IMPROVING GROUNDNUT AND MAIZE YIELDS BY ENHANCING BIOLOGICAL NITROGEN FIXATION OF FERRALSOLS AND ACRISOLS IN WESTERN KENYA

BY

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN SOIL SCIENCE, DEPARTMENT OF SOIL SCIENCE, SCHOOL OF AGRICULTURE AND BIOTECHNOLOGY, UNIVERSITY OF ELDORET, KENYA

2014

DECLARATION

DECLARATION BY STUDENT

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DEDICATION

This study is dedicated to my late father-may his soul rest in peace-whose support and encouragement brought me this far. To my mother who has always been there for me. To my brother and sisters who persistently prayed for me. Lastly to my husband and son who inspired me throughout the entire period.

ABSTRACT

Food insecurity in sub - Saharan Africa (SSA) is on the rise and this has become a global concern. One of the major contributing factors to this scenario is soil fertility depletion that culminates to low food productivity. In Kenya, nitrogen is one of the widely deficient nutrients. Biological nitrogen fixation (BNF); a symbiotic process that takes place in most leguminous crops can replenish nitrogen into the soil system. Groundnut is an important crop especially in western Kenya both for its high protein content and its economic value. Groundnut yield in this region however, is very low and there is a general concern on how to raise its production. A study was carried out in acid soils of Koyonzo and Ligala in western Kenya to determine the effectiveness of different inoculants and agricultural lime in enhancing BNF and yields of groundnuts and maize under intercropping system. Red Valencia groundnut variety was intercropped with Hybrid 513D maize variety. Two types of agricultural lime (dolomitic lime, and calcitic lime) and four rhizobia inoculants (A6w, V2w, W1w and biofix) alongside positive and negative controls were tested using the randomised complete block design (RCBD) in a split plot arrangement. There were three replications at each site. A6w, W1w and V2w were the indigenous rhizobia strains that had passed the authentication test. N treatment was the positive control where no inoculation was done but nitrogen was supplied from calcium ammonium nitrate (CAN). Maize monocrop was included in this study to act as reference crop for BNF analysis. Prior to planting, a blanket application of phosphorus and potassium were applied at the rates of 26 kg ha^{-1} and 50 kg ha^{-1}, respectively. The experiment was carried out during the long and short rainy seasons of 2011. Data collected included; nodule number and weight, soil pH, phosphorus, nitrogen, calcium, magnesium, percent nitrogen derived from the atmosphere (% Ndfa), and groundnut kernel and maize grain weights. All data was subjected to analysis of variance (ANOVA) and treatment means were separated using contrast analyses. The results showed lime significantly increased the soil pH, available phosphorus and soil calcium and magnesium. Inoculation significantly increased nodule number and weight per plant. There were significant differences among indigenous rhizobia in fixing nitrogen ($p<0.05$). Rhizobia inoculation accounted for 58.91 % and 78.95 % increase in the amount of nitrogen fixed above the control at Koyonzo and Ligala respectively. The strain that fixed the highest amount of nitrogen was A6w at both sites under the dolomitic soil amendment. Dolomitic and calcitic lime did not differ significantly ($p<0.05$) in affecting the amount of nitrogen fixed. Liming and rhizobia inoculation significantly increased both groundnut and maize yields. Liming accounted for 16.71 % and 10.55 % groundnut yield increase at Koyonzo and Ligala sites, respectively while inoculation alone accounted for 90.57 and 110.67 % groundnut yield increase at Koyonzo and Ligala sites respectively. The best treatment combination was rhizobia A6w with dolomitic lime which gave groundnut yields of 2.01 and 0.98 t ha^{-1} at Koyonzo and Ligala respectively during the 2011 SRs. Liming significantly increased maize yields. The best inoculant A6w, gave maize yields of 3.76 and 2.78 t ha^{-1} at Koyonzo and Ligala, respectively. In conclusion, soil amendment with dolomitic lime and inoculating groundnuts with rhizobia strain A6w which can be commercialized resulted in increased groundnut and maize yields. This practice can therefore be adopted by farmers in western Kenya to improve the productivity of the groundnut maize intercropping systems.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATP	Adenosine Tri-phosphate
BNF	Biological Nitrogen Fixation
DAP	Di ammonium Phosphate
DAS	Days after Sowing
DNA	Deoxyribonucleic Acid
FAO	Food and Agricultural Organization
FURP	Fertilizer Use Recommendation Project.
GOK	Government of Kenya
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
LR	Long rain
MBILI	Managing beneficial interactions in legume intercropping
NO_3^-	Nitrates
RCBD	Randomized Complete Block Design
SSA	Sub-Saharan Africa
SR	Short rain
USA	United States of America
UNICEF	United Nations International Children's Emergency Funds
WHO	World Health Organization

ACKNOWLEDGEMENT

I am grateful to God from whom all blessings flow. From his blessings I have the physical, mental and spiritual resources that have enabled me to write this thesis successfully. I express sincere gratitude to my supervisors: Prof. J.R. Okalebo, Dr. Beatrice Were and Dr. Abigael Otinga for their guidance in thesis writing. I am grateful to RUFORUM for funding this research work. Special thanks go to my parents and siblings for the support they gave during the entire period of writing this thesis.

CHAPTER ONE

INTRODUCTION

Soil nutrient depletion is a major constraint to food security in most parts of the world. In Kenya, nitrogen (N) and phosphorus (P) are the most widely deficient nutrients with N deficiencies recorded in 48% of the crop land soils (FURP, 1994). This depletion occurs mostly in the densely populated areas of western and central Kenya. To amend N deficiency, use of both organic and inorganic fertilizers in sole applications or in combinations is proposed. However, these options are limited by the spiraling costs of inorganic fertilizers that make it unaffordable to the smallholder farmers and the unsustainability of use of organic fertilizers due to the fact that these fertilizers are required in bulk to meet the crop nutrient requirements. Some legumes on the other hand are capable of fixing atmospheric nitrogen (Coyne, 1999). Depending on the type and species of the legume, these crops can be used as sources of N into a cropping system through a process known as biological nitrogen fixation (BNF). This process is facilitated by a group of microorganisms known as rhizobia (Bottomley, 1995). The BNF entails the conversion of atmospheric nitrogen (N_2) to nitrogen containing organic compounds (NO_3^- and NH_4^+) that become available to all forms of life through the nitrogen cycle (Brady and Weil, 2002). Biological nitrogen fixation is a system that maximizes use of natural ways of maintaining soil fertility and therefore has capacity for stable and sustainable crop yields in the long-term (Mugwe et al., 2007). To address the current soil nutrient depletion and low crop yield, nitrogen fixing legumes can be used and their BNF potential exploited.

