Response of Starter Nitrogen and Phosphorus Fertilizer Rates to Nodulation, Nitrogen Fixation, and Net Nitrogen Balance on Groundnut (Arachis hypogaea L.) in the Semi-Arid Tigray Region of Ethiopia

ABSTRACT

For small-scale farmers in developing countries, biological nitrogen fixation through legumes is an affordable method of reducing reliance on nitrogen fertilizer.The study aimed to look at how groundnut nodulation, biological nitrogen fixation, atmospheric nitrogen, and the net nitrogen balance were affected by starter nitrogen and phosphorus fertilizer dosages.The three times replicated treatments of three nitrogen levels (0, 15 and 30 kg N ha^{-1}*) and four phosphorus levels (0, 23, 46 and 69 kg* P_2O_5 *ha*$^{-1}$*) were arranged in a factorial combination using a randomized complete block design (RCBD). The results of the analysis demonstrated that, in comparison to plots without nitrogen fertilizer, the number of nodules per plant was significantly increased by the single application of nitrogen alone at a dose of 15 kg ha*$^{-1}$*. Compared to 69 kg* P_2O_5 *ha*$^{-1}$*, biological nitrogen fixation rose by 88.41% at 46 kg* P_2O_5 *ha*$^{-1}$*; nonetheless, it was significantly similar to zero and 23 kg* P_2O_5 *ha*$^{-1}$*. The nitrogen derived from the atmosphere (50.9%) was significantly higher in the control treatments. The interactive application of 15 kgof nitrogen ha*$^{-1}$ *and zero phosphorus resulted in a significantly greater net nitrogen balance (-4 kg ha*$^{-1}$*),whereas the co-application of 30 kg ha*$^{-1}$*ofnitrogen and zero phosphorus resulted in the lowest measurement of net nitrogen balance. Therefore, large nitrogen fertilizer application rates are not necessary to promote BNF in groundnuts.*

Keywords: Biological Nitrogen Fixation, Groundnut, Net Nitrogen Balance, Nitrogen and Phosphorus Fertilizers, Nodulation.

1. INTRODUCTION

Unbroken cereal cultivation has led to the loss of soil fertility and, thus, low yields [1, 2]. Crop rotation is more productive than continuous monoculture [3, 4]. In crop rotation, legumes play an important role in cereals due to their contribution of residual nitrogen (N) from biological nitrogen fixation (BNF). The biosphere's primary mechanism for sustaining life on earth is the BNF, ranked second in significance only to photosynthesis [5]. This is due to the role played by legumes in fixing nitrogen, restoring soil fertility through rhizobia-legume symbiosis, and breaking the cycles of pests and diseases that attack crops [6].Nitrogen derived from the atmosphere can reduce the use of synthetic N fertilizer in cereal crops in rotation after legumes [5, 6]. Legumes are therefore becoming a viable alternative to N fertilizer for resource-poor farmers [7] by constantly adding N to the soil to maintain existing N reserves in the soils [8].

Groundnut is a legume crop that produces its N via BNF and transfers large amounts of N to grains to produce protein and also provides the soil with residual N, which is essential for rotational cereal crops [9]. When residues are present and incorporated into the soil, a higher content of mineralized N is found in the soil, which subsequently ensures a promising yield for the successor cereal crops [10]. Biological nitrogen fixation is a renewable N cycle and a source of N for agriculture, and it allows host plants to grow in environments with low soil N, reducing N losses through denitrification, volatilization and leaching, thereby promoting sustainable agriculture [11]. The discovery by Bado et al. [12], confirmed that groundnut can fix N in an amount equivalent to 35 kg ha^{-1} and that subsequent cereal crops achieve higher yields when the stover residues of groundnut are returned to the soil [13]. Further findings from Yakubu et al. [14] reported that groundnut can produce 27.19 kg N ha^{-1}. Bado et al. [15] observed that groundnut sequester has an average of 8 to 23 kg N ha^{-1} and that the percentage of N derived from the atmosphere ranges from 27 to 34%.Although legumes produce N, they can suffer from N deficiency under field conditions, especially when soil N levels are very low, until nodules become functional [16, 17]. In such situations, it may be necessary to add a starter N fertilizer at a rate of 20 kg N ha^{-1} [18]. Many researchers have also reported that P fertilizer is essential for healthy root growth and profuse nodulation, which in turn may influence the BNF potential of legumes [19, 20]. Nodulation of legumes requires energy, so P nutrition is desirable to provide energy for the BNF process and plant development, including energy transfer from legumes during BNF [21], hence the application of 40 kg P_2O_5 ha^{-1} in groundnut plots increased nodule number by 160%, N content by 147% and theamount of fixed N by 169% over the unfertilized plots. An increase in soil available N was observed in P treated groundnut plots, suggesting that P increased the BNF of groundnut and consequently led to the formation of residual N in the soil [14].

