Research article

Effect of starter nitrogen and phosphorus fertilizer rates on yield and yield components, grain protein content of groundnut (*Arachis Hypogaea* L.) and residual soil nitrogen content in a semiarid north Ethiopia

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ABSTRACT

Increasing costs of chemical fertilizers, environmental concerns of their application and demand for protein foods, placed an extensive interest in growing of legume crops for human nutrition, and soil fertility replenishment. This study was conducted to investigate the effects of nitrogen (N) and phosphorus (P) fertilizers on parameters of phenology, growth performance, grain yield, yield components, grain protein content of groundnut, and residual soil nitrogen content in the northern Ethiopia during the growing season of 2017. Three levels of N (0, 15 and 30 kg ha^{-1}) and four levels of P_2O_5 (0, 23, 46 and 69 kg ha^{-1}) were set in factorial combinations of randomized complete block design with three replications. Results showed that an average total biomass yield increased by 22.5% for separate individual application of 15 kg N ha^{-1} and by 16.6% for 46 kg P_2O_5 ha^{-1} compared to control plots. Haulm yield increased by 29.17% for plots treated with N fertilization compared to control plots. Average pod yield increased by 85.4% for a combined application of 15 kg N ha^{-1} and 46 kg P_2O_5 ha^{-1} fertilizers compared to the control plots. Plots fertilized with the highest combined rates of N and P have attained lower grain yield compared to the combined application of 15 kg N ha^{-1} and 46 kg P_2O_5 ha^{-1}. The highest grain protein contents were obtained for a combined application of 30 kg N ha^{-1} and zero P, and 15 kg N ha^{-1} plus 46 kg P_2O_5 ha^{-1}. The highest N harvest index was obtained for control treatments and for plots treated with combined application of 15 kg N ha^{-1} and 46 kg P_2O_5 ha^{-1}. Residual soil N content increased by 119% on plots with combined application of 15 kg N ha^{-1} and 46 kg P_2O_5 ha^{-1} compared to control plots. Based on our results, combined application of 15 kg N ha^{-1} and 46 kg P_2O_5 ha^{-1} was recommended for increasing grain yield, grain protein content and residual soil nitrogen. The results of this study are crucial to improve groundnut productivity, grain protein content and also to provide implication on soil fertility management in a crop rotation system.

1. Introduction

Groundnut stands thirteenth in terms of food crops, 4th in terms oilseed and 3rd in terms of protein sources for humans worldwide (Taru et al., 2008). Farming of groundnut probably originated in South America (Hammons et al., 2016). This farming was introduced to Africa in the 15th and 17th century by Portugal and Spain, respectively (Nigam, 2014). Groundnut farming was introduced to Ethiopia in 1920 by Italian explorers Yebio (1984), and then widely disseminated to the warm lowlands of the country where it fits well to the prevailing agro-ecological conditions.

Groundnut was grown on 27.94 million hectares of land with annual production estimated at 47.1 million tons and an average productivity of 1.7 t ha^{-1} globally, whereas its average productivity in Africa was estimated 1.10 t ha^{-1} (FAOSTAT, 2017). In Ethiopia for instant, during the growing season in 2018/2019, the total area coverage of groundnut was estimated to be 84,237 ha and the total grain yield was 144,091.3 tons with an average productivity of 1.70 t ha^{-1} (CSA, 2019). In Tigray region, where this specific study was conducted, groundnut is produced in the warm lowland agro-ecologies zones having dominantly sandy soils such as Merebleke, and Tanqua-Abergelle districts (Assefa et al., 2012; Sibhatu et al., 2016).

Groundnut gives better yield and yield quality when good cultivars are planted on soils with an optimal soil nutrients management system (Veeramani and Subrahmaniyan, 2011). By far, N is the most needed mineral nutrient in all cropping systems, due to its key roles in different biochemical and physiological processes of the plant (Leghari et al., 2016; Pourranjbari Saghaiesh et al., 2019). Foliar or soil application of N fertilizers has been shown to significantly influence the growth, yield and quality of many crops (Dehnavard et al., 2017; Souri et al., 2019). Holl and Vose (1980) found low pea seed protein content from N deficient soils. Fields with low soil N level (<10 kg ha^{-1}) prior to planting, required application of supplementary starter N for better yield and yield quality of dry pea (Huang et al., 2017). Results from field experiment reported by Mckenzie et al. (2001) revealed that when soil N was less than 20 kg N ha^{-1}, application of starter N fertilizer improved pea yield. Conversely, the application of a higher dose of N fertilizer inhibits BNF through the development of ineffective nodules (Prasad et al., 2011). In general, higher rate of N fertilizer negatively affects nodulation and BNF of legumes crops (Dogra and Dudeja, 1993; Souri, 2016; Huang et al., 2017; Aslani and Souri, 2018). Phosphorus is also another important mineral nutrient that has different roles in plant functional metabolism (Pourranjbari Saghaiesh et al., 2019), including energy transferring of legume crops during BNF (Hussain, 2017). Hence, P can promote legume crops to produce their own N sources, but at the time of P deficiency, rates of BNF can be negatively affected due to reduced number of effective root nodules (Malhotra et al., 2018). Phosphorus nutrition is also important for groundnut crop since it improves nodulation, BNF and increase residual soil nitrogen content (Yakubu et al., 2010) and significantly contributes for healthy and efficient root growth (Mitran et al., 2018).

Farmers of the study area have long experience and interest in growing of groundnut, primarily for food, protein sources (which is equivalent protein obtained from meat and eggs) (Arya et al., 2016), and as legume-cereal crop rotation system for soil fertility restoration (Van Kessel and Hartley, 2000). Thus, poor farmers having no access to meat and egg in the study area can compensate for protein needs by integrating groundnut in their diet. However, research on groundnut is very limited as compared to most commonly grown and produced cereal crops and subsistent legume crops such as chickpea, faba bean, and lentil in Ethiopia (Asfaw et al., 2011). A few previous studies conducted on groundnut in Ethiopia have focused mainly on evaluating the adaptability of improved seeds (Ahmed et al., 2016) and management of aflatoxin (Gebreselassie et al., 2014). It has been emphasized that in the cropping systems, rotation with N fixing legume is a good strategy for replenishing soil fertility and in particular for soil N sources (Gathumbi et al., 2003; Aslani and Souri, 2018). Crop production system in a rotation where legumes are integrated is ecologically sustainable, but the amount of yield obtained from the crops is low due to limited fertilizer inputs, low inherent soil fertility and high rates of nutrient (nitrogen) leaching from sandy soils. Soils of the study area are found to be deficient with essential macronutrients such as N, P, sulfur (S), and exchangeable potassium (K) and micronutrients such as iron (Fe), zinc (Zn), and boron (B) (EthioSIS, 2014). Considering soil nutrient deficiency, balanced fertilizer types composed of NPKSZnB and NPKSFeZnB were recommended for the study area (EthioSIS, 2014). Furthermore, there is no documented information available regarding the responses of groundnut crop to N and P fertilizers and their rates application in the study area. Hence, this study was conducted to investigate and better understand the effects of various levels of starter N and P fertilizers in a separate individual and in combined applications on growth performance, grain yield, yield components, grain protein content of the groundnut and residual soil N content after harvest.

