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Quantifying the Spatiotemporal Variations of Soil Nitrogen Fixation or Absorption from Soybean, Cotton, and Maize Planted Fields to Support Sustainable Agriculture Practices

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Abstract: Sustainable agricultural practices are pivotal for environmental preservation and enhancing crop quality. Understanding soil nutrient levels is crucial in determining appropriate fertilizer application in agriculture production systems. In the 2022/23 agricultural season, an experiment that aimed to investigate the spatial distribution of nitrogen (N_2) fixation or absorption in fields cultivated with diverse crops was carried out in Mozambique. Three experimental fields were established, and the following crops were used—maize (local variety), soybean (SAN-BEIB variety), and cotton (ALBAR SZ9314 variety)—each measuring 83.35 m × 30 m. A sampling grid of 13.9 m × 10 m facilitated the collection of 24 composite soil samples per field, consisting of 5 sub-samples within 12 cells, taken at a depth of 0.0–0.20 m before planting and after harvesting, totaling 12 samples per period per field. Laboratory analysis employed the Kjeldahl method to determine total soil nitrogen levels. The Inverse Distance Weighted (IDW) method was used for mapping the spatial total soil nitrogen distribution. The results revealed distinct total soil nitrogen credit and debit patterns. Variations were notable between pre-planting and post-harvest analyses in maize and cotton, showcasing high absorption and minimal fixation. Contrary to expectations, soybeans exhibited high absorption and low fixation, challenging the determination of optimal crop rotation intervals. Quantitative results identified specific total soil nitrogen debit efficiencies of approximately 1692.29 kg ha^{-1} in cotton and 1081.5 kg ha^{-1} in maize, respectively, and a credit of 459.215 kg ha^{-1} in soybeans. Despite discrepancies, this study serves as a foundational platform for future research. As the findings of this study advocate for continued crop rotation practices to bolster soil health and enhance nutrient utilization, it provides the first novel insights into nitrogen dynamics of global key crops in Mozambique, revealing significant variations in nitrogen fixation and absorption across different crop types and fields, which is crucial for informing tailored agricultural practices and soil fertility management strategies.

Keywords: variable application rate; N spatial distribution; plant size; seed vigor; spatial distribution

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1. Introduction

In developing countries among the Sub-Saharan African Countries, about 90% of staple food production is predominantly under rainfed agriculture systems [1], in which spatial and temporal variations in the climate patterns result in significant decreases in crop productivity and production outputs [1,2]. The reduced crop production outputs will affect food security in these countries, resulting in exposure of the growing population with high vulnerability to food insecurity [3]. According to the Food and Agriculture Organization (FAO) of the United Nations (UN), the world population is projected to be 9.7 billion people by 2050 [4], with 63.04 million of these people living in Mozambique [5]. As of October 2022, the worldwide Global Hunger Index (GHI) was at a score of 18.2, which is moderate. However, for Mozambique, the Hunger Index score was classified as serious (GHI between 20 and 34.9) [6], with approximately 1.9 million people falling under high levels of acute food insecurity [7]. To feed the growing population and contribute to the achievement of the "Zero hunger" target of the UN Sustainable Development Goal (UN SDG) [8], an increase in total food production is required [4].

To increase food production, both the expansion of existing cropland and the intensification of agricultural practices on current farmland have had significant impacts [9]. However, the expansion of cropland areas is classified as an unsustainable practice to increase food production as it will lead to deforestation, loss of wildlife habitat, land degradation, and greenhouse gas (GHG) emissions [10], negatively impacting the provision of ecosystem services and goods. Thus, the intensification of the existing cropland is more sustainable than its expansion [11]. Li et al. (2014) [12] define cropping intensity as the number of cropping cycles in a specific region during a year. Despite the environmental controversy, intensive cropping plays a crucial role in meeting food demand [13], particularly in Africa, where limited land resources must support large populations [10,14]. However, the robust agriculture sector employs approximately two-thirds of the continent's workforce, contributes an average of 30% to 60% of the gross domestic product, and accounts for about 30% of export value. To achieve high annual production and productivity in this practice, a wide range of inputs—including capital, labor, pesticides, and chemical fertilizers—are utilized [15]. However, as noted by Kopittke et al. (2019) [16], this approach contributes to environmental challenges, such as agronomic land degradation.

Intensive cropping production is marked by various activities that contribute to soil degradation, such as alterations in the tillage process, the absence of continuous deep-rooting systems, limited crop functional diversity, and indiscriminate addition of chemical-based nutrients to the soil [17]. Studies suggest that intensive cropping requires an increase in soil organic matter (SOM). This can be achieved by minimizing tillage activities during land preparation and adopting integrated, multifunctional cropping rotations that incorporate forage legumes and/or small grains [18]. For instance, researchers [19–21] highlight the numerous benefits of incorporating legumes into integrated and multifunctional cropping rotations. This is because, in partnership with their associated microorganisms, legumes can fixate atmospheric nitrogen (N_2) into the soil, thereby increasing the amount of available nitrogen. The availability of N_2 is a critical factor that often limits crop productivity [22]. It is an essential component of nucleotides and proteins, it forms the backbone of chlorophyll, and it serves as one of the primary macronutrients required by plants [22]. Moreover, nitrogen in plants activates enzymes essential for primary metabolic processes, including protein synthesis, ion absorption, photosynthesis, respiration, cell multiplication, and differentiation [23]. Its absence in plants is one of the major factors limiting their growth and development [23]. Approximately 98% of the nitrogen in the soil exists in organic forms, while only about 2% is present in inorganic forms, such as ammonium (NH_4^+) and nitrate (NO_3^-), which are readily available to plants [24].

N_2 fixation, which involves a symbiotic relationship between plants and nitrogen-fixing bacteria, is the process of converting atmospheric N_2 into forms, such as NH_4^+ and NO_3^-, that plants can readily assimilate [25]. The nutrient requirements of different crops vary based on the nutrients they extract from the soil throughout their growth cycle [26]. The spatial distribution of the N_2 fixation rate, influenced by the location and agricultural season variations due to unexpected environmental changes or agricultural practices [27], significantly affects crop growth and development.

Both chemical and physical soil proprieties have been extensively mapped using Geographic Information System (GIS) techniques. Ref. [28] mapped the spatial variability of soil chemical properties in Mozambique's Cuamba district, which enabled the development of informed recommendations for suitable crop types in the region. In another study, researchers mapped soil Nitrate-N (NO_3^--N) distribution under a mixed-land use system to guide the N_2 application rate and to assess the environmental risk of NO_3^--N leaching into the groundwater [29]. In addition to its use in mapping soil properties, GIS also facilitates the creation of prescription maps, which provide recommendations for the Variable Application Rate (VAR) [30–32]. According to [33], VAR involves applying the appropriate rate of inputs based on the data generated by these prescription maps.

Although researchers have recommended crop rotation between legumes and cereals or other crops due to legumes' ability to host bacteria that fix N_2 into the soil [18,20,34], there is still limited understanding of the exact amount of nitrogen fixed or absorbed under different cropping conditions, particularly in Mozambique. Recognizing the importance of VAR practices, such as fertilizer application, this study conducted an experiment in Mozambique to map the spatial distribution of soil nitrogen fixation and absorption in fields planted with soybean, cotton, and maize. This study aimed to develop recommendations for crop rotation systems by analyzing N rates required for the growth of cotton and maize—both critical crops in Mozambique—based on their spatial distribution. This approach aims to assist farmers in managing their fields by optimizing the use of both organic nitrogen (N_2 from legumes) and chemical N_2 fertilizers, thereby enhancing productivity while minimizing environmental impacts.

2. Materials and Methods

2.1. Environmental Conditions

The study was conducted during the 2022/23 agricultural season in the Agriculture and Forest Interactions Practices section at the experimental farms of the Universidade Católica de Moçambique, Faculdade de Ciências Agronómicas (UCM FCA), in Cuamba District, Niassa Province, northern Mozambique (Figure 1). The area where the experiment was implemented was not under cultivation, and bare soil conditions had been kept for at least seven years. Therefore, the taxonomic composition of bacteria living in these soils was not affected by previous crops that could affect N fixation. The soil surface where these plots were established was flat, with a slope smaller than 1%. With an estimated area of 5.363 km^2 [35], the district of Cuamba shares borders with the districts of Metarica to the north, Mandimba in the northwest, Mecanhelas in the west, Mutuali in the east, and Gùrué in the south [35]. The region is classified based on soils, the climate, and cropping systems as Agroecological Zone 7, which corresponds to the mid-altitude areas of the Zambezia, Nampula, Tete, Niassa, and Cabo Delgado provinces [36].