Farmers in the research area typically grow groundnut in rotation with finger millet, sorghum, and Eragrostis tef because legumes increase the grain yields of subsequent crops [3]. In contrast to sorghum and wheat monoculture, Bado et al. [12] and Angus et al. [22] observed increased yields of both sorghum and wheat following legume rotation. It is estimated that American farmers might save $200 million to $300 million by employing alfalfa legumes in their crop production [23]. Legumes can generally be used to lessen impoverished farmers' reliance on artificial fertilizers [24]. The objectives of this study were to ensure proper management, offer some additional insights, and fully realize the advantages of legumes as a source of nitrogen nutrients for agricultural systems. Due to a lack of research on these subjects, this study set out to determine the impacts of starter N and P fertilizer treatments on BNF, net nitrogen balance, and groundnut nodulation.

2. MATERIALS AND METHODS

2.1. Site Description

The experiment was conducted during the 2017 growing season in Medhin village, Mereb Leke district, Tigray region of Ethiopia. Geographically, the study location was between 14° 18' 48" and 14° 25' 26" N and 38° 42' 15" and 38° 48’ 30" and with an altitude of 1390 m above sea level, and it is located 42 km from the historical administrative zone of the city of Aksum. Mixed farming, which usually includes crop and animal production, is commonly carried out on the same land unit. The area is generally known for the predominant crops such as sorghum, millet, groundnut and teff, which are grown in rotation. In addition, fruits and vegetables are regularly grown in irrigated agriculture.The study area is characterized by a hot and semi-arid agro-climatic zone and has a well-drained sandy loam soil texture with typical Luvisols soil type. The rainfall regime is unimodal, with the main rainy season concentrated from June to September. During the research year, the average annual precipitation and average monthly temperature were 636 mm and 25.3°C, respectively [25]. The optimal rainfall depth and temperature for groundnut production are between 200 and 1000 mm and 20 and 30 °C, respectively [18]. Therefore, the current weather data in the study area was in asuitable range for groundnut cultivation.

Table 1. Total rainfall and temperature during experimental period.

Month	Rainfall	Temperature (□C)		
	Total rainfall (mm)	Maximum	Minimum	Mean
January	0	34.62	15.7	25.16
February	0	35.02	12.6	23.81
March	3.1	37.8	15.18	26.49
April	27	37.92	15.6	26.76
May	53.1	37.2	18	27.6
June	78.2	36.62	17.24	26.93
July	300.1	33.58	15.1	24.34
August	78.7	32.68	14.28	23.48
September	79	34.88	16	25.44
October	16.3	35.86	14.7	25.28
November	0	35.24	13.22	24.23
December	0	35.2	12.1	23.65
Total	635.5			