2. Materials and methods

2.1. Study area

The study was conducted at Merebleke district, northern Ethiopia during the growing season of 2017 (Figure 1). The study area is located in a warm semi-arid agro-climatic zone with well-drained sandy loam soil texture and typical soil type of luvisols. It is situated at an altitude of 1390 m above sea level and at a distance of 42 km from the historical city of Axum to the north. The farming system of the study area can be characterized as mixed farming involving both crop and livestock production on the same land unit. In the area, the most dominant crops are sorghum, finger millet, groundnut and tef that are grown in a rotation system. Several vegetable types and fruits are also grown and produced in irrigated farming. The rainfall distribution of the study area is a mono-modal, with an average annual rainfall depth of 636 mm concentrated within June to September. The average monthly minimum and maximum temperatures were estimated at 15 and 35.6 °C, respectively with an average monthly temperature of 25.3 °C (Figure 2). According to Prasad et al. (2011), the optimum rainfall depth and temperature for the growing and production of groundnut are from 200 to 1000 mm and 20 to 30 °C, respectively. Therefore, the current weather data (Figure 2) of the study site was within the proposed suitable ranges of rainfall depth and temperature for groundnut production.

2.2. Experimental design and treatments

The experiment was conducted on selected farmer's field where no groundnut has been grown prior to the experiment to observe the actual effect of the treatments on groundnut production and residual soil nitrogen. The field experiment consists of a total of 12 treatments composed of three levels of N at 0, 15, and 30 kg ha^{-1} and four levels of P$_2$O$_5$ at 0, 23, 46, and 69 kg ha^{-1}. These treatments were arranged in a 3 × 4 factorial combinations and laid out in a randomized complete block design with three replications (Figure 3). The treatments with each zero level of N and P fertilizer was designated as a control treatment. All treatments were also received uniformly applied fertilizers of K at the rate of 30 kg K$_2$O ha^{-1}, S at the rate of 30 kg ha^{-1}, Zn at the rate of 2 kg ha^{-1} and B at the rate of 0.5 kg ha^{-1} to avoid yield loss due to a limited supply one of those nutrients from the soil. Fertilizers of N, P, K, and S were applied at the planting time, but the micronutrients (Zn and B) were applied later as foliar at the blooming stage of groundnut. The sources of these nutrients were urea for N, tri-superphosphate (TSP) for P$_2$O$_5$, KCl for K$_2$O, CaSO$_4$ for S, ZnSO$_4$ for Zn and borax (Na$_2$B$_4$O$_7$·10H$_2$O) for B. A local groundnut variety 'Sedi' was planted at a seed rate of 80 kg ha^{-1} using a recommended spacing between plants and rows. The size of each plot in this experiment was 3.6 m*4 m (14.4 m^2) with 9.6 m^2 net harvestable area per plot, and the spacing between blocks, plots, rows and plants was set at 1.5, 1, 0.6 and 0.1 m, respectively. In one plot, there were 6 rows and a total of 240 plants population (Figure 3).

2.3. Soil sampling and analyzed parameters

Before planting and after harvesting of groundnut, one and thirty-six disturbed composite soil samples were collected from 0 to 20 cm soil depth following zigzag sampling methods, respectively. These samples were air-dried, crushed by grinding and allowed to pass through 2 mm sieve size for analysis of selected soil physical and chemical properties. One undisturbed soil sample was also collected using core sampler for estimating dry soil bulk density. The collected disturbed samples were analyzed for soil texture, pH, electrical conductivity (EC), organic carbon (OC) content, total N, available P, cation exchange capacity (CEC), and exchangeable bases (K, Na, Ca and Mg). Soil texture was determined using the Bouyoucos hydrometer method (Bouyoucos, 1962). Soil pH
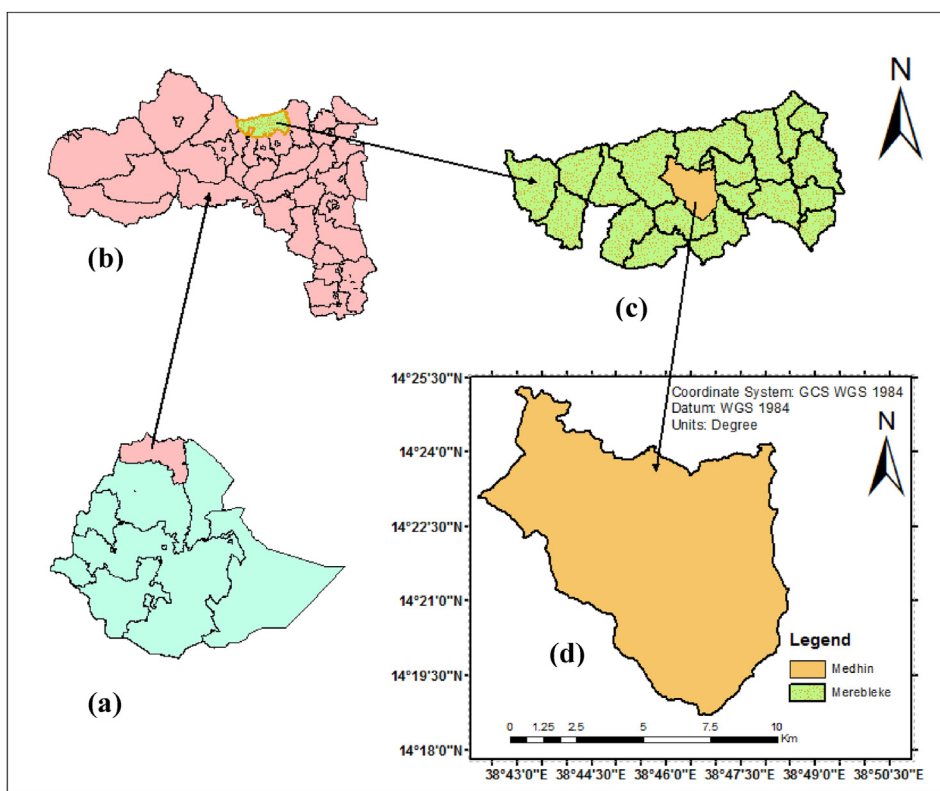
Figure 1. Location of the study area: in Ethiopia (a), in Tigray (b), in Merebleke (c) and (d) the specific study site Medhin tabia ('tabia' is the smallest administration unit).
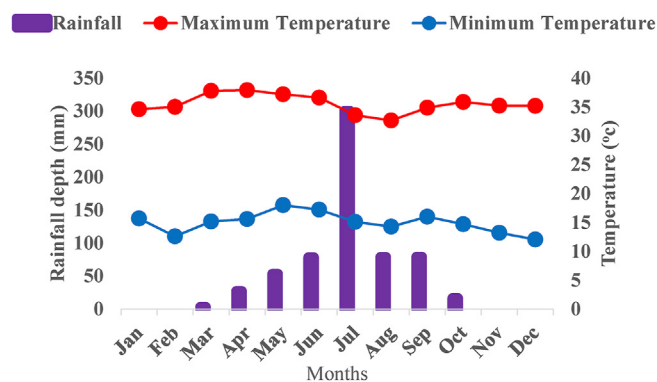