In this region, the altitude varies from 200 m in the lower Zambezi River basin to 1000 m above the mean sea level, along with the high hill areas of the Tsangano and Angónia districts [37]. The study region experiences two distinct seasons: the rainy season, which lasts from October/November to March/April and coincides with the primary cropping season (accounting for over 80% of the total rainfall), and the dry season, which extends from April/May to September/October and marks the second cropping season. The annual rainfall for this region varies from 1000 to 1400 mm, with an annual average temperature reaching 26 °C [28,35,37]. During the study duration, the daily rainfall data revealed normal to above-normal rainfall patterns (Figure 2). Notably, on the 92nd day of the year, during a critical stage of crop development, the total rainfall reached 74 mm. This precipitation level exceeded the amount recorded during the same period in 2022 and surpassed both the 15-year maximum and the 15-year average for that timeframe. This substantial increase in rainfall during a crucial stage of crop development likely influenced the overall growth and maturation of the crops.

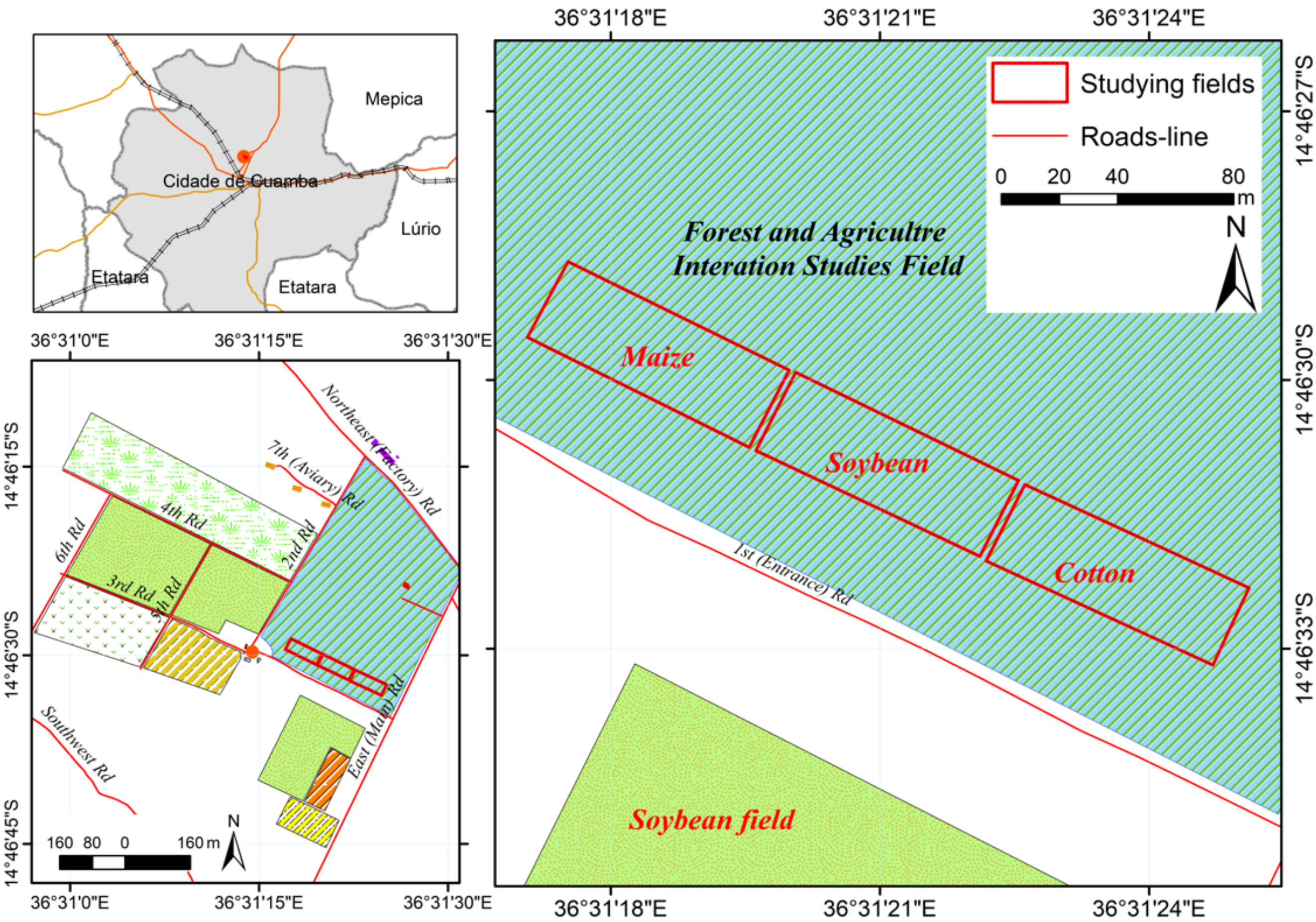

Figure 1. Geographic location of the study area in Cuamba District, Niassa Province, Mozambique.

In the Cuamba district, altitudes range from 200 to 500 m above sea level, and the landscape features undulating terrain occasionally interrupted by rocky formations [28]. This region is physiographically characterized by a low plateau area that gradually passes to a more dissected relief with steeper slopes, starting from the sub-plateau zone of transition to the lateral zone. These conditions, combined with the region's soil types—classified as Eutric Leptsols, Ferric Lixisols (also characteristic of the plot areas), and Cambic Arenosols—support the cultivation of different crops. The soil pH in the region ranges from 3.5 to 6.5, further influencing crop development. The cropping system is predominantly rainfed, with key crops including maize, sorghum, millet, cowpea, soybean, sesame, and groundnuts. In addition, cash crops, such as cotton and tobacco, are also produced, as shown in the crop calendar of major crops of the region (Figure 3). In addition to crop farming, farmers in the region also rely on livestock for food and income, raising animals like goats, chickens, ducks, pigs, sheep, and doves [38].

Upland soil collected from two sites across the study field, air-dried and sifted through a 2 mm mesh sieve, was used for the experiment. The soil texture was classified as clay loam (0–20 cm) and sandy clay (20–40 cm). At the start of the experiment, the surface soil (0–20 cm depth) exhibited the following characteristics: soil pH (H_2O), soil pH (K_2O), and electrical conductivity 1:2.5 (EC) of 7.02, 6.32, and 0.18 mS cm^{-1}, respectively. Total carbon (C) and N contents and the C:N ratio were 1.22, 0.196%, and 6.24, respectively. Available phosphate (Olsen method) was 9.40 ppm, with organic matter of 2.11%. The soil characteristics based on the soil profile survey are described in Table 1.

Table 1. Soil characteristics in the study region.

Soil Depth (cm)	Soil Texture (%)			pH		CEC ($cmol_c\ kg^{-1}$)	TC	TN	C/N
	Sand	Silt	Clay	H_2O	KCl		(%)		
00–20	38.30	22.10	39.60	7.02	6.32	13.26	1.22	0.196	6.24
20–40	51.30	13.10	35.50	6.76	5.54	11.27	0.06	0.007	9.22

CEC, cations exchange capacity; TC, total carbon; TN, total nitrogen; C/N, carbon/nitrogen ratio.

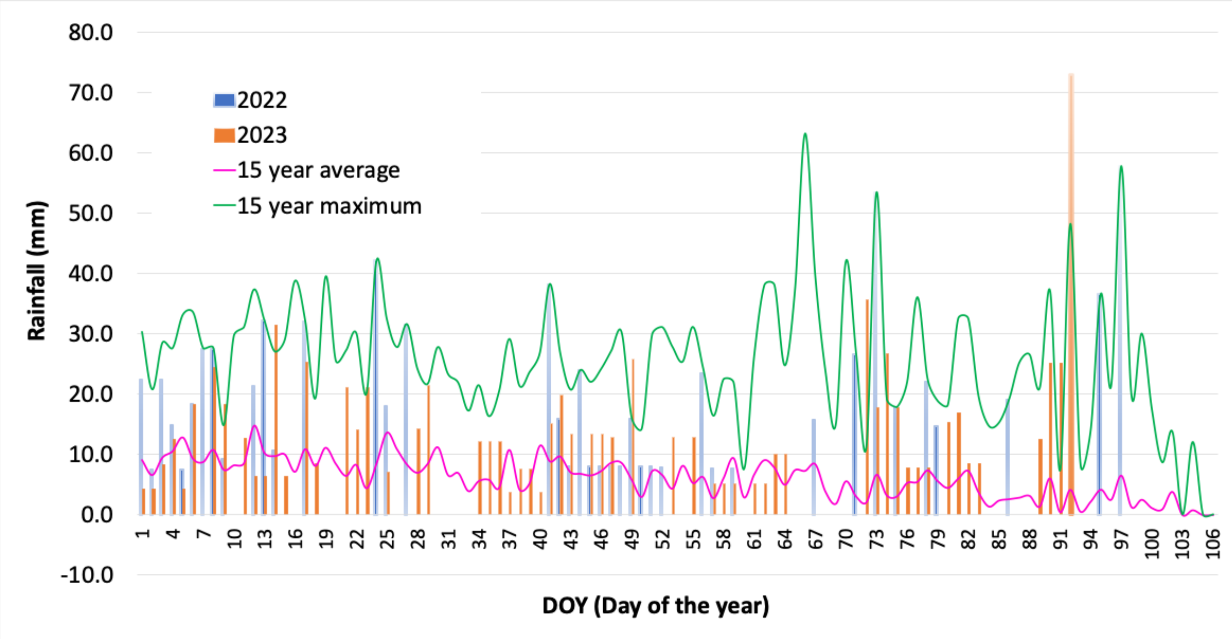

Figure 2. Rainfall profile over the study region from 1 January to 15 April 2023. Data from [39].