1.1 Biological nitrogen fixation (BNF)

Symbiotic relationship between legumes and rhizobia is responsible for the largest contribution of fixed N to the farming system (Unkovich et al., 2008). Establishment of effective N fixing symbioses between legumes and their N fixing bacteria is dependent on many environmental factors and can be greatly influenced by farm management practices (Peoples et al., 1995). One of the major factors limiting a legume's ability to fix N is the absence of sufficient numbers of effective rhizobia. Fortunately, strains of rhizobia can be introduced into the soil simply by inoculation (Giller, 2001). The process of nitrogen fixation is catalyzed by the nitrogenase enzyme whose activity can be greatly reduced by the presence of oxygen atoms. Therefore, this process takes place in anaerobic conditions. High energy is required during this process and most legumes derive this energy from oxidation of organic compounds in a process known as photosynthesis (Ribet and Drevon, 1996). Phosphorus is essential for the manufacture of ATP, an energy carrier that is used to run these processes. In acid soils, P is highly fixed and therefore unavailable for plant use (Brady and Weil, 2002). Phosphorus is essential during the BNF process as it provides the energy needed in form of ATP and therefore the BNF process is hindered by soil acidity (Postgate, 1998)

1.2 Soil acidity

Most of the soils in the tropics are characterized as acidic (Kamprath, 1984). This can be attributed to high weathering and leaching of soils in this region. Strongly acidic soils have high capacity to fix applied P fertilizer thus making it unavailable for plant growth (Currie and Christensen, 2011). The problem of soil acidity can be ameliorated by use of agricultural liming materials. The lime requirement of a particular soil varies greatly. The lime requirement of a sandy soil at pH 5.0 will be much smaller than that of a clay soil at the same pH. This is because sandy soils have smaller base exchange capacity than that of clay soil (Plaster, 2003). Similarly, soils high in organic matter are less likely to require liming because they are expected to contain an abundance of organic chelates which bind aluminium (Al) and magnesium (Mg), thus alleviating these metal toxicities in the soil (Ristow,2010). According to Sanchez (1976), the lime requirement of a soil containing 1 milli equivalent of exchangeable Al is 1.5 milli- equivalent of Ca or 1.65 t ha^{-1} of $CaCO_3$. Also the lime needed to raise topsoil pH by 1.0 point is typically 5 t ha^{-1} and needs to be repeated every 5 years (Sherpa, 2013)

1.3 Inoculation of legumes

Inoculation of legumes is an efficient and convenient way of introducing effective rhizobia to soil and subsequently the rhizosphere of legumes. Rhizobia may be introduced to legumes by inoculation of the seed or soil. Seed may be inoculated by farmers immediately prior to sowing or custom inoculated by local seed merchants with coating facilities to be sown within a week. Alternatively, legume seed may be commercially inoculated and stored prior to its sale. This product is commonly referred to as pre-inoculated seed (Clement et al., 1992). It has been reported that inoculation

increases crop yields and in crop rotations the subsequent crop following the legume has higher yields (Chelule, 2007).

There are various commercial inoculants available for different legumes. The Biofix, which is locally produced by *MEA* Ltd and sold through agrovet outlets, is recommended for beans (*Phaseolus vulgaris)*, groundnuts (*Arachis hypogea*) and soybeans (*Glycine max*) among other field legumes. Other inoculants like VAULT for groundnuts are produced by Becker Underwood in the U.S.A. (Becker, 2008).

1.4 Statement of the problem.

Increasing human population pressure on limited agricultural land is a threat to food security all over the world. In western Kenya, continuous cropping has become common on smallholder farms (Kumwenda et al., 1997). High crop yields cannot be sustained without frequent and substantial additions of mineral nutrients (Ahn, 1993), but the high cost and limited availability of these inputs means that smallholders now use little or none at all.

Groundnut is one of the principal sources of high value dietary protein and oil in western Kenya. It also plays a major role in the region as a source of income for small scale farmers. Despite these benefits low yields of 200-400 kg ha^{-1} have been reported (Nekesa et al., 1999). This is attributed mainly to low soil fertility especially nitrogen which is limiting in SSA agricultural soils (Kisinyo, 2011). In western Kenya, where groundnut is an important legume, soils are majorly deficient of both nitrogen and phosphorus (Shankarapa, 2003). Legume crops can be used to alleviate nitrogen deficiency problem via the process of BNF. Biological nitrogen fixation is enhanced using rhizobia

inoculants which boost the population of rhizobia in the soil. The use of rhizobia inoculants is an attractive and cost effective source of N for legume cultivation and requires little technical expertise. Despite this, BNF has not been as successful in substituting for chemical fertilizer as initially expected due to a number of factors, soil acidity being an example. Low soil pH negatively influences the BNF process in groundnuts by inhibiting nodulation and hence poor levels of nitrogen fixed. Various inoculants contain rhizobia strains that differ in their efficiency to fix atmospheric nitrogen (Martin et al, 1990). Phosphorus is essential in supplying the energy in form of ATP used during the biological nitrogen fixation process. In the experimental sites in western Kenya, P is limiting and therefore it means a hindrance to the BNF process of the groundnuts. Liming is known to raise soil pH to levels where fixed P can be released into the soil solution, becoming available for plant use (Kifuko, 2002). There are various sources of liming materials with different chemical reactions to raise soil pH. Little information exists on which liming material is efficient in terms of time and magnitude of reactivity. Hence, there is need to test the two commonly available liming materials and different rhizobia strains so that we can come up with the most economical combination for the farmers.

1.5 Justification of the study

Biological nitrogen fixation is an important aspect of sustainable and environmentally friendly food production method and long-term crop productivity. Groundnut is a crop that is used for human food and livestock feed. Groundnut improvement generally seeks to increase the proportion of dry matter production that goes to nut production (Ponsonnet and Nesme, 1994). Continuous use of chemical fertilizers causes

environmental pollution and imbalance in the soil microbial activity while others like ammonium fertilizers cause soil acidity (Okalebo et al., 2006). Therefore, awareness should be created on the use of organics including inoculants to sustain soil fertility and plant productivity. One alternative to reduce over-dependence on mineral fertilizers is to intercrop maize with a legume such as groundnut (*A. hypogaea* L). Groundnut has been known to fix up to 134 Kg N ha^{-1} $year^{-1}$ (Giller, 2001) depending on soil conditions. These high rates of N fixation can only be realized if we gain understanding of the environmental conditions that best support the BNF process.

1.6 Objectives

1.6.1 General objective

To determine the effectiveness of different inoculants and agricultural lime in enhancing Biological Nitrogen Fixation (BNF) and yields of groundnuts/Maize under the MBILI intercropping system in acid soils of western Kenya.

1.6.2 Specific objectives.

1. To determine effect of lime application on selected soil chemical properties over time.
2. To establish nitrogen fixation effectiveness of different indigenous rhizobia inoculants in limed soils.
3. To ascertain effectiveness of dolomitic and calcitic limes in enhancing biological nitrogen fixation potential of groundnuts.