Figure 2. Monthly rainfall depth, maximum and minimum temperatures measured during the growing season (Sources: National Metrology Agency of Ethiopia, Tigray, Mekelle branch, 2017).

Figure 3. Experimental design, plots layout and illustration of field performance of groundnut.

was measured in the supernatant suspension of a 1:2.5 soil to water ratio using pH meter (Rhoades et al., 1999). Electrical conductivity of the soil solution was measured in the saturated extract of 1:2.5 soil to water ratio using EC meter (Richards et al., 1954). Percentage of soil organic carbon (SOC) content was determined following the wet oxidation method as proposed by Walkley and Black (1934). Total N was determined using the Kjeldahl method as described by Bremner and Mulvaney (1982). Available P was determined using the Olsen method (Olsen et al., 1954). Cation exchange capacity, and exchangeable base of the soil were extracted by leaching using ammonium acetate solution and the exchangeable bases were measured by atomic absorption spectrophotometry for Ca and Mg and flame photometer for that of Na and K (Polemio and Rhoades, 1977). The soil physical and chemical properties were summarized with ratings according to different studies in (Table 1).

2.4. Residual soil nitrogen

After harvesting groundnut crop, residual soil nitrogen content of the experimental site was determined. Total nitrogen stock within sampling depth (20 cm) was then calculated to quantify the nitrate contribution of groundnut crop to the soil due to BNF.

Residual soil N after harvest (kg ha^{-1}) = area (ha) $\times$ sampling soil depth (m) $\times$ bulk density (t ha^{-1}) $\times$ N (%)

2.5. Agronomic data collected and analyzed parameters

Agronomic data such as days to 50% flowering, days to 90% physiological maturity, plant height, number of branches per plant, haulm yield (t ha^{-1}), total biomass yield (t ha^{-1}), pod yield (t ha^{-1}), grain yield (t ha^{-1}), shelling percentage, nitrogen harvest index in percent (NHI%)

Table 1. Physiochemical properties of the soil at experimental site before planting (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	Jones (1983)
pH	5.5	Moderately acid	Tekalign (1991)
ECe (dS m^{-1})	2.94	Slightly saline	Hazelton and Murphy (2007)
OC (%)	0.61	Low	Hazelton and Murphy (2007)
Total N (kg ha^{-1})	15.5	Low	Hazelton and Murphy (2007)
Available P (ppm)	6.92	Low	Hazelton and Murphy (2007)
CEC (cmol (+) kg^{-1})	13.2	Moderate	Hazelton and Murphy (2007)
Exchangeable Bases			
Exc. K (cmol (+) kg^{-1})	0.21	Low	Hazelton and Murphy (2007)
Exc. Na (cmol (+) kg^{-1})	0.16	Low	Hazelton and Murphy (2007)
Exc. Ca (cmol (+) kg^{-1})	5.8	Moderate	Hazelton and Murphy (2007)
Exc. Mg (cmol (+) kg^{-1})	6.8	High	Hazelton and Murphy (2007)

and grain protein content (%) were recorded at the appropriate growth stage of groundnut crop. Flowering was measured from the day of emergence to the date when 50% of the plants opened their flower. Maturity was recorded from the day of emergence to the date when 90% of the plants attained physiological maturity. To measure plant height and number of branches per plant, five plants were randomly selected from each plot using non-destructive sampling methods at the maximum vegetative growth stage of groundnut. Plant height was measured using a ruler and the number of branches per plant were determined through counting of the main branches and recorded for each of the selected plant. Haulm yield (t ha^{-1}), biomass yield (t ha^{-1}), pod yield (t ha^{-1}) and grain yield (t ha^{-1}) were measured for each plot after harvesting. Biomass yield was recorded from each plot, and converted into t ha^{-1} basis. Haulm yield was measured after pod yield was separated from the total biomass yield and converted into t ha^{-1} basis. Pod yield was measured by weighing the pods obtained from each harvestable net area after separating the pod from the pegs, and finally, the result was converted into t ha^{-1} basis. Grain yield for each plot was also recorded by weighing the grain obtained from each harvestable net area after separating the seeds from its pod, and finally, the grain yield was converted into t ha^{-1} basis. The shelling percentage was calculated as the ratio of grain yield to the total pod yield and multiplied by hundred.

Using sampled groundnut plants, haulm and grain were collected for determination of N content and then grain protein content and nitrogen harvest index (NHI%) were estimated. Grain protein content was calculated using the formula proposed by FAO (1970) for the N to grain protein content universal conversion factor of 6.25 was applied. Percentage nitrogen harvest index is defined as the ratio between N uptake in grain and N uptake in grain plus straw multiplied by hundred (Fageria, 2014). Samples of groundnut haulm and grain were collected at harvesting time from five non-border plants to avoid border effects. These samples were thoroughly washed using distilled water to remove adhering soils and dust particles, and then placed in a paper envelope and oven-dried at 60 °C for 68 hours. After oven drying, haulm and grain were grinded to powder and allowed to pass through a 0.5 mm mesh sieve. Determination of total N content from the plant samples was conducted using Kjeldahl methods by means of treating plant material and oxidized with concentrated sulfuric acid and distillation of an aliquot from the digest with NaOH, collecting the distillate in boric acid and titrating with 0.01 N HCl to the endpoint of the mixed indicator (Plank, 1992).

The collected soil nitrogen content and agronomic data were subjected to statistical analysis of a two-way analysis of variance. Before data analysis, the collected data was carefully checked for normality and homogeneity of variance across the separate individual levels and combination of N and P fertilizer treatments. Analysis of variance (ANOVA) was performed using SAS software. A significant difference among treatment means was assessed using the least significant difference (LSD) at 0.05 level of probability.