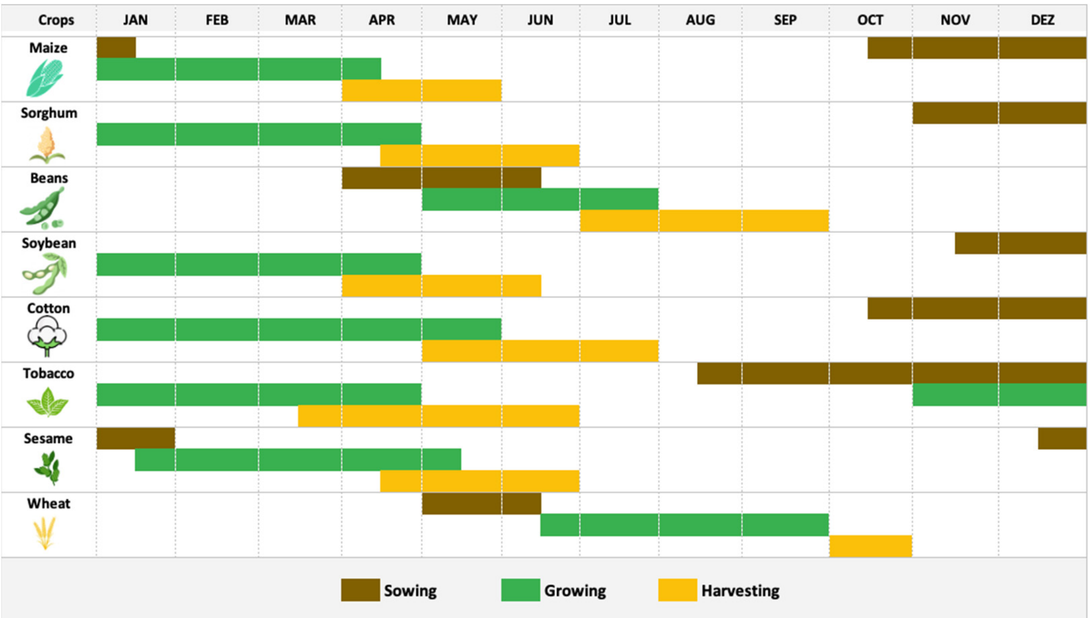

Figure 3. Crop calendar for major crops in the study region, adapted [36,40].

2.2. Seed Material and Germination Tests

For any cropping activity, the quality of the seed to be used is very important, as it can affect the stand establishment and plant population size, impacting therefore the potential yield [41,42]. For this experiment, three different crops (i.e., soybean, cotton, and maize) were studied. The seed material used herein was provided by local seed agencies and grain producers. The cotton (variety ALBAR SZ 9314), a lot of maize (local variety), and a lot of soybean seeds (local variety) were provided by the Group João Ferreira dos Santos, Sociedade Algodoeira do Niassa (JFS SAN), a company that promotes the cultivation of cotton and soybean; a second lot of soybean (variety San-Beib) was provided by the experimental agriculture department at the Universidade Católica de Moçambique, Faculdade de Ciências Agronómicas (UCM FCA); two lots of maize seed variety ZM521 (certified and non-certified) were provided by the Klein Karoo Seed Company. For the experiment, the soybean seeds were used without the application of any bacterial inoculant.

In the context of our research, we aimed to ensure uniform and robust plant establishment across the different treatments and conditions imposed in the study to reduce the impact of potential confounding factors. Therefore, before the establishment of the experiment and sowing, the seeds from different sources were submitted to laboratory tests to analyze the viability of using them in the experiment. The physical purity (PP) represents the number of pure seeds in a seed lot [43]. Based on the seed availability for tests, from each lot, 154.39 g of seed was used to separate pure and non-pure seeds. This information was then applied to Equation (1) to determine the PP. Apart from PP, other elements were also assessed. Germination capacity (GC) was determined to analyze the number of seeds that could germinate under the allocated environment in a seed lot [44–46]. The GC is also one of the elements used to determine the sowing rate, and it was calculated based on Equation (2). The speed of germination (SG) expresses the rate of germination in terms of the total number of seeds that germinate in a time interval (Equation (3)) [47]. Germination energy (GE) represents the percentage of normal seedlings found in the first counts of the germination power analysis [44,46], normally after three or four days after sowing (Equation (4)). The Mean Germination Time (MGT) measures the time it takes for the seed to germinate [46,47] (Equation (5)).

$$\text{PP } (\%) = \frac{\text{weight of pure seed}}{\text{Total seed weight}} \times 100 \quad (1)$$

$$\text{GC } (\%) = \frac{\text{Total germinated seeds}}{\text{Total number of seeds}} \times 100, \quad (2)$$

$$\text{SG} = \left(\frac{G_1}{n_1}\right) + \left(\frac{G_1}{n_1}\right) + \ldots\ldots \left(\frac{G_n}{n_n}\right) \quad (3)$$

where "G" corresponds to the total number of germinated seeds and "n" corresponds to the total number of days that the seed took to germinate.

$$\text{GE } (\%) = \frac{\text{Total germinated seeds after 4 days}}{\text{Total number of seeds tested}} \times 100 \quad (4)$$

$$\text{MGT} = \frac{\sum_{i=1}^{k} T_i \times N_i}{\sum_{i=1}^{k} N}, \quad (5)$$

where "T_i" is the time from the start of the experiment to the i-th interval, "N_i" is the number of seeds germinated in the i-th time interval (not the accumulated number, but the number corresponding to the i-th interval), and "k" is the total number of time intervals.

To determine the GC, SG, GE, and MGT, 165 grains of each crop type were used. These grains were subdivided into equal numbers of 15 and put in glass plates containing cotton, which was used to create the seed bed. After setting up the lab experiment, data of germinated seeds were recorded for 7 days. The seeds used in this experiment were selected based on the seed test results, in which PP, GC, SG, GE, and MTG were analyzed together for decision making.

Despite the cotton seeds having a physical purity of 98.9% and a mean time for germination of 36 h, their germination capacity was low (GC = 66.7%), which was somewhat influenced by their germination energy (GE = 66.8%). The two soybean lots had similar results in terms of physical purity, but the San-Beib variety had a higher germination capacity (GC = 84%) compared to the local variety. The San-Beib variety not only had a higher germination capacity but also higher results in all the other analyzed elements, with a greater emphasis on the speed of germination and germination energy, which were 81.5% and 80.0%, respectively. When comparing the three varieties of maize seed (i.e., ZM521 Certified, ZM521 Non-Certified, and Local), it was observed that the local variety performed better, with a GC of 77.6% and a mean time for germination of approximately 91 h.

Germination energy (GE) was one of the main criteria for seed selection due to its direct relevance to the overall vigor and performance of the plants in subsequent growth stages. Seeds with higher germination energy are generally associated with better establishment, faster emergence, and improved early growth compared to seeds with lower germination energy. By selecting seeds with high germination energy, we aimed to minimize potential variability attributed to variations in seed quality. This approach enhances the reliability of our findings, as it reduces the impact of confounding factors related to uneven seed germination and early seedling development. Additionally, the energy of seed germination can be an indicator of the seed's ability to mobilize stored nutrients and initiate essential physiological processes during early growth. This aligns with our interest in understanding the initial phases of plant development and how they may influence the subsequent accumulation of N_2 in the soil. Based on the seed vigor results, the following crop seed varieties were selected for planting: (1) cotton—variety ALBAR SZ 9314; (2) soybean—variety San-Beib; and (3) maize—local variety.

2.3. Plant Population Size and Crop Development Analysis

Because the plant population size (PPS) may influence the potential yield of any crop [41,42] as well as the total amount of nutrients that can be retrieved from soil [48], it is important to evaluate the plant population in the studying plots. For this study, three plots were established for the different crops (i.e., cotton, soybean, and maize). Each plot had an area of 2505 m^2 or 0.25 hectares (Figure 4A).

