# EVALUATING BIOLOGICAL NITROGEN FIXATION OF BEAN AND NITROGEN USE EFFICIENCY OF MAIZE CULTIVARS FOR IMPROVING CROP PRODUCTIVITY AND SOIL FERTILITY IN LESOTHO

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Discipline of Soil Science

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# Declaration

I, **Selebaleng Mofolo**, declare that:

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As the candidate's supervisors, we agree to the submission of this thesis:

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Prof. Pardon Muchaonyerwa (Supervisor)

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Dr. Joseph Adu-Gyamfi (Co-Supervisor)

# Abstract

Nitrogen (N) deficiency is among the most limiting factors for sustained crop yields in Lesotho, where maize (*Zea mays)* and common bean (*Phaseolus vulgaris)* intercropping is commonly practiced. Selection of varieties of common bean with high biological nitrogen fixation (BNF) potential and maize with high N use efficiency (NUE) could improve the productivity of the intercrops, and yet there are no studies that evaluated BNF potential of different legume (including common bean) genotypes and NUE of maize varieties in Lesotho. A field experiment was conducted to quantify and compare the biological nitrogen fixation (BNF) of two common bean varieties and to determine nitrogen use efficiency (NUE) of two maize varieties in the Lowlands and Foothills of Lesotho. The experiment was set up as a randomised complete block design with four (4) replications (farmer fields) each at Sakoane (Lowlands) and Machache (Foothills) and the two common bean varieties used were Pinto Nodak and NUA 45 with maize variety ZM 521 as the reference crop for BNF. The $^{15}N$ isotope dilution method was used to quantify BNF and Percent Fertilizer Nitrogen Use Efficiency (%FNUE). Urea fertilizer with 5.15% atom excess enrichment was used. The N derived from the atmosphere, fertiliser and soil were determined, followed by calculation of N budgets (i) assuming residue removal with grain and (ii) retention of residues after harvest. The two maize varieties (*Zea mays)* used for the NUE experiment were ZM 521 and ZM 523, and the experiment was done using three nitrogen fertilization levels on farmer-managed fields and research stations. . Pinto and NUA 45 derived almost the same proportion of N (29% and 28%, respectively) from fixation at flowering, while 46 (Pinto) and 29%N (NUA 45) were fixed at harvest, which were not significantly different. More N fixation was observed from Machache (52.5%) compared to Sakoane (21.6%). The mean imports and exports were estimated using N accumulated in beans. The mean N budget was 10.1 kg ha$^{-1}$ at Machache and -0.86 kg ha$^{-1}$ at Sakoane ($p<0.005$) with no difference between the two varieties when all dry matter was removed from the field (N budget 1). In a case where only grain was removed (N budget 2) the budget was 16.1 and 9.37 kg ha$^{-1}$ at Machache and Sakoane, respectively. Fertilizer N uptake increased with increasing N application rate in both dry matter and grain yield at Machache and Sakoane ($p<0.005$). Total N amount in dry matter and grain were not affected by N application rates at Machache but the ZM 523 variety took up significantly higher N in both dry matter and grain compared to ZM 521. At Sakoane, fertiliser N application at 20kg N ha$^{-1}$ significantly increased amount of total N in dry matter (9.4kg N ha$^{-1}$) and grain yield (20.7kg N ha$^{-1}$) when compared with plants that received no N and10kg N ha$^{-1}$ application. Biological nitrogen fixation by two bean varieties was not different but Pinto produced higher grain yield than NUA 45. The %FNUE of ZM521 and ZM 523 was similar but ZM523 yielded more grain in 2018/19 season and more drymatter in 2019/20 than ZM 521. Pinto would be a good food and nutritional source to farmers in addition to soil fertility benefits from BNF. The maize variety, ZM 523, could be recommended to farmers in the foothills and lowlands of Lesotho for food and nutrition security and animal feed, through the drymatter.

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# Dedication

I dedicate this work to my parents; my late father, Mr. Molete Mofolo and my mother, Mrs.Machief Mofolo, for their inspiration to follow a career in agriculture.

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# CHAPTER 1: Introduction

## 1.1. Background:

Agricultural production in Lesotho has greatly declined over the past years, resulting in food insecurity and extreme poverty (Rantšo and Seboka, 2019). Maize (*Zea mays* L.), sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum L*), beans (*Phaseolus vulgaris*) and peas (*Pisum sativum*) are the main food crops in Lesotho, and are mostly produced for home consumption under rain-fed conditions (Rasoeu *et al.*, 2006). The national production of beans and peas was 3.5 and 3.0 thousand tonnes, respectively in the early 1980s (Mokitimi, 1995), with a further decline to 0.9 and 0.4 thousand tonnes in 2018/2019 for beans and peas respectively (Statistics, 2019). Between the early 1980s and the 2000/2001(FAO/WFP, 2001) cropping season, there were declines in national production (in thousand tonnes) from 106 to 57.9 for maize, 47.7 to 10.9 for sorghum, and 17 to 5.2 for wheat. In recent years (2018/2019) cropping season, the national production decreased further to 24.6, 8.4 and 1.4 (thousand tonnes) for maize, sorghum and wheat respectively (Statistics, 2019). The decline in production could be explained by factors including among others; severe droughts, erratic rainfall, soil erosion, poor land management practices, continuous mining of nutrients by crops with little or no replenishment, and soil acidity (including aluminium and manganese toxicity) which all led to decline in soil fertility (FAO/WFP, 2005; Shisanya *et al.*, 2009).

Approximately 50% of the bean production areas in Eastern and Central Africa and 60% in Southern Africa are exposed to N deficiency as a result of both depletion of N in the soil and application of limited N fertilizer (Beebe *et al.*, 2014). Due to these factors, current bean yields in Southern Africa average only 0.6 Mg ha$^{-1}$compared to attainable yields of >1.5 Mg ha$^{-1}$ (Chianu *et al.*, 2011). Deficiency of N in Lesotho is among the most limiting factors for increased crop yields and management of N inputs is major challenge for increased agricultural production and attainment of sustainability (Zoundjl *et al.*, 2016).

Although application of inorganic fertilizers helps to address the limitations of essential nutrients, most resource-poor farmers cannot access the fertiliser due to financial constraints (Nyemba and Dakora, 2010). Farmers do not apply the recommended fertiliser rates for maize and other grains due to high costs resulting in large yield gap compared to yield potential. Recently tremendous increase in national production (in thousand tonnes) to 173 for maize, 33.9 for sorghum, 8.85 for wheat, 6.64 for beans and 6.02 for peas during 2016/2017 summer cropping season (Statistics, 2017), was due to an increase in production area, recommended fertilizer application rates related to Government’s block farms and improved seed varieties. The Government of Lesotho introduced an input subsidy program, which made both seed and fertilizers affordable and accessible to some farmers. This intervention has helped increase the use of fertilizer but challenges relating to broad accessibility and efficient use of fertilizers persists. Moreover, the long-term sustainability of this approach depends on continuous funding from Government. Increased use of other N sources like organic residues and biological nitrogen fixation (BNF) within the context of integrated soil fertility management can ensure food security in areas where N fertilizer is too expensive or simply not available.

The incorporation of leguminous crops in an intercropping system with cereals improves the use of soil N resources and reduces the requirement for synthetic fertilizer N. This is a low input agricultural strategy for food and environmental security as it helps to achieve increased crop yields in the cropping systems while minimizing the use of N fertilizer (Bagayoko *et al.*, 2000; Bationo and Ntare, 2000; Adu-Gyamfi *et al.*, 2007).  Grain legumes and cereals can complement one another in human nutrition as legumes provide high levels of protein and cereals supply carbohydrates. Cereal-legume intercropping also guarantees reduced risk where one crop failure may occur, it ensures household nutrition, income, and enhances productivity (Himmelstein *et al.*, 2017). In Lesotho, maize-common bean intercropping systems are common on smallholder farms. Selecting common bean cultivars with high BNF and maize cultivars with high nitrogen use efficiency (NUE), can significantly improve productivity of the intercrop.

The nitrogen fixing ability of common bean in association with rhizobia is often characterized as poor compared to other legumes (Reinprecht *et al.*, 2020). This may be attributed to competition between inoculant rhizobia (the symbiotic N2–fixing bacteria) and those present in the soil (Chekanai *et al.*, 2018). Another reason for poor fixation is explained by Graham (1981) as the inherent characteristics by different cultivars. Graham and Halliday (1977) discovered variation in common bean cultivar for symbiotic N2–fixation where determinate bush cultivars were weaker in this trait compared to indeterminate or climbing cultivars. Other studies have been conducted in Rwanda, DR Congo, Kenya, Malawi and Zimbabwe for screening of common bean cultivars with ability to fix N2 with different rhizobium strains under different biophysical conditions (Baijukya *et al.*, 2010). In Zambia, Mozambique and Malawi 20 genotypes were tested by Monyo Emmanuel and Laxmipathi Gowda (2018) where they discovered that genotypes with high number of nodules resulted in high shoot biomass and yield.

Quantities of N fixed by legumes have long been studied by many authors in other countries (Adu-Gyamfi *et al.*, 2007; Vera-Nunez *et al.*, 2008; Giambalvo *et al.*, 2011b; Tauro *et al.*, 2013; Sarr *et al.*, 2016; Zoundjl *et al.*, 2016). Currently, legumes such as soybean, common bean, groundnuts, pigeon peas, chickpeas, cowpeas, etc. are fixing approximately 11 million tonnes of N in developing countries (International Atomic Energy Agency, 2008). Grain legumes or green manure legumes can fix up to 300 kg N/ha in a season although P and K deficiencies can reduce BNF (International Atomic Energy Agency, 2008). Despite that most farmers use legumes in intercropping or rotation with non-leguminous crops, no studies have been conducted in Lesotho to quantify the amount of N fixed by legumes using the stable $^{15}$N methodology. Bean is the most commonly cropped legume either as a sole crop, in intercropping or in rotation with other crops. Bean is an important source of affordable protein in countries with little animal protein supply, like Lesotho. It provides dietary fibre, starch and minerals such vitamin B6 and folic acid in diets affordable by the poor (Garden-Robinson and McNeal, 2013), thiamin, zinc,(Murphy *et al.*, 1975), iron (Sgarbieri *et al.*, 1979) , and potassium (Meiners *et al.*, 1976). Therefore, nutritionally, it is important and can be used for national food and nutrition security program while also benefiting soil fertility enhancement by fixing nitrogen.

The bean variety that has been used by Basotho farmers since ancient times is Pinto Nodak,while the variety NUA 45 (biofortified) has been newly introduced. The two bean varieties are early maturing, high yielding, and drought tolerant. NUA 45 is an Andean line developed by CIAT Colombia-breeding line. It's classified as Calima bean. Pinto Nodak is developed in Washington by North Dakota. Comparing $N_2$ fixation of the two varieties using 15N isotopic method will be helpful to better advice farmers on which one they can use for improving soil fertility while increasing yield at the same time. While the best non-fixing reference crop is non-nodulating lines of the test legume (Okito *et al.*, 2004), non-fixing reference mono or dicotyledonous crops (Reiter *et al.*, 2002) or non-legume weeds (Schwenke *et al.*, 1998) can be used in the absence of non-nodulating lines of the test legume. Maize will therefore be used in compare the $N_2$ fixation of the two varieties because it has no ability to fix nitrogen, but its ability to extract and uptake nitrogen from the soil is almost similar to grain legumes and their maturity period is almost the same. In addition to improving yield of the main protein source, there is the need to also improve productivity of main staple crop, through growing varieties that efficiently use the limited fertiliser resources. The N use efficiency or recovery of applied $^{15}$N fertilizer by non-fixing staple food crops like maize is not clear in Lesotho. Two maize genotypes, ZM 521 and ZM 523 are open pollinated varieties and have been newly introduced to farmers in Lesotho. They originate from Zimbabwe. They can produce high yield under water-stressed conditions, they are open pollinated varieties and they are resistant to common maize diseases, although they differ in maturity by 20 days with ZM 523 maturing later. There is a need to carry out a study that can identify the maize variety that has higher N use efficiency for the benefit of farmers, who usually apply insufficient fertiliser rates.

There is no information on studies conducted to evaluate the quantity of N fixed by different legume genotypes and nitrogen use efficiency (NUE) of maize varieties from traditional cropping systems using either isotopic ($^{15}$N enrichment method) or non-isotopic method on farmers' fields and agricultural research stations in Lesotho. The findings from such studies can be useful for management of N supply and N utilization in these cropping systems. Hence the study aimed to determine the levels of BNF of common beans (NUA 45 and PintoNodak) and NUE of maize varieties (ZM 521 and 523) using nuclear technology by conducting participatory trials on farmers' fields. The findings could provide farmers with technologies that can restore soil fertility and increase productivity.

## 1.2. Aim:

The aim of this study was to evaluate two bean cultivars for biological nitrogen fixation and two maize cultivars for nitrogen use efficiency to restore the fertility of selected soils in Lesotho using isotopic techniques. The specific objectives of this study were to:

(i) To quantify and compare the biological nitrogen fixation (BNF) and yields of two common bean varieties on farmer-managed fields.
(ii) To determine nitrogen use efficiency (NUE) and yields of two maize varieties on farmer-managed fields in two agro-ecological zones.

## 1.3. Hypotheses:

(i) Common bean varieties fix different amounts of nitrogen from the atmosphere.
(ii) Maize varieties differ in %FNUE when grown on farmer managed fields in Lesotho.

# CHAPTER 2:
# Nitrogen use efficiency in maize and other grain crops: A Review

## 2.1. Introduction

Worldwide, maize is one of the most important crops with high potential for increased yields when supplemented with nitrogen (N) fertilizer (Panda *et al.*, 2004). Maize production occurs in almost all countries, on an area of about 160 million hectares (Da Silva *et al.*, 2017). Maize is the main staple food crop in Africa, especially in Southern Africa. It constitutes the smallholder cropping systems in the continent. It is either grown as a monoculture, intercropped or rotated with legumes. Essential as it is, maize production is often inhibited by drought and low rainfall. Apart from these, maize low production on farmers' fields maybe attributed to other abiotic factors (Lengwati *et al.*, 2020) such as soil nutrient availability.

Nutritionally maize is rich in carbohydrate, mostly in a form of starch, proteins, lipids, vitamins and minerals (de Oliveira *et al.*, 2014). One of the main attributes of grains is that they can be directly consumed, without processing them to remove the hull like with other cereals, such as rice and wheat (Langner *et al.*, 2019). Nitrogen absorption is important for plant growth, and fertilization increases uptake of N and yield of cultivated crops like maize (*Zea mays* L.) (Panda *et al.*, 2004).The use of N fertilizer can help farmers to achieve food security at household level especially if they plant crop varieties with high N use efficiency.

Genotype and its interaction with N fertilization level governs the crop responsiveness to N availability (Chardon *et al.*, 2010). The crop-N demand is affected by biophysical factors such as climate, soil type, water availability and cropping seasons (Cassman *et al.*, 2002; Dorsey, 2014; Guttieri *et al.*, 2017). For example, under irrigated farming system, the yield potential for a certain crop variety is largely influenced by solar radiation and temperature while in dryland farming, the amount of rainfall and temporal distribution influence yield potential (Cassman *et al.*, 2002).

Global nitrogen use efficiency (NUE) is approximately 33% for cereal production including wheat (*Triticum aestivum* L.); maize (*Zea mays* L.); rice (*Oryza sativa* L. and *0. glaberrima* Steud.); barley (*Hordeum vulgare* L.); sorghum (*Sorghum bicolor* (L.) Moench); millet, (*Pennisetum glaucum* (L.) R. Br.); oat, (*Avena sativa* L.); and rye, (*Secale cereale* L.) (Rahman *et al.*, 2011). The major goal of conducting research in

NUE is to maximize the proportion of N recovered from the soil (REN) and to obtain an enhanced efficiency (Shejbalová *et al.*, 2014). Decreasing NUE due to high N input obeys the law of diminishing marginal returns (Raun and Johnson, 1999). Thus, interventions in increasing NUE and reducing N inputs will be useful for farmers and help farmers to make the best use of limited fertilisers and benefit from growing crops with the highest NUE especially for cereal crops like maize, wheat and sorghum which are important for food security globally. Currently, crop production in Lesotho is not concerned about reducing N inputs by using varieties that have high NUE. The recommendations for different varieties is based on disease and drought resistance. Hence there is a need to evaluate the NUE of different maize varieties since there is no information about NUE of different maize varieties recommended to farmers.

Meeting the global challenge for increased food demand and protection of environmental quality requires synchrony between N supply and crop demand without excess or deficiency in order to improve trade-offs amongst yield, profit, and environmental protection (Cassman *et al.*, 2002). Therefore, the objective of this section is to discover the NUE of different grain crop species or varieties planted using different cropping systems and their ability to absorb and use nitrogen to produce carbohydrates and proteins.

## 2.2. Factors affecting fertilizer nitrogen use efficiency (FNUE)

Fertilizer N use efficiency (FNUE) is defined as the amount of fertilizer N absorbed by the plant per kg of N applied as fertilizer (IAEA, 2008). Determining FNUE in crop plants is an essential method in accessing the fate of applied chemical fertilizers and the role they play in improving crop yields (Fageria, 2012). Fertilizer Nitrogen use efficiency is affected by environmental factors such as climate, soil type, preceding crops, type of fertilizer, placement and timing of N fertilization and N fertilizer application rate, different plant varieties and different farming systems. In a study conducted by Adu-Gyamfi *et al.* (1996), timing of N application resulted in higher grain yield and NUE, whereby urea application was delayed by 45 days after sowing. Apart from timing of N fertilizer, fertilizer placement methods were tested, and deep placement resulted in increased grain yield by 21-23% (6.7Mg ha $^{-1}$) and NUE by 44% compared to broadcasting in rice production (Baral *et al.*, 2020). In another study, band application method of N fertilizer at 50kg ha$^{-1}$ to sorghum resulted in 36% N recovery compared with 13% which was broadcasted.

In addition, different N doses were applied to wheat at different growth stages and N uptake was maximized at 120kg ha $^{-1}$ which was divided into 3 equal portions and applied as one-third basal during planting, one-third as top-dressing during crown initiation and the rest one-third top dressed at first node stage (Rahman *et al.*, 2011). Greater NUE was observed in AC Gehl oat variety which was newly released due to improvement of N-translocation efficiency which resulted in more N accumulation in the vegetative tissues than Prescott (Zhao *et al.*, 2012). Nitrogen use efficiency increased by 60% in a study conducted by Westermann *et al.* (1988), where 15N-labeled fertilizer was applied before planting on Russet Burbank potatoes. Additionally, conservation farming system resulted in high NUE compared to conventional farming in a study conducted in Zambia using different maize varieties (Simunji *et al.*, 2018).

Another major factor which is not easily manageable is climate, it affects both soil and fertilizer changes and, subsequently, their availability and uptake by plants, and thus crop growth and development. Climatic conditions vary with location and determine different pathways through which fertilizer N is lost to surface water by runoff, to groundwater by leaching (downward movement within the soil profile) and to the atmosphere as gaseous (NH3, N2, N2O, NO) emissions. It is therefore important to consider the change with time in climatic conditions in order to develop appropriate strategies to improve FNUE.

### 2.2.1. Nitrogen losses through different pathways from the soil

Low NUE in cereal grain production is due to different reasons, including the release of $NH_3$ from plant tissues, denitrification and loss of fertilizer N resulting from surface runoff or generally when mineral N ($NH_4$ and $NO_3$) is present in excess of plant needs (Raun and Johnson, 1999). Different studies conducted reported low NUE due to N loss of 52% to 72%(Francis *et al.*, 1993), 14% to 51%(Reddy and Reddy, 1993) and 0% to 51% (Jokela and Randall, 1997)as $^{15}$N in maize and 7% to 40% in winter wheat (Bronson *et al.*, 1991; Fillery and McInnes, 1992; Recous *et al.*, 1988; Van Cleemput *et al.*, 1981).

Gaseous plant N loss greater than 45 kg N ha$^{-1}$ yr$^{-1}$ was reported in soybean *(Glycine max*(L.) Merr.) (Stutte *et al.*, 1979). In Aulakh *et al.* (1982), fertilizer N losses through denitrification was 9.5% in winter wheat, 10% in lowland rice (De Datta *et al.*, 1991), 10% in corn planted under conventional tillage, and 22% in no till practice (Hilton *et al.*, 1994). Fertilizer N lost through surface runoff ranged from 1% (Blevins *et al.*, 1996) to

13% (Chichester and Richardson, 1992) of the total N applied. These losses reduce the amount of fertiliser N utilised by the plants, thus resulting in low NUE. Figure 2.1 shows a simple Nitrogen cycle showing the typical fate of 100 pounds of N fertilizer applied to a maize field.