4. To determine yield response of groundnuts and maize to liming and rhizobia inoculation.

1.7 Hypotheses

1.7.1 General hypotheses

There are differences in the effectiveness of different rhizobia inoculants and agricultural limes in enhancing biological nitrogen fixation of groundnuts and yields of groundnut and maize.

1.7.2 Working hypotheses

1. Different liming materials differ in their effects on soil chemical properties.
2. There exist differences in performance among indigenous rhizobia in enhancing Biological Nitrogen Fixation (BNF) of groundnuts in limed soils.
3. Calcitic and dolomitic limes differ in their effects on Biological Nitrogen Fixation (BNF) of groundnuts.
4. Calcitic and dolomitic lime affect groundnut and maize yields differently.
5. There exist differences among rhizobia inoculants in increasing groundnut and maize yields.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical aspects of groundnut

The groundnut (*A. hypogaea*), is a species in the legume or "bean" family (Fabaceae). It is an annual herbaceous plant growing 30 to 50 cm tall. The leaves are opposite, pinnate with four leaflets (two opposite pairs; no terminal leaflet), each leaflet is 1 to 7 cm long and 1 to 3 cm broad. The flowers are a typical pea flower in shape, 2 to 4 cm across, yellow with reddish veining. The name *hypogaea* means "under the earth"; after pollination, the flower stalk elongates causing it to bend until the ovary touches the ground. Continued stalk growth then pushes the ovary underground where the mature fruit develops into a legume pod. Pods are 3 to 7 cm long, containing 1 to 4 seeds (Young, 2006).

There are mainly four seed types and common groundnut varieties: Runner, Virginia, Spanish and Valencia. Each of these peanuts is distinctive in size and flavor. Runners have attractive kernel size range; a high proportion of runners are used for peanut butter. Virginia has the largest kernels and account for most of the peanuts roasted and eaten as "inshells." When shelled, the larger kernels are sold as salted peanuts. Spanish-type peanuts have smaller kernels covered with a reddish-brown skin. They are used predominantly in peanut candy, with significant quantities used for salted nuts and peanut butter. They have higher oil content than the other types of peanuts which is advantageous when crushing for oil. Valencia usually has three or more small kernels per

pod. They are very sweet peanuts and are usually roasted and sold in the shell; they are excellent for fresh use as boiled groundnuts (Knauft and Gorbet, 1989).

Groundnut grows well in warm tropics and subtropics below 1500 M above sea level. Optimum daily growing temperature requirements is 30º C and growth stops at 15º C. The plant does not tolerate frost. Cooler temperatures delay flowering and seed formation. Water requirements are 500 to 600 mm well distributed throughout the growing season for good growth. However the crop is drought resistant and can survive severe lack of water but yields are reduced. The crop grows well on a pH range of 5.5 to 7.0 (Parker, 2004).

Maturity period is 90-130 days depending on the variety. At maturity the inside of the pods is grey with a rattling sound when shaken and mature nuts should be firm and dry as well as brown on the outside. During harvesting, the nuts are dug up with care to avoid breaking off and remaining in the ground. The drying period is 2-3 days, and then the nuts are removed from the plants and dried on mats for 7-10 days, to a moisture content of 10 %. Shelling is usually done by hand followed by sorting to remove the broken, dirty, damaged nuts which lower the quality and consequent selling price. Storage of dried nuts is done in clean dry conditions to avoid growth of *Aspergillus spp* of fungi which releases afflatoxin chemicals deleterious to human health particularly the liver (Gitau and Wanene, 2012).

2.2 Importance of groundnut

The groundnut seeds are rich in oil 38-50%, protein 25%, calcium, magnesium, phosphorus, potassium and vitamins. They are reported to have medicinal value

particularly in the treatment of diarrhoea and heamophilia. Groundnuts are used to help fight malnutrition and are high-protein, high-energy and high-nutrient. Groundnut-based pastes are developed to be used as a therapeutic food to aid in famine relief. The World Health Organisation (WHO), (United Nations International Children's Emergency Fund (UNICEF), Project groundnut Butter and Doctors Without Borders have used these products to help save malnourished children in developing countries (Bret, 2011).

Research studies have shown that peanuts contain high concentrations of a polyphenolic antioxidant, primary p-coumaric acid. This compound has been thought to reduce the risk of stomach cancer by limiting the formation of carcinogenic nitrosamines in the stomach. Peanuts are excellent source of resveratrol, another polyphenolic antioxidant. Resveratrol has been found to have protective function against cancers, heart disease, degenerative nerve disease and viral/fungal infections (FAO, 2008).

Most of the world groundnuts are processed into oil used for cooking. The cake that comes out of oil press is ground into flour and used in many human foods as it is rich in protein. The seeds are eaten raw, as roasted snack, used in confectionery, used in soups and made into sauces to accompany meat and starchy dishes. In Africa the plant is grown by small scale farmers both for cash and subsistence (Yao, 2004).

2.3 Groundnut production.

China leads in production of groundnuts, having a share of about 41.5% of overall world production, followed by India (18.2%) and the United States of America (6.8%) (FAO, 2008). According to FAO (2008), West Africa leads in the production of groundnut seed cake while Eastern Africa (Kenya being one of these countries) is the second largest

producer. However, this production has not been steady (FAO, 2008). In Kenya groundnut production comes third after soybean and common bean among the legume crops. According to FAOSTAT (2012), groundnut production has dropped for a period of ten years. The average yield of groundnuts in 2002 was 1100 Kg ha^{-1} and in 2010 the yield was slightly above 500 Kg ha^{-1}. Currently, farmers' yield of groundnuts ranges between 400 and 700 Kg/ha (ICRISAT, 2007). Under good husbandry these yields can be doubled. Groundnut varieties differ in their yield potentials (Table 1)

Table 1: Average yield potential of different groundnut varieties under research in Kenya.

Groundnut variety	Mean kernel yield kg ha^{-1}
"Red Valencia"	1500
"Serere 116" (white)	1250
"Bukene"	1530
"Manipintar"	2450
"Makulu Red"	2720
"Homa Bay"	770
"Asirya Mwitunde"	1300

(Source: ICRISAT 2007)

2.4 Maize production in kenya.

Maize is a staple food and an important source of carbohydrates in Kenya. It provides a large proportion of calorie needs to majority of consumers in rural and urban areas

(Mahmound et al., 1992). A large proportion of maize production comes from small scale farm holders although majority of them retain the produce for consumption (Economic Survey, 2001). The national average maize yield per hactare is estimated at 1.8 tones per hactare (Barrett and Michael, 1994). In western Kenya yields of upto 3 tones per hactare have been reported under good husbandry (Awour, 2001). However in some infertile soils yields as low as 0.5 tones per hactare have been realised (Ingosi, 2005).