3. Results and discussion

3.1. Phenological parameters

The results indicated that separate individual levels and combinations of N and P fertilizers rates did not affect phenological parameters of groundnut such as time of flowering and maturity (Table 2). This might be due the fact that biological N fixation produced enough N for groundnut plants to initiate flowering and maturation. Nevertheless, these phenological traits are more under genetic control and adaptation in some plant species to environmental stresses, and less responsive to management practices (Souri and Hatamian, 2019; Fattahi et al., 2019). Similarly, application of P fertilizer and manure had no significant effect on flowering and maturity of Werer 962 and Oldhale groundnut varieties (Melese and Dechassa, 2017). In eastern Ethiopia (Babile) application of manure alone has no significant effect on flowering and maturity of Werer 962 and Oldhale groundnut varieties (Melese and Dechassa, 2017). This indicated that phenological parameters of groundnut are independent of mineral and organic fertilizer applications and controlled by other factors.

3.2. Growth parameters

The average height of groundnut plant was significantly affected by separate individual applications of each the N and P fertilizer levels and their combinations (Table 3). Combined application of N and P significantly increased plant height. At the highest levels of both N and P$_2$O$_5$ (30 and 69 kg ha^{-1}), plant height increased by 11.10 cm compared to average plant height from the control treatments. A similar increase in plant height, but in a lower scale, was also observed for other N and P combinations (except 0 levels). This is mainly due to growth promotion via rapid meristematic activities induced by the application of combined N and P in a nutrient deficient soil. Balanced fertilizers application can promote plant cell division and cell elongation, resulting in improved vegetative growth and ultimately increase plant height, number of branches per plant and number of nodules per plant. Here, the most deriving force for increasing plant height was N fertilizer through increased vegetative growth. Nitrogen is the most highlighted nutrient in

Table 2. Effect of N and P fertilizers on days to 50% flowering and days to 90% maturity of groundnut plant.

N (kg ha^{-1})	P$_2$O$_5$ (kg ha^{-1})				
	0	23	46	69	Mean
	Flowering (days)				
0	53	52	51.3	51.7	52
15	52.7	52	51.3	52.7	52.2
30	51.7	53.3	53	52.7	52.7
Mean	52.5	52.4	51.9	52.3	
CV (%)		2			
LSD (P = 0.30) for N		NS			
LSD (P = 0.65) for P		NS			
LSD (P = 0.18) for N*P		NS			
N (kg ha^{-1})	P$_2$O$_5$ (kg ha^{-1})				
	0	23	46	69	Mean
	Maturity (days)				
1	85	85	85.3	84.7	85
15	86.3	86.7	85	85.7	85.9
30	86.3	85	86.7	85	85.6
Mean	85.9	85.6	85.7	85.1	
CV (%)		2.3			
LSD (P = 0.49) for N		NS			
LSD (P = 0.86) for P		NS			
LSD (P = 0.87) for N*P		NS			

Table 3. Plant height and number of branches per plant for the various levels of N and P fertilizer treatments.

N (kg ha^{-1})	P$_2$O$_5$ (kg ha^{-1})				
	0	23	46	69	Mean
	Plant height (cm)				
0	45.0^E	50.1^{CD}	50.7^{DC}	49.3^D	48.8^C
15	50.7^{CD}	52.3^{BC}	52.9^{BC}	55.1^{AB}	52.7^B
30	54.7^{AB}	53.8^{AB}	54.5^{AB}	56.1^A	54.8^A
Mean	50.2^B	52.1^A	52.7^A	53.5^A	
CV (%)		3.2			
LSD (P < 0.0001) for N		1.4			
LSD (P = 0.002) for P		1.6			
LSD (P = 0.04) for N*P		2.8			
N (kg ha^{-1})	P$_2$O$_5$ (kg ha^{-1})				
	0	23	46	69	Mean
	Number of branch per plant (Numbers)				
0	6.7^C	10.0^B	11.0^B	11.0^B	9.7^B
15	11.0^B	11.0^B	14.0^A	12.0^{AB}	12.0^A
30	12.0^{AB}	11.3^B	12.0^{AB}	11.0^B	11.6^A
Mean	9.9^C	10.8^{BC}	12.3^A	11.3^{AB}	
CV (%)		11.3			
LSD (P = 0.0003) for N		1.1			
LSD (P = 0.0003) for P		1.2			
LSD (P = 0.04) for N*P		2.1			

Where, means followed by the same letters are not significantly different at (P ≤ 0.05).

increasing plant height as it enhances vegetative growth through cell division and cell elongation (Souri and Hatamian, 2019; Souri et al., 2019). Besides, it has also been shown that application N fertilizer can significantly improve the uptake and assimilation of other nutrients and internal re-translocations (Souri and Hatamian, 2019). An increased plant height for groundnut was also reported because of increased application of NPK fertilizer when applied either at basal or split doses

together with farmyard manure (Sengupta et al., 2016). Better plant height of soybean was also observed when 11.5 kg N ha^{-1} and 46 kg P$_2$O$_5$ha^{-1}are applied in a combination and inoculated with Bradyrhizobium Japonicum used in an integrated manner (Tarekegn and Kibret, 2017).

Our results show that the highest average plant height of 54.8 cm was recorded for plots treated with 30 kg N ha^{-1}compared to average height

for control plots (no N) and plots treated with 15 kg N ha^{-1}. The shortest average plant height of 48.8 cm was recorded for the control plots without N fertilization. Gohari and Niyaki (2010), and Tenywa et al. (2016) reported that the application of N fertilizer remarkably increased the plant height of groundnut. Nitrogen fertilizer has a pronounced effect on the plant height of pea due to increased growth performances (Achakzai, 2012). When plots of groundnut were fertilized with 23, 46 and 69 kg P$_2$O$_5$ kg ha^{-1} average values of plant height were clearly increased when compared to an average plant height from the control plots (no P), though the differences remain statistically insignificant. Erman et al. (2009) also reported a taller plant height of field pea with fertilization of P$_2$O$_5$ as it enhances nitrogen fixation. The results indicate that P fertilizer enhance vigorously and healthy plant growth performance and yield and yield components.