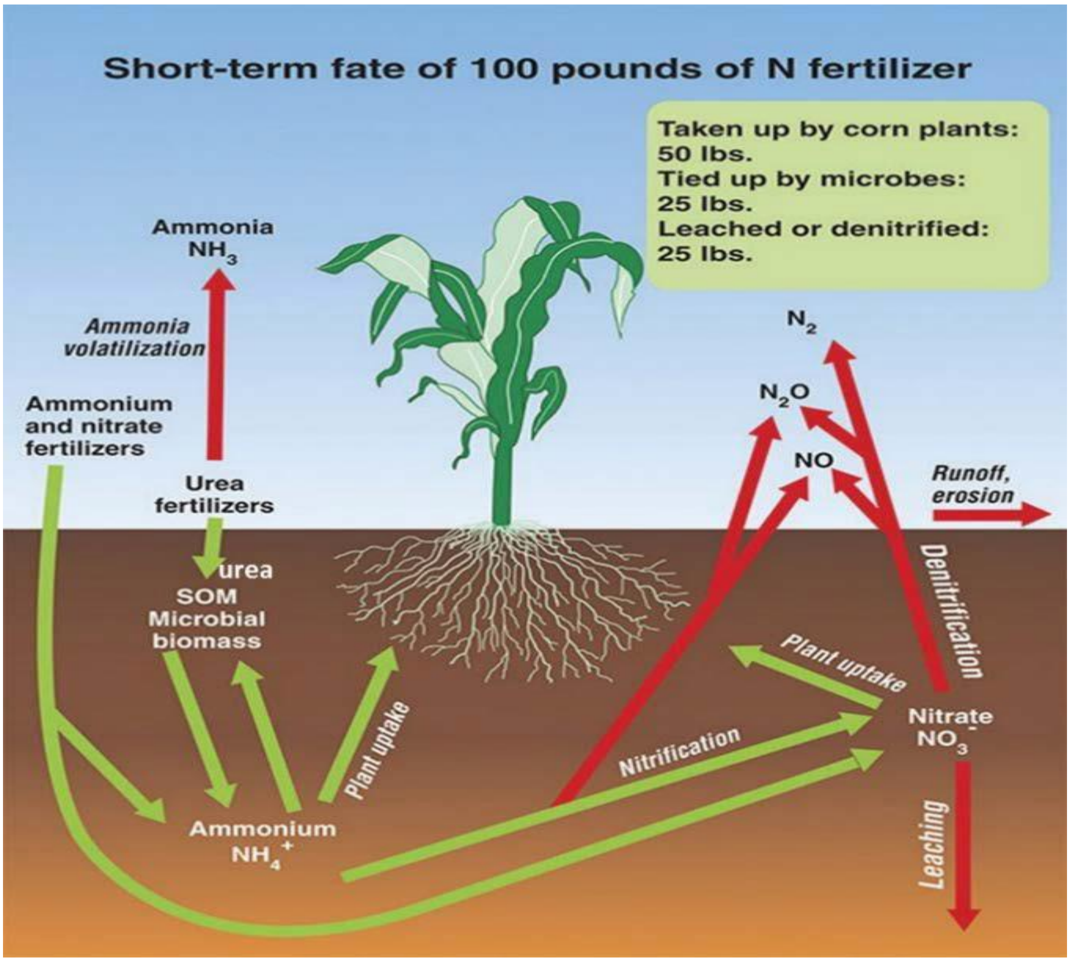

*Figure 2.1: The fate of 100 pounds of N fertilizer applied to a maize field (Millar et al., 2014)*

### 2.2.2. Plant factors

Fertilizer nitrogen use efficiency is affected by other plant factors such as the nitrogen recovery efficiency/nitrogen uptake efficiency (NRE/NuPE) and nitrogen internal efficiency/nitrogen utilization efficiency (NIE/NUTE) (Moll *et al.*, 1982). Nitrogen recovery efficiency/nitrogen uptake efficiency is the capability of aboveground plant parts to recover N from the applied N fertilizer, and NIE/NUtE is the capacity of plants in transforming the N taken up by the crop into grain (Coque and Gaflais, 2007). Examination of N uptake, assimilation, translocation and remobilization is necessary in achieving NIE (Moll *et al.*, 1982). Figure 2.2 shows the key traits for nitrogen use efficiency, where the blue colour shows the most commonly used definitions applicable to cereal crops, critical biochemical processes are shown in yellowish colour and the green coloured boxes indicate the final breeding goals of high yield and high grain N (protein) content. The Studies conducted by (Carlone and Russell, 1987; Ma *et al.*, 1998) revealed that the NRE component significantlydominated where there was high N supply; whereas the NIE component was more essential in low N availability environments. The findings by Ciampitti *et al.* (2012) revealed that leaf chlorophyll (SPAD readings) and plant stem volume at silking stage were good indicators of final plant N uptake, grain yield and NUE of maize.

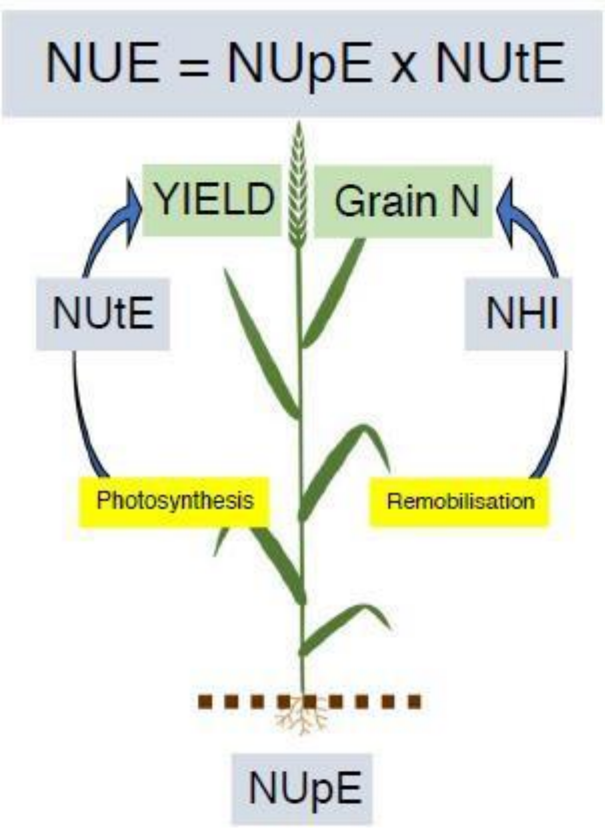

*Figure 2.2: The key traits for nitrogen use efficiency.(Hawkesford and Griffiths, 2019)*

## 2.3. Methods of measuring fertilizer N recovery

Fertilizer N recovery in the cropping system can be estimated by using the non-isotopic difference method and the isotopic method. The latter can use depleted or enriched method (Raun and Johnson, 1999).

### 2.3.1. Non isotopic difference method

This method calculates the fertilizer recovery using a number of different N rates and unfertilized plots as the differences in N taken up by plants in fertilized and non-fertilized plots per amount of N applied (IAEA, 2008a). This method assumes that the quantity of available soil N in fertilized plots is equivalent to that in unfertilized crop (Adu-Gyamfi *et al.*,1996). However, this assumption was found to be incorrect by (Jenkinson *et al.*, 1985; Knopke and Towner, 1992) as N uptake from the soil by fertilized crop is normally higher than soil-N for unfertilized crops, as such, this method results in overrated fertilizer recovery (Adu-Gyamfi *et al.*, 1996).

### 2.3.2. Isotopic/plant recovery method

This method uses labelled fertilizers to distinguish between N from fertilizer and N from soil.It gives an accurate determination of fertilizer-N recovered by the crop (N recovery) as calculated from the total N uptake and fertilizer N uptake. Although this method is more precise compared to the difference/apparent recovery method, it has some constraints associated with mineralization or immobilization turnover as the fertilizer-N applied to the soil undergoes exchange with the native soil (Adu-Gyamfi *et al.*, 1996), as a result, the application of N-labelled fertilizer will cause the loss of N by immobilization and plants gain non-labelled native soil-N by mineralization (Strong, 1995).

## 2.4. Varietal differences in nitrogen utilization efficiency

There is large variation in the physical characteristics of different crops and they each have different nitrogen use efficiencies. Fertilizer N uptake by plants is only 20 to 50% of N applied to soil for cereal crop production. Nitrogen use efficiency in rice production varies from 30% or lower, under intensive maize production, it can be up to 70% (Cassman *et al.*, 2002). Nitrogen use efficiencies of potatoes and wheat are quite different (Raun and Johnson, 1999; Zebarth *et al.*, 2009). Despite large differences between wheat and vegetable crops, Muurinen *et al.* (2006) showed that most cereal crops have very similar NUEs. The cereal crops used in their study were wheat, barley, and oats, which are similar grain-producing grasses. They found no statistical difference in NUE among the different species, but within the species themselves there were differences among varieties.

Moreover, Foulkes *et al.* (2009) discovered that wheat cultivars suited for feed and bread have different traits for improved NUE. They reviewed that increased root length density at depth and a high capacity for N accumulation in the stem can result in increased NUpE. While low leaf lamina N concentration, effective post-anthesis remobilization of N from stems to grain, and less efficient remobilization of N from leaves to grain and delayed senescence increased NUtE. Generally, they concluded that low N concentration is vital for increased NUE when planting wheat for feeding purposes while the opposite is required in wheat production for bread. Additionally, Zhao *et al.* (2012)reported that two oats genotypes demonstrated differences in NUE where cv. AC Gehl had greater NUEthan cv. Prescott, mainly because of high recovery efficiency in the shoots. Giambalvo *et al.* (2010) discovered differences in NUE for different wheat cultivars which were planted under different interspecific weed completions at no N application and 80kg ha$^{-1}$. Valbelice cultivar recorded the highest NUE compared to Russello and Simeto varieties where there was no interspecific weed competition and with interspecific weed competition.

The results obtained by Abera *et al.* (2016) where open pollinated and hybrid maize varieties were compared in Ethiopia suggested that hybrid maize varieties; Jibat, Wenchi and Webii had higher nitrogen uptake efficiency compared to open pollinated variety (Horra). So was the same for NUE where Jibat had the highest NUE, followed by Wenchi, Webii and Horra. Rochiman and Purnobasuki (2013) also evaluated ten maize genotypes at four nitrogen application levels, where five of them were open pollinated and the other five were hybrids. They also concluded that hybrid varieties have high NUE compared to open pollinated varieties. Sorghum (*Sorghum bicolor* (L.) Moench) was studied by Gardner *et al.* (1994) and genetic diversity for NUE was confirmed by different varieties which were compared for their ability to survive under limited nitrogen.

## 2.5. Nitrogen requirement and management for cereal crops

The main objective to enhance cereal nitrogen management is to match the nitrogen supply to the nitrogen demand (Bock and Hergert, 1991). The nitrogen requirement by the crop is determined by the level of crop growth, and the cereal yield and quality can be affected by nitrogen availability (Doltra *et al.*, 2011). The nitrogen supply for a cereal crop comes from fertilizer and manure mineralization (Dorsey, 2014). Mineralization is the release of plant available nitrogen from soil organic matter and crop residues as a result of soil microbial activity (Sullivan *et al.*, 2020). Crop growth is also affected by management practices, variety, planting date, soil and climatic conditions (Bock and Hergert, 1991).

Doltra *et al.* (2011) conducted a study in Denmark where conventional and organic farming systems were used on different soil types and climatic conditions, cereal yields were affected by soil type with greater drymatter and grain yield on loamy soil and amount of fertilizer N used. Low amount of N on organic systems negatively affected cereal production. Adu-Gyamfi *et al.* (1997) studied the effect of time of N application on Alfisols using intercropping (sorghum and pigeonpea) and sole systems and the results revealed that delayed N application until 40 days after planting increased drymatter and grain yield in sorghum. Furthermore,a combination of fresh crop residues and N inorganic fertilizer was examined, where rotation of guar and wheat was done on Vertisols, the results showed that more N was recovered on plots where crop residues were added compared to plots with no crop residues (Mubarak *et al.*, 2015).

The optimum fertilizer nitrogen rate for crops varies from field-to-field and from year-to-year due to variation in both crop nitrogen demand and soil nitrogen supply. Nitrogen requirement of crops produced under irrigated and dryland farming also differs. Experiments conducted in western Nebraska, Colorado, and Montana by Smika *et al.* (1969) revealed that moisture stored in subsoil in the dry regions was the determining factor for yield and how crops responded to applied or residual N. Grain crop production under dryland farming requires relatively lower fertilizer N rates than in humid area. When moisture content is low with abundant N supply, intensive vegetative growth occurs due to late season moisture, thereby resulting in low yield production (Olson, 1984). Excessive N application rates of 60 kg ha$^{-1}$ are rarely required on soils except for sandy and fallowed eroded soils with the most possible economic rate in the order of 20 to 30 kg ha$^{-1}$(Olson, 1984). Good nitrogen management results in good and healthy environment with sustainable agricultural production for intensive farming worldwide.

# CHAPTER 3: Biological nitrogen fixation by crop plants: A meta-analysis

## 3.1. Introduction

Biological nitrogen fixation (BNF) is a process whereby nitrogen gas ($N_2$) from the atmosphere is incorporated into the soil and converted to readily available form of N obtainable by selected group of plants known as legumes through mutual relationship with a number of bacterial species which infect the roots of the plants and form structures known as nodules (Giller, 2001). All plants require large amounts of N for proper growth and development (Abdel-Aziz *et al.*, 2014). Low soil N fertility is one of the main limitations to crop production, farmers include grain legume crops in their cropping systems to improve the soil fertility status since it is a huge challenge for small holder farmers to supply N to crops as mineral fertilizer, because they are inaccessible to resource-poor farmers due to their high cost. As such, they include legumes as sole crops, intercrops in rotations with main crops or green manures in order to improve soil N fertility and increase crop yields for enhanced food and nutritional security through BNF (Somado and Kuehne, 2006).

It is estimated that legumes provide 20% of food protein worldwide and there are predictions that BNF may be used as a major source of nitrogen for plant protein production (Montañez, 2000). The effectiveness of BNF could be affected by different types of legumes and other biophysical factors such as soil nutrient availability (Somado and Kuehne, 2006). Studies conducted in India, Canada, Austria, Venezuela and Kenya whereby soybean, common bean, forage, peas and trees were evaluated for BNF respectively revealed different percentages of nitrogen derived from the atmosphere, ranging between 46% in common beans to 82% in soybean under different climatic conditions (Ståhl *et al.*, 2005; España *et al.*, 2006; Ardakani *et al.*, 2009; Hossain *et al.*, 2016; Milkha *et al.*, 2017).

Different methods are used to estimate BNF and FNUE and the precise estimates depend on the type of method used to quantify them. The most frequently used non-isotopic methods include N difference, relative ureides abundance, acetylene reduction assay, while the 15N natural abundance and 15N enrichment or dilution methods are the commonly used isotopic methods (Forrester *et al.*, 2006; Peoples *et al.*, 2009).

Among them, the 15N enrichment method is the most accurate in quantification of nitrogen turnover in the main processes of the nitrogen cycle. This method directly measures N through 15N isotope tracer and does not depend on yield (Chalk *et al.*, 2014). It has been used in different parts of the world, either on farmers' fields or in research stations and different findings were obtained where the amount of $N_2$ derived from the atmosphere through symbiotic relationship and the nitrogen budget were estimated using different cropping systems (Ankomah *et al.*, 1996; Okito *et al.*, 2004; Bado *et al.*, 2006; Adu-Gyamfi *et al.*, 2007; Zoundjl *et al.*, 2016). The 15N labelled single-treatment fertility design is the dominating means for evaluating the importance of fertilizer management practices such as timing, placement and sources and it measures FNUE without plant–fertilizer interaction (IAEA, 2008b). Given these differences, there is a need to synthesise the information on BNF in order to make global generalisations and to determine the crops that are likely to give the greatest benefits for different localities.

A global literature review (meta-analysis) was done to have a background information about BNF by different legume crops. Furthermore, to determine how the identified factors (different soil class, order and pH conditions and organic carbon class) that affect BNF, under different climatic regions and geographical location will compare/contrast with results that will be obtained from the current study.

## 3.2. Materials and Methods

The investigation on biological nitrogen fixation was done using a meta-analysis based on studies conducted in the field and greenhouse using 15N isotope dilution method. The studies used in the meta-analysis covered 35 countries (Austria, Bangladesh, Benin, Brazil, Burkina Faso, Cameroon, Canada, China, Colombia, Cote D'Ivoire, Denmark, Egypt, Ethiopia, Ghana, India, Ireland, Italy, Japan, Kenya, Malawi, Mexico, NewZeland, Pakistan,Peru, Portugal, Senegal, Sri Lanka, Sweden, Syria, Tanzania, Thailand, USA, Venezuela and Zambia). These resulted in a total of 56 papers with 539 comparisons for legume types,334 for soil texture, 384 for soil orders, 432 for soil pH, 323 for soil organic carbon, 510 for climatic regions and 324 for latitude and longitude coordinates. Different common bean varieties *(Phaseolus vulgaris) were*, put in one category as beans.

Different pea varieties *(Pisum sativum)* were grouped as peas, the grass or fodder legumes were categorised as forage while herbs such as *Fenugreek and Crotalaria* *ochroleuca* and ornamental plant legumes such as shruby *(Medicago arborea)* were categorised as herbs and soybean was not under any category. These group descriptions were used for the convenience of the current data analysis. All studies included in the database reported on percentage amount of nitrogen derived from the atmosphere (%Ndfa). Table 3.1 shows a summary of information on author, study location on which the studies were conducted and different factors that affect BNF (different legumes planted, soil orders, soil textural class, soil conditions, soil organic matter and climatic conditions).

Different legumes fix nitrogen depending on their ability to form root nodules which fix N and access the available N in the root zone before they fix N. Soil orders such as Oxisols fix low amounts of N because they are highly weathered soils and highly acidic. At low pH, the rhizobial activity is negatively affected and some macronutrients like Phosphorus are not readily available to plants, as a result, legume root development is restricted which in turn affects the fixing ability of such crops. Soils with higher organic C are likely to have high BNF because of availability of nutrients and water, as a result of organic matter, supporting microbial activity, including rhizobia species involved in BNF. Fine textured soils like clay could have high BNF due to high nutrient and moisture retention of these soils which support microbial survival and activity. Different climatic zones such as the tropics might have low BNF due to highly weathered and leached soils found in such areas.

## 3.3. Data generation

Data was generated from different journals obtained from electronic databases searched using Google, Google scholar, Springer link and Science direct. Key words such as BNF, $^{15}$N dilution techniques, symbiotic nitrogen fixation, legumes and common beans were used to search for journal articles published from 1985 to 2018. The database included information on author name(s), year of paper publication, country and location where the trial was conducted. Other information includes soil physical and chemical properties where the studies were conducted, different environmental factors include mean annual precipitation (MAP), rainfall data during the cropping season, mean annual temperature (MAT), altitude, geographical location with the description based on latitude and longitude. Duration of the trials was also recorded. Illustrations of variables used in classifying the experimental conditions are shown in Table 3.2. The climatic regions were extracted directly from the papers and classified based on MAP and MAT. These were tropical regions which are normally hot and wet with greater than

1000mm rainfall and temperature greater than 20$^{o}$C, Sub-tropical region which are warm and arid-humid, received 300-1000mm with temperature between 10-20 $^{o}$C, and temperate regions which are cool to moist, received less than 800mm rainfall with temperature below 10. On papers where MAP and MAT were not given, the world map was used to identify different regions from which countries belong.

Soil texture was extracted directly from the papers, soil pH conditions which indicated the degree of acidity or alkalinity of the soil with genuine ranges from highly acidic (pH less than 5), acidic (optimum pH) which is the suitable range for most crops (5.1-6.5), Alkaline (6.6-8) ( to highly alkaline (pH above 8), and organic carbon were derived from the journal articles and grouped based on the amount obtained from each study where any amount below 1% was categorised as very low, from 1% to 3% was categorised as medium and above 3% as high (Mutema *et al.*, 2015; Abdalla *et al.*, 2016; Mathew *et al.*, 2017). Soil orders were obtained using the USDA taxonomic group using world soil database to match different taxonomic groups. Different bean varieties were put in one category as beans, while fodder and grasses were grouped as forage. Herbs are all edible plants and ornamental plants.

