and atmospheric N to N uptake by cowpea and millet in the treatments. In the homefields, the local variety cowpea fixed 40.5 kg ha^{-1} of N from the atmosphere and assimilated a total of 71.8 kg ha^{-1}. It therefore absorbed 31 kg ha^{-1} of mineral N from the soil. The total quantities of N fixed and assimilated by the selected cowpea variety in the homefields were significantly lower. In the outfields, the quantities of N fixed and assimilated by the cowpea were very low compared with the homefields (Figure 2).

Table 1: Variation in cowpea and millet performance according to the technical options tested**.** Values in brackets are standard deviations of means. Homefields are regularly amended with manure and outfields rarely amended over the last decade *: P≤0.05; **: P≤0.01; ***: P≤0.001; ns: P> 0.05

Variables measured	Local cowpea / Homefields	Selected cowpea / Homefields	Local cowpea/ Outfields	Selected cowpea / Out fields	Significance of P-values		
					Field type	Variety	Field type * Variety
δ^{15}N Cowpea (‰)	4.5 (0.7)	3.6 (0.6)	3.1 (0.8)	1.4 (0.4)	**	*	ns
N % Cowpea (%)	2.53 (0.12)	2.60 (0.20)	3.12 (0.30)	3.13 (0.21)	**	ns	ns
Cowpea biomass at flowering (kg ha^{-1})	3158 (858)	1053 (286)	195 (23)	65 (8)	***	*	*
Total N Cowpea at flowering (kg ha^{-1})	71.8 (16)	27.4 (8.2)	5.9 (0.7)	2 (0.3)	***	*	*
Ndfa Cowpea at flowering (%)	61.1 (4.4)	66.2 (3.9)	69.8 (4.8)	80.1 (2.3)	**	*	ns
N Cowpea from symbiotic fixation (kg ha^{-1})	40.5 (7.7)	16.3 (4.2)	4.2 (0.7)	1.6 (0.2)	***	**	*
N % Millet at flowering (%)	0.50 (0.04)	0.80 (0.03)	0.53 (0.04)	0.88 (0.10)	ns	***	ns
Biomass Millet at flowering (kg ha^{-1})	6975 (888)	7091 (710)	3321 (627)	3506 (768)	***	ns	ns
Total N Millet at flowering (kg ha^{-1})	38.3 (8.3)	56.3 (5.8)	18.6 (4)	25.8 (3.2)	***	*	ns
Number of nodules per plant at flowering cowpea	15 (4)	16 (2)	11 (2)	12 (2)	ns	ns	ns
Cowpea grain yield at the end of the cycle (kg ha^{-1})	651 (51)	279 (16)	142 (19)	51 (2)	***	***	***
Millet grain yield at end of cycle (kg ha^{-1})	793 (91)	927 (68)	678 (117)	530 (111)	*	ns	ns

3.2. Variation in cowpea grain yield (kg ha^{-1}) as a function of organic input and the variety grown

Grain yields measured at harvest were significantly higher in homefields and for the local variety. The variety effect on this variable was only significant in homefields (Table 1). Cowpea straw yields at harvest were not measured, but straw yields measured at flowering showed the same trends as grain yields at harvest.

3.3. Variation in nitrogen uptake and yield of millet associated with cowpea as a function of organic input and the variety grown

Millet associated with cowpea in homefields assimilated significantly more nitrogen than in outfields. It took up more nitrogen when combined with the research-derived variety than when combined with the local variety (Figure 2). The same trend was found in both homefields and outfields. Grain yields of millet at harvest were significantly higher in homefields than in outfields. They were higher in the intercropping with the selected variety than with the local variety, but the differences were not statistically significant (P>0.05).