The number of branches per plant were significantly affected by the application of N and P fertilizers at a separate individual and combined rates (Table 3). The combined application of N and P fertilizers at the rate of 15 kg N ha^{-1} and 46 kg P$_2$O$_5$ ha^{-1} has resulted in largest number of branches per plant (Table 3). The smallest average numbers of branches per plant of 6.7 was measured from the control plots. The average number of branches per plant for plots treated with a combined rate of 15 kg N ha^{-1} and 46 kg P$_2$O$_5$ ha^{-1} fertilizers exceeded the corresponding average number of branches per plant from control treatments by 52.4%. The results revealed that starter N at 15 kg ha^{-1} combined with 46 kg P$_2$O$_5$ ha^{-1} promoted cell division and cell elongation, ultimately increased the number of branches per plant, nodulation and yield of groundnut. The N requirement of legume is low compared to non-nitrogen fixing crops while their requirement for P is high. This is because, legume crops can fix atmospheric nitrogen into ammonia form through BNF processes for which application of P is required in large amount as sources of ATP for BNF. The research results reported by Achakzai (2012) showed that significantly increasing number of branches per plant of pea crop with an increased rate of N fertilizer application. Plant growth, in terms of plant height or number of branches, is a function of the interaction and balance of physiologically important metabolites such as carbohydrates and phytohormones toward

induction of dominant apical growth or branching (Mardanluo et al., 2018; Souri, 2016). Several studies also reported an increased number of branches per plant for faba bean due to soil fertilization and management through application of both organic and inorganic soil amendments (Fekadu et al., 2018). Applications of 15 kg N ha^{-1} has a similar effect on number of branches per plant of groundnut compared to application of 30 kg N ha^{-1}. The results indicated that average number of branches per plant are also significantly affected by a separate individual application of P (Table 3). This is related to the contribution of P in plant nutrition and growth performances. In line with our results, Majeed et al. (2014) and Shafi et al. (2015) also reported that the number of valuable tillers of the wheat crop was appreciably enlarged as the rates of P fertilizer increases.

3.3. Yield and yield attributes

The yield and yield attribute results showed that the total biomass yield of groundnut was significantly affected by separate individual rates of N and P fertilizer application, but there was no interaction effect (Table 4). Average total biomass recorded from plots treated with 15 kg N ha^{-1} and 30 kg N ha^{-1} were significantly different compared to average biomass yield from the control plots. The maximum total biomass yield of 7.73 t ha^{-1} was obtained from 15 kg N ha^{-1}, but it was insignificant when compared to the biomass yield obtained from 30 kg N ha^{-1} i.e.7.46 t ha^{-1}. The minimum average biomass yield was achieved without N fertilizer application and was 6.31 t ha^{-1}. This entails that effect of N and P was independent for the observed variation in total biomass yield. Similar to our findings, Melese and Dechassa (2017) also reported that combined application P and manure have a non-significant effect on biomass when applied to the Werer 962 and Oldhale groundnut varieties. Nitrogen application enhances the number of meristematic cells, and cell growth, consequently results in large plant biomass production (Lawlor, 2002). Too much application of N and K often resulted in excessive vegetative growth of groundnut and larger biomass yield (Annadurai et al., 2009). Blumenthal et al. (2008) reported that adding N fertilizer to crop resulted in higher biomass yields, protein content of the yield and N

Table 4. Total biomass and haulm yield of groundnut at different rates of separate N and P fertilizers and combined applications.

N (kg ha^{-1})	P$_2$O$_5$ (kg ha^{-1})				
	0	23	46	69	Mean
	Total biomass yield (t ha^{-1})				
0	5.47	6.32	6.91	6.54	6.31[B]
15	7.11	7.74	8.59	7.46	7.73[A]
30	7.24	7.64	7.62	7.35	7.46[A]
Mean	6.61[B]	7.23[AB]	7.71[A]	7.12[AB]	
CV (%)	10.8				
LSD (P = 0.0003) for N	0.7				
LSD (P = 0.05) for P	0.8				
LSD (P = 0.81) for N*P	NS				
N (kg ha^{-1})	P$_2$O$_5$ (kg ha^{-1})				
	0	23	46	69	Mean
	Haulm yield (t ha^{-1})				
0	2.86	3.14	3.2	3.28	3.12[B]
15	4.04	4.12	3.75	4.1	4[A]
30	4.3	3.78	3.91	4.13	4.03[A]
Mean	3.73	3.68	3.62	3.84	
CV (%)	13.7				
LSD (P = 0.0002) for N	0.4				
LSD (P = 0.84) for P	NS				
LSD (P = 0.80) for N*P	NS				

Where, means followed by the same letters are not significantly different at (P ≤ 0.05).

concentration in plant tissue. Similar to our findings, Meena et al. (2011) also reported an enhanced biomass yield of groundnut from plots treated with higher doses of N fertilizer compared to the condition without N fertilizer application. Research results reported by Tarekegn and Kibret (2017) also confirm that the application of 23 kg N ha^{-1} resulted in higher biomass yield of groundnut when compared to average biomass yield from control plots and plots treated with 11.5 kg N ha^{-1}. The results also indicated that application of P fertilizer had a significant effect on total biomass yield when applied at 46 kg P_2O_5 ha^{-1} compared to control treatments (Table 4). Similar to the case with effect of N fertilizer, the smallest biomass yield for groundnut was also measured for control plots compared to the plots treated with P indicating that P is also a limiting nutrient in the soils of the study area. Singh (2004) stated that P application had brought a significant increase in total biomass yield of groundnut in calcareous soil receiving 50 kg P_2O_5 ha^{-1}. Vegetative and reproductive growth and development of crops are negatively affected when planted on soils deficient in P (Zhang et al., 2014).

Haulm yield of groundnut was significantly affected by the application N fertilizer, but not affected due to the applications of N in combinations with P and separate individual application of P (Table 4). Treatments with separate individual applications of N fertilizer at 15 and 30 kg N ha^{-1} had a significant advantage over the control treatments. The maximum average haulm yield of 4 t ha^{-1} was recorded from treatments with 30 kg N ha^{-1} and 15 kg N ha^{-1} application rates, whereas; the minimum average haulm yield of 3.1 t ha^{-1} was recorded for the control (no N) treatments. Similarly, the highest haulm yield of groundnut was obtained from the application of N at 40 kg ha^{-1} (Meena et al., 2011). Most of the time haulm yield of groundnut consistently increased with increasing application rates of N fertilizer up to 30 kg N ha^{-1}, after which haulm yield starts declining slightly (Lombin et al., 1985). Similarly, Singh et al. (2007) reported that application of 30 kg N ha^{-1} significantly increased straw yield of cowpea compared to the yield from the corresponding control plots.