*Table 3.1: References included in the database, including factors affecting biological nitrogen fixation, where studies were conducted*

| No. | Author | Country | Legume Type | Soil order | Soil tex. class | Soil Condition | OC Class | Climatic Region |
|---|---|---|---|---|---|---|---|---|
| 1 | Abdel-Aziz et al (2014) | Egypt | Bean |  | Clay | Highly basic | Medium | Sub-tropical |
| 2 | Adu-Gyamfi et al (2007) | Malawi | Pea | Inceptisol |  |  |  | Tropical |
| 3 | Al-Chammaa et al (2014) | Syria | Soybean | Aridisol | Clay | Basic | Low |  |
| 4 | Amanuel et al (2000) | Ethiopia | Bean |  |  | Acidic | Medium | Sub-tropical |
| 5 | Ankomah et al (1996) | Austria | Pea | Vertisol | Sand | Acidic | Medium | Tropical |
| 6 | Ardakani et al (2009) | Austria | Forage | Mollisol |  | Basic | Medium | Temperate |
| 7 | Asare et al (2015) | Ghana | Bean | Ultisol | Sand | Basic | Medium | Sub-tropical |
| 8 | Ashworth et al (2015) | United States of America | Pea | Ultisol | Loam | Acidic |  | Tropical |
| 9 | Bado et al (2006) | Burkina Faso | Groundnut | Ultisol | Sand | Acidic | Low | Tropical |
| 10 | Bado et al (2018) | Burkina Faso | Pea | Ultisol | Sand | Acidic | Low | Tropical |
| 11 | Burchill et al (2014) | Ireland | Forage |  | Loam | Acidic | High | Tropical |
| 12 | Cadisch et al (1989) | Colombia | Forage | Oxisol |  | Highly acidic | Medium | Tropical |
| 13 | Carranca et al (1999) | Portugal | Bean |  |  | Basic | Low | Temperate |
| 14 | Cazzato et al (2012) | Italy | Bean |  | Clay | Basic |  | Sub-tropical |
| 15 | Duque et al (1985) | Brazil | Bean |  |  | Acidic |  | Tropical |
| 16 | Espana et al (2006) | Venezuela | Pea | Ultisol | Sand | Highly acidic | Low | Tropical |
| 17 | Franzini et al (2013) | Brazil | Bean | Oxisol | Loam | Highly acidic | Medium | Tropical |
| 18 | Giambalvo et al (2011) | Italy | Forage | Vertisol | Clay | Highly basic |  | Sub-tropical |
| 19 | Guene et al (2003) | Senegal | Bean |  | Sand | Basic |  | Tropical |
| 20 | Hafeez et al (2000) | Pakistan | Lentil |  | Loam | Basic | Low | Tropical |
| 21 | Haque et al (2012) | Bangladesh | Lentil |  | Sand | Acidic | Medium | Sub-tropical |
| 22 | Hardarson et al (1991) | Austria | Bean | Inceptisol |  | Highly basic | High | Temperate |
| 23 | Hossain et al (2016) | Canada | Pea | Mollisol | Loam | Basic | Medium | Temperate |
| 24 | Jensen (1986) | Denmark | Pea |  |  | Basic |  | Temperate |
| 25 | Kihara et al (2011) | Kenya | Soybean | Oxisol | Clay | Acidic |  | Tropical |
| 26 | Kipe-Nolt and Giller (1993) | Colombia | Bean | Mollisol |  |  |  | Tropical |
| 27 | Kumar and Goh (2000) | New Zealand | Forage | Inceptisol | Loam | Acidic | Medium | Sub-tropical |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 28 | Kurdali (2010) | Syria | Plant/herb | Oxisol | | Basic | Low | |
| 29 | Li et al (2015) | Denmark | Forage | | Sand | Acidic | | Temperate |
| 30 | Lonati et al (2015) | Italy | Forage | | | Highly acidic | | Temperate |
| 31 | Manrique et al (1993) | Peru | Bean | | | | | Tropical |
| 32 | Milkha et al (2017) | India | Soybean | Inceptisol | Sand | Highly basic | Low | Sub-tropical |
| 33 | Mohammad et al (2010) | Pakistan | Bean | | Loam | Basic | Low | Sub-tropical |
| 34 | Muller and Pereira (1995) | Brazil | Bean | Oxisol | | Acidic | Medium | Tropical |
| 35 | Munyinda et al (1988) | Zambia | Soybean | Alfisol | Loam | Acidic | | Tropical |
| 36 | Ndiaye et al (2000) | Senegal | Pea | Entisol | Sand | Basic | Low | Tropical |
| 37 | Okito et al (2004) | Brazil | Soybean | Ultisol | | Acidic | | Tropical |
| 38 | Ruschel et al (1982) | Brazil | Bean | | | | | Tropical |
| 39 | Saia et al (2016) | Italy | Plant/herb | Vertisol | Clay | Highly basic | | Sub-tropical |
| 40 | Samba et al (2002) | Senegal | Plant/herb | | | Basic | | Tropical |
| 41 | Sanginga et al (1990) | Austria | Tree | Inceptisol | Loam | Highly basic | High | Temperate |
| 42 | Sarr et al (2008) | Japan | Pea | Alfisol | | | Low | Temperate |
| 43 | Senaratne et al (1995) | Sri Lanka | Bean | Alfisol | | | | Tropical |
| 44 | Somado and Kuehne (2006) | Cote D'lvoire | Forage | Ultisol | | Acidic | | Tropical |
| 45 | Stahl et al (2005) | Kenya | Tree | Alfisol | Loam | Acidic | Medium | Sub-tropical |
| 46 | Sulas et al (2016) | Italy | Forage | Ultisol | Sand | Acidic | | Sub-tropical |
| 47 | Sylla et al (1998) | Senegal | Tree | Entisol | Sand | Basic | Low | Tropical |
| 48 | Tauro et al (2013) | Austria | Pea | Inceptisol | Loam | Acidic | Medium | Temperate |
| 49 | Toomsan et al (1995) | Thailand | Groundnut | Ultisol | Sand | Acidic | Low | Tropical |
| 50 | Vasquez-Arroyo et al (1998) | Mexico | Bean | | Sand | Basic | Low | Sub-tropical |
| 51 | Vera-Nunez et al (2008) | Mexico | Bean | Ultisol | Loam | Highly acidic | High | Tropical |
| 52 | Wanjiku et al (1997) | New Zealand | Pea | | | | | Temperate |
| 53 | Wivstad et al (1987) | Sweden | Forage | | Loam | Acidic | High | Temperate |
| 54 | Wolyn et al (1991) | Canada | Bean | | Sand | | | Temperate |
| 55 | Xie et al (2015) | China | Forage | Inceptisol | Basic | Low | | |
| 56 | Zoundji et al (2016) | Benin | Soybean | Oxisol | Sand | Acidic | Low | Tropical |

*Table 3.2: Variables used to categorize different experimental conditions with reference from (Mutema et al., 2015; Mathew et al., 2017)*

| Factor | Remarks | Categories | Symbol | Factor class |
|---|---|---|---|---|
| Climatic region | | Precipitation>1000 mm Temp>20 °C | Hot and wet | Tropical |
| | | Precipitation 300–1000 mm; Temp10–20 °C | Warm and arid-humid | Sub-tropical |
| | | Precipitation<800 mm Temp<10 °C | Cool and arid to moist | Temperate |
| Clay content (%) | Soil texture based on the clay content | >32% | Texture | Clay |
| | | 20–32% | | Loam |
| | | <20% | | Sand |
| pH | Soil pH as cited in the paper | < 5 | Soil pH | Highly acidic |
| | | 5.1-6.5 | | Acidic |
| | | 6.6-8.0 | | Basic |
| | | > 8.0 | | Highly basic |
| Organic carbon (g $kg^{-1}$) | Soil carbon as cited in the paper | <10 | SOC | Low |
| | | 10-30 | | Medium |
| | | >30 | | High |

## 3.4. Data analyses

The sample size was determined and stratified by legume type, different soil variables and climatic regions (Table 3.3 and Table 3.4). The variability and distribution of datasets for the different factor strata (Table 3.3) were explained using box-plots (Figure 3.1 to Figure 3.7). Each box-plot captured the minimum, maximum, median, mean, Q1 and Q3 values after checking and removing outliers from the boxplots. Summary statistics, described by minimum, maximum, median, standard deviation (SD), skewness, 25th quartile (Q1) and 75th quartile (Q3), kurtosis and coefficient of variation, were produced for plant grain yield, environmental factors, percentage of N derived from air (%Ndfa), %OC, pH, nitrogen concentration and total nitrogen (Table 3.5).

## 3.5. Results

### 3.5.1. Global variation of environmental, soil and plant variables

Global variability of environmental, soil and plant factors are shown and summarized in Table 3.5. Most of the studies were conducted in the Northern hemisphere with average annual temperature and rainfall of 21.17 ± 0.56$^{o}$C (n=113) and 989 ± 44.13mm (n=182), respectively. The lowest temperature for all studies, 7.6$^{o}$C was in Denmark under forage production (Li *et al.*, 2015), while the highest was 28$^{o}$C in Japan under pearl millet-cowpea intercropping (Sarr *et al.*, 2008). The maximum and minimum rainfall received were 2271mm in Mexico (Vera-Nunez *et al.*, 2008) and 114mm in Syria (Al-Chammaa *et al.*, 2014). There was a great variation in percent Ndfa, with the minimum 0.01% in Piracicaba Sao Paulo, Brazil where common beans were planted in Oxisols under acidic conditions. The maximum 99.9% was in Nyambi Malawi on an inceptisol (Adu-Gyamfi *et al.*, 2007). On average Ndfa was 52.05 ± 1.25% for 539 observations. The most/highly acidic soil used across all experimental sites had pH 4.5 (Table 3.5) in Italy and Brazil where trifolium alpinum and different common bean cultivars were planted, respectively (Franzini *et al.*, 2013; Lonati *et al.*, 2015). The highly basic soils10.6 were aridisols in Syria where sole and mixed cropping of fodder shrubs was done (Kurdali, 2010). The mean pH for all studies was 6.37 ± 0.07 for(n=429).

The highest grain yield (GY) 4870kg ha$^{-1}$ was obtained in Potenza Italy where fababeans (Table 3.5) were planted in sub-tropical regions (Cazzato *et al.*, 2012) while the lowest GY, 7.89 kg ha$^{-1}$ was obtained in Batatta Sri Lanka on Alfisols where mungbean was intercropped with maize (Senaratne *et al.*, 1995). The mean GY was 977.87 ± 66.51 kg ha $^{-1}$ (n=190). The maximum N concentration was 8.5% in white lupine and the minimum 0.00% in cowpeas (Ankomah *et al.*, 1996; Sulas *et al.*, 2016) on field and greenhouse experiments conducted in Italy and Austria, respectively. The mean N concentration was 2.8% ± 0.15 for (n=122). The maximum total nitrogen (TN) was 456 kg ha$^{-1}$ across all experimental sites, where beans were planted at different P rates on ultisols (Vera-Nunez *et al.*, 2008) in Mexico while the minimum TN was in cowpeas 0.00 kg ha$^{-1}$ planted on entisols in tropical regions of Senegal(Ndiaye *et al.*, 2000). On average, TN was 76.03 ± 4.65 kg ha $^{-1}$ (n=368) for all studies analysed (Table 3.5).

*Table 3.3: Values of %Ndfa for different climatic regions and soil factors affecting biological nitrogen fixation*

| | | **Sample** | **Mean** | **Maximum** | **Minimum** |
|---|---|---|---|---|---|
| Hemisphere | Northern hemisphere | 221 | 58.9 | 96.2 | 0.22 |
| | Southern hemisphere | 73.0 | 23.7 | 98.5 | 0.01 |
| Climatic regions | Sub-tropical | 92.0 | 58.7 | 96.2 | 0.01 |
| | Temperate | 109 | 54.0 | 98.5 | 0.72 |
| | Tropical | 312 | 49.0 | 99.9 | 0.01 |
| Soil texture | Clay | 56 | 63.6 | 92.0 | 0.01 |
| | Loam | 153 | 35.8 | 90.0 | 0.01 |
| | Sand | 124 | 50.7 | 96.2 | 0.22 |
| Soil class | Alfisol | 24 | 50.8 | 85.2 | 2.70 |
| | Aridisol | 14 | 59.9 | 79.4 | 38.8 |
| | Entisol | 26 | 61.9 | 95.0 | 33.3 |
| | Inceptisol | 68 | 59.7 | 99.9 | 0.60 |
| | Mollisol | 34 | 50.0 | 79.7 | 0.72 |
| | Oxisol | 109 | 36.9 | 99.7 | 0.01 |
| | Ultisol | 78 | 66.8 | 96.2 | 27.1 |
| | Vertisol | 30 | 75.3 | 92.0 | 41.6 |
| Soil pH condition | Acidic | 148 | 55.0 | 96.2 | 0.03 |
| | Basic | 118 | 56.2 | 96.2 | 0.60 |
| | Highly acidic | 97.0 | 33.3 | 93.0 | 0.01 |
| | Highly basic | 68.0 | 58.4 | 92.0 | 0.01 |
| Organic C class | High | 48.0 | 56.3 | 89.0 | 19.8 |
| | Low | 121 | 53.8 | 84.1 | 23.2 |
| | Medium | 154 | 35.2 | 90.0 | 0.01 |

*Table 3.4: Values of %Ndfa for different legume types as affecting biological nitrogen fixation*

| | | **Sample** | **Mean** | **Maximum** | **Minimum** |
|---|---|---|---|---|---|
| **Legume type** | Bean | 203 | 36.6 | 96.2 | 0.01 |
| | Forage | 100 | 71.3 | 98.5 | 0.60 |
| | Groundnut | 12 | 70.0 | 87.9 | 37.0 |
| | Lentil | 13 | 26,4 | 68.5 | 0.31 |
| | Pea | 105 | 64.9 | 99.9 | 7.90 |
| | Plant/herb | 10 | 65.1 | 90.0 | 47.0 |
| | Soybean | 63 | 53.5 | 79.8 | 0.50 |
| | Tree | 33 | 46.4 | 79.4 | 6.00 |

*Table 3.5: Statistical summary of plant and environmental factors used in the study*

| **Statistic** | **GY kg ha$^{-1}$** | **Lat** | **LON** | **MAP (mm)** | **MAT °C** | **Nfda** | **N conc** | **OC** | **T N kg ha$^{-1}$** | **pH** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **---------------------%------------------ -** | | | | |
| n | 190 | 294 | 285 | 182 | 113 | 539 | 122 | 323 | 368 | 429 |
| Mean | 978 | 15.0 | 9.09 | 989 | 21.2 | 52.1 | 2.84 | 1.54 | 76.0 | 6.37 |
| Median | 850 | 11.1 | -4.33 | 900 | 25.5 | 56.1 | 2.65 | 1.04 | 51.0 | 6.10 |
| Min | 7.89 | -43.5 | -108 | 114 | 7.60 | 0.01 | 0.00 | 0.20 | 0.00 | 4.50 |
| Max | 4870 | 80.0 | 390 | 2271 | 28.0 | 99.9 | 8.50 | 5.12 | 456 | 10.6 |
| Q1 | 129 | 0.12 | -47.6 | 581 | 16.5 | 34.1 | 2.00 | 0.58 | 3.85 | 5.10 |
| Q3 | 1400 | 37.5 | 16.7 | 1400 | 27.0 | 74.2 | 3.56 | 2.03 | 111 | 7.68 |
| SD | 917 | 29.2 | 96.1 | 595 | 6.14 | 29.1 | 1.67 | 1.22 | 89.2 | 1.36 |
| SEM | 66.5 | 1.70 | 5.69 | 44.1 | 0.58 | 1.25 | 0.15 | 0.07 | 4.65 | 0.07 |
| %CV | 93.8 | 194 | 1057 | 60.2 | 29.0 | 56.0 | 58.9 | 79.2 | 117 | 21.3 |
| Skew | 1.44 | -0.05 | 2.59 | 0.83 | -0.49 | -0.43 | 0.54 | 1.20 | 1.55 | 0.10 |
| Kurtosis | 2,65 | -0.77 | 7.87 | 0.04 | -1.14 | -0.80 | 0.78 | 0.58 | 2.27 | -1.28 |

n=number of observations, Min and Max =minimum and maximum, Q1 and Q3= first and third quartile, SD = standard deviation, SEM = standard error of mean and CV =coefficient of variation, GY = grain yield, Lat = latitude, Lon = longitude, MAP = mean annualprecipitation, MAT=mean annual temperature, Ndfa = Nitrogen derived from atmosphere, N conc = nitrogen concentration, OC = organic carbon, TN = total nitrogen.

### 3.5.2. Comparison of Ndfa under different hemispheres

There were more studies conducted in the northern hemisphere (n= 221) with average Ndfaof 59.0%, which was higher than in the southern hemisphere with 23.7% for n=73 (Table 3.3, Figure 3.1). However,the maximum Ndfa 98.5% and the minimum 0.01% were obtained in the southern hemisphere as shown on Figure 3.1.

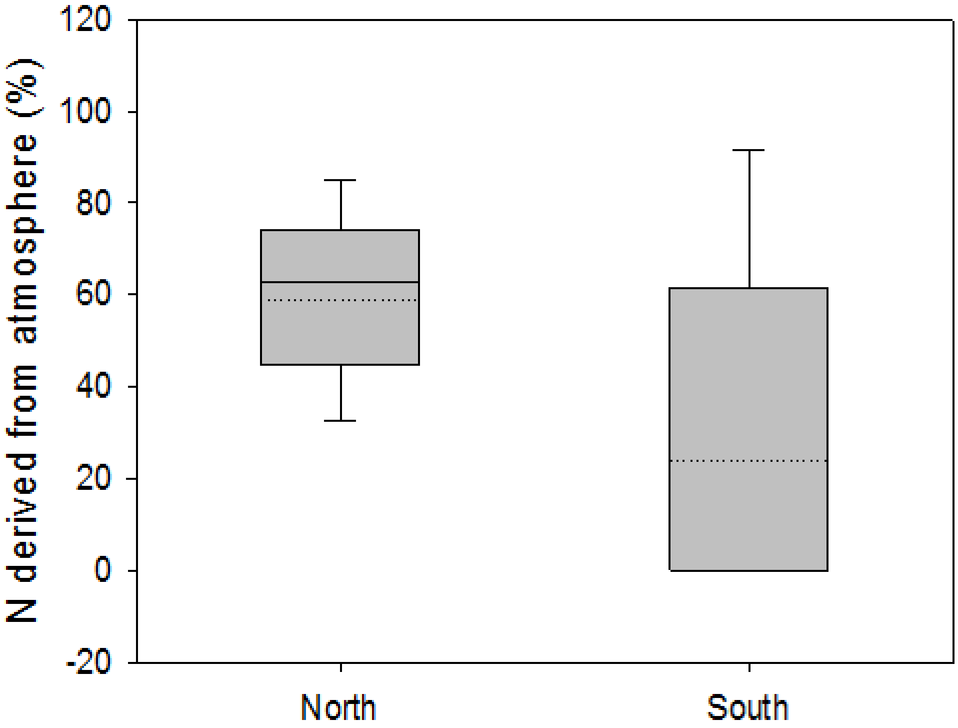

*Figure 3.1: %Nitrogen derived from the atmosphere in the northern and southern hemisphere. Illustration of minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%), values is shown in each box. The dotted and solid lines represent mean and median respectively*.

#### 3.5.2.1. Comparison of Ndfa under different climatic regions

The studies conducted in sub-tropical regions resulted in the highest Ndfa 59% on average(n=92), while the temperate region had 54% mean Ndfa (n=109). The lowest Ndfa (49%) was for studies conducted in the tropical regions (n= 312), with the largest variation (Figure 3.2).