2.5 Nutrition of groundnuts

The nutritional needs of groundnuts must be satisfied to attain maximum yields. To obtain maximum pod yield, adequate supply of every essential nutrient as per plant requirement at different growth stages has to be ensured. The availability of plant nutrients in the soil depends on factors such as soil pH, moisture content, cropping pattern, rate of release of micronutrients from the soil mineral and the presence of other ions in the soil (Ikisan, 2000). Groundnuts require phosphorus, nitrogen, potassium, sulphur, calcium, magnesium, zinc, copper, boron, iron molybdenum, sodium and chlorine.

2.5.1 Phosphorus and Nitrogen

It is an important nutrient for groundnut crop. It stimulates the setting of pods and decreases number of unfilled pods. It also hastens maturity of the crop. Single super phosphate and triple super phosphate are the common inorganic sources of phosphorous in the soil and furrow placement is the best for the phosphate fertilizers (Ikisan, 2000). Groundnut being a leguminous crop, it does not respond to heavy application of nitrogen.

It is however, the major limiting nutrient in the proper growth of a groundnut crop. In the early stages of growth nitrogen is very much in demand and application in two equal split doses is recommended (Nekesa et al., 1999).

2.5.2 Calcium and Sulphur

These two nutrients are taken up from the pod zone by the pegs and developing pods. Calcium requirement of the groundnut crop is heavy and availability of this nutrient in adequate quantities is very essential. It is mostly required during pod filling and if deficient, kernels with dark plumule as well as shrivelled pod are obtained. Sulphur is directly involved in the biosynthesis of groundnut oil. It also plays an important role in chlorophyll formation and its deficiency results into chlorosis in the young and middle leaves. The source of these two elements is gypsum (Kaye and Laby, 1995).

2.5.3 Potassium, Magnesium and Boron

Potassium is also important in groundnut production and is required in large quantities. It can be supplied regularly in form of potassium sulphate. Magnesium is essential in chlorophyll formation and its deficiency leads to chlorosis which is experienced on older leaves beginning at the margin and spreading towards the midrib. Boron is an important micronutrient whose deficiency leads to depressed and discoloured groundnut cotyledons. Necrosis can also be realized near the leaf margins (Rehm and Schmitt, 2002).

2.6 The biological nitrogen fixation in groundnuts

Legumes can obtain nitrogen from the atmosphere for their own needs through the process of biological nitrogen fixation (BNF) in nodules. Nodules are growth on roots or

stems of legume plants where bacteria reside. During the infection process the rhizobia are curled up with root hair thus the rhizobia penetrates this root hair cells with an infection thread that grows through the root hair into the main root. This causes the infected cells to divide and form a nodule. The rhizobia can now begin nitrogen fixation. Since plants cannot use atmospheric N, it will be fixed. Fixation occurs when soil bacteria such as Bradyrhizobia takes atmospheric nitrogen and fixes it into ammonia (NH_4). The level of ureide nitrogen in a plant is correlated with the amount of fixed nitrogen the plant takes up. The amount of nitrogen fixed annually is about 44-66 million tones worldwide providing almost half of nitrogen used in agriculture (Giller, 2001).

The process that replenishes most available nitrogen to biological systems is symbiotic and asymbiotic nitrogen fixation. Even though there are industrial sources of fixed N they still account for less than half of the total nitrogen fixed by biological systems (Coyne, 1999). Nitrogen fixation is a reductive process. For this reason, the microorganisms must have a ready supply of electrons for significant nitrogen fixation to occur. Nitrogen fixation requires an enzyme complex called nitrogenase. This operates in a microenvironment protected from oxygen. Nitrogenase is composed of two soluble proteins; the Fe protein dinitrogen reductase and MoFe protein dinitrogenase. Nitrogen fixation does not occur when ammonia or nitrates or organic nitrogen are readily available. It has been hypothesized that the reason for this is that the presence of available nitrogen may direct electrons from nitrogenase and therefore no reduction and hence no fixation (Rynne et al., 1994).

The overall equation for catalyzed nitrogen fixation is;

$$N_2 + 16\ ATP + 2H^+ \longrightarrow 2NH_4^+ + 16ADP + H_2$$

(Source Havlin et al. , 2005)

Nitrogen is very much in demand when nitrogen fixation is still in the initial stages. In this case a starter dose of 15-20 kg N ha^{-1} should be applied to encourage N fixation by rhizobia inoculation (Masrivani, 2009). Top dressing may not be necessary but any need of top dressing should be assessed by examining the nodules and nodulation for efficient nitrogen fixation. Root nodules that show red color when cut open are evidence of effective biological N fixation (Brady and Weil, 2002). If the nodulation and nitrogen fixation is low or poor then the crop should be applied with 30-40 kg ha^{-1} at 30-45 days from sowing (Masrivani, 2009).

2.7 Quantification of nitrogen fixation

The different approaches that have been used to quantify N_2 fixation by crop, pasture and woody legumes and non-nodulating plants have been extensively reviewed (e.g. Boddey, 1987; Boddey et al. 2000; Chalk 1985; Chalk et al. 2002; Giller 2001; Peoples et al. 1995; Shearer and Kohl 1986; Unkovich and Pate 2000;). The acetylene reduction and hydrogen evolution methods measure the activity of nitrogenase, the enzyme responsible for N_2 fixation (Unkovich et al., 2008).

One of the most commonly used techniques for quantifying fixed nitrogen is the ^{15}N abundance method. There are several advantages associated with this method; it is a simple, low-cost method that can be applied when facilities for only dry matter determinations and total N analyses are available. Potential limitations of this method include; the method requires a non N_2-fixing control to be included in the experimental

design. Differences between N_2-fixing and non N_2-fixing plants in root morphology and rooting depth can result in different capacities to use soil N (Chalk 1998). Moreover, there may be errors in accurately quantifying total N accumulated by the N_2-fixing plants and control plants. Despite these limitations the technique is likely to be most reliable under conditions of low plant available N and where there are large differences in N yield between the N_2-fixing plants and non N_2-fixing control (Unkovich et al., 2008).

2.9 Soil acidity and liming

The activity of hydrogen ions in soil solution determines the acidity and alkalinity of the solution. Acidic soils have a high concentration of hydrogen ions (Spark, 2003). Acidity in soils comes from H^+ and Al^{3+} ions in the soil solution and sorbed to soil surfaces. Many causes contribute to the formation of acid soils including rainfall, use of high nitrogen synthetic fertilizers, plant root activity and weathering of primary and secondary soil minerals. Acid soils can also form due to presence of pollutants such as acid rain and mine spoilings.

Aluminium (Al^{3+}) is important in acid soils because between pH 4 and 6, Al^{3+} reacts with water (H_2O) forming $AlOH^{2+}$ and $AlOH^+$ releasing extra H^+ ions. Every Al^{3+} ions can create 3 H^+ ions (Brady and Weil, 2002). Soil acidity negatively influences crop production either directly or indirectly. Plants grown in acid soils can experience a variety of symptoms including aluminium (Al), hydrogen (H), and/or manganese (Mn) toxicity as well as potential nutrient deficiencies of calcium (Ca) and magnesium (Mg) (Lippert,2000). Aluminium toxicity is the most wide spread problem in acid soils. When aluminium is present in the soil solution, it enters into the plant roots passively through osmosis. Aluminium damages roots whereby it interferes with uptake of calcium. It also

binds with phosphate and interferes with production of Adenosine tri phosphate (ATP) and deoxyribonucleic acid (DNA) both of which contain phosphate (Osmond et al., 2002). Aluminium can also restrict cell wall expansion causing roots to become stunted (Brady and Weil, 2002). Soils with high content of manganese containing minerals can cause manganese toxicity whose classic symptom in plants is crinkling or cupping of leaves.