The results of pod yield for groundnut revealed a significant effect of N and P fertilizers both in separate individual and in combined applications (Table 5). The highest average pod yield of 4.84 t ha^{-1} was obtained from plots treated with combined application of 15 kg N ha^{-1} and 46 P_2O_5 kg ha^{-1} and followed by the combined application of 30 kg N ha^{-1} and 23 kg P_2O_5 ha^{-1} (Table 5). The lowest average pod yield of 2.61 t ha^{-1} was recorded from the control treatments. Average pod yield produced from the combined application of 15 kg N ha^{-1} and 46 P_2O_5 kg ha^{-1} was the highest and exceeded the average pod yield obtained from control plots by 130.5%. It is clear from the results that groundnut crop requires starter N to initiate nodulation and BNF, which ultimately enhances the yield and yield components including pods. Starter N played a critical role in plant growth leading to higher photosynthetic activity and translocation of photosynthates to the sink and pods. Similarly, Kumar et al. (2015) also reported that combined application of N and P fertilizers significantly affected the pod yield of groundnut. It was also reported that promising pod yield of groundnut was obtained from a combined application of organic, inorganic and bio-fertilizers (Vala et al., 2017). Separate individual application of N fertilizer also brought a significant effect on pod yield of groundnut (Table 5). Pod yield from plots treated with N fertilizer at 15 kg ha^{-1} brought higher pod yield as compared to controlled plots and plots treated with 30 kg N ha^{-1}. Similar to the effects of N, pod yield of groundnut is also significantly affected by the separate individual application of P fertilizer (Table 5). The application of P fertilizer at the level of 46 kg P_2O_5 ha^{-1} has resulted in highest pod yield and significantly higher compared to the other levels of P_2O_5 applications. Similar to our results, Kabir et al. (2013) also reported the highest pod yield for groundnut from plots treated with the application of 50 kg P_2O_5 ha^{-1} compared to the control treatments, which is probably due to differences in soil fertility status of the experimental sites.

The results in Table 5 revealed that application of N and P fertilizer at separate individual rates and different combinations significantly increased grain yield of groundnut crop. The highest average grain yield of 2.66 t ha^{-1} was attained from the combined applications of 15 kg N ha^{-1} and 46 P_2O_5 kg ha^{-1} followed by average yield obtained from plots treated with 30 kg N ha^{-1}and no P application i.e. 2.48 t ha^{-1}. Combined application of 30 kg N ha^{-1}and 69 P_2O_5 kg ha^{-1} fertilizers resulted in

Table 5. Effect of N and P fertilizers on pod and grain yields of groundnut.

N (kg ha^{-1})	P_2O_5 (kg ha^{-1})				
	0	23	46	69	Mean
	Pod yield (t ha^{-1})				
0	2.61^E	3.19^{CD}	3.71^{BC}	2.99^{DE}	3.12^C
15	3.067^{DE}	3.62^{BC}	4.84^A	3.37^{BCD}	3.72^A
30	2.94^{DE}	3.86^B	3.71^{BC}	3.22^{CD}	3.43^B
Mean	2.87^D	3.56^B	4.09^A	3.19^C	
CV (%)	9.6				
LSD (P = 0.0007) for N	0.3				
LSD (P < 0.0001) for P	0.3				
LSD (P = 0.05) for N*P	0.6				
N (kg ha^{-1})	P_2O_5 (kg ha^{-1})				
	0	23	46	69	Mean
	Grain yield (t ha^{-1})				
0	1.78^E	1.91^{DE}	2.22^{BCD}	1.81^E	1.93^B
15	1.98^{DE}	2.01^{CDE}	2.66^A	1.98^{DE}	2.16^A
30	2.48^{AB}	2.27^{ABCD}	2.40^{ABC}	1.68^E	2.21^A
Mean	2.08^B	2.06^B	2.43^A	1.82^C	
CV (%)	11.4				
LSD (P = 0.02) for N	0.2				
LSD (P = 0.0002) for P	0.2				
LSD (P = 0.04) for N*P	0.4				

Where, means followed by the same letters are not significantly different at (P ≤ 0.05).

lowest grain yield of 1.68 t ha^{-1} which was lower by 59% compared to grain yield obtained from plots treated with combined application of 15 kg N ha^{-1} and 46 P$_2$O$_5$ kg ha^{-1}. It was also observed that the effect of N and P fertilizers on growth performance and grain yield largely depend on each other. Application of balanced nutrients played a very important role in enhancing grain yield, developing prolific nodule and healthy root system for better BNF and enhanced absorption of soil water and nutrients. This in turn gives better plant growth leading to higher photosynthetic activity and translocation of photosynthates products to the sink. Similarly, it was also reported that integrated application of mineral fertilizers with manure enhanced grain yield of groundnut (Melese and Dechassa, 2017). Bala and Nath (2015) also indicated that integrated soil nutrient management as strategy to optimize groundnut productivity. Similar to our findings, the research results reported by Vala et al. (2017) also revealed that integrated application of organic, inorganic and bio-fertilizers as nutrient sources resulted in higher grain yield of groundnut compared to the condition without fertilizer applications. Our results indicated that the highest grain yield i.e. 2.66 t ha^{-1} obtained at experimental site is even higher when compared to the average national grain yield of 1.72 t ha^{-1} for groundnut reported by CSA (2019).

The result of grain yield obtained in this study is in line with the results reported for different leguminous crops from different parts of the world. This indicates that even nitrogen-fixing leguminous crops require starter nitrogen to produce higher yield compared to the yield condition without fertilizer application. Nitrogen-deficient soils can result in low grain yield of legume crops and therefore application of starter N can enhance yield of legume, but when the soil has enough N level (equivalent to starter nitrogen) there is no need of supplementing legumes with N fertilizer (Huang et al., 2017). As the application rates of N fertilizer increases there is a tendency for reduced pod and grain yield even though this enhances haulm production (Lombin et al., 1985). Similarly, when soils contain less than 20 kg N ha^{-1}, application of N fertilizer increased pea yield (Mckenzie et al., 2001). A higher kernel yield of groundnut was found from plots treated with N fertilizer (Gohari and Niyaki, 2010).

Annadurai et al. (2009) found that application of 25 kg N ha^{-1} to groundnut plots as an optimal and cost-effective rate.

Nitrogen requirement of groundnut crop is supplied through BNF, but at low soil fertility a starter N at the rate of 20–30 kg ha^{-1} is desirable to feed the plant until the plant develops nodule formation and harbor bacteria for BNF (Roy et al., 2006). Promising grain yield of cowpea was also found with the application of 30 kg ha^{-1} starter N compared to the condition with no N fertilizer application (Singh et al., 2007). When only P fertilizer is applied to the plots of groundnut, the highest grain yield of 2.43 t ha^{-1} was obtained from plots treated with 46 kg P$_2$O$_5$ ha^{-1}. The smallest average grain yield of 1.82 t ha^{-1} was attained with no P application i.e. control plots. In addition to N, Annadurai et al. (2009) reported that grain yield and yield attributes of groundnut were significantly increased with application of P$_2$O$_5$ up to 40 kg ha^{-1}.