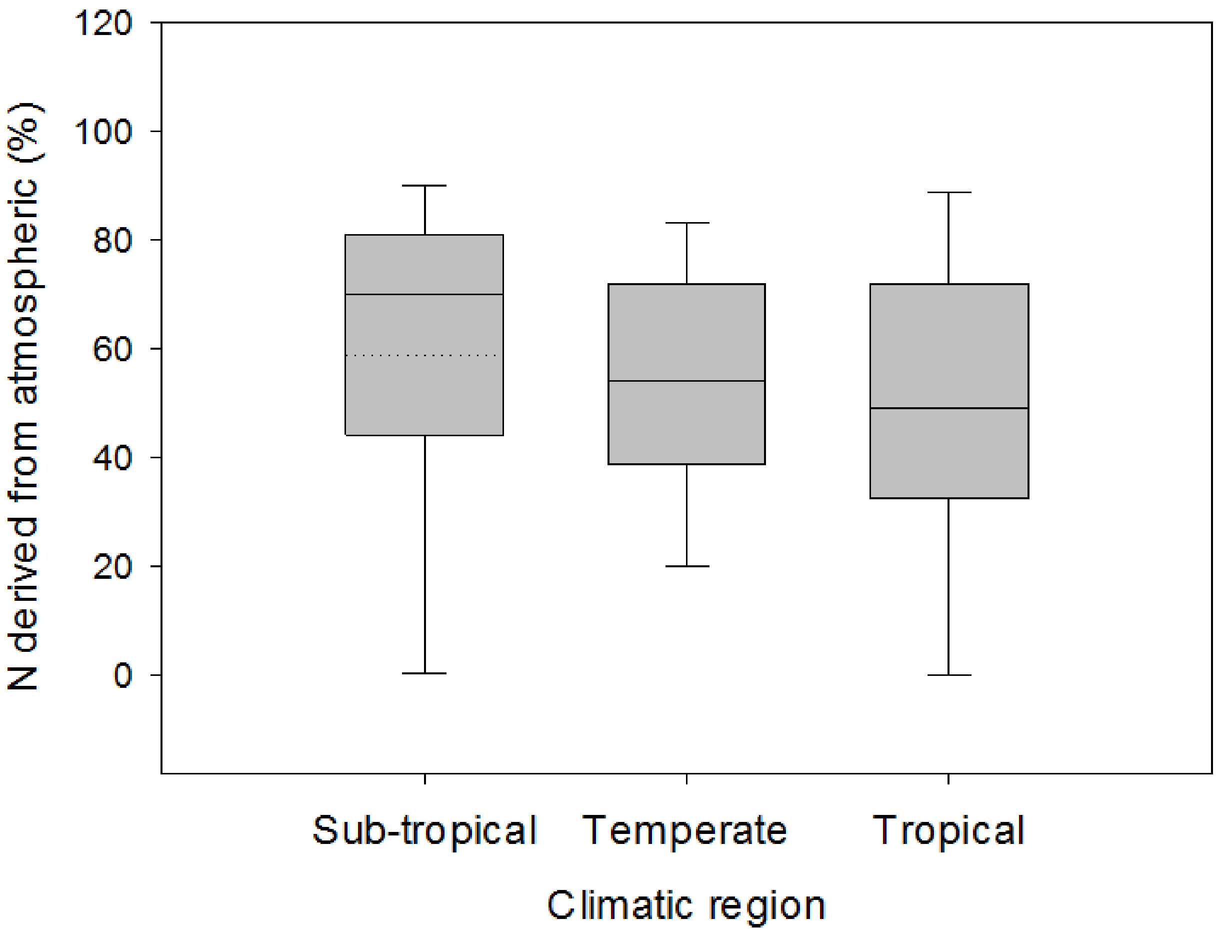

*Figure 3.2: %Nitrogen derived from the atmosphere in different climatic regions. Illustration of minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%), values is shown in each box. The dotted and solid lines represent mean and median respectively*

#### 3.5.2.2. Comparison of NDFA under different soil orders

Vertisols had the highest mean Ndfa across all experimental sites, with 75.3% (n=30), followed by 66.8% for ultisols (n = 78) depicted on Figure 3.3. On average, the least Ndfa was recorded in Oxisols with 36.9% although there was a great variation in Ndfa compared to other soil orders, (n=109). Furthermore, the minimum Ndfa for all soil orders, 0.01% was in oxisols (n=109) while the maximum Ndfa 99.9% was in inceptisols (n=68).

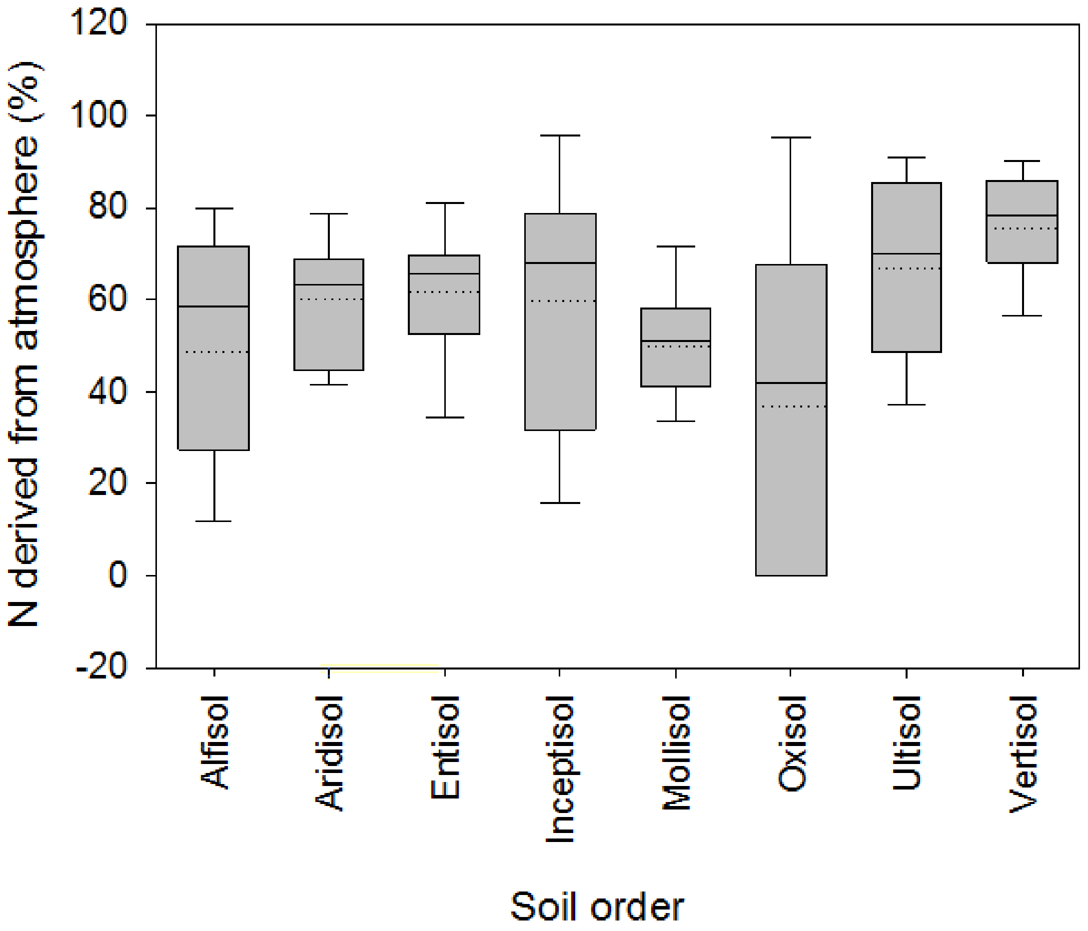

*Figure 3.3: %Nitrogen derived from the atmosphere in different soil orders. Illustration of minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%), values is shown in each box. The dotted and solid lines represent mean and median respectively*

#### 3.5.2.3. Allocation of Ndfa to different soil textural classes and pH conditions

Highly basic pH conditions exhibited the highest mean Ndfa value of 58% (n=68) with the maximum value of 92% and the minimum of 0% Ndfa (Figure 3.4). Basic and acidic conditions follow with 56% (n=118) and 55% (n=149) mean values respectively, with the maximum value of 96% in both conditions and 1% and 0% minimum values for basic and acidic conditions, respectively. Nitrogen fixation decreased with decrease in pH conditions as indicated by the Ndfa mean value of 33% (n=97) with the highest value of 93% and the lowest value is 0% (Figure 3.4). For studies on clay soils more nitrogen was fixed from the atmosphere with the highest mean value of 64% (n=56), and the highest value of 92% and lowest 0% Ndfa, followed by sand with 51% mean Ndfa (n=124), with the highest Ndfa valueof 96% and the lowest of 0%. On average, loam shows the least value of Ndfa 36% although it consists of the largest sample size (n = 153). The highest Ndfa value of Ndfa is 90% and the lowestvalue is 0.3% (Figure 3.5.

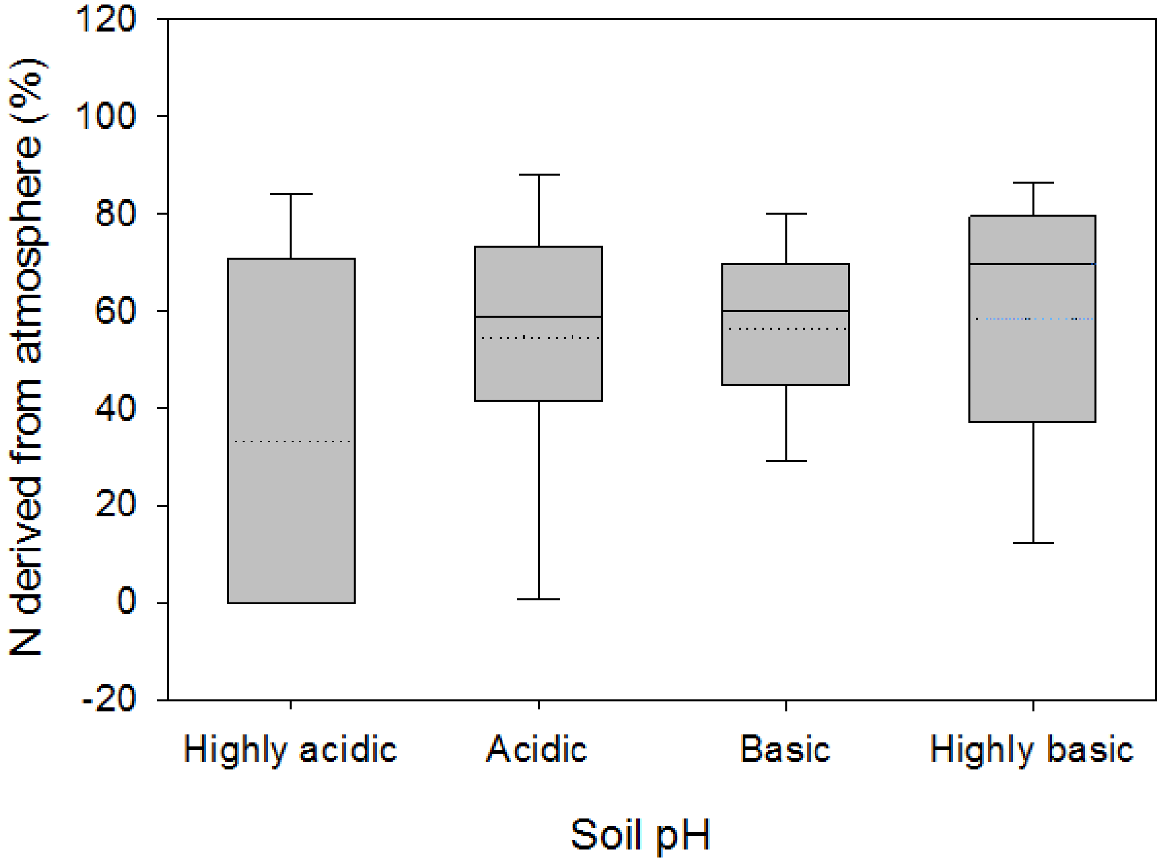

*Figure 3.4: %Nitrogen derived from the atmosphere in different soil pH ranges. Illustration of minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%), values is shown in each box. The dotted and solid lines represent mean and median respectively.*

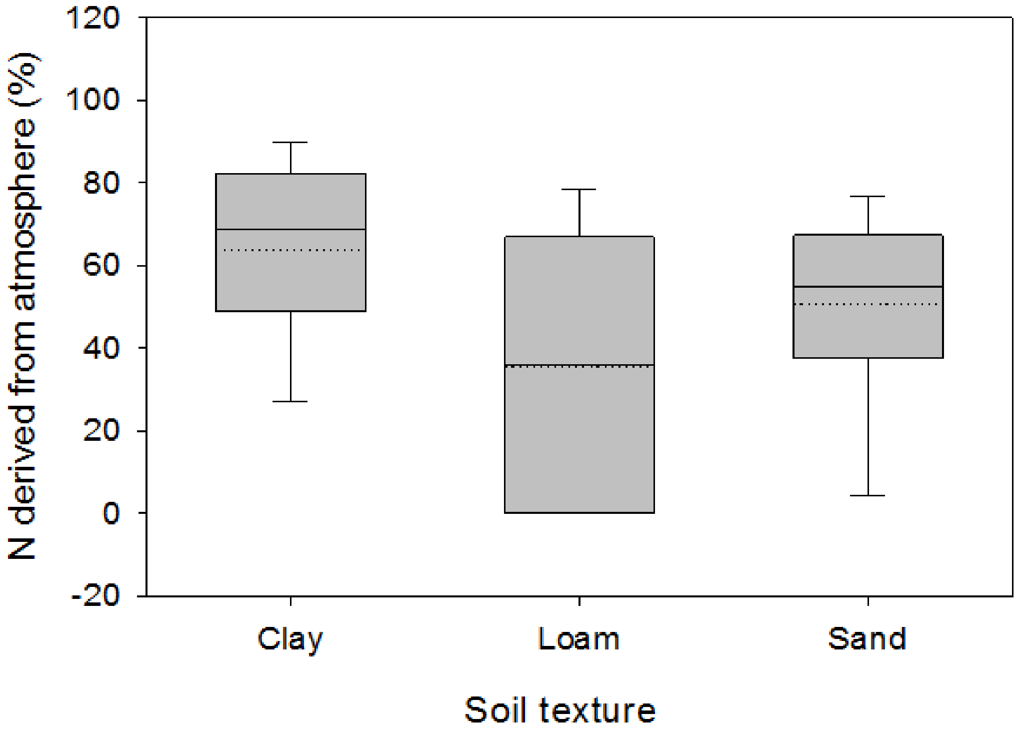

*Figure 3.5: %Ndfa in different soil texture. Illustration of minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%), values is shown in each box. The dotted and solid lines represent mean and median respectively.*

### 3.5.2.4. Comparison of soil organic carbon with Ndfa

The global studies reveal that soils high in organic carbon have the highest capacity to fix nitrogen from the atmosphere, as indicated by mean Ndfa value of 56% (Figure 3.6) although the sample size is the lowest (n=48). The highest Ndfa under this soil condition is 89% and the lowest is 6%. The low OC soil organic carbon follows with 53.8% on average(n=121), with the maximum value of 84% and minimum of 1%. The medium soils had the lowest mean Ndfa of 35% across all experimental sites with the highest sample size of 154,and the maximum value of 90% and minimum value of and 0%.

### 3.5.2.5. Response of different legume types to nitrogen fixation

Out of eight different legume types, forage fixed highest percentage of nitrogen from the atmosphere as shown by the mean Ndfa value of 71% (n=100) (Figure 3.7), with the maximum being 99% and the minimum 1%. Groundnut was the second with 70% on average (n=12), with maximum and minimum values of 88% and 37%, respectively. Herbs and peas fixed 65% on average for 10 and 105 sample sizes, respectively, while Trees andbeans fixed 46% (n=33) and 37% (n=203) on average. The highest value of Ndfa was 79% for trees and 96% for beans. The lowest values were 6% in trees and 0% in beans. Soybeans fixed 54% on average (n=63), while Lentil accumulated the least with mean Ndfa value equivalent to 26% with the maximum and minimum values of 69% and 0.3% respectively. Soybean derived 54% N from the atmosphere with 91% maximum and 1% minimum values (Figure 3.7).

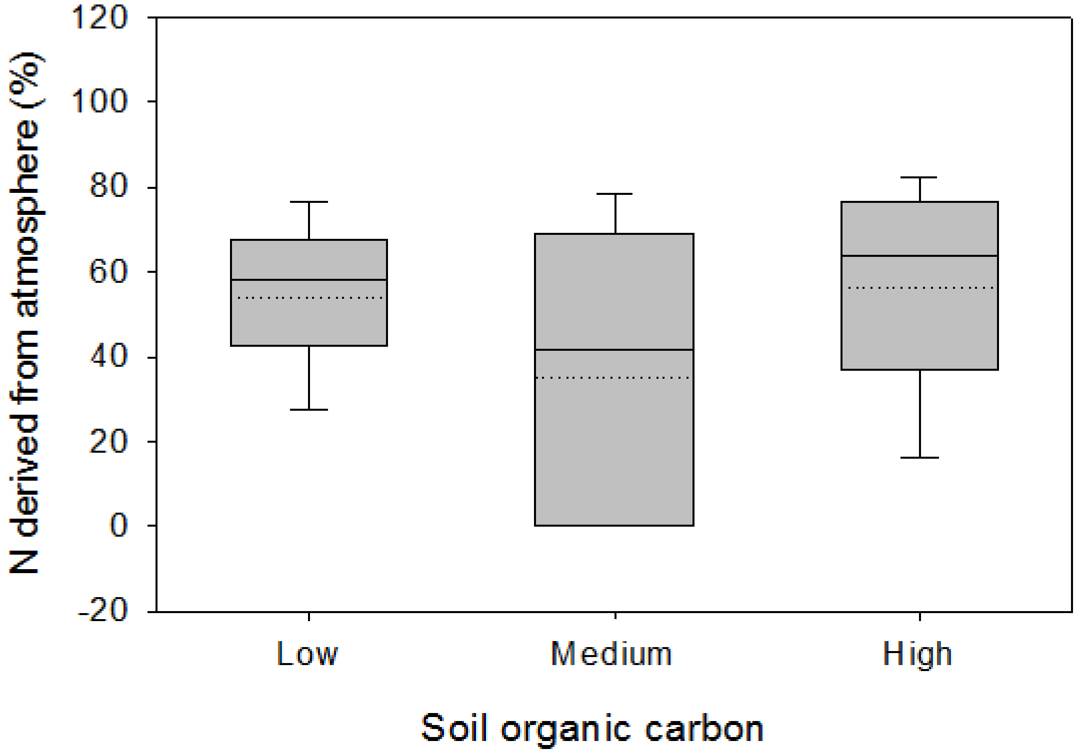

*Figure 3.6: %Nitrogen derived from the atmosphere in different soil organic carbon. Illustration of minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%), values is shown in each box. The dotted and solid lines represent mean and median respectively.*

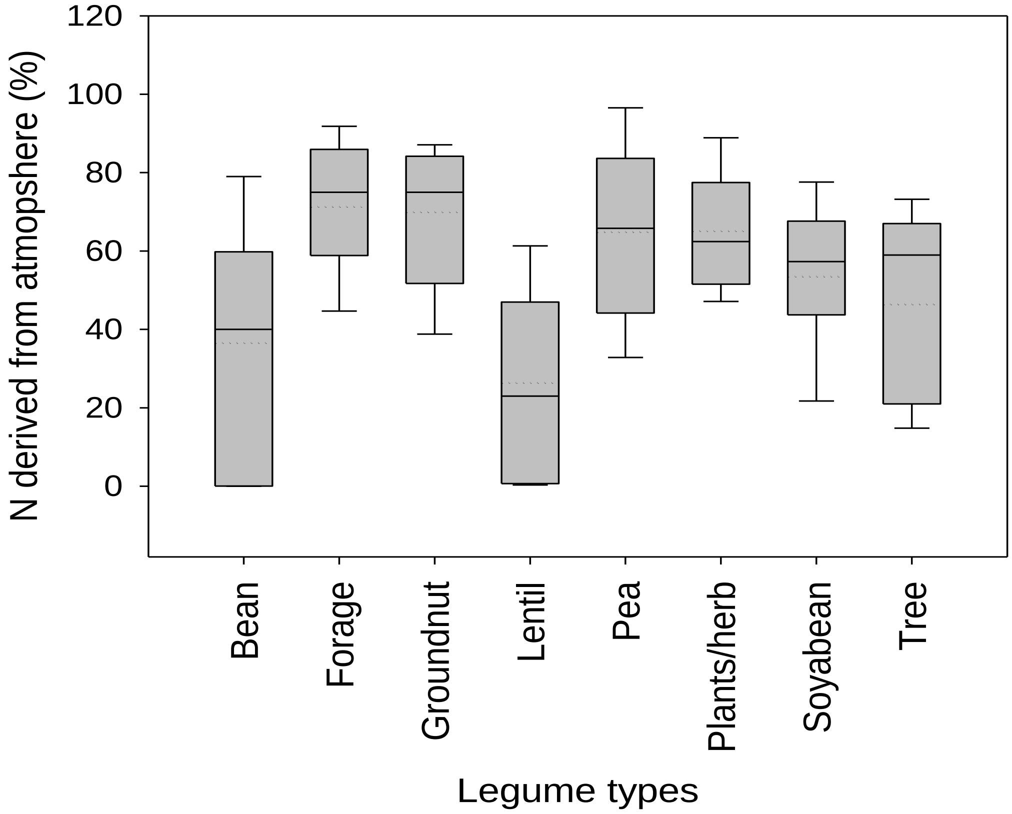

*Figure 3.7: %Nitrogen derived from the atmosphere in different legume types. Illustration of minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%), values is shown in each box. The dotted and solid lines represent mean and median respectively.*

## 3.6. Discussions

The higher average Ndfa for the Northern Hemisphere (Carranca *et al.*, 1999; Hafeez *et al.*, 2000; Guene *et al.*, 2003; Burchill *et al.*, 2014; Ashworth *et al.*, 2015) could be because the work done in the Southern Hemisphere was done in tropical soils that are highly weathered and acidic, due to high extent of weathering and leaching. The evidence is shown on studies conducted in Tanzania, Malawi, Zambia, and Brazil (Duque *et al.*, 1985; Munyinda *et al.*, 1988; Müller and Pereira, 1995; Adu-Gyamfi *et al.*, 2007; Franzini *et al.*, 2013) in the tropical region of the Southern Hemisphere. The lower Ndfa in tropical soils could be explained by highly weathered and leached soils which are acidic, as shown in a study by Bado *et al.* (2018), where BNF for local and improved cowpea varieties was compared. These soils negatively affect biological nitrogen fixation in legumes since the cells of Rhizobium bacterial species become smaller, the synthesis of extracellular polysaccharides decreases, and the formation of nodules is inhibited under low pH values and Ca and Mg deficiency occurs (Cunningham and Munns, 1984). The sub-tropical soils are warm moist but not as highly acidic as tropical soils, which explain the higher BNF, while cooler temperate region could reduce activity of the rhizobium when compared to sub-tropical regions. Studies conducted in subtropical region resulted in high Ndfa (Amanuel *et al.*, 2000; Mohammad *et al.*, 2010; Asare *et al.*, 2015; XIE *et al.*, 2015) compared to the one conducted in the temperate regions (Jensen, 1986; Sanginga *et al.*, 1990; Wolyn *et al.*, 1991; Ardakani *et al.*, 2009).