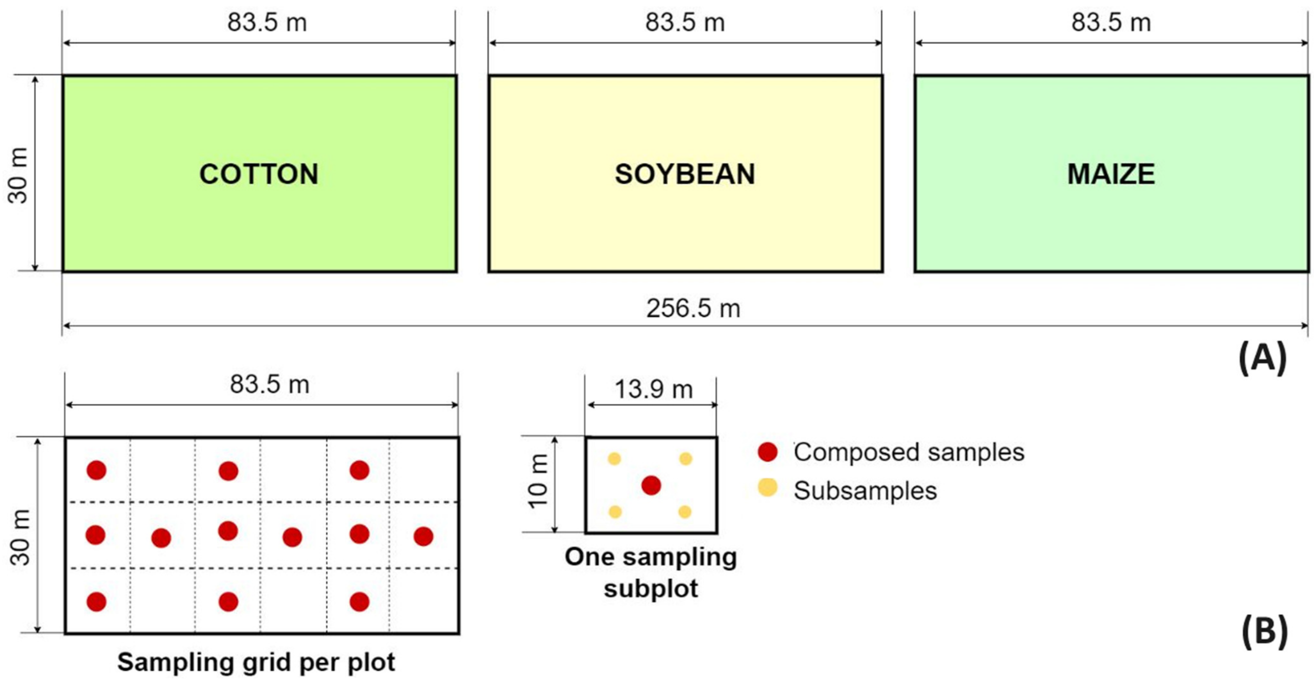

Figure 4. Field design (**A**) and sampling grids (**B**).

Based on the plant spacing for each crop (i.e., maize: 90 × 30 cm; soybean: 50 × 20 cm; and cotton: 80 × 20 cm), which was selected according to local farming practices, the plant population size was analyzed at three different periods: 15 days after sowing, 45 days after sowing, and two weeks before the harvesting period. For each period, the total number of plants within the usable area of each plot was counted. This number was then adjusted to represent the plant population per hectare using Equation (6).

$$\text{PPS (ha)} \frac{\text{cv} \times \text{N}}{\text{Plot area(ha)}} \tag{6}$$

where PPS corresponds to the plant population size, N corresponds to the total number of plants in the usable area in the plot, and cv is the correction value (in our study, 1 was used as the correction value).

In this study, alongside analyzing the plant population size across different periods, an assessment of crop development conditions was also performed. This involved analyzing the development of pests and diseases and implementing measures to prevent their spread throughout the fields to mitigate potential crop damage. An organic approach was employed to effectively manage pests and diseases. Organic approaches prioritize environmentally friendly and sustainable techniques, aiming to reduce reliance on synthetic chemicals and promote natural ecosystem balance [49]. By employing such methods, the study aimed to ensure the health and productivity of the crops while minimizing negative impacts on the surrounding environment. As such, our study relied on the use of garlic for pest and disease control thanks to its natural insecticidal properties (i.e., Allicin and Allin) [50,51], as it showed better results than other crops, such as ricinus and neem [52–55]. The yield assessment was conducted by measuring the harvested grain of maize and soybean, as well as the fiber of cotton, for each plot (in kilograms per plot). These measurements provided an initial yield value specific to each plot. To estimate the yield per hectare, the yield obtained per plot was converted to tons per hectare using Equation (7). This conversion was essential to standardize the results and to facilitate comparison across different plots and treatments. The conversion process involved adjusting the plot-specific yield values by a correction factor, which accounted for the plot size and enabled the extrapolation of the yield to a per-hectare basis.

$$\text{Yield}\left(\frac{\text{t}}{\text{ha}}\right) = \frac{\text{cv} \times \text{N}}{\text{Plot area}(sqm)}/1000 \tag{7}$$

where N corresponds to the total seed weight per plot and cv is the correction value. In our study, 10,000 m^2 (which corresponds to 1 hectare) was used as the correction value, and 1000 was used as the conversion value from kilograms per hectare to tons per hectare.

2.4. Soil Sampling Design and Data Collection

2.4.1. Soil Sampling Strategy

A grid-based soil sampling method [56,57] was used to collect soil samples for N_2 analysis. As the success of the grid soil sampling is dependent on several factors, including the grid size and the number of sampling points, we followed the recommendation of [58] for spatially explicit sampling for soil fertility analyses. The recommended approach involves collecting a composite sample within a grid ranging from 0.4 to 2.0 hectares in area, with sub-samples ranging from 8 to 10 samples. We adjusted these numbers based on the size of our fields, dividing them into 18 grids of equal size of 139 m^2 (10 m × 13.9 m). To ensure soil heterogeneity, we only used 12 of these grids and selected 5 sampling points within each grid (Figure 4B). Soil samples were collected in two different periods—before seeding and after harvesting—resulting in a total of 24 samples from each field. Using a soil auger, samples were taken to a depth of at least 20 cm [59,60]. Samples collected within each grid were combined to create a composite sample.

The geographic information (latitude, longitude, and altitude) for the central point of each grid was collected using the GVG (GIS, Camera, and GPS) mobile app [61]. This allowed for precise geolocation of each composite soil sample. The composite samples were then analyzed in the laboratory to extract the total amount of N_2 in the soil in the two periods. The grid soil sampling method offered a systematic approach to soil sampling, ensuring the collection of representative and unbiased samples. This technique has been widely used in various fields, including agriculture [57,62,63].

2.4.2. Soil Nitrogen Measurement

A total of 72 samples, collected from three different fields over two distinct periods, were submitted to the laboratory for total soil nitrogen analysis. Nitrogen exists in various chemical forms, including organic N_2 compounds, ammonium (NH_4^+), nitrate (NO_3^-), and other inorganic forms [64]. Total nitrogen (TN) analysis considers the entirety of these N_2 constituents. The method used for soil nitrogen analysis was the Kjeldahl method, which is known for its accuracy and precision, and it is widely used in soil science research and agriculture to assess soil fertility and nutrient management [65–67]. This method relies on a series of chemical reactions to convert all forms of N_2 in the soil into ammonium ions (NH_4^+), which can then be quantified [66]. In this process, a soil sample was digested with concentrated sulfuric acid and a catalyst, typically a mixture of copper sulphate and selenium, in a Kjeldahl digestion flask. This resulted in the mineralization of organic N_2 compounds and the reduction of NO_3^- and nitrite (NO_2^-) forms to NH_4^+. The ammonium ions were subsequently converted to ammonia gas through alkaline distillation. The released ammonia gas was then collected in a boric acid solution and titrated with a standardized acid solution (i.e., hydrochloric acid) to determine the ammonia content. The TN content of the soil sample was then calculated as %N based on the amount of ammonia detected.

After obtaining the TN for the 72 samples, it was subsequently converted to kilograms per hectare (kg ha^{-1}) using data gathered during the sample collection process. This data included the soil depth, bulk density, and the percentage of fine soil content in a volume of soil within one hectare. The obtained results were then used in the interpolation process to map the spatial distribution of nitrogen content before the sowing and after the harvesting period. Then, the differences in nitrogen levels were calculated to assess temporal variations in soil N_2.