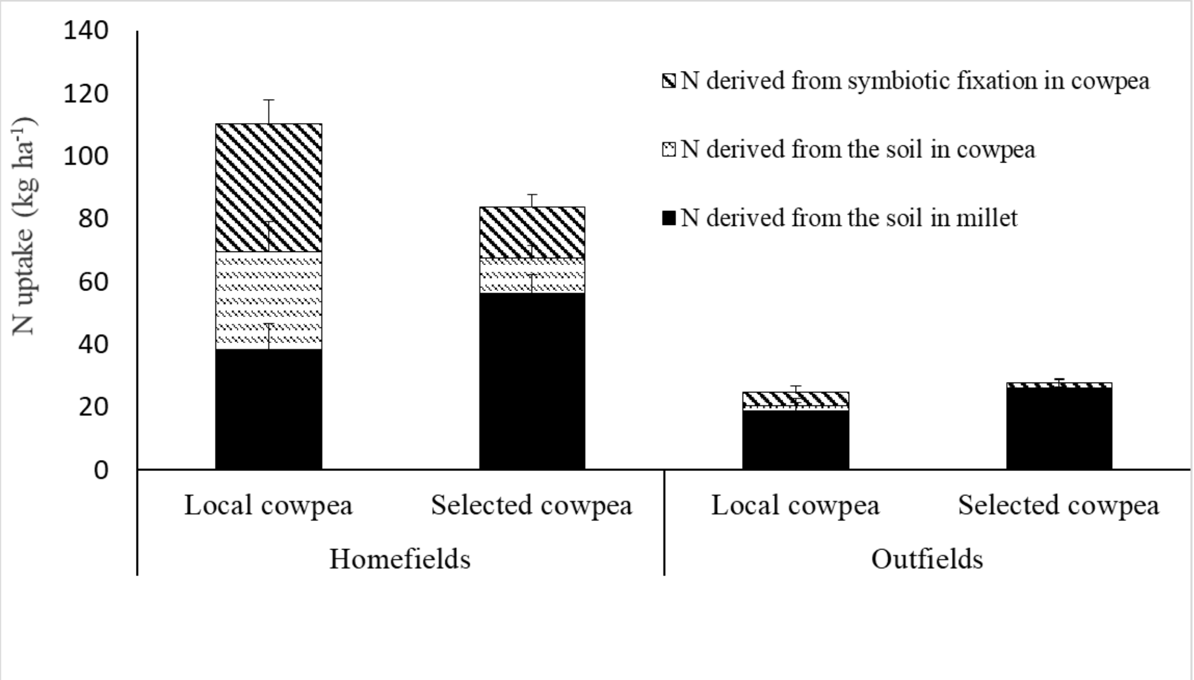

Figure 2: Quantities of nitrogen (N) either from the soil (for cowpea and millet) or from symbiotic fixation (for cowpea), the two species being grown in association. Local cowpea indicates the local creeping cowpea variety (*Baye Ngagne*) and Selected cowpea indicates the research-derived short-cycle cowpea variety (*Melakh*). The homefields are regularly amended with manure and the outfields have rarely been amended over the last decade.

4. Discussion

Cowpea nodulation was much lower than that obtained under controlled conditions by Fall et al. (2003) for the research-derived "Melakh" variety. This suggests that the growing conditions in farmers' plots may have been less favorable to nodulation than in the greenhouse trials considered by these authors. As indicated by the % Ndfa values, cowpea consistently met a substantial portion of its nitrogen needs through symbiotic fixation across all studied conditions. The significant increases in % Ndfa values for cowpea in the outfields (not amended with manure) and with the Melakh variety could be justified by the greater competition for soil nitrogen exerted by the intercropped millet on cowpea crops in these situations (Sánchez-Navarro et al., 2024).

The increase in cowpea N fixation (expressed in kg ha^{-1}) in homefields was not associated with higher values of % Ndfa in homefields than in outfields. Our results indicate that this is mainly due to higher cowpea biomass production per unit area in homefields. The increase in cowpea biomass production due to manure input in homefields is consistent with references showing that organic fertilization plays a fundamental role in the productivity of the agroecosystems studied: it is the main source of essential nutrients for crops (Tounkara et al., 2020), it improves soil structure and water retention capacity (Tounkara et al., 2020; Liu et al., 2024), and through the development of soil life and diversity, it promotes the natural control of crop pests (Mbengue et al., 2024).

Compared with the selected cowpea variety, the local variety significantly increased the nitrogen fixation of cowpea (expressed in kg ha^{-1}). As in the case of the homefields, this result is mainly attributable to the higher cowpea biomass production of the local variety. A major reason for the higher biomass production of the local variety could be that it is better adapted to the many biophysical constraints of these agroecosystems. For example, its creeping habit, staggered flowering and root characteristics (spatial distribution, root length density, exudates) could have led to greater water, nutrient and light efficiency in association with millet. This hypothesis is in line with farmers who describe this variety as 'elastic', in reference to its tolerance to water stress. It is also in line with Casañas et al (2017), who stress that local varieties, with their inherent genetic diversity shaped by natural selection, are likely well-suited to the specific soil and climate conditions of their region. Furthermore, farmers' own selection practices would have adapted them to better meet individual needs and preferences. The local variety 'Baye gagne' deserve to be evaluated in greater depth in future research, and could be of interest to varietal improvement programs run by breeders.

The choice of cowpea variety has a significant impact on cowpea competition with millet grown in intercropping. The local variety, which is more productive in terms of biomass, exerts greater competitive pressure on millet than the selected variety. These results suggest that cowpea sowing density played a crucial role in the dynamic of competition between local cowpea and millet. To optimize the production of both crops while favoring biomass production and nitrogen fixation by cowpea, it is likely that the sowing density of local cowpea needs to be adjusted downwards. This hypothesis aligns with local practices where farmers reduce the sowing density of the local cowpea in intercropping (which is about three times higher in this study than typical local rates) to minimize the cowpea competition and favor millet growth.

The variability in competitive relationships between millet and cowpea, depending on cowpea varieties, suggests that the most interesting varieties for growing in pure stands may not be the same for intercropping systems. Traditional trials, focused on pure crop stands, overlook interspecies interactions and farmers' motivations regarding both varietal choice and sowing density. Here, the dual use of cowpea (fodder and grain) likely motivated the choice of the local variety. Moreover, farmers' prioritization of millet probably leads them to cultivate cowpea at lower sowing densities than we tested. This study highlights the limitations of a reductionist approach to varietal evaluation, particularly in a context of intercropping and environmental and socioeconomic constraints.

5. Conclusion

Organic fertilization and varieties are two levers that can be used to improve nitrogen fixation by cowpeas grown in intercropping with millet. The local variety produces more biomass, enabling it to fix about three times more nitrogen than the research-derived variety, but this high biomass production is associated with significant competition from the local cowpea on the intercropped millet. With a view to reducing this competition farmers generally sow cowpea at a density three times lower than in our study. The local variety 'Baye Ngagne' appears to be a genetic resource particularly suited to this production context. The results of this study highlight the need to co-construct cowpea variety evaluation protocols with farmers, in order to integrate a wider range of criteria and better meet farmers’ needs.

Ethics

The authors declare that the experiments were carried out in compliance with the applicable national regulations.

Declaration on the availability of data and models

The data supporting the results presented in this article are available on request from the author of the article.

Declaration on Generative Artificial Intelligence and Artificial Intelligence Assisted Technologies in the Drafting Process.

The authors have used artificial intelligence-assisted technologies to translate from French to English.

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Declaration of interest

The authors declare that they have no conflict of interest in the publication of this article.

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