Plants that grow in heavy textured soils (high clay content) mostly known as Vertisols are healthier compared to plants grown in medium and light soils owing to their ability to retain nutrients as they have high cation exchange capacity. As such, they are known to be fertile soils. The lower Ndfa in Oxisols than Vertisols could be explained by the highly acidic conditions in these soils. The Oxisols are formed in regions of high rainfall and with high average temperatures, where extreme chemical weathering occurs, and the bases are leached leaving $Al^{3+}$ and $H^{+}$ ions dominating the exchange site of soils. The high concentration of $H^{+}$ ions causes the solubility of Mn, and Fe, which strongly prohibits BNF (Whelan and Alexander, 1986). Similarly the findings from separate studies revealed that Ndfa decreases with decrease in soil pH due to sensitivity of bacteria to soil acidity. Bacteria, including rhizobia bacterial species, are more active under basic and close to neutral pH and their activity decreases with increase in acidity (decline in pH), (Samba *et al.*, 2002). In the experiment conducted by Evans *et al.*

(1980), the efficient nodulation and nitrogen fixation did not occur at pH 4.8 and below due to shortened roots and swollen root hairs which occur in most legumes growing on acid soils (pH < 5.0), and these phenomenon complicate or completely suppress the infectious process and the formation of efficient nodules (Lapinskas, 2007).

On the other hand, Vertisols and other young soils have pH close to neutral and support growth and activity of bacteria, including rhizobia which supports the higher Ndfa in these soils than Oxisols. The findings by (Kumar and Goh, 2000; Giambalvo *et al.*, 2011b; Saia *et al.*, 2016) revealed that the lowest Ndfa was 57%, 69% and 77% for pH 8.4, 5.7 and 8.2 respectively in Vertisols and Inceptisols while lowest Ndfa in Oxisols was 42% and 44% at pH 5.1 and 4.8 respectively (Cadisch *et al.*, 1989; Kihara *et al.*, 2011). In the same way, the higher Ndfa in clayey soils than other textures (Kurdali *et al.*, 2003; Giambalvo *et al.*, 2011a; Cazzato *et al.*, 2012; Tauro *et al.*, 2013; Al-Chammaa *et al.*, 2014; Saia *et al.*, 2016), could be explained by nutrient, and moisture retention of these soils which support microbial survival and activity and the higher Ndfa in soils with higher organic C could be because of availability of nutrients and water, as a result of organic matter, supporting microbial activity, including rhizobia species involved in BNF. The differences in soil types (vertisols to oxisols), with variations in pH and clay content, could affect BNF in farmer’s fields in Lesotho, where such variation occurs, and currently, there is no literature on the level of BNF from different legume types, considering the different soil characteristics in the Foothills and Lowlands where legumes are commonly intercropped with maize.

Different legumes are able to fix different amounts of nitrogen depending on their ability to form root nodules which fix N and access the available N in the root zone before they fix N (Haque *et al.*, 2012). The formation of the nodules is also controlled by soil temperature since the activity of nitrogenase is different for different species. For example, soybean (*Glycine max (L.)* Merr.), produces nodules at an early stage at 25$^{o}$C (Lindemann and Ham, 1979) while clover (*Trifolium repens L.*) requires10–35 $^{o}$C (Richardson and Syers, 1985; Whitehead, 1995) regardless of the varieties and the rhizobia strains, common beans require up to 35$^{o}$C (Piha and Munns, 1987). The low BNF in beans and soybeans compared to forage legumes may be attributed to genetic differences. Forage legumes planted in warm moist and cooler temperate regions showed high BNF ranging from 34-99% (Wivstad *et al.*, 1987; Wanjiku *et al.*, 1997; Sylla *et al.*, 1998).

### 3.7. Conclusion

The meta-analysis revealed that, globally, there was a great variation in %Ndfa, between different climatic regions, soil characteristics and legume crop types. The BNF is higher for subtropical environments, followed by temperate, with tropical environments having the lowest. Soil type, as affected by the extent of weathering and leaching, has strong effects on BNF with the highest in vertisols and lowest in oxisols. Soil with higher fertility status including higher pH and organic matter promote BNF compared to those of poor fertility. Legume type showed strong variations in BNF with the highest in forage legumes and groundnut, and the lowest in lentils and beans. The wide range of %Ndfa for bean (0-60%) in between the first and third quartile, indicate that there could be major differences in been genotypes in terms of BNF. As such, studies on variation in BNF of different bean genotypes need to be carried out especially in Lesotho where no such studies on BNF have been documented, yet legumes are important components of agro-ecosystems.

# CHAPTER 4:
# Biological nitrogen fixation of bean and nitrogen use efficiency of maize cultivars in Lesotho

## 4.1. Introduction

The agricultural production potential of Lesotho is mainly in the Lowlands and the Foothills (Ministry of Agriculture and Food Security, 1995). The soils of the Foothills are derived mainly from basalts of the Lesotho formation, and are dominated by kaolinite and sesquioxides (Schmitz and Rooyani, 1987) while the Upper Lowlands soils are derived from sandstone of Clarens formation, and usually have a coarse texture, low pH, low organic matter, and are deficient in nitrogen (N), phosphorus (P) while potassium (K) deficiency is rare (Marake and Molumeli, 1999; Molete et al., 2005). Like in other African countries, deficiency of N is among the most limiting factors for increased crop yields (Nyemba and Dakora, 2010), and management of N inputs is major challenge for increased agricultural production and attainment of sustainability (Zoundjl et al., 2016). The decline in agricultural production in Lesotho has resulted in food insecurity and extreme poverty (Rantšo and Seboka, 2019). The main food crops in Lesotho are maize (Zea mays L.), sorghum (Sorghum bicolor), wheat (Triticum aestivum L), beans (Phaseolus vulgaris) and peas (Pisum sativum) and are mostly produced for home consumption under rain fed conditions (Rasoeu et al., 2006). The national production of beans, peas, maize, sorghum and wheat has extremely declined from the 1980s to 2018 (Statistics, 2019). The decline in production could be explained by severe droughts, erratic rainfall, soil erosion, poor land management practices, continuous mining of nutrients by crops with little or no replenishment (FAO/WFP, 2005; Shisanya et al., 2009).

Although application of inorganic fertilizers helps to address the limitations of essential nutrients, most resource-poor farmers cannot access the fertiliser due to financial constraints (Nyemba and Dakora, 2010). In Lesotho, farmers do not apply the recommended fertiliser rates due to high costs resulting in large yield gap compared to yield potential. Increased use of other N sources like organic residues and biological nitrogen fixation (BNF) within the context of integrated soil fertility management can ensure food security in areas where N fertilizer is too expensive or simply not available. The incorporation of leguminous crops can achieve increased crop yields in the cropping systems (Bagayoko et al., 2000; Bationo and Ntare, 2000; Adu-Gyamfi et al., 2007). Quantities of N fixed by legumes have long been studied by many authors in other countries (Adu-Gyamfi et al., 2007; Vera-Nunez et al., 2008; Giambalvo et al., 2011a; Tauro et al., 2013; Sarr et al., 2016; Zoundjl et al., 2016), and there is evidence that some legumes such as soybean, common bean, groundnuts, pigeon peas, chickpeas, cowpeas, can fix up to 11 million tonnes of N in developing countries (International Atomic Energy Agency, 2008). Nitrogen fixation up to 300 kg N/ha in a season has been reported for grain or green manure legumes, although P and K deficiencies can reduce BNF (International Atomic Energy Agency, 2008). Although most farmers use legumes in intercropping or rotation with non- leguminous crops, no studies have been conducted in Lesotho to quantify the amount of N fixed by legumes using the stable 15N method.

Bean is the most commonly cropped legume either as a sole crop, in intercropping or in rotation with other crops and is an important source of affordable protein in addition to thiamin, zinc, (Murphy et al., 1975), iron (Sgarbieri et al., 1979), and potassium (Meiners et al., 1976) in Lesotho. In addition, it also benefits in soil fertility enhancement by fixing nitrogen. Basotho farmers have used the Pinto Nodak variety over the decades, while the variety NUA 45 (biofortified) has been newly introduced, and the two varieties are early maturing, high yielding, and drought tolerant. Comparing N2 fixation of the two varieties using 15N isotopic method will be helpful to better advice farmers on which one produce higher yield while improving soil fertility at the same time. While the best non-fixing reference crop is non-nodulating lines of the test legume (Okito et al., 2004), non-fixing reference mono or dicotyledonous crops (Reiter et al., 2002) including maize can be used in the absence of non-nodulating lines of the test legume. Maize has no ability to fix nitrogen, but its ability to take up nitrogen from the soil is almost similar to grain legumes and their maturity period is almost the same.

In addition to improving yield of the main protein source, there is the need to also improve productivity of main staple crop, through growing varieties that efficiently use the limited fertiliser resources. The N use efficiency maize is not clear in Lesotho, especially for the two maize genotypes, ZM 521 and ZM 523 that have newly been introduced to farmers in Lesotho because of their ability to tolerate drought. They differ in maturity by 20 days with ZM 523 maturing later. There is a need to carry out a study that can identify the maize variety that has higher N use efficiency for the benefit of farmers, who usually apply insufficient fertiliser rates. The findings from such studies can be useful for management of N supply and N utilization in these cropping systems. The findings could provide farmers with technologies that can restore soil fertility and increase productivity.

## 4.2. Aim:

The aim of this study was to evaluate two bean cultivars for biological nitrogen fixation and two maize cultivars for nitrogen use efficiency to restore the fertility of selected soils in Lesotho using isotopic techniques. The specific objectives of this study were to:

(i) To quantify and compare the biological nitrogen fixation (BNF) and yields of two common bean varieties on farmer-managed fields.

(ii) To determine nitrogen use efficiency (NUE) and yields of two maize varieties on farmer-managed fields in two agro-ecological zones.

## 4.3. Description of study sites

The study was conducted on two agro-ecological zones of Lesotho, namely; Lowlands represented by Sakoane village and the Foothills represented by Machache village, as indicated in Figure 4.1. The two agro-ecological zones were chosen because of high maize and bean production that occurs in these areas. Apart from that, they are easily accessible, for this reason monitoring and data collection were not constrained by resources.

### 4.3.1. Machache study area

The Machache study area is found in Maseru District on the Foothills of Lesotho, approximately, 55 km north-east of Maseru. It lies at 29° 21' 46.08 36'' S and 27° 53' 49.8264'' E and at 1854 m above sea level. The Foothills (1,800 to 2,000m above sea level) are found between the Lowlands and the Highlands, and occupy an area of about 4 600 km$^2$ and form 15% of the total land area. Annual rainfall is approximately 600mm. Soils are of Alfisols group derived mainly from basalts of the Lesotho Formation (Carroll and Bascomb, 1967). The soils are high in organic matter with low base saturation (45%). Cultivation on steep slopes without conservation measures and over-grazing make the soils susceptible to wind and rainwater erosion. Maize, sorghum, beans and summer peas are common crops.

### 4.3.1. Sakoane study area

The Sakoane study area is found in the Berea District in the northern Lowlands of Lesotho,approximately 35 km north of Maseru, and is located at 29° 10' 28.8192'' S and 27° 49' 52.1148'' E and at 1500 m above sea level. The Lowlands, lying between 1,400m and 1,800m above sea level, form the western part of the country, occupying about 5 200 km$^2$, which is 17% of the total surface area. The annual rainfall is approximately 650mm. Soils are of Inceptisols derived from sandstone of Clarens formation. These soils are acidic, low in organic matter and are susceptible to sheet and gully erosion by wind and rainwater dueto steep (Carroll and Bascomb, 1967). Maize, sorghum, beans, winter wheat and vegetables are the common crops. Rainfall data recorded in the long term and during 2018/2019 and 2019/2020 cropping seasons for Machache and Sakoane are shown in Table 4.1.

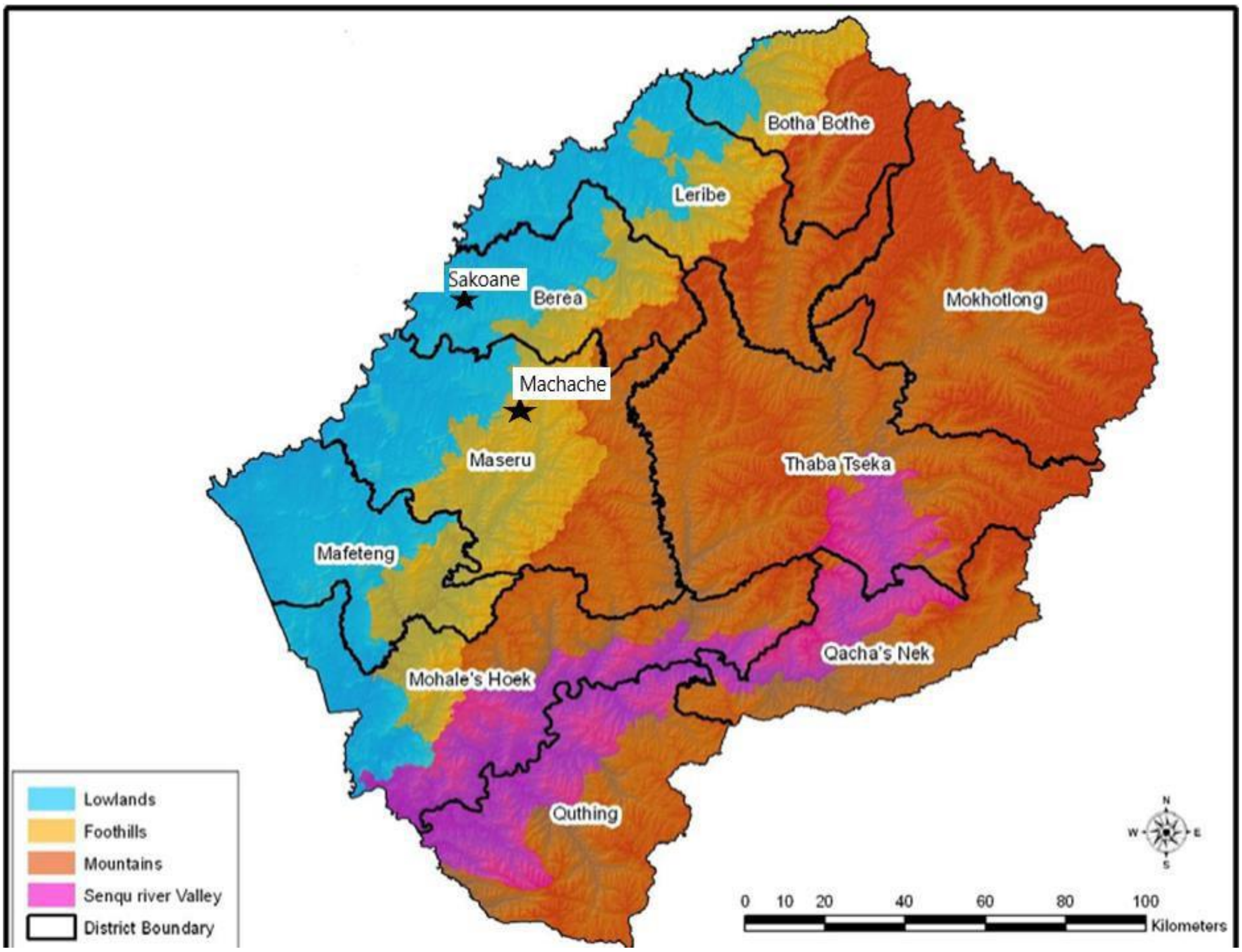

*Figure 4.1: Location of study sites in two agro-ecological zones of Lesotho (Statistics, 2010)*

*Table 4.1 Rainfall during 2018/2019 and 2019/2020 cropping seasons at Machache and Sakoane*

| **Month** | **Rainfall (mm)** | | | |
|---|---|---|---|---|
| | **Machache** | | **Sakoane** | |
| | **2018/2019** | **Long-term *** | **2018/2019** | **Long-term*** |
| January | 23.0 | 135 | 18.0 | 139 |
| February | 185 | 107 | 165 | 113 |
| March | 136 | 105 | 124 | 99.8 |
| April | 183 | 65.5 | 93.0 | 71.8 |
| May | 42.0 | 25.0 | 18.0 | 29.9 |
| **TOTAL** | **569** | **438** | **418** | **454** |
| | | | | |
| | **2019/2020** | | **2019/2020** | |
| January | 139 | | 208 | |
| February | 219 | | 134 | |
| March | 127 | | 51.0 | |
| April | 122 | | 93.0 | |
| May | 0.00 | | 0.00 | |
| **TOTAL** | **607** | | **486** | |

*Long-term data averaged for a 30-year period starting from 1988 to 2018

## 4.4. Farm selection, soil sampling and analysis

### 4.1.1. Selection of farms

Public gatherings were conducted in the two locations in July and August 2018 to sensitize the community about the project. Thereafter, the area extension officer, local chief and a counsellor helped with identification of farms (farmers) and they considered the most dedicated farmers to farming every season. Three smallholder farms were selected and a fourth one was at the research station for each location, and each of the farms served as a replicate.

### 4.1.2. Soil sampling and analysis

Soil samples were collected from the 0-20cm depth for all selected farms in both locations prior to planting, for characterization. There were 15 subsamples collected on each farm and composited per farm. The area of farms varied between 0.95 to 1.5ha. The samples were air-dried, sieved through a 2 mm sieve and analysed for physicochemical properties (Table 4.2 and Table 4.3). Available phosphorus, potassium, zinc, copper and manganese were analysed after Ambic-2 extraction (Hunter, 1975; Farina, 1981). The Ambic-2 extracting solution which consisted of 0.25 *M* $NH_4CO_3$ + 0.01 *M* $Na_2EDTA$ + 0.01 *M* $NH_4F$ + 0.05 g L-1 Superfloc (N100), adjusted to pH 8 with a concentrated ammonia solution was prepared and 25 mL of this solution wasadded to 2.5 mL soil, and the suspension was stirred at 400 r.p.m. for 10 minutes using a multiple stirrer. The extracts were filtered using Whatman No.1 paper. Phosphorus was determined on a 2 mL aliquot of filtrate using a modification of the Murphy and Riley (1962) molybdenum blue procedure (Hunter, 1975).

Potassium was determined by atomic absorption on a 5 mL aliquot of the filtrate after dilution with 20 mL de-ionised water. Zinc, Cu and Mn were analysed by atomic absorption on the remaining undiluted filtrate. Soil pHwas determined in 1 M KCl solution at a 1:2.5 soil to solution ratio. Extractable calcium, magnesium was determined after extraction with 1 M KCl solution, where the solution wasadded to 2.5 mL soil and the suspension was stirred at 400 r.p.m. for 10 min using a multiplestirrer. The extracts were filtered using Whatman No.1 paper and 5 mL of the filtrate was diluted with 20 mL of 0.0356 *M* $SrCl_2$, and Ca and Mg determined by atomic absorption. Fordetermining extractable acidity, 10 mL of the filtrate was diluted with 10 mL of de-ionized water and 2-4 drops of phenolphthalein were added, then titrated with 0.005 *M* NaOH.

Percent acid saturation was calculated as "extractable acidity" x 100 / (Ca + Mg + K + "extractable acidity"). Total carbon (C) and nitrogen (N) were analysed by the Automated Dumas dry combustion method using a LECO CNS 2000. Particle size distribution of soils was done using the pipette method according to Day (1965) and soil textural class was determined from a textural triangle. A 20 g soil sample (<2 mm) was treated with hydrogen peroxide to oxidize the organic matter. The sample was made up to 400 ml with de-ionized water and left overnight. The clear supernatant was siphoned off and the sample puddled. A further addition of de-ionized water was done, the sample stirred and left overnight. The clear supernatant was again siphoned off. Dispersing agents (NaOH and sodium hexametaphosphate) were added and the sample stirred on Hamilton Beach stirrers. The suspension was made up to 1L in a measuring cylinder and the clay (<0.002 mm) and fine silt (0.002-0.02 mm) fractions measured with a pipette after sedimentation. Fine silt plus clay was measured after 4-5 min (exact time depends on temperature) at 100 mm, and clayafter 5-6 h at a depth of 75 mm. Sand fractions included very fine sand (0.05 - 0.10 mm), fine sand (0.10 - 0.25 mm), medium sand (0.25 - 0.50 mm) and coarse sand (0.50 - 2.0 mm) which were determined by sieving. Coarse silt (0.02-0.05 mm) was estimated by difference. Machache soils have a fine loamy texture with low phosphorus and high base cations. Both Machache and Sakoane soils are acidic with exchangeable acidity and percent acid saturation higher at Machache compared to Sakoane. Sakoane soils are sandy with slightly higher available P and low base cations compared to Machache soils.