2.2. Experimental Design and Treatments Set up

The experiment was conducted in the field of a selected farmer who had not grown groundnut in the previous growing season to determine the existing effects of the treatments on nodulation, BNF and net soil nitrogen balance of groundnut. In this experiment, groundnut and sorghum crops were planted side by side in the same soil. Sorghum and groundnut crops were planted as non-nitrogen fixers and nitrogen fixers, respectively, in order to estimate BNF. The same treatments were applied to sorghum (anon-nitrogen fixer) and groundnut (anitrogen fixer). The field trials included a total of 12 treatments, with three N levels at doses of 0, 15 and 30 kg ha^{-1} and four P_2O_5 levels at doses of 0, 23, 46 and 69 kg ha^{-1}. These treatments were arranged in 3 × 4 factorial combinations and in RCBD with three replicates. The treatment with zero N and P fertilizer content was designated as thecontrol treatment. All treatments were uniformly enriched with balanced fertilization containing potassium (K) at 30 kg K_2O ha^{-1}, sulfur (S) at 30 kg ha^{-1}, zinc (Zn) at 2 kg ha^{-1} and boron (B) at 0.5 kg ha^{-1} to avoid BNF loss due to a limited supply of one of those nutrients from the soil.According to Cisse [26], a balanced fertilization schedule is important to avoid yield-limiting factors and nutrient balance should be considered beyond N, P and K and include secondary elements and micronutrients when the soil is facing nutrient deficiencies. The fertilizers N, P, K and S were mainly applied during the sowing period, while the microelements (Zn and B) were applied during the flowering period of groundnut. The source of these nutrients was urea for N, Trisuperphosphate (TSP) for P_2O_5, KCl for K_2O, $CaSO_4$ for S, $ZnSO_4$ for Zn, and borax ($Na_2B4O_7 \cdot 10H_2O$) for B. A landrace Sedi groundnut variety was planted at a seed rate of 80 kg ha^{-1} as a biological nitrogen-fixing plant. The *melkam* sorghum variety was planted as a non-nitrogen fixing plant at a seed rate of 15 kg ha^{-1}. The plot size for growing groundnut (biological nitrogen fixer) was 3.6 m × 4 m (14.4 m^2) with a net harvestable area of 9.6 m2, and the distance between blocks, plots, rows and plants for groundnut cultivation was set at 1.5, 1, 0.6 and 0.1 m, respectively. One plot had 6 rows of groundnut and a total stand of 240 groundnut plants. For sorghum cultivation, the distances between blocks, plots, rows and plants were 1.5 m, 1 m, 0.75 m and 0.2 m, respectively. The sorghum plot consisted of 5 rows and a total population of 100 plants.

2.3. Soil Sampling Procedures and Analysis

Before planting, a composite sample of the disturbed soil was collected with an auger sampler at a depth of 0–20 cm using the zigzag method according to the experimental setup. This pre-planting composite sample was used to characterize the history of the site. Before planting, an additional sample of undisturbed soil was taken using a core sampler to determine the dry bulk density of the soil. After harvesting both crops, 36 soil samples were collected at similar depths for thesorghum and groundnut crops in each plot of the two crops. Post-harvest samples were deliberately used for the BNF determination. These disturbed composite soil samples were air-dried, ground and passed through a 2 mm sieve to analyze some chemical and physical properties of the soil. Soil samples collected after harvest were analyzed primarily to estimate residual soil N contents. The collected disturbed samples were analyzed for soil texture, pH, electrical conductivity (EC), organic carbon (OC), total N, available P, cation exchange capacity (CEC), and exchangeable cations (K, Na, Ca and Mg). Soil texture was determined using the Bouyoucos hydrometric method [27]. The soil pH was measured in the supernatant suspension at a soil/water ratio of 1:2.5 using a pH meter [28]. The electrical conductivity of a soil solution in a saturated extract with a soil/water ratio of 1:2.5 was measured with an EC meter [29]. The percentage of soil organic carbon (SOC) was determined using the wet oxidation method proposed by Walkley and Black [30]. Total nitrogen was determined using the Kjeldahl method described by Bremner and Mulvaney [31]. Available P was determined using the Olsen method [32]. The cation exchange capacity and exchangeable cations of the soil were extracted by leaching an ammonium acetate solution, and the exchange cations were measured by atomic absorption spectrophotometry for Ca and Mg and aflame photometer for Na and K [33]. The soil parameters analyzed for the above parameters are listed in Table 2.

Table 2. Physiochemical properties of experimental soil before sowing (NA-not applicable)

Parameters	Values	Rating	References
%Clay	8	NA	
%Silt	39	NA	
%Sand	53	NA	
Textural Class	Sandy loam	NA	
Bulk density (g cm^{-3})	1.46	Moderate	[34]
pH	5.5	Moderately acid	[35]
EC(dS m^{-1})	2.94	Slightly saline	[34]
CEC (cmol(+) kg^{-})	13.2	Moderate	[34]
OC (%)	0.61	Low	[34]
Total N (%)	0.053	Low	[34]
Available P (ppm)	6.92	Low	[34]
Exc.K (cmol(+) kg^{-1})	0.21	Low	[34]
Exc.Na (cmol(+) kg^{-1})	0.16	Low	[34]
Exc.Ca (cmol(+) kg^{-1})	5.8	Moderate	[34]
Exc.Mg (cmol(+) kg^{-1})	6.8	High	[34]