N and P fertilizers significantly affected the shelling percentage of groundnut when applied as a separate individual and in combined applications (Table 6). Combined application of 30 kg N ha^{-1} without P fertilizer had the highest shelling percentage of 85% compared to all other treatments. It is known that shelling percentage increase when the numbers of the sound kernel or filled pod increase, thus a combination of 30 kg N ha^{-1} without P fertilizer application had brought filled pod, i.e. a pod with a marketable grain of groundnut. This revealed that N fertilizer had a greater influence on the shelling percentage of groundnut in the absence of P containing fertilizer. Gohari and Niyaki (2010) also conducted that addition of N fertilizer in combination with iron (Fe) fertilizer have resulted in a better shelling percentage of groundnut in northern Iran. The main effect of N and P fertilizers had revealed a significant effect on the ratio of grain to pod yield. Accordingly, plots treated with N fertilizer at the rate of 30 kg N ha^{-1} have resulted in highest shelling percentage of 65%; however, it was not statistically different compared to the shelling percentage obtained from control plots (without N fertilizer). In line with our findings, Meena et al. (2011) also reported that the highest shelling percentage of groundnut was observed for application of large amount of N fertilizer than a relatively smaller rates. Control plots of groundnut (without P fertilizer) application resulted in

Table 6. Shelling percentage and grain protein content of groundnut under different rates of N and P fertilizers application.

N (kg ha^{-1})	P$_2$O$_5$ (kg ha^{-1})				
	0	23	46	69	Mean
	Shelling percentage (%)				
0	68^B	60^{BCD}	60^{BCD}	62^{BCD}	63^{AB}
15	65^{BC}	55^{CD}	55^{CD}	59^{BCD}	58^B
30	85^A	59^{BCD}	64^{BC}	52^D	65^A
Mean	73^A	58^B	60^B	58^B	
CV (%)	10.2				
LSD (P = 0.05) for N	5.4				
LSD (P < 0.0001) for P	6.2				
LSD (P = 0.02) for N*P	10.7				
N (kg ha^{-1})	P$_2$O$_5$ (kg ha^{-1})				
	0	23	46	69	Mean
	Grain protein content (%)				
0	26.39^H	29.51^{CDE}	29.85^{CD}	28.54^{EF}	28.57^B
15	28.09^{FG}	28.85^{DEF}	33.11^A	30.40^{BC}	30.11^A
30	33.75^A	31.17^B	27.70^{FG}	27.08^{GH}	29.92^A
Mean	29.41^B	29.84^{AB}	30.22^A	28.67^C	
CV (%)	2.37				
LSD (P < 0.0001) for N	0.6				
LSD (P = 0.006) for P	0.7				
LSD (P < 0.0001) for N*P	1.2				

Where, means followed by the same letters are not significantly different at (P ≤ 0.05).

highest shelling percentage of 73% compared to plots treated with application of P at 23, 46 and 69 kg P_2O_5 ha^{-1}.

The results of grain protein content of groundnut treated with different separate individual levels and combined application of N and P fertilizers are presented (Table 6). Grain protein content of groundnut was significantly affected by N and P fertilizers applications. The highest grain protein content of 33.75% was recorded from plots treated with 30 kg N ha^{-1} and without P application, but this was not statistically different compared to grain protein content from plots treated with combined application of 15 kg N ha^{-1} and 46 P_2O_5 kg ha^{-1}. The smallest average grain protein content of 26.39% was recorded from the grains harvested from the controlled treatments. Our result showed that, the grain protein content of groundnut was in the range of 26.39–33.75% for the different treatments with a general increasing trends with increasing N fertilizer rates. Groundnut production in developing countries is increasingly attractive as income, food and protein sources where protein from animal sources is less accessible by the community. Nitrogen and phosphorus are necessary for protein biosynthesis in plant tissues. There is always positive response of grain or leaf protein content to N application particularly under soil with N deficiency (Souri and Aslani, 2018; Aghayie Noroozlo et al., 2019). Integrated fertilizer application and soil management improved protein content of the groundnut (Ola et al., 2013). Similarly, Daramy et al. (2016) indicated that the highest total grain N and crude protein content of groundnut were recorded from a combined application of 30 kg N ha^{-1} and without P application. Inoculation of Rhizobium and P application increased the crude protein content of cowpea grain (Kyei-Boahen et al., 2017). Application of P and foliar spraying of Zn fertilizers to plots of groundnut have exerted a positive effect on grain protein content (Gobarah et al., 2006). In contrast, keeping N at zero level and increasing the levels of P_2O_5 from (0–46) kg ha^{-1} showed slightly increasing trends of average grain protein content, but it decreased at the level of 69 kg P_2O_5 ha^{-1} (Table 6). In line with our results, Awadalla and Abbas (2017) also reported that grain protein content of groundnut increased with increasing rates of N fertilizer. Havlin et al. (2005) also reported that P also improves crop quality

through increased grain protein contents and improved proportion of marketable yields.

Similarly, application of N and P fertilizers at separate individual rates significantly affected grain protein content of groundnut (Table 6). This is attributed to the fact that N is a key component of amino acid, which is the building block of protein. Higher grain protein content of dry pea was also reported from application higher rate of N as urea and slow-release polymer-coated N fertilizers (Huang et al., 2017). Plots fertilized with 46 kg P_2O_5 ha^{-1} had highest grain protein content of 30.22% and significantly larger compared to plots without P and plots treated with 69 kg P_2O_5 ha^{-1}. The highest grain protein content obtained from 46 kg P_2O_5 ha^{-1} was attributed to enhanced BNF and its utilization by the groundnut. Cowpea grain protein content also increased with increasing rates of P fertilization due to the fact that P is crucial in the process of biological N fixation (Singh et al., 2007).

The nitrogen harvest index (NHI%) was significantly affected by single and combined application of N and P fertilizers (Table 7). The results also showed that the interaction effect of N and P at the rate of 15 kg N ha^{-1} and 46 kg P_2O_5 ha^{-1} had the largest NHI of 58.4%. However, this effect of combined N and P applications remain statistically insignificant compared to treatments with no N and 23 kg P_2O_5 ha^{-1}, no N with 46 kg P_2O_5 ha^{-1} and including control treatments. The highest NHI for control treatments is attributed to assimilation of large part of biologically fixed ammonia to grain nitrogen by the groundnut (Table 7). The largest N content in the grain also indicate the conversion of N from the haulm to grain is higher.

However, NHI decreased when plots were treated with the highest level of combined application of N and P fertilizers. Treatments with small values of NHI may indicate that the largest portion of N content is stored in the haulm of the plant than in the grain. Bulman and Smith (1994) reported that the larger NHI shows increased conversion of N from the haulm to the grain. Nitrogen harvest index is a useful indication of N partitioning in the crop and gives an indication of how efficiently the crop is in utilizing obtained N for grain production (Fageria, 2014). Enhanced N use efficiency of grain is fundamental in reduce cost of crop

Table 7. Nitrogen harvest index (NHI) and residual soil nitrogen content of groundnut as affected by N and P fertilization.