*Table 4.2: Physicochemical properties (mean ± standard deviation) of soils used in two locations for different farms in 2018/2019 and 2019/2020*

| | P | K | Ca | Mg | TC | EA | %AS | Zn | Mn | CU |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | mg $kg^{-1}$ | | | | | | |
| **MAC** | | | | | | | | | | |
| **2018/19** | 3.76 ±1.87 | 136 ±16.5 | 1224 ± 514 | 504 ± 357 | 11.0 ± 5.01 | 11.0 ± 5.00 | 5.25 ± 6.08 | 7.23 ±4.52 | 25.4 ±11.4 | 8.86 ± 2.53 |
| **2019/20** | 4.85 ± 0.79 | 260 ± 63.3 | 1935 ± 1487 | 583 ± 367 | 15.6 ± 10.0 | 0.50 ± 0.50 | 5.13 ± 5.36 | 1.70 ± 0.38 | 63.1 ± 18.6 | 10.8 ± 4.04 |
| **SAK** | | | | | | | | | | |
| **2018/19** | 3.64 ± 1,88 | 80.8 ± 25.4 | 343 ± 57.2 | 78.3 ± 11.0 | 2.74 ± 0.38 | 0.17 ± 0.07 | 6.13 ± 2.39 | 0.68 ± 0.32 | 11.9 ± 4.07 | 1.09 ±0.26 |
| **2019/20** | 8.01 ± 3.49 | 177 ± 54.4 | 468 ± 128 | 92.7 ± 36.9 | 3.62 ± 1.07 | 0.07 ± 0.05 | 2.00 ± 1.41 | 1.24 ± 0.65 | 33.5 ± 6.00 | 1.33 ± 0.28 |

MAC = Machache, SAK = Sakoane, P= phosphorus K= exchangeable potassium; Ca= exchangeable calcium Mg =exchangeable magnesium, TC = total cations, EA = exchangeable acidity, AS = acid saturation, Zn = Zinc, Mn = Manganese, Cu =Copper extractable with Ambic-2 solution

*Table 4.3: Physicochemical properties (mean ± standard deviation) of soils used in two locations for different farms in 2018/2019 and 2019/2020 season*

| | **OC** | **N** | **Sa** | **Si** | **Cl** | **pH (KCl)** |
|---|---|---|---|---|---|---|
| | | | **%** | | | |
| **Machache** | | | | | | |
| **2018/2019** | 1.59 ± 0.72 | 0.12 ± 0.07 | 50.0± 20.0 | 33.4 ± 18.4 | 19.6± 5.45 | 4.18± 0.39 |
| **2019/2020** | 1.94 ± 1.24 | 0.11 ± 0.10 | * | * | * | 4.28 ± 0.40 |
| **Sakoane** | | | | | | |
| 2018/2019 | 0.69 ± 0.23 | 0.05 ± 0.00 | 82.9 ± 2.90 | 9.36 ± 2.00 | 7.70 ± 1.38 | 4.21 ± 0.23 |
| 2019/2020 | 0,65 ± 0.17 | 0.07 ± 0.01 | * | * | * | 4.40 ± 0.12 |

OC = organic carbon, Sa = sand, Si = silt, Cl = clay, * = particle size analysis was not donefor 2019/2020 season.

## 4.5. Experimental design and management for biological nitrogen fixation

For estimating BNF, beans and maize (as a reference crop) were planted on 280.8m$^2$ area with a micro plot area of 35.1m$^2$ (3m*11.7m). The two crops were planted on the same date, with maize planted as an intercrop between beans (Figure 4.2). Two common bean varieties, viz; NUA 45 and Pinto Nodak were planted with interrow spacing of 0.9m and 0.4m in-row plant spacing, while for maize the spacings were 0.9m and 0.45m, respectively. The Nodak Pinto and NUA 45 bean varieties were developed for their shorter growing season (90 days) and high productivity. The Nodak Pinto was developed by the USDA and has a vine type growth habit and is resistant to bean rusts, and yields up to 3.5 t ha$^{-1}$. The NUA 45 was developed by CIAT Colombia-breeding, it is classified as Calima bean, and is a determinate bush type with white flowers and medium green leaves, it is resistant to common bean rust, angular leaf spot, and bacterial blight and can produce up to 2.9 t ha$^{-1}$.

There was a total of 429 bean plants with 56 plants in the micro plot, while there were 348 maize plants with 42 plants in the micro plot. The micro plot was fertilised with $^{15}$N labelled urea at 20 kg N ha$^{-1}$ of 5.15% atom excess and main plots received $^{14}$N urea at 20kg N ha$^{-1}$ respectively. All plots across different farms received phosphorus in a form of calcium phosphate and potassium in a form of potassium chloride (muriate of potash) and lime at recommended rates based on soil test results as indicated in Table 4.4 and Table 4.5 respectively, for the two cropping seasons. Beans were inoculated with nitrasec Rhizobiumtropici U808 and U809 inoculant mixture with $2*10^9$ microbial strength 24 hours prior to sowing in 2018/2019. The beancap Rhizobium leguminosarum biovar phaseoli with JD5 $5*10^6$ colony forming units/gram was used in 2019/2020 cropping season. The previous supplier for 2018/2019 inoculant couldn’t provide the inoculant in 2019/2020 season and the inoculant was not available in all other suppliers. Therefore, a different inoculant was used in the 2019/2020 season. Three seeds were planted per hole and thinned to one plant 14 days after germination. Weeding was done manually using a hand hoe when necessary, in order to maintain weed free plots. Cutworm bait was applied as basal at planting, storm and whole wheat bait were used to treat pests and rodents. The study was carried out under rainfed conditions in two summer cropping seasons: 2018/2019 and 2019/2020 on the same farms but different plots. The rainfall data for the two seasons are presented on Table 4.1.

*Table 4.4: Summary of fertilizers applied for BNF (maize and beans) in two locations for different farms in 2018/2019*

| **Loc.** | **Farms** | **P applied (kg ha$^{-1}$)** | | **K applied (kg ha$^{-1}$)** | | **Lime (L ha$^{-1}$)** |
|---|---|---|---|---|---|---|
| | | **Maize** | **Beans** | **Maize** | **Beans** | |
| **MAC** | Maseru | 60.0 | 50.0 | 0.00 | 0.00 | 75.0 |
| | Tsólo | 60.0 | 60.0 | 20.0 | 0.00 | 0.00 |
| | Ntanana | 60.0 | 60.0 | 0.00 | 0.00 | 0.00 |
| | Research site | 60.0 | 45.0 | 0.00 | 0.00 | 75.0 |
| **SAK** | Mantsema | 60.0 | 60.0 | 110 | 60.0 | 25.0 |
| | Lekoti | 60.0 | 60.0 | 20.0 | 0.00 | 25.0 |
| | Malelu | 60.0 | 60.0 | 75.0 | 25.0 | 25.0 |
| | Research site | 60.0 | 60.0 | 170 | 120 | 0.00 |

Loc = Location, MAC=Machache, SAK= Sakoane

*Table 4.5: Summary of fertilizers applied for BNF (maize and beans) in two locations for different farms in 2019/2020*

| **Loc.** | **Farms** | **P applied (kg ha$^{-1}$)** | | **K applied (kg ha$^{-1}$)** | | **Lime (L ha$^{-1}$)** |
|---|---|---|---|---|---|---|
| | | **Maize** | **Beans** | **Maize** | **Beans** | |
| **MAC** | Maseru | 60.0 | 60.0 | 0.00 | 0.00 | 0.00 |
| | Tsólo | 60.0 | 60.0 | 0.00 | 0.00 | 0.00 |
| | Ntanana | 60.0 | 60.0 | 0.00 | 0.00 | 75.0 |
| | Research site | 60.0 | 60.0 | 0.00 | 0.00 | 75.0 |
| **SAK** | Mantsema | 45.0 | 30.0 | 0.00 | 0.00 | 0.00 |
| | Lekoti | 60.0 | 60.0 | 0.00 | 0.00 | 0.00 |
| | Malelu | 70.0 | 55.0 | 0.00 | 0.00 | 0.00 |
| | Research site | 60.0 | 45.0 | 0.00 | 0.00 | 0.00 |

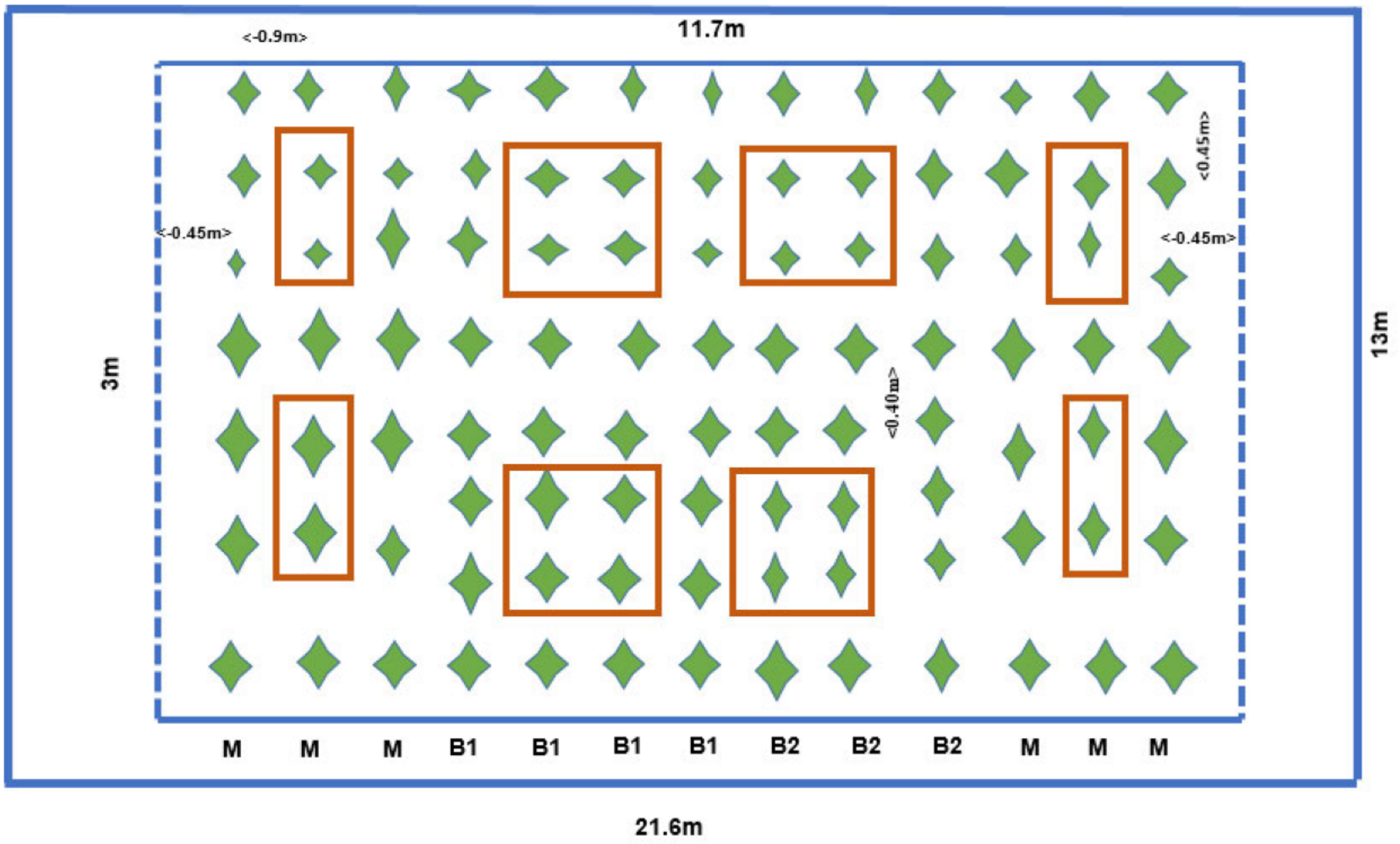

*Figure 4.2: Micro plot layout for BNF experiment using N15 with plants sampled at flowering/tasselling and maturity stage, M = Maize, B1= Bean variety 1(pinto), B2 = bean variety 2 (NUA 45), ◆ = plant stand, ▭ = sampled plants*

### 4.5.1. Plant sampling and analysis of plant materials

Plant sampling was done at 50% flowering and when beans have reached maturity at harvest at 127days after planting (DAP) at Machache and 115DAP at Sakoane. At Sakoane the plants grew faster and reached physiological maturity earlier than at Machache. From each plot, four plants were randomly sampled per bean variety and for maize, using a knife and sickle to cut them at ground level and roots were uprooted with a spade on both the N-15 and the N-14 plots per sampling stage. The four plant samples were mixed to form a composite sampleper variety for each plot. Roots were washed to remove adhering soil, followed by drying ofall samples at 70$^{o}$C to constant weight. Mass of both fresh and oven dried samples was recorded. At the final sampling (maturity), samples were divided into grain (in maize), pods (in beans), haulms (stems and leaves).

The harvest index (HI) was calculated as the ratio of grain to dry matter.

HI = $\frac{grainyield(kgha-1)}{drymatteryield(kgha-1)}$ (Equation 1)

The samples were ground up using IKA MF 10 basic micro fine grinder drive and total N content was determined using the LECO TruMac CNS auto analyser, while the $^{15}$N isotope analysis was conducted from the International Atomic Energy Agency (IAEA) laboratory using an isotope mass ratio spectrometer. However, lockdown restrictions due to COVID- 19 pandemic resulted in challenges for tissue analysis of the samples from the 2019/2020 season. The BNF assessment was done using the isotope dilution method, which assumes that N from the atmosphere fixed by legumes results in dilution of $^{15}$N tracer in the plant.

### 4.5.2. Estimation of biological nitrogen fixation and yields

The percentage of plant N derived from BNF (%Ndfa) was calculated using equation 2 (Peoples *et al.*, 1989; Adu-Gyamfi *et al.*, 2007; Tauro *et al.*, 2013; Zoundjl *et al.*, 2016).

%Ndfa =1- $\frac{atom\%N-15(fc)}{atom\%excessN-15(nfc)}\ 1-\frac{atom\%N(fc)}{atom\%N(nfc)}1-\frac{atom\%N(fc)}{atom\%N(nfc)}$*100 (Equation 2)

Where Ndfa: plant N derived from atmosphere, fc: fixing crop, nfc: non-fixing crop.

In order to calculate the quantity of nitrogen fixed by the entire plant in kg/ha, i.e., the amountof N fixed by beans through symbiosis = %Ndfa * total N in plant samples. The proportion of plant N derived from fertilizer (%Ndff) was estimated using Equation 3.

$$\%Ndff = \frac{atom\%excessN-15(crop)}{atom\%excessN-15(fertilizer)} * 100 \qquad \text{(Equation 3)}$$

The atom % excess in fertilizer was corrected by subtracting 0.366, which is the natural abundance from the air.

$$\%Ndfa + \%Ndff \text{ and } \%Ndfs = 100 \qquad \text{(Equation 4)}$$

Where %Ndfs is the plant N derived from soil and is calculated using Equation 5.

$$\%Ndfs = 100 - (\%Ndfa + \%Ndff) \qquad \text{(Equation 5)}$$

In order to get the amount of N from fertilizer, soil and fixation, the %Ndff, %Ndfs and %Ndfawere multiplied by the total N amount as shown in equation 6, 7and 8.

$$Ndff = N_{total} * \%Ndff \qquad \text{(Equation 6)}$$

$$Ndfs = N_{total} * \%Ndfs \qquad \text{(Equation 7)}$$

$$N_{fixed} = N_{total} * \%Ndfa \qquad \text{(Equation 8)}$$

Where $N_{total}$ was calculated using Equation 9.

$$TN\ (kg\ ha^{-1}) = Grain\ yield\ (kg\ ha^{-1}) * \%N_{crop} \qquad \text{(Equation 9)}$$

### 4.5.3. Nitrogen budgets calculations

The N balance at the soil surface is the difference between the N added to the soil as inputs/imports and the total quantity of N that has been removed from the soil (outputs/exports) each year (Vassiliki *et al.*, 2013). The total N inputs for agricultural production include inorganic and organic fertilizers, BNF and the wet and dry deposition from the atmosphere. Nitrogen removal is the total N taken up by crops at harvest or by grazing (Vassiliki *et al.*, 2013). For this experimental trial, the inputs were fertilizer and N fixation by the legume (bean) and the outputs were total N amount in harvested crops. Nutrient losses such as leaching, erosion, overland and lateral transport of nutrients were not considered (Adu-Gyamfi *et al.*, 2007; Zoundjl *et al.*, 2016) since there was no fertilizer application on the farms for the past two seasons before the trial was conducted. In order to calculate the N balance, the assessment of two situations was considered. Simulation of nutrient budget was based on farmers practice during harvesting. The first situation (budget 1) was based on assumption that farmers harvested all the aboveground biomass leaving only fallen leaves, which will be incorporated into the soil at ploughing, using Equation 10. Another scenario (budget 2) assumed that only grain was harvested while stover and fallen leaves are incorporated in the soil, using Equation 11 (Adu-Gyamfi *et al.*, 2007; Zoundjl *et al.*, 2016).

Budget 1 = (Amount of N applied + Amount of N fixed) – (Amount of N in shoot dry matter +Amount of N in harvested grain). (Equation 10)

Budget 2 = (Amount of N applied + Amount of N fixed + Amount of N in shoot dry matter) - Amount of N in grains (Equation 11)

## 4.6. Experimental design and management for nitrogen use efficiency

Two maize varieties (ZM 521 and ZM 523) were used for estimation of NUE. The two maize varieties are developed by CIMMYT and they were bred for drought tolerance and high yielding abilities. They were chosen as test crops because they mature faster and they are open pollinated varieties. The same attributes were considered for the two bean varieties. The same farms used for 2018/2019 cropping season were used in 2019/2020 season although different plots were constructed in each farm. The soil was amended with all required P and K based on the recommendations from soil tests (Table 4.6 and Table 4.7) except for nitrogen. All plots across different farms in both locations received phosphorus in the form of calcium phosphate and potassium in a form of potassium chloride (muriate of potash). There were three N rates. For each N rate, there was a micro plot established with an area of 28.8m$^2$ (4m * 7.2m), row spacing of 0.9m and plant spacing of 0.45m, (Figure 4.3). The N rates were N0 (no N applied), N1 with 10 kg N ha$^{-1}$ of 5.15% atom excess applied at half the recommended rate) and N2 with 20 kg N ha$^{-1}$ of 5.15% atom excess (applied at recommended rate) for each micro plot, respectively. On the main plot, urea $^{14}$N was applied at the same rate as in the micro plot. Three seeds were planted per hole and thinned to one plant 14 days after germination. For each rate, there were 459 plants per plot with 72 plants in the micro plot. The study was carried out in two summer cropping seasons; 2018/2019 and 2019/2020., with plots made on a different land from the previous season plots. Management of experimental plots was mostly done by farmers although the planting patterns were influenced by researchers, who also collected all data and processed it at the research station.