2.5. Nitrogen Distribution Mapping

The laboratory results of soil N_2 content from the different samples were used as training samples for spatial interpolation. The Inverse Distance Weighting (IDW) method was applied to predict nitrogen content at unobserved locations within each field. IDW is a deterministic spatial interpolation technique that considers only distance relationships [68]. This method is widely used for estimating values at unknown locations based on the known values at surrounding locations [28,69]. We used a power of two, which is the most common value for this parameter. This method is sometimes referred to as Inverse Square Distance. The interpolation procedure was carried out for each target location within the field, resulting in a continuous spatial representation of nitrogen distribution. ESRI's mapping software ArcMap, version 10.7, was used to perform the interpolation.

By applying the IDW method, this study aimed to identify and analyze the spatial patterns of nitrogen credit or debit in the soil, influenced by the different crop types under investigation. The difference between nitrogen levels before sowing and after harvest was calculated to determine the credit or debit of nitrogen in the soil. This approach enabled an assessment of the impact of crop cultivation on the soil's nitrogen content.

IDW is a local interpolator because it relies on the values of nearby samples when making predictions. We established a search neighborhood in the form of a circular area that encompassed the entire study domain. Within this circle, we determined fixed ranges for the minimum and maximum number of sample points. This approach ensured that the radius of the circle remained small in areas with a high density of sample points and expanded in regions with fewer points. To mitigate the influence of data clusters, the circular area was further subdivided into equal-angle sectors. Various combinations of minimum and maximum sample numbers within these sectors were then systematically assessed by analyzing prediction error statistics obtained through cross-validation. The most suitable modeling parameters for local neighborhoods were determined based on their ability to yield a Mean Error (ME) value closest to zero (Equation (8)), and the smallest Root Mean Square Error (RMSE) (Equation (9)). The ME is used to ascertain if predictions exhibit bias, while RMSE evaluates prediction accuracy.

$$\mathrm{ME} = \frac{1}{\mathrm{N}}\sum_{\alpha}^{\mathrm{N}}[\mathrm{Z}^{*}(\mathrm{X}\alpha) - \mathrm{Z}(\mathrm{X}\alpha)] \tag{8}$$

$$\mathrm{RMSE} = \sqrt{\frac{1}{\mathrm{N}}\sum_{\alpha}^{\mathrm{N}}[\mathrm{Z}^{*}(\mathrm{X}\alpha) - \mathrm{Z}(\mathrm{X}\alpha)]^{2}}, \tag{9}$$

where “Z*(Xα)” represents the predicted values at specific sample locations “Xα”, while “Z(Xα)” represents the measured values at those same sample locations.

3. Results

3.1. Plant Population Size, Crop Development, and Yield Assessment

The summary of the plant population size results is presented in Table 2. The results indicate that 15 days after sowing, soybean with a plant spacing of 50 × 20 m exhibited the highest plant density per hectare (36,571.00 plants) compared to the other crops. However, this count was still 56.06% lower than the expected number of plants based on the designated plant spacing. Similar patterns were observed for cotton and maize crops, which, despite having wider plant spacing, showed lower plant densities per hectare, with 26,878.00 and 23,980.00 plants, respectively (representing reductions of 55.14% and 34.04%, respectively, compared to the expected counts). To address this issue, an overseeding activity was conducted after the initial count, involving planting additional seeds in areas with poor plant emergence or unsatisfactory plant population. Then, 30 days later (45 days after sowing), a second count revealed 47,558.00 cotton plants, equivalent to 79.22% of the total expected plant population (estimated at 60,036 plants). This represented a 34.37% increase compared to the first count conducted 15 days after sowing. Similar increases were observed for soybean and maize, with plant populations of 51,838 and 26,239.00 plants, respectively (representing 62.17% and 72.04% of the expected plant population). These numbers corresponded to an increase of 18.23% and 6.08% for soybean and maize, respectively, compared to the first count.

Table 2. Summary of the plant population size of different crops in different periods (plants ha^{-1}).

Crop	15 Days After Sowing		45 Days After Sowing		2 Weeks Before Harvesting		
	Count	Difference (%) *	Count	Difference (%) **	Count	Difference (%) ***	Difference (%) ****
Cotton	26,878	−55.14	47,558	+34.37	43,168	−9.23	+60.61
Soybean	36,571	−56.06	51,838	+18.23	36,865	−28.88	+0.80
Maize	23,980	−34.04	26,239	+06.08	19,268	−26.57	−19.65

* Difference from expected emergency percentage. ** Difference between the second and the first count. *** Difference between the third and the second count. **** Difference between the third and the first count.

Two weeks prior to harvesting, a third count showed a decrease in plant populations. There were 43,168.00 cotton plants, representing a 9.23% decrease from the second count and a 60.61% increase from the first count. For soybean, there were 36,865.00 plants, indicating a 28.88% decrease from the second count and a 0.80% increase from the first count. As for maize, there were 19,268 plants, representing a 26.57% decrease from the second count and a 19.65% decrease from the first count. The decrease observed in the third count compared to the second count can be attributed to heavy rains that occurred in late March 2023 and early April, significantly impacting crop development. Figure 2 provides the daily rainfall observations in the study area from January to the first quarter of April 2023. Another factor contributing to the reduction in plant populations was the occurrence of pests and diseases. Although organic methods for pest and disease control were implemented in this study and yielded some positive results, their effectiveness did not fully align with previous research recommendations. Certain pests and diseases persisted throughout the plant cycle, particularly in the cotton field. Figure 5 illustrates some of the pests and diseases observed during the study.

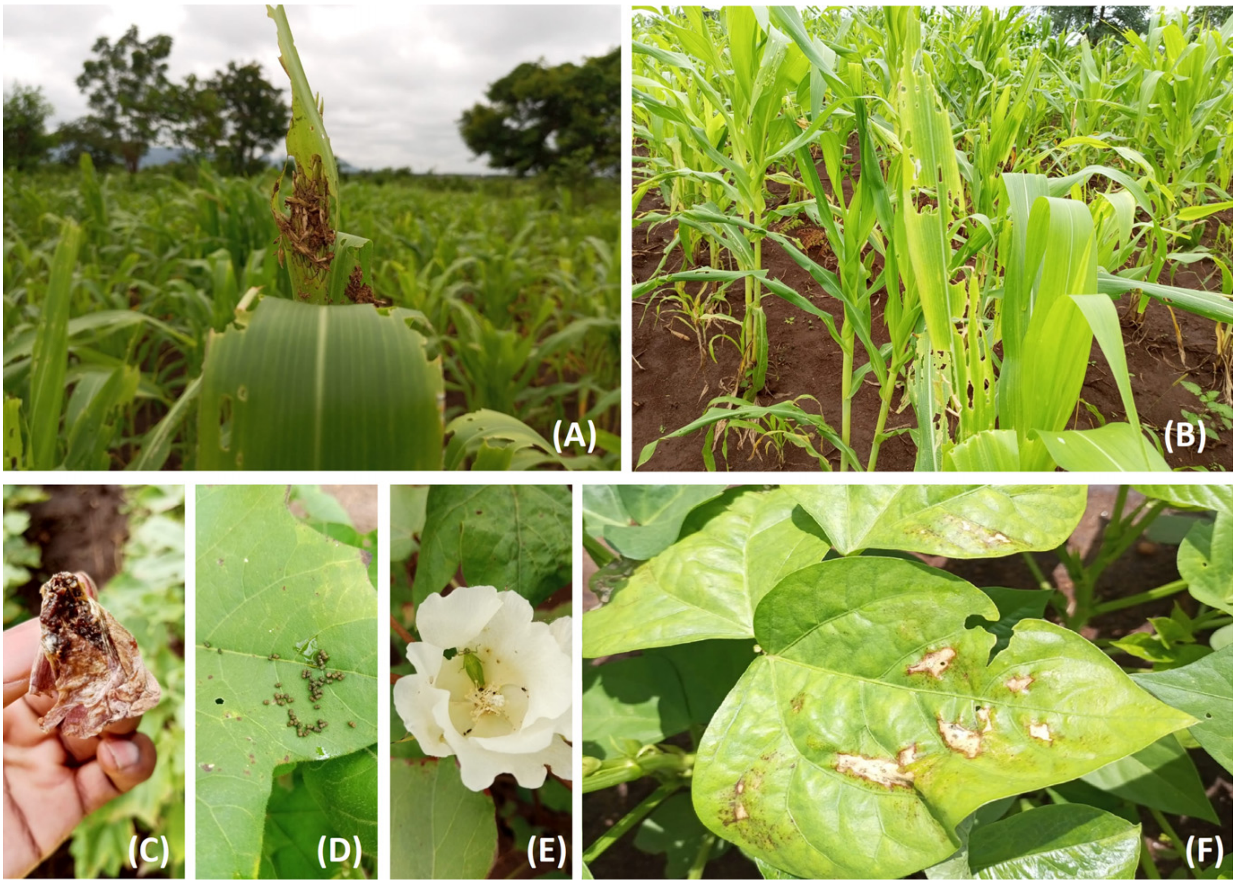

Figure 5. Different pest and diseases observed in the maize field ((**A**) *Spodoptera frugiperda* and (**B**) *grasshoppers*), the cotton field ((**C**) *Pectinophora gossypiella*, (**D**) *Aphis gossypii*, and (**E**) *grasshoppers*), and soybean ((**F**) *Cercospora kikuchii*).