Lime is applied to acid soils to neutralize excess acidity and maintain a soil pH of 6.0-6.5 below which crop yields diminish. Soil acidity can be corrected easily by liming the soil, or adding basic materials to neutralize the acid present. The most commonly used liming material is agricultural limestone. Limestone is the most important and abundant sedimentary rock, formed by the compaction of the remains of coral animals and plants on the bottoms of oceans around the world. It is composed of the mineral calcite (calcium and magnesium carbonate) along with small amounts of other minerals (Blanchini and mallarino, 2002). The limestone is not very water –soluble, making it easy to handle. The acidity reacts with the carbonate (CO_3) to form carbon dioxide (CO_2) and water (H_2O). The result is a soil that is less acidic (Lungati et al., 2004). Lime is a product derived from burned (calcite) limestone. Calcium carbonate is probably the most commonly available and least expensive lime product that is in powder form. The finer the particle size of lime, the faster it will have an effect on soil pH. Some lime products are powders that are turned into pellets for ease of application and much less mess. Lime powders, while less expensive than palletized products, can be very messy and difficult to spread (Gitau and Wanene, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental sites

The experiment was conducted in two sites of western Kenya, Koyonzo in Matungu district (latitude 0^o 25.3' N and longitude 35^o 04' E) and Ligala in Ugenya district (latitude 0^o 03'N and longitude 34^o 25' NE) of the Greenwich meridian (GOK, 1997). Matungu district receives an annual rainfall of range between 1250 and 1800 mm distributed in two rainy seasons, the long rains starting from March to June and the short rains from August to October. The mean annual temperature varies between 21 and 25^oC. The soils are developed from volcanic rocks, mainly basalt, which are well drained deep to very deep and vary from dark red Nitisols and Ferralsols to dark brown Acrisols (GOK, 1997). Ugenya district also receives a bimodal rainfall pattern, with long rains starting in March to June with the peak in April. Short rains start from September to December with peak in October. The annual rainfall ranges from 880-2000 mm; the annual mean maximum temperature ranges between 27 and 30^oC while annual mean minimum temperatures vary between 15 and 17^oC. The soils are developed from basalt, of volcanic origin and the soils are well drained, deep and friable. The predominant soils in this district are Nitisols, Ferralsols and Acrisols (GOK, 1997).

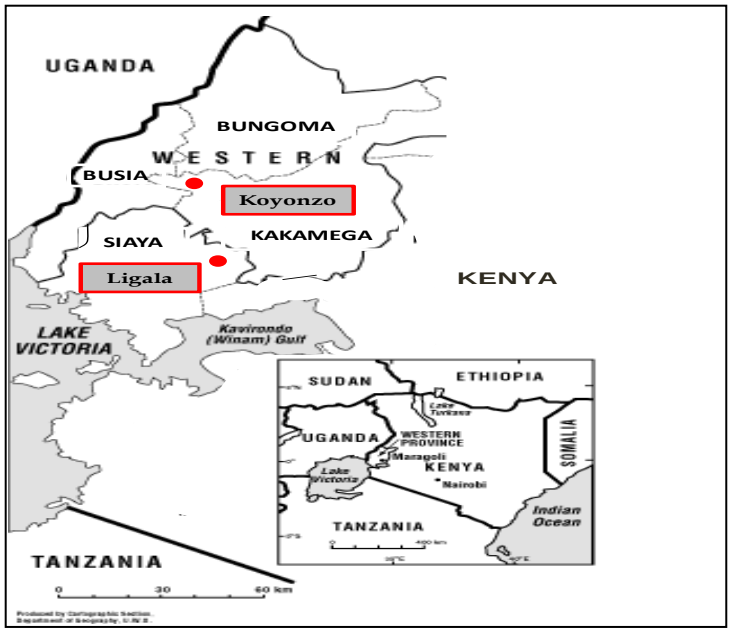

Figure 1: A map of western Kenya showing the study sites: Koyonzo in Matungu district, Kakamega County and Ligala in Ugenya district, Siaya County.

Modified from Abwunza, J. (1995)

3.2 Field experiment

3.2.1 Materials

One maize variety (H513D) bought from a Kenya seed company stockist was used to intercrop a groundnut variety known as Red Valencia which was obtained from Kenya Agricultural Research Institute (KARI) at Kisii. Liming materials used were calcitic lime (90 % $CaCO_3$) and dolomitic lime (50 % $CaCO_3$ and 21% $MgCO_3$) both from Athi River. Planting fertilizer TSP (46% P_2O_5) and a top dressing fertilzer CAN (27%N) were used. Inoculants used were: Biofix obtained from *MEA* limited, Rhizobia isolates from groundnut nodules coded as A6w, V2w and W1w (Table 2) of groundnut plants collected from Rabango, Emasatsi and Vivalo regions of western Kenya respectively. These

indigenous rhizobia strains were isolated and characterized in the laboratory and were found to be morphplpgically and biochemically different (Onyango, 2013).

Table 2: Morphological and biochemical characterization of rhizobia strains used for seed inoculation in this experiment.

Rhizobia strains					
Characteristic	Duration (hours)	A6w	V2w	Biofix	W1w
Growth rate	24	√	√	X	X
	48	√	√	√	√
Growth elevation		Pulvinate	raised	raised	Raised
Citrate utilization	24	√	√	√	√
Acid tolerance (pH 3.5)	24	√	√	X	X
	48	√	√	√	√
Aluminium tolerance (150 µM)	24	√	√	X	X
	48	√	√	√	√

Source: BNF project report- University of Eldoret, Biotechnology center, 2011

3.2.2 Treatments and treatment application

The experiment was laid out in two seasons: 2011 LR (Long rain) and 2011 SR (Short rain). Treatments were laid down in a randomised complete block design (RCBD) in a split plot arrangement. The experiment was replicated three times. The main plots consisted of the lime treatments: Dolomitic lime (L1), calcitic lime (L2) and the control (L0). The sub-plots were composed of the inoculants treatment: commercial inoculant (biofix), indigenous rhizobia isolates viz; A6w, V2w and W1w. A positive control (sole mineral N application at 34 kg ha^{-1}) and a negative control (0 kg N ha^{-1}) were also

included in the sub-plots. The N fertilizer was supplied as calcium ammonium nitrate (CAN) in a split application at planting and at six weeks after planting.