2.4. Collected Plant Data, Sampling Procedures and Analysis

Nodulation was assessed at the mid flowering of thegroundnut. With destructive sampling, five randomly selected plants were taken from each plot, and a nodule count per plant was performed to determine effective nodules. The whole plant was carefully uprooted using a fork so as to obtain intact roots and nodules. From the uprooted plants, the number of nodules per plant was recorded by counting the number of nodules from five plants and averagingthe number of nodule per plant. Nodules with a pink to dark-red color were considered effective nodules. Nodules with agreen colorwere identified as non-effective nodules. At the time of harvesting of groundnut and sorghum crops, five plant samples were collected from 36 plots for each groundnut crop (haulms and grains) and sorghum crop (stovers and grains) to determine the N accumulated in the plants. The groundnut plants were dug up, depodded and the pods taken away. The five sorghum plant samples taken from the inner rows, were cut near the ground. Nitrogen determination in sorghum plant tissues was used as a non-nitrogen fixing plant to evaluate groundnut BNF. All nitrogen fixing and non-nitrogen fixing plant samples were thoroughly washed with distilled water to remove adhered contaminants and dust particles, then placed in a paper envelope and oven-dried at 60 °C for 68 h. After drying in the oven, haulms and grains of groundnut and stover and grains of sorghum were crushed and passed through a 0.5 mm sieve. The determination of the total N content of the plant samples was carried out by the Kjeldahl method using the treatment of plant materials and oxidation with concentrated sulfuric acid and distillation of an aliquot from the digest with NaOH, collection of the distillate in boric acid and titration with 0.01 N HCl to the endpoint of the mixed indicator [36].

2.5. Biological Nitrogen Fixation

The amount of biological nitrogen fixation by groundnut was calculated from the total nitrogen difference (TND).The TND method considers the cultivation of nitrogen-fixing legumes with adjacent non-nitrogen-fixing crops grown on the same land with the same soil fertility status and assumes that the difference in nitrogen in plant tissue at harvest between crops was the quantity of fixed N_2. In the current experimental case, groundnut was the nitrogen-fixing crop, while sorghum was the non-nitrogen-fixing crop. This method assumes that legumes and non-legumes absorb the same amount of N from the soil. The reason for choosing sorghum is due to its agroecological needs and root morphology, which is similar tothat of groundnut. For each plot, the TND method was used based on the requirement for the same treatments. The BNF of groundnut was calculated according to the method described by Peoples et al [37] proposed formulaused to quantify the amount of BNF by legumes in short-term cropping systems(Eq 1).

$$\text{BNF} = [\text{N yield (legume)} - \text{N yield (nonlegume)}] + [\text{N soil (legume)} - \text{N soil (nonlegume)} \text{….Eq (1)}$$

All parameters included in the formula expressed by kg ha^{-1}.

2.6. Proportion of Nitrogen Derived from the Atmosphere (%Ndfa)

The %Ndfa is the pure N which is contributed from the atmosphere by the N_2 fixing legume crop. The %Ndfa was calculated by adopting the formula of Rennie [38], which is used to estimate the %Ndfa (Eq 2).

$$\%\,Ndfa = \frac{[\text{N yield (legume)} - \text{N yield (nonlegume!)}]}{\text{N yield (legume)}} \times 100 \text{….Eq (2)}$$

2.7. Net Nitrogen Balance (NNB).

Net nitrogen balance (NNB) is the difference between the biologically fixed nitrogen in legumes and the nitrogen removed from legume grains. This study assumed how much nitrogen would be added to the soil from biologically fixed nitrogen if groundnut stover residues were incorporated into the soil. Therefore, the NNB of the groundnut stover residues from biologically fixed nitrogen was calculated by subtracting the nitrogen removed by the groundnut grains from the biologically fixed nitrogen in legumes [39].

$$\text{NNB (kg per ha)} = \text{BNF (kg per ha)} - \text{N removed by grain (kg per ha)} \ldots\text{.Eq (3)}$$

2.8. Statistics Analysis

The collected data were statistically analyzed using a two-way analysis of variance (ANOVA). Before data analysis, the collected data were tested for normality and homogeneity of variance at individual levels and in the combination of N and P fertilizers. An analysis of variance was performed using the Genstat 18 edition. A significant difference between treatments was assessed using the Fisher's testforleast significant difference (LSD) at a probability level of 0.05.