Table 3 summarizes the yield assessment results for cotton, soybean, and maize, highlighting seed weight per plot, yield per hectare, and national average yield ranges. The cotton yield of 0.65 t ha^{-1} surpasses the national average range of 0.5 to 0.6 t ha^{-1}, indicating superior performance in the assessed plots. Conversely, the soybean yield of 1.13 t ha^{-1} falls below the national average range of 1.6 to 2.5 t ha^{-1}, suggesting suboptimal performance. Similarly, maize yield is notably low at 0.28 t ha^{-1} compared to the national average range of 0.9 to 1.2 t ha^{-1}, indicating significantly poorer performance in the assessed plots.

Table 3. Summary of yield assessment results (kg ha^{-1}).

Crop	Seed Weight (kg plot^{-1})	Yield (t ha^{-1})	National Average Yield (t ha^{-1})
Cotton	163.25	0.65	0.5–0.6 [70]
Soybean	281.30	1.13	1.6–2.5
Maize	70.80	0.28	0.9–1.2 [71]

3.2. Temporal Variations of Soil Nitrogen

Laboratory analysis results of variations in soil N content measured in kilograms per hectare (kg ha^{-1}) across different sample points within each field are presented in Table 4. The analysis of various agricultural fields—cotton, soybean, and maize—provides insights into soil N_2 dynamics across their growth cycles. In the cotton field, initial N_2 levels before sowing ranged from 2584.5 to 3876.8 kg ha^{-1}. After harvest, values varied from 1033.8 to 2584.5 kg ha^{-1}, indicating an overall decline in N_2 concentration. The most significant decrease observed was 1809.2 kg ha^{-1}. Notably, while most areas experienced reduced N_2 levels after harvesting, one location showed an increase of 258.5 kg ha^{-1}, suggesting nitrogen fixation. However, in multiple instances, a negative difference indicated decreased soil N_2 levels after harvest, suggesting an overall reduction in soil N_2 in the cotton field.

Table 4. Summary of lab results for soil nitrogen content determination (kg ha^{-1}).

Sample	Cotton Field			Soybean Field			Maize Field		
	Before Sowing	After Harvesting	Difference	Before Sowing	After Harvesting	Difference	Before Sowing	After Harvesting	Difference
1	2584.5	2584.5	0,0	1601.4	1143.8	−457.5	2467.2	1727.0	−740.2
2	3101.4	2326.1	−775.4	1372.6	1372.6	0.0	1480.3	1233.6	−246.7
3	2843.0	2326.1	−516.9	1372.6	1601.4	+228.8	1973.7	1480.3	−493.4
4	4910.6	3618.3	−1292.3	1601.4	1143.8	−457.5	1480.3	1727.0	+246.7
5	3359.9	2584.5	−775.4	1372.6	1830.1	+457.5	1727.0	1480.3	−246.7
6	3618.3	3359.9	−258.5	2287.7	1372.6	−915.1	2220.5	2713.9	+493.4
7	3359.9	3101.4	−258.5	1372.6	1143.8	−228.8	2467.2	1973.7	−493.4
8	2584.5	2843.0	+258.5	1830.1	2287.7	+457.5	1233.6	3700.8	+2467.2
9	2843.0	1033.8	−1809.2	1372.6	1601.4	+228.8	2220.5	3454.0	+1233.6
10	3876.8	2584.5	−1292.3	1830.1	915.1	−915.1	2220.5	2713.9	+493.4
11	3359.9	1550.7	−1809.2	1372.6	1143.8	−228.8	2220.5	2960.6	+740.2
12	2584.5	1033.8	−1550.7	1601.4	915.1	−686.3	2220.5	2467.2	+246.7

In the soybean field, initial N_2 levels ranged from 1372.6 to 2287.7 kg ha^{-1} before sowing. After harvest, values ranged from 1143.8 to 1830.1 kg ha^{-1}, showcasing a general decrease in soil N_2. Interestingly, amid this decline, there was a remarkable exception: a notable increase in N_2 levels, specifically in the southern region of the field, in areas close to samples 5 and 8 (Table 3). Analyzing the disparities highlighted the complexity of N_2 dynamics, revealing its significant fixation (457.5 kg ha^{-1}) and substantial absorption (915.1 kg ha^{-1}) throughout the soybean crop cycle. Concerning maize, initial N_2 levels in the soil spanned from 1233.6 to 2467.2 kg ha^{-1} before sowing. After harvest, values ranged from 1233.6 to 3700.8 kg ha^{-1}, with some instances showing a substantial increase of +2467.2 kg ha^{-1}. However, despite this overall increase, specific sampled points indicated a reduction in TN content (e.g., sample 1 = −740.2 kg ha^{-1}). This suggests significant N_2 fixation by maize during its growth cycle, as maize plants likely extracted nitrogen from the soil to support their growth needs. Consequently, this difference implies a decline in soil N_2 levels across the entire period of maize cultivation.

3.2.1. Cotton Field

Figure 6 depicts how N_2 content in the soil changes before and after the cotton cultivation cycle. This map offers valuable insights into how N_2 levels fluctuate during different stages of the process. Before planting cotton, the map showed a wide range of N_2 distribution, varying from 2100.1 to 4908.65 kg ha^{-1}. The highest N_2 concentration was found in the northwest part of the field, while the southwest and southeast areas had notably lower values.

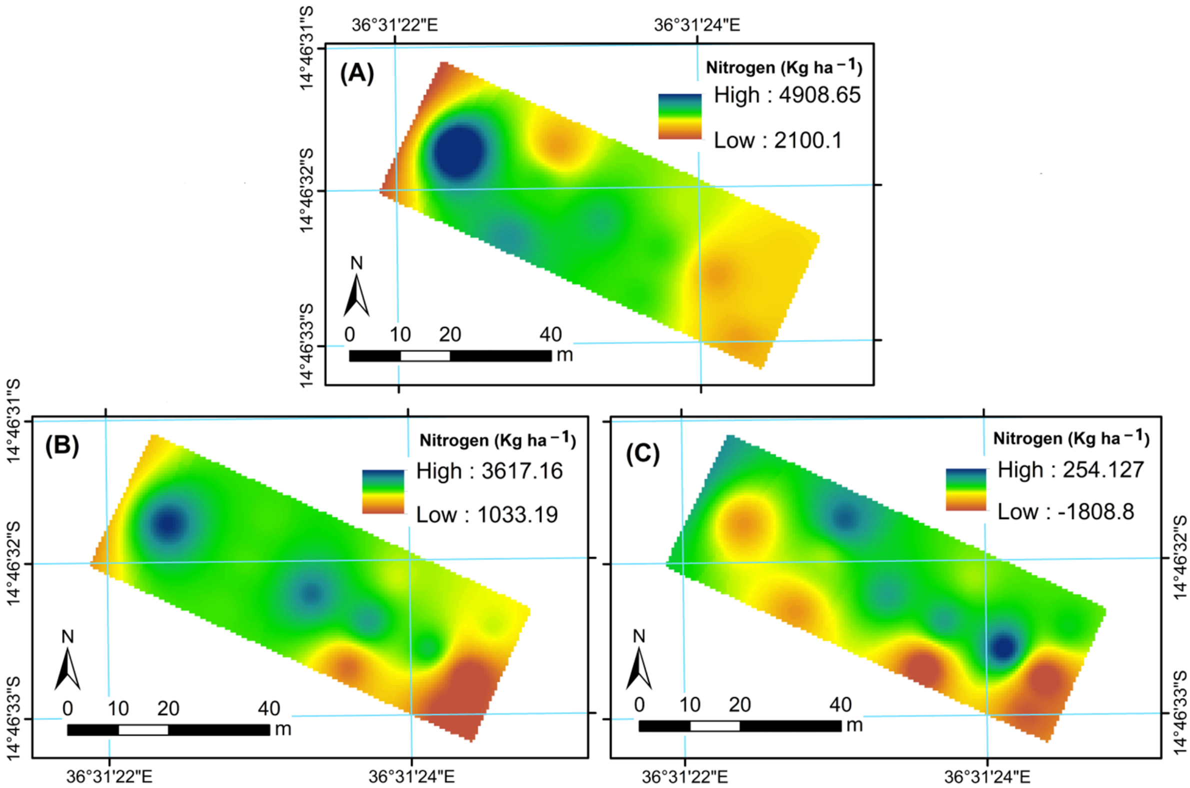

Figure 6. Spatial distribution of soil nitrogen in the cotton field before sowing (**A**) and after harvesting (**B**), and the differences (**C**) in the cotton field.