Lime was applied one month prior to planting to give time for the reaction within the soil. The lime was applied at the rate of 2 t ha^{-1} (Mupagwa and Tagwira, 2005) and was applied only at the beginning of 2011 LR (first season). There was a blanket application of P and K fertilizers during planting at the rate of 26 and 50 kg ha^{-1} respectively (FURP, 1994). This was done so as to eliminate any limitations on the treatments as a result of P and K deficiencies. Using sisal straps, planting furrows were made following the MBILI (Managing Beneficial Interactions in Legume Intercrops) system of intercropping (Otinga, 2007). Maize rows were spaced at 30 cm within rows and at 50 cm pairs that are 100 cm apart ('the gap'). Two rows of groundnuts were planted within the gap at 33 cm row spacing. Inoculation of groundnut seed with different inoculant treatments was done on the planting date under shade to ensure viability of the inoculant. This was done by sprinkling gum arabic on wetted groundnut seeds so as to act as a sticker. Different inoculants were introduced to the groundnuts as per the treatment layout and mixed thoroughly for even distribution of the inoculant (Onyango, 2013).

3.2.3 Field layout

Plot sizes of 5 * 4.5 m were laid out in a finely dug field. A path of 0.5 m was left between plots within a block and 1 m path between blocks. Plots of monocrop maize were included to act as reference crops during BNF analysis using the ^{15}N natural abundance method.

3.2.5 Data collection

3.2.5.1 Soil characterization

Initial soil sampling was done for the initial characterization of the experimental sites. Periodic soil sampling was done on a 30 day-interval up to harvesting for chemical analysis in the laboratory for soil pH, N, P, Ca and Mg nutrients using the laboratory soil manual by Okalebo et al., 2002 (appendix 10). Dilutions of a soil sample (taken before planting) were subjected to most probable number (MPN) and counts done in growth pouches using Red Valencia as host; colony counts were done on yeast extract mannitol agar cultures.

3.2.5.2 Nodulation data

Data on nodule number and weight were taken by sampling groundnut plants randomly at 42 days after sowing (DAS). Using a shovel three groundnut plants were uprooted carefully to avoid detaching of root nodules from each plot. Slowly the roots were washed through running water to remove soil. Counting of root nodules was done manually and recorded. Fresh weights of these nodules were taken using an electronic weighing machine. The nodules were then dried in the oven at 37^0C. Dry weight was taken and recorded.

3.2.5.3 The estimation of BNF potential using ^{15}N natural abundance method

The proportion of N fixed by groundnuts through the process of BNF was measured from the above ground biomass. Three shoot stems were picked from every plot and chopped into small sizes then air dried in the greenhouse. The dry groundnut biomass was finely

ground and 5 g of the ground material was sent to TSBF in Nairobi for further processing and micro balancing. These samples were then sent to KULeuven Soil Science laboratory in Belgium to be analyzed for the amount of N fixed using the ^{15}N natural abundance method.

The calculation of the proportion of N in groundnuts derived from the air (% Ndfa) was performed using the equation of Shearer and Kohl (1986) and Unkovich et al. (2008) and was calculated as follows:-

$$\%Ndfa = \frac{(\delta\,^{15}N \text{ reference plant} - \delta\,^{15}N \text{ legume})}{\delta\,^{15}N \text{ reference plant} - \beta \text{ value}} * 100$$

Where:

$\delta\,^{15}N$ reference plant-the $\delta^{15}N$ value for the reference plant

$\delta\,^{15}N$ legume- the $\delta\,^{15}N$ value for the total N in the groundnut grown under conditions in which atmospheric N_2 and N from other sources are available.

β value-is the isotopic discrimination of fixed N in the groundnuts as measured in the groundnut that is forced to solely depend on fixed N by growing them hydroponically with N-free nutrient pots (Shearer and Kohl, 1986). The β value used was -1.41 obtained from Okito et al. (2004).

Nitrogen yield and amount on nitrogen derived from the atmosphere were calculated as follows:

$$\text{N Yield (kg ha}^{-1}) = \frac{\text{Total N} \quad x \quad 1000}{\text{Effective area}}$$

$$\text{Amount of nitrogen fixed (kg ha}^{-1}) = \frac{\%\text{Ndfa x N yield}}{100}$$

3.2.5.4 Crop yield data

Harvesting of groundnut and maize was done by discarding two outer rows per plot and two plants at the ends of each row. Thus, four inner rows per plot were harvested from an effective area of 13.5 m^2. In the harvest area, total weights of unshelled maize grain were taken. Maize was shelled by hand and grain weights recorded for each plot. The stovers were cut at ground level and its fresh weights taken. Sub-samples (6 stalks per plot) from the stover were taken and cut into small pieces (3-5 cm) and mixed thoroughly. Sub-samples of the chopped stovers were taken and their fresh weight recorded. These stovers (chopped) were sun dried to obtain dry stover weight. For groundnuts all pods were removed from the plants and fresh shoot weight taken. A sub sample was taken for pod fresh and dry weight, drying and shelling to obtain grain yields. All plant tissue samples were ground for plant tissue analysis to determine N, P, Mg and Ca contents.

3.3 Statistical analysis

All data was subjected to ANOVA using GenStat computer package, 12th edition. Mean separation was done using contrast analysis.

The model used in data analysis was as follows:

$X_{ijk} = \mu + \alpha_i + \beta_j + £_{ij} + \lambda_k + Y_{ik} + £_{ijk}$

Where X_{ijk} = observation

μ = overall mean

α_i = Treatment effect (Liming effect)

β_j = Block effect

$£_{ij}$ = Error 1

λ_k = sub plot effect (Inoculation effect)

Y_{ik} = Interaction (Lime * Inoculation)

$£_{ijk}$ = Experimental error (Error 2)

CHAPTER FOUR

RESULTS

4.1 Initial soil characterization for the study sites

Table 3 shows the initial soil characterization of the study sites at Koyonzo and Ligala. Based on the relative proportions of sand, clay and silt, the Koyonzo and Ligala soils were classified as sandy clay loam and sandy loams respectively. Both soils were characterized by low pH. However, the Ligala site was more acidic than Koyonzo. Both sites had very low nitrogen in the soil. Carbon content in the soil for the two sites was moderate. Both sites had low available phosphorus as their values were below the minimum of 10 mg kg^{-1} of soil required for optimal plant growth. Phosphorus deficiency was far more severe at Ligala compared to Koyonzo. Soils from both sites showed calcium deficiencies as the levels were all below 1.25 $cmol_c kg^{-1}$ soil. In contrast, Mg was found to be adequate since its levels were above the critical level of 0.30 $cmol_c kg^{-1}$ (Okalebo, 2002). Results for the most probable number (MPN) indicated that both sites had very low rhizobia population in the soil. Koyonzo recorded a population of 1.4×10^6 rhizobia counts in 1 kg of dry soil while Ligala had 1.2×10^6 rhizobia counts in 1 kg of dry soil.