3. RESULTS AND DISCUSSION

3. 1. Before Planting Soil Physiochemical Properties of the Experimental Site

The result of the physicochemical analysis of the soil at the test site is shown in Table 2. The soil texture class of the test site was sandy loam, which was moderately suitable for plant growth. Therefore, sandy loam is suitable for growing groundnut because groundnut is usually grown on light-textured soils. The soil in the study area has a bulk density of 1.46 g cm^{-3}, which was classified as moderate (1.3–1.6g cm^{-3}) [34]. The moderate bulk density of the soil with a sandy-loamy texture allows groundnut roots and pegs to easily penetrate the soil, absorb nutrients, absorb water and form pods. Furthermore, the pH value at the study site was 5.5, which corresponds to the moderately acidic category described by Tekalign[35]. As Murata and colleagues [40] studied, groundnut root and peg growth, pod formation, and overall plant development were best when soil pH was greater than or equal to five and declined rapidly when the pH value fell below this value. The low pH value of the soil also affects the nodulation ability of the groundnut [41]. The organic carbon content in the soil of the research area was 0.61% (Table 2). As stated by Hazelton and Murphy [34], a SOC value between 0.4 and 0.6% was considered low, and therefore the current value of soil organic carbon was low. Furthermore, the TN content in the research center was measured at 0.053% (Table 2), which is a low estimate [34]. The low SOC and TN content of the experimental site may have negatively affected groundnut production and it was necessary to add fertilizer to these soils. As shown in Table 2, theavailable soil phosphorus at the test site is 6.92 ppm. According to Hazelton and Murphy [34] review of soil test interpretations, the current result for soil available phosphorus is a low value (5–10ppm). At the research area, soil EC was measured at 2.94 dS m^{-1} (Table 2), which belongs to slightly saline soils (2-4 dS m^{-1}) and at this level, saline soils can negatively affect the yield of salt-sensitive plants [34]. The content of exchangeable Na cations in the study area was 0.16 cmol (+) kg^{-1}, which is considered low (0.1–0.3 cmol (+) kg^{-1}) [34]. The exchangeable K content of the soil at the research site was 0.21 cmol (+) kg^{-1} (Table 2). According to the interpretation of the soil analysis by Hazelton and Murphy [34], the current exchangeable K result is in the low category(0.2–0.3 cmol (+) kg^{-1}). The exchangeable Ca and Mg contents in the soil at the study area were 5.8 and 6.8 cmol (+) kg^{-1}, respectively (Table 2). According to the categoryrecommended by Hazelton and Murphy [34] for interpreting soil analysis results, the exchangeable Ca content in the experimental area is in the moderate category (5–10 cmol (+) kg^{-1}), while the value of the exchangeable Mg is high (3-8 cmol (+) kg^{-1}). Therefore, the Ca and Mg exchangeable cations of the experimental site are suitable for groundnut cultivation. The CEC of the research area was 13.2 cmol (+) kg^{-1}, which was considered moderate (12–25 cmol (+) kg^{-1}) [34]. The presence of moderate CEC in the soil creates a suitable soil environment for better groundnutgrowth.

Table 3. Analysis of variance for groundnut nodulation, biological nitrogen fixation, Nitrogen derived from the atmosphere and net nitrogen balance as affected by starter N and P fertilizers and their interaction effects.

Source of variation	Groundnut traits	DF	SS	MS	P level	SE	CV (%)
N fertilizer	NN	2	2197.1	1098.6	0.05	17.78	17.8
	BNF	2	1699.2	849.6	0.093	17.97	24.9
	Ndfa	2	1250.61	625.30	<.001	7.81	21.2
	NNB	2	3866.5	1933.2	<.001	4.62	16.8
P fertilizer	NN	3	698.2	232.7	0.54	17.78	17.8
	BNF	3	9090.9	3030.3	<.001	17.97	24.9
	Ndfa	3	1939.37	646.46	<.001	7.81	21.2
	NNB	3	2146.7	715.6	<.001	4.62	16.8
N * P	NN	6	1137.8	189.6	0.73	17.78	17.8
	BNF	6	809.3	134.9	0.86	17.97	24.9
	Ndfa	6	443.93	73.99	0.33	7.81	21.2
	NNB	6	6586.7	1097.8	<.001	4.62	16.8
Residual	NN	24	7583.3	316.0			
	BNF	24	7747.2	322.8			
	Ndfa	24	1463.36	60.97			
	NNB	24	511.93	21.33			