The central region averaged around 3000 kg ha^{-1}. The N_2 levels in the soil ranged significantly, well above the cotton crop's requirements. This indicates that the soil had more than enough N_2 to support the crop's growth, providing an ideal environment for healthy development. After harvesting, there was a notable change. The field showed variations in N_2 content, ranging from 1033.19 to 3617.16 kg ha^{-1}. Compared to before planting, there was an average reduction of around 1000 kg ha^{-1}. The visual representation indicates substantial reductions in N_2 content, especially in the southeast part of the field, where the decrease exceeded 1800 kg ha^{-1}, highlighting changes in its level. However, the northern and northwestern regions experienced smaller reductions, below 500 kg ha^{-1}.

3.2.2. Soybean Field

Before sowing, the analysis of N_2 content in the soil revealed a notable diversity in values in the soybean field (Figure 7), ranging from 1372.02 to 2286.78 kg ha^{-1}. The highest nitrogen content of 2286.78 kg ha^{-1} was observed in the central region of the field, while the region with the lowest nitrogen values was located along a vertical line extending from the northeast to the southeast, spanning from the northern to the southern border of the study area. These notable variations in N_2 content highlight the diversity of N_2 levels prior to sowing. The central region of the field recorded average N_2 levels of 1700 kg ha^{-1}. The field exhibited a wide variation in TN content after soybean harvesting, ranging from 915.19 to 2284.9 kg ha^{-1} (Figure 7B). This demonstrates a significant reduction in soil N_2 content, whereas an increase was expected through biological fixation.

Analysis of the differences between the two periods, aiming at understanding the amount of nitrogen fixed in the field (Figure 7C), revealed an unexpected result. Significant nitrogen fixation was observed only in the southwest region of the field, with values ranging from 663.69 kg ha^{-1} to 914.78 kg ha^{-1}. This notable fixation phenomenon warrants further investigation and attention.

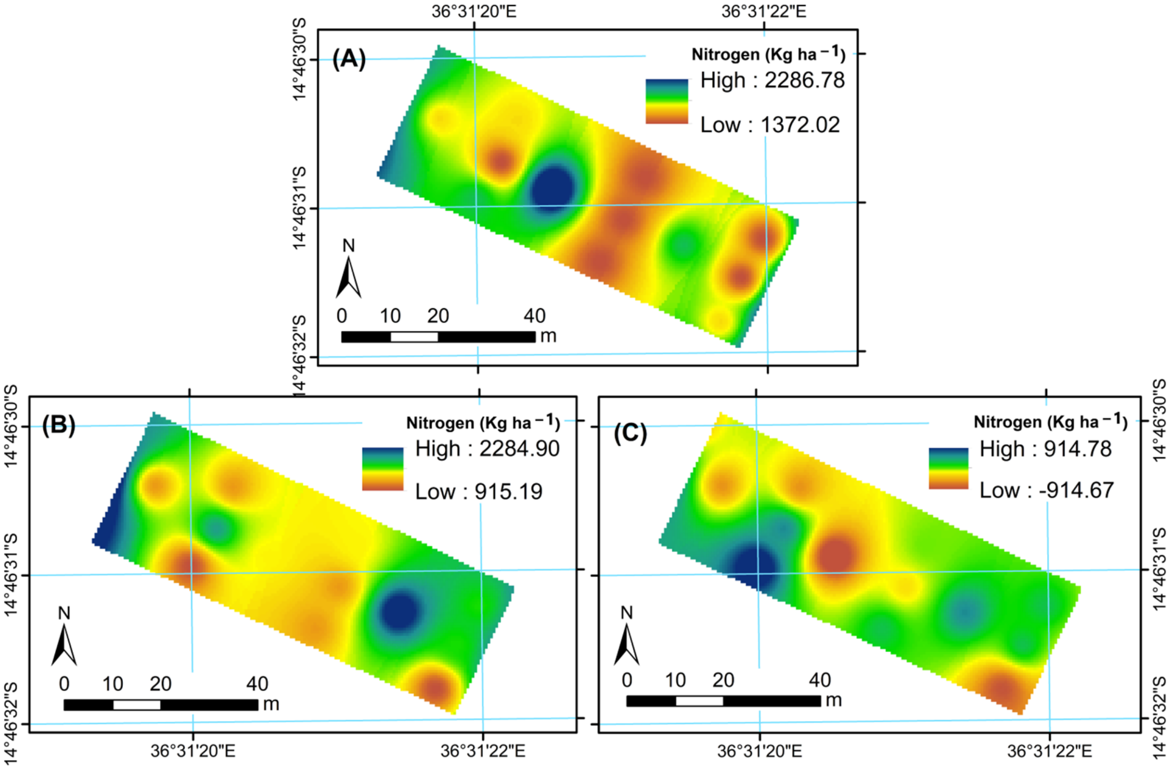

Figure 7. Spatial distribution of soil nitrogen in the soybean field before sowing (**A**) and after harvesting (**B**), and the differences (**C**) in the soybean field.

On the other hand, the southeast and northeast regions showed values not exceeding 118.43 kg ha^{-1}, suggesting minimal fixation in those areas. Additionally, the remaining regions of the field registered negative values, below 3.64 kg ha^{-1}, indicating a scenario of nitrogen absorption. This unusual pattern reveals an uneven distribution of the nitrogen fixation process in the field, with the southwest standing out as the region with the most significant fixation, while the southeast and the northeast present moderate values, and the other regions show an absence of fixation, possibly due to unfavorable conditions or other specific environmental variables.

3.2.3. Maize Field

Before planting the maize crop, the analysis of N_2 content in the soil revealed a notable diversity in the obtained values (Figure 8). This variability in soil N_2 levels reflects the complex interaction of various factors that play a fundamental role in determining the fertility of this essential environment for plant growth. Within the maize field area, nitrogen values ranged from 1235.11 to 2466.02 kg ha^{-1}, indicating significant variability across the field. The highest N_2 content, 2466.02 kg ha^{-1}, was noted in the northwest region of the field, while the region with the lowest N_2 value (1235.11 kg ha^{-1}) was identified in the eastern portion of the field. These variations highlight the variability of N_2 content before sowing. In the central region of the field, particular attention should be given to the recorded average nitrogen content, which reached a value of 2118.00 kg ha^{-1}. The results confirm that the field was suitable for maize cultivation, as the N_2 levels available before sowing were sufficient to support the establishment and growth of the maize crop. After the maize harvest, a notable difference in N_2 levels was observed compared to the beginning of the study, as illustrated in Figure 8C.

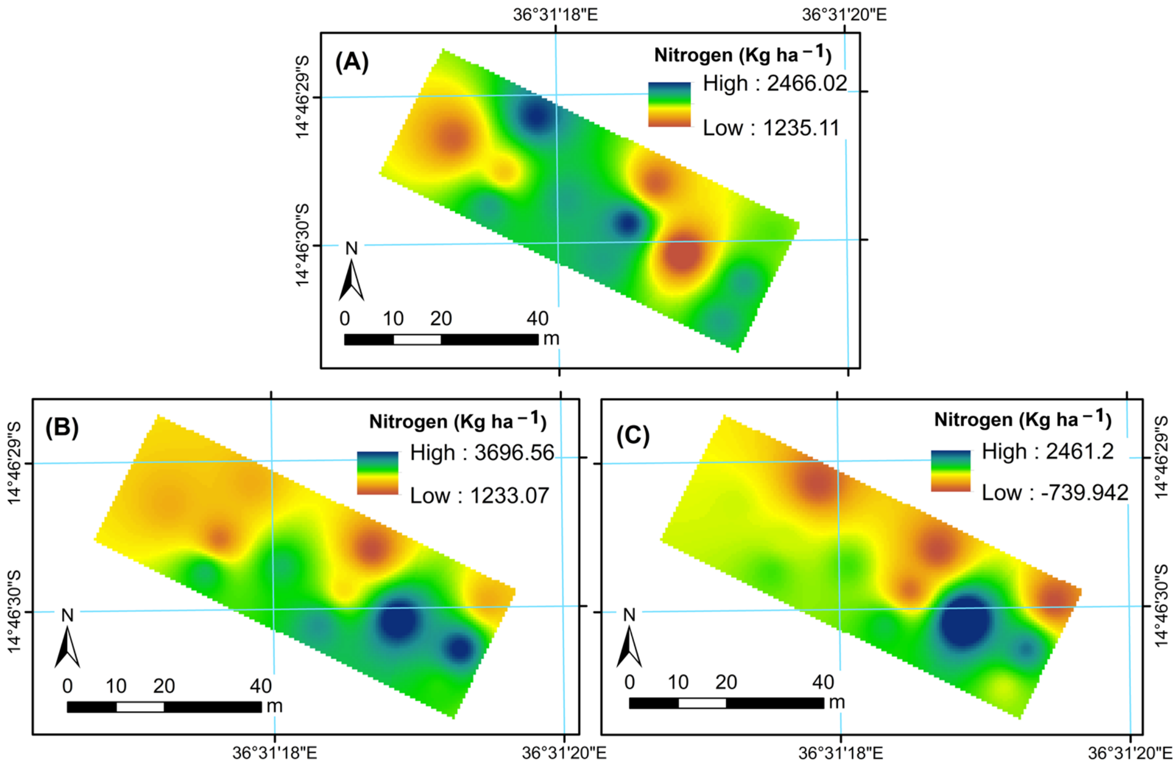

Figure 8. Spatial distribution of soil nitrogen in the maize field before sowing (**A**) and after harvesting (**B**), and the differences (**C**) in the maize field.