Generally, Koyonzo was more fertile compared to Ligala. However, both sites qualified for use in the present study since their soils were acidic, deficient in several major plant nutrients and had a low population of rhizobia insufficient to achieve significant nitrogen fixation by groundnut.

Table 3: Selected physical and chemical properties of soil samples collected from the top 0-15 cm at Koyonzo and Ligala study sites before the installation of the experiment during the 2011 LR.

Soil property	Site	
	Koyonzo	**Ligala**
% sand	66.10	52.60
% clay	23.40	44.50
% silt	12.40	10.40
Textural class	Sandy clay loam	Sandy clay
pH (1:2.5 soil : water)	5.20	4.60
% N	0.09	0.08
% C	1.32	1.26
Olsen P (mg kg -1)	9.20	2.20
Ca($cmol_c$ kg^{-1})	0.72	0.77
Mg($cmol_c$ kg^{-1})	0.40	0.30

Data are means of 54 replications of measurements.

4.2 The effects of lime on different soil parameters at different sampling times

4.2.1 Soil pH

Application of lime increased the soil pH at both sites (Figures 2a and 2b). There was no significant difference ($p<0.05$) between calcitic and dolomitic lime in their effect on raising soil pH. Dolomitic lime performed better than calcitic lime at both site. During 2011 LR, the highest pH value was obtained at 60 days after sowing (DAS) where a pH value of 6.5 and 5.4 were recorded at Koyonzo and Ligala sites, respectively. During the 2011 SR the highest pH value was realized at 150 DAS in both sites. It was also noted that the soil pH values declined at the end of each planting season (90 and 180 DAS) at both sites (Figures 2a and 2b).

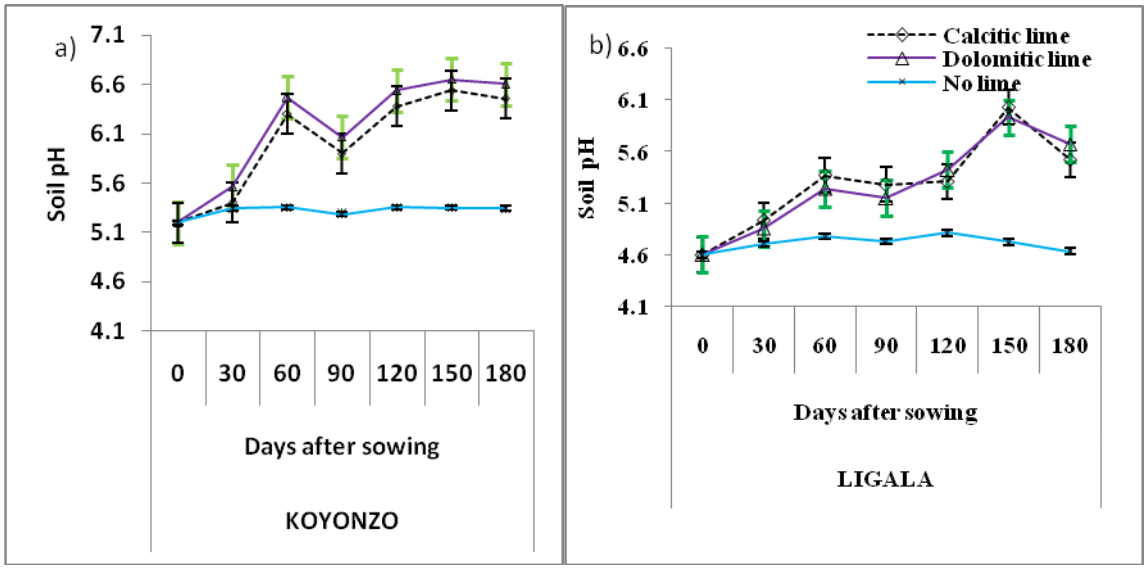

Figure 2: Changes in soil pH at a) Koyonzo and b) Ligala during two rainy seasons of 2011 following amendment with calcitic or dolomitic limes.

Lime was only applied before the onset of the 2011LR and time 0-21 are days before lime application. Error bars show standard error of the differrence (SED).

4.2.2 Soil available phosphorus (Olsen P)

Liming led to an increase in available phosphorus at both sites (Figures 3a and 3b). At Koyonzo, the available P content increased to values of 10.9-12.8 mg kg $^{-1}$ soil depending on lime type and sampling time. Although an increase in available P was also observed upon liming at Ligala, the amount (3.55-7.07 mg kg $^{-1}$ soil) did not reach the optimum for crop production. The two types of lime did not differ significantly although the quantity was higher in treatments with dolomitic lime than under calcitic lime at both sites.

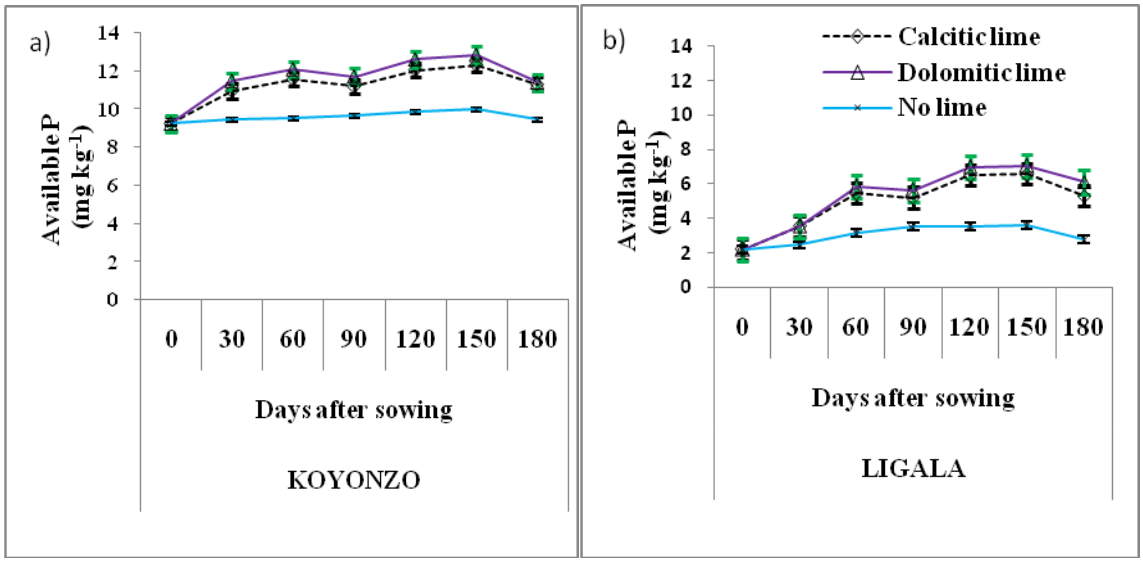

Figure 3: Changes in soil available phosphorus at a) Koyonzo and b) Ligala during two rainy seasons of 2011 following amendment with calcitic or dolomitic limes.