Total	NN	35	11616.4
	BNF	35	19346.6
	Ndfa	35	5097.27
	NNB	35	13111.73

NN-number of nodules, DF- degree of freedom, SS- sum of squares, MS- mean squares, P level-provability level, SE- standard error and CV- coefficient of variation.

3.2. Nodulation

For the average number of nodules per plant, a significant effect was only observed for independently applied N fertilizer, while insignificant effects were observed for P fertilizer alone and the interaction of N fertilizers and P (Tables3 and 4). The largest number of nodules per plant were observed in the plots treated with 15 kg N ha^{-1}, with no significant differences at the dose of 30 kg N ha^{-1}. The average number of nodules per plant decreased by 21.16% in the unfertilized plots compared to plots treated with 15 kg N ha^{-1}. Applicationof a higher dose of N fertilizer on groundnut plants can accelerate the disintegration of nodules, which is due to the inhibitory effect of nitrates on groundnut nodules and their development [42]. Application of high doses of N to legumes resulted in poor root growth and a reduction in flavonoid synthesis, thereby inhibiting rhizobia recognition and infection of legumes [43]. Confirming the current results, Li et al. [44] and Salvagiotti et al. [45] also observed a reduction in nodule number in faba beans and soybeanswith increasing nitrogen rates. For nodule initiation of common bean, 20 kg ha^{-1} starter N was required [46]. Consistent observations from different studies have shown the negative effects of higher N doses on thenodulation and BNF of legumes [17].

Table 4. Groundnut nodulation, nitrogen derived from the atmosphere and net nitrogen balanceaffected by N fertilizer.

N (kg ha^{-1})	NN	Ndfa (%)	NNB (kg ha^{-1})
0	89.65^{b}	42.99^{a}	-17.34^{a}
15	108.62^{a}	38.72^{a}	-23.25b
30	101.33^{ab}	28.91^{b}	-41.67^{c}
LSD (0.05)	14.98	6.58	3.89

Means followed by the same letters in the same column are not significantly different at (P≤0.05), NN-number of nodules per plant, LSD-least significant difference.

Table 5. Groundnut biological nitrogen fixation, nitrogen derived from the atmosphere and net nitrogen balanceaffected by N fertilizer.

P_2O_5 (kg ha^{-1})	BNF (kg ha^{-1})	Ndfa (%)	NNB(kg ha^{-1})
0	71.30a	40.02^{a}	-28.11^{b}
23	82.98^{a}	42.71^{a}	-15.64a

46	87.75^{a}	40.48^{a}	-28.61^{b}
69	46.68^{b}	24.28^{b}	-37.31^{c}
LSD (0.05)	17.48	7.60	4.493

Means followed by the same letters in the same column are not significantly different at ($P \leq 0.05$), LSD-least significant difference.

3.3. Biological Nitrogen Fixation (BNF)

No significant effect was observed on the interaction of N and P or on the separate effect of nitrogen fertilizer, while only P fertilizer had a significant effect on the BNF of groundnut legume (Tables3 and 5). When applying P_2O_5 at a dose of 46 kg ha^{-1}, a higher average BNF (87.75 kg ha^{-1}) was observed, which was not significantly different from the application of P_2O_5 (0.23 kg ha^{-1}). Mean BNF consistency increased for plots receiving 46 kg P_2O_5 but decreased significantly at 69 kg P_2O_5 ha^{-1}. The average BNF achieved after the application of 46 kg P_2O_5 ha^{-1} was 87.98% and higher than the P_2O_5 applied at a dose of 69 kg ha^{-1}. In fact, legumes can capture nitrogen from the atmosphere through a mutualistic symbiosis with some bacteria. However, to take advantage of this role, legumes require optimal phosphorus for energy conversion in the nodules. In addition, P plays an important role in the development of a healthy root, thereby improving plants' water and nutrient absorption. Confirming the current result, Hernandez et al. [47] reported that P significantly influences the growth and metabolism of legumes. Tropical legumes enriched with phosphate fertilizers have ahigher BNF [46]. Although BNF from legumes has played an undeniably importantrole in global food security, itsmaximum utilization is limited by P availability [11]. Under conditions of low soil P availability, the effectiveness of BNF in legumes is reduced [49–5]. Accordingly, Ssali and Keya [52] found increased nitrogen and BNF yields in beans after P application.