This difference is particularly striking because the field exhibited a wide variation in TN content, ranging between 1233.07 and 3696.56 kg ha^{-1}. This reveals a significant increase in soil nitrogen content, with an average of 2000 kg ha^{-1}. Consequently, the visual representation of the differences between these two time periods clearly illustrates the more substantial increases in soil nitrogen content, mainly concentrated in the southeast region of the field, where reductions exceed 1900 kg ha^{-1}, providing a vivid picture of nitrogen dynamics. In contrast, the northern and northwestern regions experienced relatively modest increases, with values below 300 kg ha^{-1}.

4. Discussion

Soil fertilization significantly influences the growth and yield of various crops globally by altering soil nutrient availability, including nitrogen, phosphorus, and potassium [72,73]. Farmers have traditionally relied on chemical fertilizers to boost yields, yet studies [74,75] have highlighted their significant environmental impact. Consequently, organic farming has been widely recommended. This study aimed to quantify the total nitrogen fixable from different crop types: cereals (i.e., maize), legumes (i.e., soybean), and fiber crops (i.e., cotton). Seed quality is crucial in crop production, impacting germination and emergence [76]. Understanding seed vigor is vital for successful crop establishment. While conventional wisdom prioritizes the physical purity of seeds, this research emphasizes that high physical purity alone does not ensure optimal germination and subsequent crop yield. Studies by [77,78] align with these findings, emphasizing the multifaceted nature of seed vigor, where factors like germination energy and speed significantly influence seed performance.

The N_2 variations in plant population size in the field influenced the total amount of nitrogen, highlighting how nutrient concentrations can vary. Despite the promising seed quality, achieving the expected plant populations during the early stages of plant development proved to be challenging. These difficulties are consistent with broader agricultural trends highlighted in previous research. Studies by [79,80] emphasize the profound impact of adverse weather conditions and pest infestations on plant populations

(Figure 2). These findings align with the observed reductions in plant counts near harvest in this study, which were attributed to inclement weather and persistent pest challenges. Pest and disease infestation, adverse climatic factors, such as excessive rainfall (Figure 5), inadequate light levels, and poor soil quality, such as acidity, impacted plant germination and growth. These findings are consistent with the observations reported by [79,81–83]. The adoption of overseeding as a strategy to address suboptimal plant emergence aligns with practices recommended by [84,85], demonstrating its efficacy in improving plant populations. This is further supported by the findings of this research. In this study, pests, diseases, and climate variations, as shown in Figures 2 and 5, not only reduced the total population size but also significantly affected the total yield, particularly for cotton and maize.

Different crops exhibit varying N_2 requirements for optimal growth. The total amount of N_2 to be applied is influenced not only by the type of crop but also by various factors such as the specific crop variety, growth stage, environmental conditions, and soil characteristics. The temporal fluctuations in soil N_2 content observed in this study, concerning the total nitrogen credit or debit across fields with different crops, align with prevailing trends highlighted in agricultural research. Studies by [86,87] highlight the dynamic nature of soil N_2 levels across various crop cycles, showcasing the intricate interplay between biological N_2 fixation, plant uptake, and environmental influences. The spatial distribution of N_2 content within the fields displayed notable variations, demonstrating the heterogeneous nature of soil N_2 levels across agricultural landscapes. These findings align with prior research by [88,89], emphasizing the significance of spatial heterogeneity in soil N_2 content. The observed patterns in N_2 distribution before and after crop cultivation revealed diverse ranges of N_2 content across different fields. Interestingly, following the harvest, the maize field exhibited substantial N_2 fixation, resulting in an increase in soil N_2 content [90,91]. However, specific sampled points showed a reduction in TN content, indicating substantial nitrogen assimilation by maize during its growth cycle, as reported by [90]. Conversely, the soybean field showcased a decrease in soil N_2 content after harvesting. Remarkably, there was significant nitrogen fixation (457.5 kg ha^{-1}) and substantial N_2 absorption (915.1 kg ha^{-1}) throughout the soybean crop cycle. This variability could be attributed to localized factors influencing nitrogen dynamics in different areas of the field [92–94].

One important aspect to note was the use of one year of experiment data. The use of a one-year timeframe was fundamental to providing a foundational understanding of nitrogen dynamics within the specified crops. Recognizing the need for a more comprehensive view, this study acknowledges the importance of longitudinal studies to capture interannual variations. As such, future research should incorporate multi-year experiments, accounting for climate fluctuations, pest pressures, and crop management practices. This approach will facilitate a nuanced assessment of nitrogen dynamics' sustainability, offering valuable insights to optimize agricultural practices over time. Extending experimental durations allows researchers to discern long-term effects, contributing to the development of resilient and sustainable agricultural systems. There is still a knowledge gap in how much nitrogen is fixed or assimilated by crops under various farming practices, particularly in Mozambique. Our findings shed light on the intricate dynamics of nitrogen distribution, unveiling variations across diverse fields and crop types. The observed patterns, including significant nitrogen fixation in maize fields and substantial nitrogen absorption in soybean fields, highlight the importance of adopting tailored approaches in agricultural practices. We assert that our study adds to the existing body of knowledge by providing quantifiable insights into the nitrogen dynamics of key crops in Mozambique. This research serves as a foundation for further investigations into crop-specific interventions, seed selection strategies, and soil fertility management techniques. By elucidating the spatial heterogeneity of nitrogen content and the different mechanisms of fixation and assimilation within specific crops, this study contributes to the advancement of sustainable agricultural practices, aiming to optimize productivity and ensure soil health in the context of Mozambique's predominant crops.

5. Conclusions

This research delved into multifaceted seed germination, plant population size, and soil N_2 content across diverse crop types—cereals (maize), legumes (soybean), and fiber crops (cotton). The study revealed essential insights into the interplay of seed quality, crop development, and soil fertility, shedding light on key factors critical for sustainable agricultural practices. Understanding the complex interplay between germination capacity, energy, and speed provided nuanced insights into seed performance, consistent with findings from previous studies. Moreover, the challenges in achieving expected plant populations during early development reflected broader agricultural trends, shaped by adverse weather conditions, persistent pest issues, and suboptimal environmental factors. The temporal and spatial analysis of soil nitrogen content highlighted the dynamic nature of N_2 flux across diverse crop cycles. While the maize field exhibited significant nitrogen fixation post-harvest, certain areas revealed a reduction in TN content. This may reflect soil N_2 extraction by the maize crop or other factors, such as leaching beyond the sampled soil depth. Conversely, the soybean field displayed a decrease in soil N_2 content after harvesting, accompanied by substantial nitrogen fixation and absorption throughout the soybean crop cycle. These findings underscored the intricate N_2 dynamics within specific crops and emphasized the need for tailored agricultural approaches.

This research consolidates diverse insights, reaffirming the importance of addressing challenges in seed selection, crop management, and soil health to foster sustainable agricultural practices. Future research should focus on innovative strategies to mitigate environmental adversities, optimize crop-specific interventions, and refine soil fertility management techniques. Examples of such strategies are the development and testing of flood-tolerant crop varieties to counteract extreme weather events, and utilizing remote sensing and Artificial Intelligence to monitor soil moisture and predict climate risks, enabling timely interventions. Moreover, future studies in Mozambique should focus on refining fertilizer application rates and timing for specific crops to maximize nutrient efficiency while minimizing overuse and environmental impact.

By leveraging a holistic understanding of seed vigor, plant population dynamics, and soil nitrogen content, agricultural practices can be optimized, contributing significantly to global food security efforts and sustainable agricultural development. As such, this study not only elucidated key aspects of seed germination, plant population dynamics, and soil nitrogen content but also advocated for a holistic and tailored approach in agricultural practices to ensure sustained productivity, soil health, and global food security in the long term.

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