*Table 4.6: Summary of fertilizers applied on NUE plots in two locations for different farms in 2018/2019*

| Site | Farms | P applied (kg $ha^{-1}$) | K applied (kg $ha^{-1}$) | Lime (L $ha^{-1}$) |
|---|---|---|---|---|
| **MAC** | Maseru | 60.0 | 0.00 | 75.0 |
| | Tsolo | 60.0 | 20.0 | 0.00 |
| | Ntanana | 60.0 | 0.00 | 0.00 |
| | Rsrch site | 60.0 | 0.00 | 75.0 |
| **SAK** | Mantsema | 60.0 | 110 | 25.0 |
| | Lekoti | 60.0 | 20.0 | 25.0 |
| | Malelu | 60.0 | 75.0 | 25.0 |
| | Rsrch site | 60.0 | 170 | 0.00 |

MAC = Machache, SAK = Sakoane

*Table 4.7: Summary of fertilizers applied on NUE plots in two locations for different farms in 2019/2020*

| Site | Farms | P (kg $ha^{-1}$) | K applied (kg$ha^{-1}$) | Lime (kg$ha^{-1}$) |
|---|---|---|---|---|
| **MAC** | Maseru | 60 | 0 | 0 |
| | Tsolo | 60 | 0 | 0 |
| | Ntanana | 60 | 0 | 75 |
| | Rsrch site | 60 | 0 | 75 |
| **SAK** | Mantsema | 45 | 0 | 0 |
| | Lekoti | 60 | 0 | 0 |
| | Malelu | 70 | 0 | 0 |
| | Rsrch site | 60 | 0 | 0 |

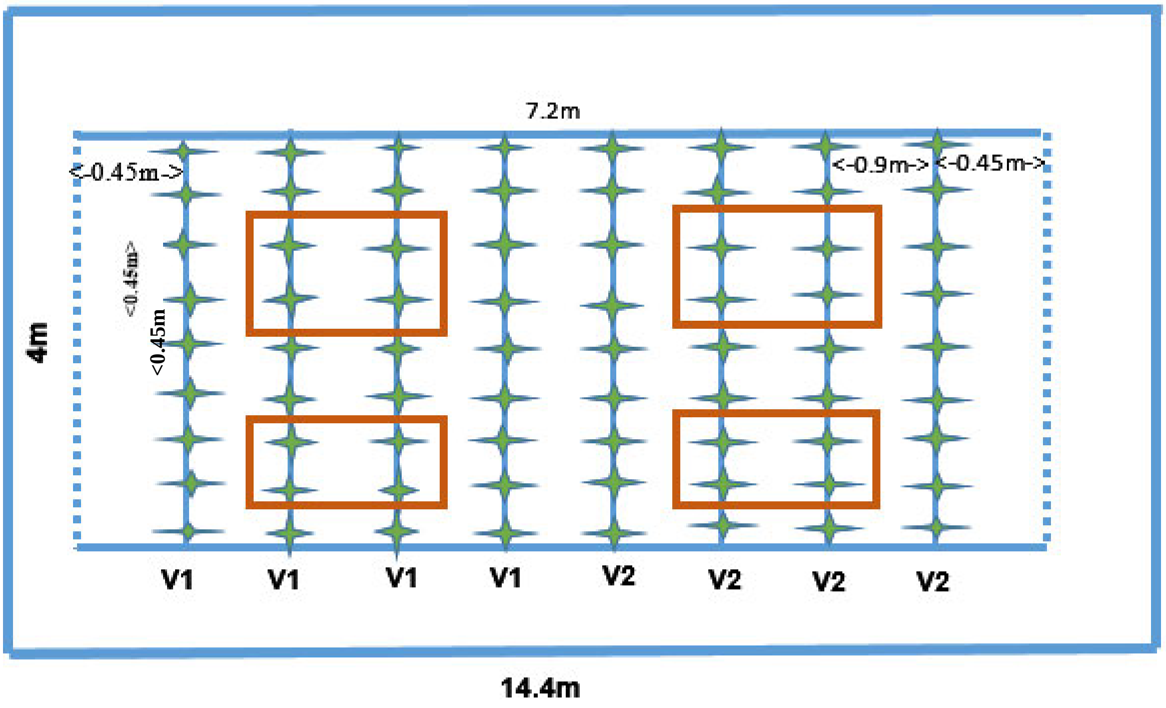

*Figure 4.3: Micro plot layout for nitrogen use efficiency showing plants sampled at harvest stage V1 = maize variety 1 (ZM 521), V2 =maize variety 2 (ZM523)* ✧ *= plant stand,* 🟧 *= sampled plants*

## 4.7. Determination of NUE from two maize varieties

Plant sampling at harvest was done at 175 and 163 DAP for Machache and Sakoane. Eight plants were sampled in the micro and main plot. The stalk was cut at ground level using a sickle and roots were uprooted with a spade. Roots were washed with water to remove soil followed by washing with 1M HCl. Fresh biomass was recorded then samples were divided into cobs and stalks prior to oven drying at 70°C to constant weight. After oven drying, cob husk was removed before threshing afterwards. Grain, husk and stover weights were recorded, before being ground using IKA MF 10 basic micro fine grinder drive. Total N and carbon content were determined using the LECO TruMac CNS auto analyser, whereas $^{15}$N isotope analysis was done from IAEA laboratory using an isotope mass ratio spectrometer.The lockdown restrictions as a result of COVID-19 resulted in challenges for tissue analysis of the samples for the 2019/2020 season.

Fertilizer nitrogen use efficiency (FNUE) or the fertilizer N recovery (FRN) was estimated using equation 12 (Adu-Gyamfi *et al.*, 1997)

$$\text{FNUE} = \frac{\text{amount of N in crop derived from fertilizer (Ndff)}}{\text{amount of N added to the soil (Napplied)}} * 100 \qquad \text{(Equation 12)}$$

(Check equation 6 for calculation of Ndff)

## 4.8. Statistical analysis

Collected data were analysed using GenStat statistical software 18th version. Analysis of variance (ANOVA) was conducted by running a full model across different farms for study location and varieties tested for BNF, while for NUE the model was across the varieties and fertilizer N rates for each location. Means were separated using the least significant difference (LSD) and by Turkey test at $p \leq 0.05$.

## 4.9. RESULTS

### 4.9.1. Biological nitrogen fixation by Pinto and NUA 45

#### 4.9.1.1. N budgets for two bean varieties planted at Sakoane and Machache

The N budgets for two scenarios of two bean varieties planted at Machache and Sakoane are on Table 4.8. Harvesting all the aboveground dry matter (N budget 1) differed significantly between the two sites, with Machache showing higher N budget (10.1 kg $ha^{-1}$), while Sakoane gave a mean N budget of -0.86kg $ha^{-1}$. However, there is no difference between the two varieties when all dry matter is removed from the field. If only grain yield was harvested, leaving other plant materials on the field (budget 2), the budget was improved for both sites and varieties indicating that more N availability in the soil for the next cropping season/ crop. Even though there was an improvement in N budget 2 when compared to N budget 1, there is no significant difference between the two sites and the two varieties (**Error! Reference source not found.**).

*Table 4.8: N inputs, and budget for Pinto and NUA 45 at Machache and Sakoane.*

| Factor | N inputs | N outputs | N budget 1 | N budget 2 |
|---|---|---|---|---|
| **Variety** | | | | |
| Pinto | 32.0 | 25.6 | 6.29 | 11.2 |
| NUA | 27.2 | 24.2 | 2.95 | 14.2 |
| **Site** | | | | |
| Machache | 33.0 | 22.7 | 10.1b | 16.1 |
| Sakoane | 26.2 | 27.1 | -0.86a | 9.37 |

Letters a and b = the significant difference between Machache and Sakoane; where 'a' shows the lower value and 'b' shows the higher value

#### 4.9.1.2. Drymatter, grain yields and harvest index

Site and variety did not affect the bean stover yield at flowering in 2018/2019 season but the site effect was significant in 2019/2020, with a higher amount of stover at Machache (1104 kg ha$^{-1}$) than Sakoane (747 kg ha$^{-1}$). There was no difference in drymatter (DM) yield between the two bean varieties (Table 4.9). At harvest, the DM was not affected by variety and site in both seasons, while variety affected harvest index (HI), in both seasons and grain yield (GY) in second season. Pinto had higher GY, in the second season, and HI in both seasons, than NUA 45 (Table 4.10). There were no differences in GY, DM and HI between the two sites (Table 4.10).

*Table 4.9: Drymatter yield of bean varieties at Machache and Sakoane in 2018/2019 and 2019/2020 seasons at flowering stage*

| Factor | DM yield (kg ha$^{-1}$) | |
|---|---|---|
| | 2018/2019 | 2019/2020 |
| **Variety** | | |
| Pinto | 211 | 930 |
| NUA 45 | 353 | 921 |
| **Site** | | |
| Machache | 287 | 1104b |
| Sakoane | 277 | 747a |

*DM = drymatter, letters a and b = the significant difference between Pinto and NUA 45; where 'a' shows the lower value and 'b' shows the higher value*

*Table 4.10: Grain and drymatter yields of bean varieties at Machache and Sakoane in 2018/2019 and 2019/2020 at harvest*

| **Factor** | **Grain** | **DM** | **HI** | **Grain** | **DM** | **HI** |
|---|---|---|---|---|---|---|
| | | **2018/2019** | | | **2019/2020** | |
| **Variety** | | | | | | |
| Pinto | 457 | 881 | 0.70b | 1443b | 2493 | 0.58b |
| NUA 45 | 312 | 662 | 0.62a | 889a | 1994 | 0.45a |
| **Site** | | | | | | |
| Machache | 314 | 652 | 0.67 | 1045 | 2093 | 0.50 |
| Sakoane | 454 | 890 | 0.65 | 1286 | 2394 | 0.53 |

DM = dry matter, HI = harvest index, letters a and b = the significant difference between Pinto and NUA 45; where ‘a’ shows the lowest value and ‘b’ shows the higher value

#### 4.9.1.3. Biological nitrogen fixation by beans

There were no significant interaction effects of site and variety on N percentages derived from soil (Ndfs), from fertiliser (Ndff) and from air (Ndfa). Sites and varieties did not affect the concentrations of N from the different sources. Figure 4.4 shows the percent N budget for Pinto and NUA 45 at flowering and harvest. Pinto derived 28.6% of the N in plant from the air at flowering and 45% at harvest, while NUA 45 derived about 28% from the air at both sampling times. Both varieties derived more of the N from the soil followed by the atmosphere, with the least from fertilizer at flowering. NUA 45 followed the same trend at harvest while Pinto derived more N from air with the least from the fertilizer at harvest. The N fixation by the bean varieties at Machache during flowering (29.5%) and harvest (52.5%) was higher than at Sakoane. At Sakoane, more N was derived from soil, air and fertilizer while at Machache, more N was derived from air and soil with the least from the fertilizer (Figure 4.5.).

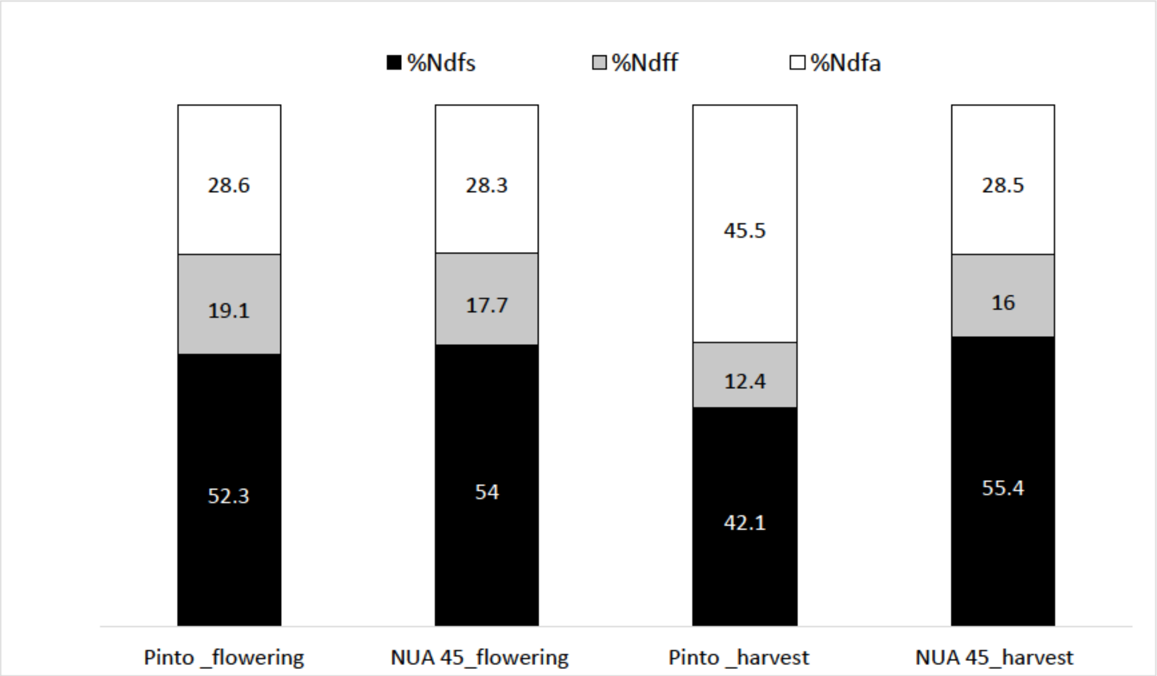

*Figure 4.4: Percent N budget for pinto and NUA 45 at flowering and harvest*

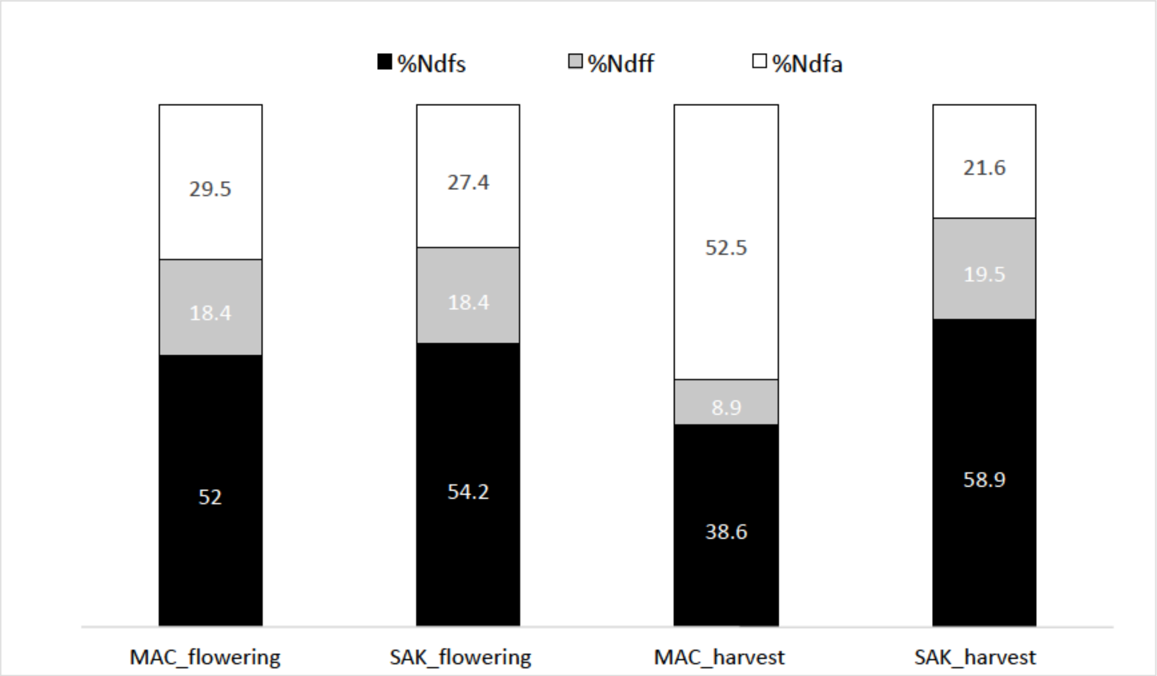

*Figure 4.5: Nitrogen budget (%) of beans varieties at Machache and Sakoane during flowering and harvest*

#### 4.9.1.4. Nitrogen accumulation in beans at flowering

Both site and variety did not affect concentrations of N from the different sources at flowering (Table 4.11). Only the amount of N derived from the soil and total N amount were affected by bean variety but not by site. The bean variety NUA 45 took up more N from the soil (5.52 kgha$^{-1}$) than Pinto (2.92 kg ha$^{-1}$). The results of total N amount followed the same trend as N amount derived from the soil, with NUA 45 taking up more (10.8 kg ha$^{-1}$) than Pinto (5.70kgha$^{-1}$), at flowering (Table 4.11). At harvest, all the parameters were not significantly affected by interaction of site and bean variety (Table 4.11). There were no significant differences between bean varieties on all the parameters studied at harvest. The Sakoane site had higher N amount derived from fertiliser and soil than the Machache site.

*Table 4.11: Amount of N (kg ha-1) derived from the atmosphere, fertilizer and soil of bean varieties at Machache and Sakoane at flowering and harvest*

| **Factor** | **N amount (kg ha$^{-1}$)** | | | |
|---|---|---|---|---|
| | **Ndfa** | **Ndff** | **Ndfs** | **Total** |
| | | **Flowering** | | |
| **Variety** | | | | |
| Pinto | 1.43 | 1.40 | 2.92a | 5.70a |
| NUA 45 | 3.32 | 1.92 | 5.52b | 10.8b |
| **Site** | | | | |
| Machache | 2,46 | 1.55 | 3.99 | 8.00 |
| Sakoane | 2.29 | 1.76 | 4.45 | 8.50 |
| | | **Harvest** | | |
| **Variety** | | | | |
| Pinto | 12.0 | 3.08 | 10.5 | 25.6 |
| NUA 45 | 7.20 | 3.81 | 13.1 | 24.2 |
| **Site** | | | | |
| Machache | 13.1 | 1.74a | 7.90a | 22.7 |
| Sakoane | 6.20 | 5.14b | 15.7b | 27.1 |

Ndfa (N derived from air), Ndff (N derived from fertilizer) and Ndfs (N derived from soil), letters a and b = the significant difference between Pinto and NUA 45 or between Machache and Sakoane; where ‘a’ shows the lower value and ‘b’ shows the higher value

### 4.9.2. Comparison of nitrogen use efficiency in ZM521 and ZM523

#### 4.9.2.1. Grain yield, drymatter yield and grain harvest index for two maize varieties

Grain yield and harvest index were not affected by N rate, at Machache in the 2018/2019 season (Table 4.12). The ZM523 produced higher grain yield in comparison to ZM 521 at Machache in the same season. Stover yield increased significantly at 10kg ha$^{-1}$ and 20kg ha$^{-1}$ N application compared to no N application. There was no difference between 10 and 20kg ha$^{-1}$ application, in the 2018/2019 season. In the 2019/2020 season, at the Machache site, both N rate and variety did not affect grain yield, and harvest index (Table 4.13). Drymatter yield was higher in ZM 523 compared to ZM521, but no effect was observed between N rates at Machache.

At the Sakoane site, grain and drymatter yields were significantly higher at 10 and 20 kg N ha$^{-1}$ rates compared to where no N was added, but there were no differences between 10 and 20 kg N ha$^{-1}$ rates, in the 2018/2019 season (Table 4.12). Harvest index was increased significantly when 20 kg N ha$^{-1}$ was applied compared to 0 to 10kg N ha$^{-1}$ at Sakoane in the 2018/2019 season. There were no differences between the two varieties in grain, drymatter yields and HI, at Sakoane (Table 4.12) in the 2018/2019 season. The results of drymatter yield and harvest index, at Sakoane site, followed the same trend as those for the same site in the 2019/2020 season, (Table 4.13), where they increased significantly when N rate was increased to 10 and 20 kg N ha$^{-1}$ compared to where no N was added. However, there was no difference between 10 and 20 kg N ha$^{-1}$ application rates, and both varieties had no impact on drymatter and harvest index at Sakoane (Table 4.13) however, ZM 523 at 20 kg N ha$^{-1}$ accumulated higher grain yield, than at other rates and also when compared with ZM521 at all N rates (Table 4.14) in 2019/2020 season.

*Table 4.12: Yield data and grain harvest index for two maize (ZM 521 and ZM 523) varieties for different N application rates at Machache and Sakoane in 2018/2019*

| | MAC | | | SAK | | |
|---|---|---|---|---|---|---|
| **Factors** | **GY** | **DM yield** | **HI** | **GY** | **DM yield** | **HI** |
| **N rate (kg ha$^{-1}$)** | | | | | | |
| **0** | 909 | 1881a | 0.48 | 1068a | 2277a | 0.47a |
| **10** | 1108 | 2102b | 0.55 | 1513b | 3080b | 0.48a |
| **20** | 1162 | 2191b | 0.53 | 1894b | 3276b | 0.57b |
| **Variety** | | | | | | |
| **ZM 521** | 774a | 1843 | 0.44 | 1464 | 2750 | 0.52 |
| **ZM 523** | 1345b | 2274 | 0.60 | 1519 | 3005 | 0.50 |

MAC= Machache, SAK= Sakoane, GY= grain yield, DM= drymatter, HI = harvest index, letters a and b = the significant difference for different parameters analysed; where 'a' shows the lower value and 'b' shows the higher value

*Table 4.13: Yield data and grain harvest index for two maize varieties for different N application rates at Machache and Sakoane in 2019/2020*

| **Factor** | **Machache** | | | **Sakoane** | |
|---|---|---|---|---|---|
| | **GY** | **DM yield** | **HI** | **DM yield** | **HI** |
| **N rate (kg ha$^{-1}$)** | | | | | |
| **0** | 2172 | 6044 | 0.45 | 2496a | 0.38a |
| **10** | 3295 | 5932 | 0.54 | 3875b | 0.44b |
| **20** | 3273 | 6369 | 0.53 | 4823b | 0.44b |
| **Variety** | | | | | |
| **ZM 521** | 2774 | 5204a | 0.52 | 3447 | 0.43 |
| **ZM 523** | 3052 | 5932b | 0.50 | 4017 | 0.40 |

GY= grain yield, DM = Drymatter, HI = harvest index, letters a and b = the significant difference between different N rates or varieties; where 'a' shows the lower value and 'b' shows the higher value

*Table 4.14: Grain yield for two maize (ZM 521 and ZM 523) varieties for different N application rates a Sakoane in 2019/2020*

| **Factor** | **Sakoane** | |
|---|---|---|
| | **Grain Yield** | |
| **N rate(kg ha-1)** | **ZM521** | **ZM523** |
| **0** | 1458a | 1501a |
| **10** | 1705a | 1698a |
| **20** | 2496a | 2496b |

Letters a and b = the significant difference between Pinto and NUA 45; where ‘a’ shows the lowest value and ‘b’ shows the higher value

#### 4.9.2.2. Percentages and amounts of N from fertilizer and soil, in drymatter, and recovery efficiency of applied N in two maize varieties at Machache and Sakoane

There were significant differences in N application rates on percent N derived from soil (Ndfs) and fertilizer (Ndff), fertilizer N amount and percent N use efficiency in maize stover at Machache (Table 4.15). Increasing N application rate increased Ndff and fertiliser N amount and decreased Ndfs. Fertilizer nitrogen use efficiency (%FNUE) was increased where fertiliser was added but there was no significant difference between 10 and 20 kg N ha$^{-1}$ application rates at the Machache site. There were no significant variations between the two maize varieties analysed, except total N which was higher in ZM523 than ZM521 at Machache (Table 4.15). At Sakoane, fertiliser N application at 10 and 20kg N ha$^{-1}$ significantly increased amount of Ndff and %FNUE and decreased Ndfs when compared with plants that received no N application. However, there was no difference between 10 and 20kg ha$^{-1}$ application although there was a positive trend with increase in N application. There were no differences between the varieties, except for FN, where ZM521 had higher levels than ZM523 (Table 4.155).