3.4. Proportion of Nitrogen Derived from the Atmosphere (%Ndfa)

A significant influence on the %Ndfa was found when N and P fertilizers were applied separately, but not when N and P fertilizers were applied in combination (Tables 3, 4 and 5). As the N fertilizer dose increased, the %Ndfa continuously decreased. A significantly higher amount of %Ndfa was found in plots not fertilized with N compared to plots receiving 30 kg N ha^{-1}; however, no significant difference with plots receiving 15 kg N ha^{-1}. Plots treated with 30 kg N ha^{-1} were characterized by a lower Ndfa of 48.70 and 33.93% compared to unfertilized plots and plots treated with 15 kg N ha^{-1}, respectively. Based on the results, it was observed that the N fixing capacity of groundnut plants decreases when fertilized with a higher dose of N and P fertilizers because the nutrient requirement of groundnut plants depends on the fertilizer applied. Therefore, Table 7 shows that BNF and Ndfa have a positive connection.Legumes sequester more N from the atmosphere when grown with little or no N fertilizer. The amount of %Ndfa can be negatively affected by increasing fertilizer rates, although groundnut kernel yield was higher with higher N fertilizer rates [9]. The BNF of alfalfa was reduced and delayed due to high N input [53]. The inclusion of legumes in agricultural production, such as crop rotation and green manure, can reduce the dependence on the use of N fertilizers as they have the potential to fix atmospheric N [54]. Legumes fix more N from the atmosphere when the soil is nutrient poor [1,55). Legumes grown on N-richsoils or fertilized with large amounts of N fertilizer inhibited the growth of legume nodules and thus reduced BNF [1, 45).

Likewise, aseparate application of P fertilizer had a significant impact on Ndfa percentage. Unfertilized plots and plots with 23 and 46 kg P_2O_5 ha^{-1} remained significantly similar but were characterized by a higher Ndfa of 64.83, 75.91 and 66.72%, respectively, compared to plots with 69 kg P_2O_5 ha^{-1}. When soil P deficiency was alleviated by adding chemical P fertilizers, the %Ndfa in legumes improved [56]. Phosphorusnutrientsignificantly influences the growth of legumes and their symbiotic relationship with soil rhizobia due to the specific effect of P on the growth of rhizobia in soil, their survival and their nodulation ability [19].

Table 6. Interaction effect of N and P fertilizer rates on net nitrogen balance of groundnut.

N (kg ha^{-1})	P_2O_5 (kg ha^{-1})			
	0	23	46	69
	Net nitrogen balance			
0	-9.18^{a}	-8.33^{a}	-19.32^{b}	-32.53^{c}
15	-4.04^{a}	-6.32^{a}	-35.73^{c}	-46.90^{d}
30	-71.12^{e}	-32.28^{c}	-30.79^{c}	-32.51^{c}
LSD (0.05)	7.78			

Means followed by the same letters across all columns and rows are not significantly different at ($P \leq 0.05$), LSD-least significant difference.