N (kg ha^{-1})	P_2O_5 (kg ha^{-1})				
	0	23	46	69	Mean
	Nitrogen Harvest Index (%)				
0	58.2[A]	54[ABC]	55.5[AB]	48.9[CDEF]	54.1[A]
15	47.9[DEF]	46.4[EF]	58.4[A]	46.3[EF]	49.8[B]
30	52.8[BCD]	51.6[BCDE]	45.6[F]	38.1[G]	47.0[C]
Mean	53[A]	50.7[A]	53.1[A]	44.4[B]	
CV (%)	6.3				
LSD (P < 0.0001) for N	2.7				
LSD (P < 0.0001) for P	3.1				
LSD (P = 0.0003) for N*P	5.4				
N (kg ha^{-1})	P_2O_5 (kg ha^{-1})				
	0	23	46	69	Mean
	Soil Nitrogen (kg ha^{-1})				
0	25.7[DE]	27.4[CD]	30.4[BC]	26.0[ED]	27.4[B]
15	30.7[ABC]	31.2[AB]	33.9[A]	24.5[DEF]	30.1[A]
30	22.2[F]	23.7[EF]	23.1[F]	22.8[EF]	22.8[C]
Mean	26.3[BC]	27.4[AB]	29.2[A]	24.5[C]	
CV (%)	7.6				
LSD (P < 0.0001) for N	1.8				
LSD (P = 0.0005) for P	2				
LSD (P = 0.013) for N*P	3.5				

Where, means followed by the same letters are not significantly different at (P ≤ 0.05).

production and also environmental pollution resulting from nitrate leaching or nitrate loading in surface water while at the same time maintaining high quality grain production (Fageria, 2014). In view of soil fertility, Peoples and Craswell (1992) observed that a higher value of NHI resulted in a lower amount of residual soil N to be returned to the soil through addition of crop residue as the N stored in the grain is completely harvested and removed from the field.

3.4. Residual soil nitrogen

The results of residual soil N after harvest are presented (Table 7). The results showed that the total N content of the soil after harvest was significantly affected by a separate individual and combined applications of N and P fertilizers (Table 7). The highest soil N of 33.9 kg ha^{-1} was observed from plots treated with combined application of 15 kg N ha^{-1} and 46 kg P$_2$O$_5$ ha^{-1}, and followed by plots treated with a combined application of 15 kg N ha^{-1} and 23 kg P$_2$O$_5$ ha^{-1}. There was no significant difference among treatments combination with respect of residual soil nitrogen among 15 kg N ha^{-1} and 46 kg P$_2$O$_5$ ha^{-1}, 15 kg N ha^{-1} and 23 kg P$_2$O$_5$ ha^{-1}, and 15 kg N ha^{-1} and without P. The lowest residual soil N content of 22.2 kg ha^{-1} was recorded for plots treated with combined application of 30 kg N ha^{-1}and without P. This implied that groundnut crop satisfies its N requirement from the applied N fertilizer, until BNF starts, but application of large amount of N fertilizer inhibit BNF through development ineffective nodules and leadings to lower residual soil nitrogen after harvest. The average soil N of the experimental site before planting of groundnut crop was found to be 15.5 kg ha^{-1} (Table 1). After crop harvest the level of soil nitrogen content increased by 119% indicating that groundnut crop plays an important role in BNF and increase soil nitrogen content after harvest favoring subsequent crops.

In general, soil N content after harvesting groundnut showed increasing trends in all experimental plots compared to the soil N content before planting. This is due to the application of starter N and combined P fertilizers that have promoted BNF by groundnut crop. Application of increasing levels of P$_2$O$_5$ fertilizer from 0 to 46 kg ha^{-1}, while keeping N at 15 kg ha^{-1} resulted into consistently increasing residual soil N content after harvest. Total amount soil N content ranging from 14.6 to 43.8 kg ha^{-1} is considered as low level of soil N (Hazelton and Murphy, 2007; Tekalign, 1991). Hence, the highest total N content of the soil in the study area was low even after harvesting of groundnut. This is attributed to sandy soils with low soil organic matter content and leaching of nitrate below the rooting depth. Previous studies e.g. Adeleke and Haruna (2012) indicated that residual soil N content after harvesting of groundnut crop can reach up to 200% compared to the level of soil N before planting. Similarly, it has been shown that residual soil N increased significantly with the application of DAP fertilizer i.e. 46 kg P$_2$O$_5$ ha^{-1} combined with 18 kg N ha^{-1} at Babile site in Ethiopia (Argaw, 2017). Chalk (1991) indicated that when N fixing crops are included in crop rotation system, the plant can satisfy all or at least part of its N requirements through BNF and the amount of fixed N in excess of the current plants requirement will be stored in the soil and benefit subsequent corps. The amount of residual nitrate recorded for plots treated with 15 kg N ha^{-1}resulted into higher residual soil N of 30.7 kg ha^{-1} compared to the application of 30 kg N ha^{-1} and no N application. Similarly, separate individual application of P fertilizer at the rate of 46 kg P$_2$O$_5$ ha^{-1} resulted in average soil N content of 29.2 kg ha^{-1}. It is a fact that under poor soil conditions, application of N and P fertilizers at small rates can increase plant root growth and development, as well as BNF efficiency (Pourranjbari Saghaiesh et al., 2019; Aslani and Souri, 2018; Naiji and Souri, 2018). Our results indicated that with the application of starter N and P fertilizers residual soil N is enhanced after harvesting of groundnut. Similar, to our results the study conducted by Eisa et al. (2011) was also indicated that with application of P fertilizer and fertilizers containing micronutrients such as Fe, Zn and Mn, the residual soil N is enhanced after harvesting groundnut. Including legumes in crop rotation system has multiple advantages from the point of views of cropping diversification, improved diet and nutrition and ecologically sustainable soil fertility restoration.

4. Conclusions

The plant phenology was not affected by single or combined application of N and P fertilizers. However, plant growth was significantly affected by separate and combined application of N and P fertilizers. Plant growth parameters such as plant height and number of branches were increased by higher N and P fertilizers application rates. Plant biomass yield significantly increased when higher doses of both N and P fertilizers were separately applied. Haulm yield was only increased by higher rates of N application. Combination of N and P at 15 kg N ha^{-1} and 46 kg P$_2$O$_5$ ha^{-1} resulted in higher pod yield, grain yield, grain protein content and residual soil N contents after harvest. Nitrogen harvest index was also found to be significantly affected by the main and interaction effect of both N and P fertilizers. The results indicate that increased productivity, grain quality of groundnut and enhancing the soil nitrate content after harvest with application of both N and P fertilizers. In addition to enhancing productivity and grain quality of groundnut, the study is also very relevant in demonstrating ecological soil fertility replenishment in a crop rotation system involving legumes.

Declarations

Author contribution statement

Kinfe Tekulu: Conceived and designed the experiments; Performed the experiments; Collected the data; Analyzed and interpreted the data; Wrote the paper.

Gebeyehu Taye: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Dereje Assefa: Conceived and designed the experiments; Performed the experiments.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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