*Table 4.15: Proportion and amount of N from different sources and %FNUE of drymatter of the two maize varieties for different N application rates at Machache and Sakoane in 2018/2019*

| **Factors** | **%Ndff** | **%Ndfs** | **TN** | **FN** | **%FNUE** |
|---|---|---|---|---|---|
| | | | **Machache** | | |
| **N rate (kg ha$^{-1}$)** | | | | | |
| **0** | 0.00a | 100c | 12.0 | 0.00a | 0.00a |
| **10** | 6.00b | 94.0b | 15.8 | 0.99b | 9.89b |
| **20** | 13.0c | 87.1a | 16.0 | 1.96c | 9.82b |
| **Variety** | | | | | |
| **ZM 521** | 6.81 | 93.2 | 11.7a | 0.86 | 5.47 |
| **ZM 523** | 5.76 | 94.2 | 17.5b | 1.11 | 7.69 |
| | | | **Sakoane** | | |
| **N rate (kg ha$^{-1}$)** | | | | | |
| 0 | 0.00a | 100b | 5.87a | 0.00a | 0.00a |
| 10 | 16.6b | 83.4a | 6.36a | 0.88b | 8.82b |
| 20 | 22.7b | 77.4a | 9.42b | 1.95c | 9.76b |
| **Variety** | | | | | |
| ZM 521 | 13.8 | 86.2 | 7.26 | 1.09b | 7.01 |
| ZM 523 | 12.4 | 87.6 | 7.17 | 0.80a | 5.37 |

Ndff= percent nitrogen derived from fertilizer, Ndfs = percent nitrogen derived from soil,TN= total amount of nitrogen, FN= amount of fertilizer N in drymatter, letters a and b = the significant difference between different parameters analysed; where 'a' shows the lowest value and 'b' shows the higher value

#### 4.9.2.3. Percentages and amounts of N from fertilizer and soil in grain, and recovery efficiency of applied N in two maize varieties at Machache and Sakoane

The results of different parameters of grain at Machache followed the same trends as those of stover. Increasing N application rate increased Ndff, and fertiliser N amount and decreased Ndfs in the grain. The FNUE in the grain increased significantly when 10 and 20kg N ha$^{-1}$ were added compared to where no N was added, with no difference in FNUE between 10and 20 kg N ha$^{-1}$ rates, at Machache site (Table 4.16). Total N amount in grain was not affected by N application rates at Machache but the ZM 523 variety took up significantly higher N in the grain compared to ZM 521 (Table 4.16).

Similarly, to Machache, the results of different grain parameters at Sakoane followed the same trends as those of stover. Nitrogen derived from soil was significantly reduced by increase in fertiliser N rate while Ndff and fertiliser N amount in plant significantly increased. Moreover, similarly to Machache, the FNUE increased significantly with N additions at 10 and 20kg N ha$^{-1}$ compared to where no N was added, but there was no difference between 10 and20 kg N ha$^{-1}$ rates, at Sakoane site (Table 4.16). Total N amount was significantly increased by the 20 kg N ha$^{-1}$ application rate compared to where no N was applied. The two varieties were not affected by any of the parameters analysed (Table 4.16)

*Table 4.16: Proportion and amount of N from different sources and FNUE of grain for two maize varieties at Machache and Sakoane in 2018/2019*

| **Factors** | **%Ndff** | **%Ndfs** | **TN** | **FN** | **%FNUE** |
|---|---|---|---|---|---|
| | | **Machache** | | | |
| **N rate (kg/ha)** | | | | | |
| 0 | 0.00a | 100c | 12.9 | 0.00a | 0.00a |
| 10 | 5.96b | 94.0b | 14.0 | 0.82b | 8.19b |
| 20 | 13.1c | 86.9a | 15.5 | 1.92c | 9.60b |
| **Variety** | | | | | |
| ZM 521 | 6.88 | 93.1 | 11.1a | 0.87 | 5.29 |
| ZM 523 | 5.83 | 94.2 | 17.1b | 0.96 | 6.57 |
| | | **Sakoane** | | | |
| **N rate (kg/ha** | | | | | |
| 0 | 0.00a | 100c | 10.7a | 0.00a | 0.00a |
| 10 | 14.5b | 85.5b | 13.8a | 1.60b | 16.0b |
| 20 | 21.2c | 78.8a | 20.7b | 3.50c | 17.5b |
| **Variety** | | | | | |
| ZM 521 | 12.0 | 88.0 | 14.8 | 1.78 | 12.0 |
| ZM 523 | 11.8 | 88.2 | 15.3 | 1.62 | 10.4 |

Ndff= percent nitrogen derived from fertilizer, Ndfs = percent nitrogen derived from soil, TN=total amount of nitrogen, FN= amount of fertilizer N in stover, *letters a, b and c = the significant difference between different N rates; where 'a' shows the lowest value and 'c' shows the highest value*

### 4.9.2.4. Percentages and amounts of N from fertilizer and soil in whole plants, and recovery efficiency of applied N in two maize varieties at Machache and Sakoane

Total N amount in the whole plants was not affected by either N rates or variety at both sites (Table 4.17). Fertiliser N amount in whole plants was increased by N rate at both sites (Table 4.17). Fertiliser N use efficiency was increased by fertilisation at 10 and 20kg N ha$^{-1}$ when compared to no N application at both sites but no significant difference was observed between 10 and 20kg N ha$^{-1}$ application rates. There were no differences between the two varieties in fertiliser N amount and use efficiency for whole plants at both sites (Table 4.17).

*Table 4.17: Total N (kg N ha-1), fertilizer N amount (kg ha-1), %FNUE of the whole plant at Machache and Sakoane in 2018/2019 season*

| | **Machache** | | | **Sakoane** | | |
|---|---|---|---|---|---|---|
| **Factors** | **TN** | **FN** | **%FNUE** | **TN** | **FN** | **%FNUE** |
| **N rates (kg N ha$^{-1}$)** | | | | | | |
| **0** | 24.2 | 0.00a | 0.00a | 16.6 | 0.00a | 0.00a |
| **10** | 29.7 | 1.81b | 9.04b | 20.7 | 2.49b | 12.4b |
| **20** | 31.9 | 3.89c | 9.71b | 28.2 | 5.45c | 13.6b |
| **Variety** | | | | | | |
| **ZM 521** | 28.8 | 1.72 | 5.38 | 22.6 | 2.87 | 9.50 |
| **ZM 523** | 28.4 | 2.07 | 7.12 | 21.1 | 2.42 | 7.87 |

*TNU and FNU represent total nitrogen amount and fertiliser nitrogen amount (kg ha-1), respectively. %NUE represents percentage nitrogen use efficiency, letters a, b and c = the significant difference between different N rates; where ‘a’ shows the lowest value and ‘c’ shows the highest value*

## 4.10. DISCUSSIONS

### 4.10.1. Biological nitrogen fixation

#### 4.10.1.1. Drymatter, grain yield and harvest index

The high drymatter (DM) yield at Machache (1104kg ha$^{-1}$) compared to Sakoane (747 kg ha$^{-1}$) at the flowering stage could be explained by the high rainfall at Machache during the 2019/2020 (Table 4.1). Another aspect could be fine loamy textured soils at Machache, with higher organic C and N than Sakoane soils (Table 4.3), which contributed in water retention and nutrient supply. High clay content at Machache results in high cation exchange capacity which is important for nutrient availability, hence the presence of higher N in Machache compared to Sakoane soils, resulting in increased drymatter.

Although the rainfall was also lower at Sakoane than Machache, except in January, in the 2019/2020 season, the difference was not high enough to result in variation in stover yield at flowering. At harvest, when the whole plants had reached the maximum level of growth and maturity, the lack of differences in DM as affected by location/site and variety, suggests that the two varieties produce similar drymatter accumulation when grown under the same conditions, and that the site effects were not significant on DM in 2018/2019. The lack of differences between the two sites may be because of soil and management differences between replicate fields, causing large measure of residual error, reducing sensitivity of side effects, such that the differences in rainfall did not affect the overall yields.

Although there was no difference in grain yield (GY) during 2018/2019 season, the higher grain yield in the 2019/2020 season and HI in both seasons for the Pinto variety than NUA 45, could be explained by genetic differences. When compared to Pinto, NUA45 produced less grain for the same amount of drymatter, which suggests that NUA45 allocated less resources towards grain production. These results concur with those obtained in a study conducted by (Morojele and Sekoli, 2016) who analysed the variability in yield and yield components among common bean genotypes in Lesotho. In the study by (Morojele *et al.*, 2016), Pinto and NUA 45 recorded higher GY (7450 kg ha$^{-1}$) and (7150 kg ha$^{-1}$) respectively, compared to the results obtained in the current study for both seasons because of high fertilizer applied, (250kg ha $^{-1}$) of 2:3:2(22) and the field was irrigated twice a week due to severe drought which prevailed during the study period. In another study NUA 45 produced 180-940 kg $ha^{-1}$ where it was studied for its nutrient response in America (LIL, 2016) and Pinto yield (kg $ha^{-1}$ ranged between 2001 and 2164 in two consecutive years (Williston, 2014). NUA 45 results are in line with the findings in study although Pinto results are lower compared to findings from different authors. This may be attributed to the difference in rainfall between the two countries where studies were conducted (America and Lesotho).

These high grain yields in Pinto suggest that it could help alleviate food security challenges, than NUA45. However, NUA 45 was found to be more nutritious in terms of Fe (≥102mg/kg) and Zn (≥35 ppm) by (Morojele *et al.*, 2017) as compared to Pinto with 54 mg Fe $kg^{-1}$ and 24 mg Zn $kg^{-1}$ (Moraghan and Grafton, 2001). In addition to the nutritional composition, farmers at both Machache and Sakoane reported better taste of NUA45 at the end of the trials. This may suggest that Pinto is better for food security (food quantity), while NUA45 could be better for nutritional quality. As such farmers may need to produce both varieties to satisfy food and nutrition requirements. The food and nutrition security benefits of the bean varieties will also be associated with soil fertility through biological nitrogen fixation.

#### 4.10.1.2. Biological Nitrogen Fixation by two bean varieties

Pinto demonstrated higher proportion of nitrogen derived from fixation (45.5% Ndfa) while NUA 45 fixation was only 28.5% of the total N. However, total N in the Pinto tissue was lower (25.4 $kgha^{-1}$) that NUA 45 (24.2 kg $ha^{-1}$), indicating that N fixed by Pinto was 11.5 kg $ha^{-1}$, while that of NUA 45 was 6.90 kg $ha^{-1}$. The higher N fixation could explain the greater harvest index (HI) of Pinto than NUA 45 in the 2018/2019, and possibly grain yield and HI in the 2019/2020. However, biological N fixation was not measured in the 2019/2020 season, due to challenges presented by lockdowns related to the COVID-19 pandemic. Similar increase in N fixed was observed in studies conducted by (Hardarson *et al.*, 1993; Adu-Gyamfi *et al.*, 2007; Franzini *et al.*, 2013). Similar %Ndfa estimates of different cultivars common beans were also observed in several studies (Ruschel *et al.*, 1982; Wolyn *et al.*, 1991; Kipe-Nolt and Giller, 1993; Giller, 2001; Manrique *et al.*, 1993; Müller and Pereira, 1995). Although the results of %Ndfa for the second season are not presented, the high harvest index for Pinto during the 2019/2020 season suggests that there is a likelihood to have high N fixation after the analysis of the second season data. According to (Giller, 2001), P availability is a prerequisite for N fixation, and the critical tissue P concentration for common beans below which normal plant growth may not occur is 0.2% (Thung, 1991). The soil properties for two sites where the study was conducted demonstrated low P; 3.76 and 4.85 mg $kg^{-1}$ at Machache during 2018/2019 and 2019/2020and 3.64 and 8.01 mg $kg^{-1}$ at Sakoane for two cropping seasons (Table 4.2). The higher N fixed by Pinto compared to NUA 45, suggests that P requirement for this variety to fix more N is low, possibly because of the visibly extensive root system for Pinto allowed the crop to access P in deeper soil layers than NUA 45. The low biomass in NUA 45 might be due to low P in the soil and as a result, crop allocated a large proportion of Net C assimilation to the production of roots rather than photosynthetic tissues. Similar results were obtained by Adu-Gyamfi *et al. (*2007) in their study of biological nitrogen fixation and phosphorus budgets in farmer-managed intercrops of maize–pigeon pea.

Pinto and NUA 45 fixed 12kg $ha^{-1}$ and 7.2kg $ha^{-1}$, respectively, which compares favourably with results reported by several authors using different common bean cultivars. (Duque *et al.*, 1985; Wolyn *et al.*, 1991; Manrique *et al.*, 1993). Common beans are considered to have low ability to form nodules and fix atmospheric nitrogen compared to other grain legumes (Vásquez-Arroyo *et al.*, 1998). Although higher N amount from fertiliser and soil was observed at Sakoane than Machache at harvest, this difference did not affect total N amount, drymatter and grain yield. The balance (Ndfa) was generally higher at Machache. The lower amount of Ndff at Machache in 2018/2019 is explained by the large soil pool as indicated by soil tests, (Table 4.3).This may also be related to high available carbon in the soil which might have resulted in immobilization of labelled N isotope. As such, the proportion of N fixed at Machache (at harvest) increased to 52.5% compared to 21.6% at Sakoane, as beans were forced to source out N from fixation in order to meet their N requirement because the available soil N might have diminished before the plant reached physiological maturity.

#### 4.10.1.3. Nitrogen budgets of two bean varieties planted at Sakoane and Machache

The difference in N budget 1 between the two sites indicate that the N output was less than the inputs at Machache suggesting that the initial N input was enough for the crop demand. Although the results are in accordance with findings by (Laberge *et al.*, 2009; Schipanski *et al.*, 2010), they however reported that a positive N balance resulted when Ndfa was above 60% in soybeans. At Sakoane, the plants removed more N than the initial input to the soil, suggesting that crops depleted soil N in the scenario when all aboveground materials were harvested, leaving no N available to be used by the successive crop. Sakoane results compare favourably with results reported in studies conducted by (Adu-Gyamfi *et al.*, 2007; Zoundjl *et al.*, 2016) where soybean and pigeon pea were studied. If only the grain is removed at harvest (N budget 2), more N will remain in the soil at both sites because of the addition of the above ground biomass, which could be incorporated thereby reducing the C:N ratio, that is increasing total soil N and making a positive contribution to soil fertility at both sites irrespective of variety. Similar results were reported in Thailand (Toomsan *et al.*, 1995), where groundnut stover improved rice growth and grain yield up to 26% and increased total dry matter upto 31%. Many studies conducted later were also in agreement with earlier findings (Sanginga, 2003; Adu-Gyamfi *et al.*, 2007; Jaynes, 2008; Zoundjl *et al.*, 2016). The harvesting of the DM and GY in bean could have significant effects on N budgets and soil fertility. Based on the results, N budget 2, where only grain is removed would be better for soil fertility than N budget 1, where both grain and residues are removed. This in turn could help resource poor farmers at Machache and Sakoane who apply little or no fertilizers.

### 4.10.2. Yields and nitrogen use efficiency of maize varieties

The higher grain yield (GY) in the 2018/2019 season and higher drymatter (DM) yield in the 2019/2020 season at Machache for the ZM523 could be explained by genetic differences between the two maize varieties. The findings by the Department of Agricultural Research Lesotho through (CIMMYT, 2005) reported the same for ZM 523 where screening of early maturing maize varieties was done at different agro-ecological zones of Lesotho. These findings suggest that ZM523 maize may be more efficient at utilising N, other nutrients, and possibly water than the ZM521. Production of ZM 523 by Machache and Sakoane farmers can result in increased productivity, poverty alleviation and improvement of livelihoods, although farmers are slow to adopting new varieties.

The higher DM where N fertiliser was applied indicated that greater N availability increased biomass accumulation at Machache in the 2018/2019 season. Similar results were observed for GY and DM yield and HI at Sakoane for both seasons. These findings suggest that addition of N fertiliser increases biomass and grain accumulation, which would be beneficial for food security. Similar results were obtained by (Abera *et al.*, 2016; Adu-Gyamfi *et al.*, 1996) where sorghum and maize were planted at different N application rates respectively.The increase in Ndff and fertiliser N (FN) and decline in Ndfs in stover and grain with increase in fertiliser rate at both Machache and

Sakoane, indicates that addition of fertiliserN increases N availability and uptake from the fertiliser and limits uptake of inherent soil N. Application of fertiliser increases NUE for stover, grain and both combined, resulting in increase of either stover yield, grain yield or both.

These results compare well with those obtained for sorghum and wheat (WB Gunnison) by (Adu-Gyamfi *et al.*, 1996; Ondoua and Walsh, 2017) respectively where %NUE increased with increasing N application rate up to150kg N ha $^{-1}$, although the application rates used in our study were far below those used by these authors. The difference was due to the high costs of $^{15}$N fertilizer used in the field experiment, so the recommended rate was 20kg N ha $^{-1}$. It is therefore assumed that if higher recommended rate (60kg ha$^{-1}$) and above recommended N rates were used, the %NUE could decrease with increasing N application especially above 50kg ha$^{-1}$ for some studies and 150kg ha$^{-1}$ for most of the studies as reviewed from several authors who used different cereal crops like maize (Abera *et al.*, 2016), rice,(Choudhury and Yousop, 2009; Rahman *et al.*, 2009) wheat (Vida) (Ondoua and Walsh, 2017), barley and sorghum (Harmsen and Moraghan, 1988; Kaizzi *et al.*, 2012).

The higher total N amount in stover at Machache and in grain at Sakoane in the ZM523 in 2018/2019 could explain the higher stover and/or grain yield than that of the ZM521. This indicates that this variety takes up more combined N from soil and fertiliser, resulting in greater biomass production. Higher total N in ZM523 compared to ZM 521 (at Machache) implies that farmers can benefit from planting this variety especially when their residues are retained and incorporated in the soil as this will increase soil fertility for the subsequent crop by adding more N to the soil. It can also improve animal nutrition if the stover is removed and used as animal feeds.

## 4.11. Conclusion and recommendations

The study showed that the effectiveness of bean for BNF depends on the genotype. Production of beans by resource poor farmers without means to ameliorate soil fertility problems, using bean variety that can fix more nitrogen and produce higher yield may benefit farmers by achieving both economic and environmental sustainability. Biological nitrogen fixation by two bean varieties was not different but Pinto produced higher grain yield than NUA 45. Pinto would be a good food and nutritional source to farmers in addition to soil fertility benefits from BNF.

The FNUE of ZM521 and ZM 523 was similar but ZM523 yielded more grain in 2018/19 season and more drymatter in 2019/20 than ZM 521. The maize variety, ZM 523, could be recommended to farmers in the foothills and lowlands of Lesotho for food and nutrition security and animal feed, through the drymatter. This study introduces the BNF of common beans and NUE of maize varieties which aim at improving soil fertility and as food security in Lesotho. The results from this study will add value to studies which will be conducted by different researchers on BNF and NUE, in variety screening to address climate change issues and other biotic and abiotic factors that hinder greater agricultural production. The identification of crop plants with more efficient nitrogen usage is important in research in achieving greater agricultural sustainability and adoption of Climate Smart Agricultural (SMA) technologies. BNF provides an economically smart and ecologically sound ways in reduction of external nitrogen input and improving the quality and quantity of internal resources. Future studies on BNF should be designed such that replication is done on the same farms in different locations/areas. Furthermore, more common bean varieties need to be tested for screening the best varieties for BNF. Higher N application rates such as 60kgha$^{-1}$, than were used in this study be tested for their effects on maize yield and harvest index.

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