3.5. Net N Balance (NNB)

It was noted that applying N and P fertilizers separately and combining them resulted in a significant change in the groundnut's net soil nitrogen balance(Tables 3, 4, 5 and6). It is important to note that after applying 15 kg ha^{-1} of nitrogen ata zero P level, a decrease in N export from groundnut seeds was observed. This dosage was then followed by a combined application of 15 kg N ha^{-1} and 23 kg P_2O_5 ha^{-1}, but was still very close to the combined application of zero N with four P_2O_5 values (0, 23, 46 and 69 kg ha^{-1}) and a combined application of 30 kg N ha^{-1} with three P_2O_5 levels (23, 46 and 69 kg ha^{-1}). The greatest removal of N by groundnut kernels was observed at fertilization of 30 kg N ha^{-1} at a constant P zero level. The current result shows that the application of high N fertilizers to groundnut plants reduced the amount of biologically fixed N since legumes rely on the chemical fertilizer used. The smaller negative numbers suggested that groundnut plants used little N from the soil and that most of the N requirement came from biologically fixed N in the atmosphere. Positive values provide a useful indication of the potential input of atmospheric nitrogen into the system, but negative values indicate the removal of N from the soil. Current results show that all biologically fixed N and some soil N are exported by the groundnut grains. A positive net addition of N to the soil by legumes can be expected if the Ndfa percentage is above the nitrogen harvest index (NHI) by grains. Opposing the current results, Kermah et al. [1] reported that groundnut contributes an average of 2 kg ha^{-1} of net nitrogen to the soil when only grains are exported. The negative N balance in legume soil reflects a lower Ndfa percentage than NHI [57]. Peoples and Craswell [58] found that although the amount of BNF produced by legumes may be high, it is still not sufficient to compensate for the N exported by the removed seeds. Higher values often mean that while the groundnut kernel removed substantial amounts of N from the soil has been absorbed rather than contributed to the soil system.

Similar to the interactions between N and P fertilizers, N fertilizer alone had a significant influence on the net N balance of groundnut (Tables 3 and 4). Lower N removal by groundnut kernels was observed in the unfertilized groundnut plot and was significantly similar to that applied at a rate of 15 kg N ha^{-1}. The net N balance measured in plots not treated with N fertilizer increased by 140% compared to plots fertilized with 30 kg N ha^{-1}. The decreasing trend in net N balance compared to groundnut with increasing N fertilization could be because the high N content in the root zone of groundnut kills the nodules, thus reducing the BNF, and the crop also depends on the N fertilizer applied for growth. A small nitrogen fertilizer application rate boosted plant growth, nitrogenase activity, nodule development, and BNF [59]. The overuse of chemical fertilizers on legume crops has caused a serious disruption in the nitrogen cycle, leading to nitrate accumulation in soil and water and nitrogen oxide pollution of the atmosphere [60]. The soil's NNB was significantly impacted by P's main effect. Plots receiving 23 kg ha^{-1} P_2O_5 showed better soil NNB, but plots receiving 69 kg ha^{-1} P_2O_5, which is the higher rate, demonstrated low soil NNB. P availability in the legume crops' rhizosphere is essential for increasing BNF, which improves soil NNB. [61]. Low soil N mining occurred from the stimulation of groundnut BNF by P fertilizer applied at 23 kg ha^{-1}. Table 7 displays the positive correlation between BNF and NNB.

Table 7. Correlation between groundnut nodulation, biological nitrogen fixation, nitrogen derived from the atmosphere and net nitrogen balance.

	NN	*BNF*	*Ndfa*	*NNB*
NN	1			
BNF	0.18^{NS}	1		
Ndfa	0.07^{NS}	0.80^{***}	1	
NNB	0.08^{NS}	0.39^{*}	0.73^{***}	1

*Stars indicate significant effects *p (probability level)* $\leq$ 0.05, ***p* $\leq$ 0.01, ****p* $\leq$ 0.001, *NS- non-significant, NN- number of nodules per plant.*

4. CONCLUSION

In conclusion, based on the results obtained, it was observed that groundnut nodulation, BNF, %Ndfa and net N balance improved with little or no fertilizer application. Adding large amounts of nutrients to groundnut likely reduced the potential ability to fix atmospheric nitrogen, due to groundnut growth is dependent on applied fertilizers. Therefore, legumes can reduce N fertilizer costs for smallholder farmers in developing countries. Furthermore, the risk of environmental pollution is low because legumes are included in the agricultural system due to low demand for external inputs. Therefore, overfertilizing groundnut crop excessively results in environmental contamination and incurs extra costs.

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COMPETINGINTERESTS

The author have declared that there is no any conflict of interests.

AUTHOR CONTRIBUTION STATEMENT

Kinfe Tekulu initiated the research, wrote the thesis research proposal, conducted the research, did data entry and analysis, and wrote the manuscript.

DATAAVAILABILITY

The authors state that, depending on the request, they can submit the data whenever they want. Upon a reasonable request, the corresponding author will provide the available datasets used and analyzed during the study.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author declared that no generative AI tools, such as text-to-image generators or large language models (ChatGPT, COPILOT, etc.), were used in the writing or editing of the paper.

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