Lime was only applied before the onset of the 2011LR and time 0-21 are days before lime application. Error bars show standard error of the differrence (SED).

During the 2011 LR, the peak P level was realized at 60 DAS and a drop in the P levels was seen at harvesting 90 DAS. This same scenario repeated itself during the 2011 SR where the peak P level was at 150 DAS and a drop at 180 DAS. Such variability in available P levels during the cropping season was not evident in the lime control at either site.

4.2.3 Nitrogen in the soil

Soil nitrogen increased gradually in the limed plots at the two sites until 60 and 150 DAS for the 2011 LR and 2011 SR, respectively (Figures 4a and 4b). Similar to soil available P, after these sampling times, soil N began to decline. At the end of each cropping season there was a drastic drop in soil N at both sites. Generally, higher values of % N were

obtained under dolomitic lime treatment compared to Calcitic lime. In the non-limed plots, the N content appeared similar throughout the experimental period. Unlike P, the N amount recorded for matching sampling times was similar for the two sites.

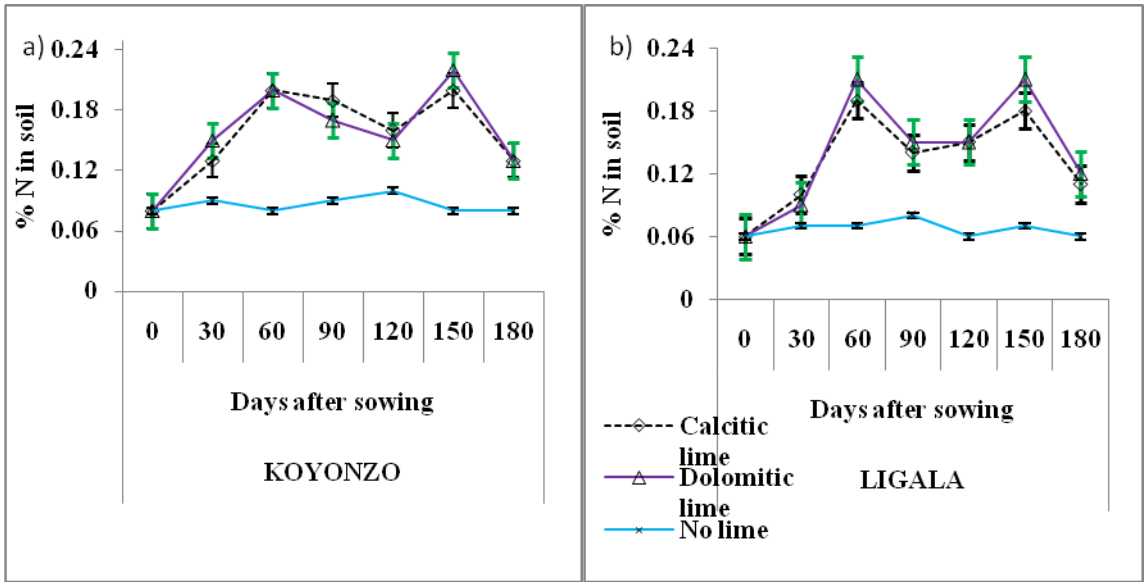

Figure 4: Changes in soil nitrogen at a) Koyonzo and b) Ligala during two rainy seasons of 2011 following amendment with calcitic or dolomitic limes.

Lime was only applied before the onset of the 2011LR and time 0-21 are days before lime application. Error bars show standard error of the differrence (SED).

4.2.4 Calcium in the soil

When the soil was limed, there was a sharp increase in Ca at 30 DAS (Figures 5a and5b) at the Koyonzo site. After that, the levels of soil Ca remained similar throughout the two cropping seasons. For Ligala, the sharp increase in soil Ca happened between 30 and 60 DAS, but the rest of the experimental period maintained almost the same quantity. Soil Ca was slightly lower in the 2011 SR season as compared to 2011 LR season at Koyonzo. The Ca level in the soil was higher in calcitic lime treatments compared to those with dolomitic lime.

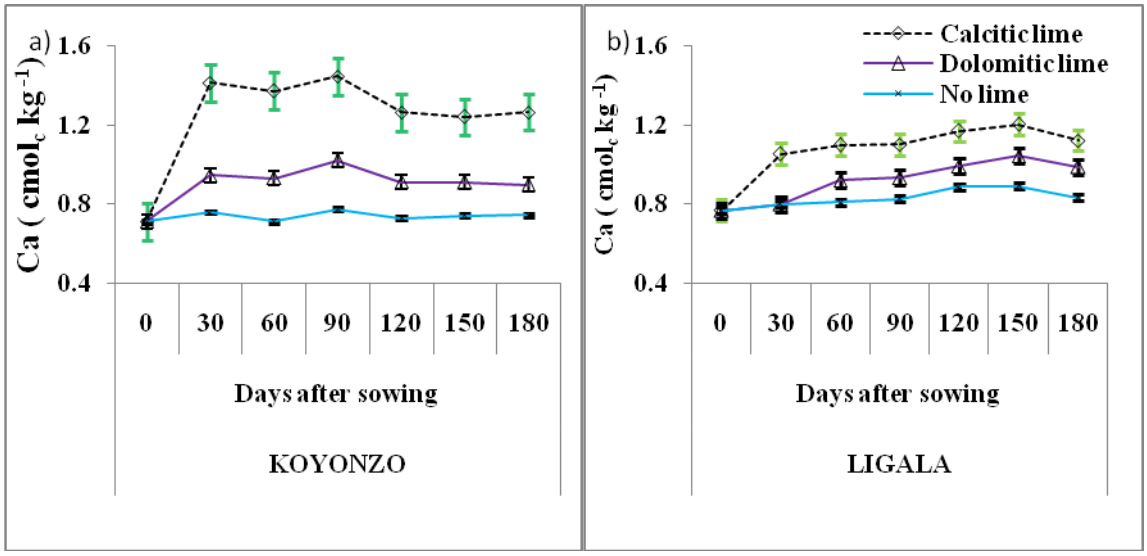

Figure 5: Changes in soil calcium at a) Koyonzo and b) Ligala during two rainy seasons of 2011 following amendment with calcitic or dolomitic limes.

Lime was only applied before the onset of the 2011LR and time 0-21 are days before lime application. Error bars show standard error of the difference (SED).

4.2.5 Magnesium in the soil

Dolomitic lime raised magnesium in the soil to levels significantly higher than those obtained with calcitic lime. There was a peak in the magnesium level at 60 DAS at Koyonzo and at 30 DAS at Ligala site as depicted by the dolomitic lime treatment (Figure 6a and 6b). At both sites the magnesium levels in the soil decreased towards the end of the growing season. Similar to calcium, the levels of magnesium were lower in the 2011 SR as compared to 2011LR season at Koyonzo while less notable variation between the seasons could be seen at Ligala.

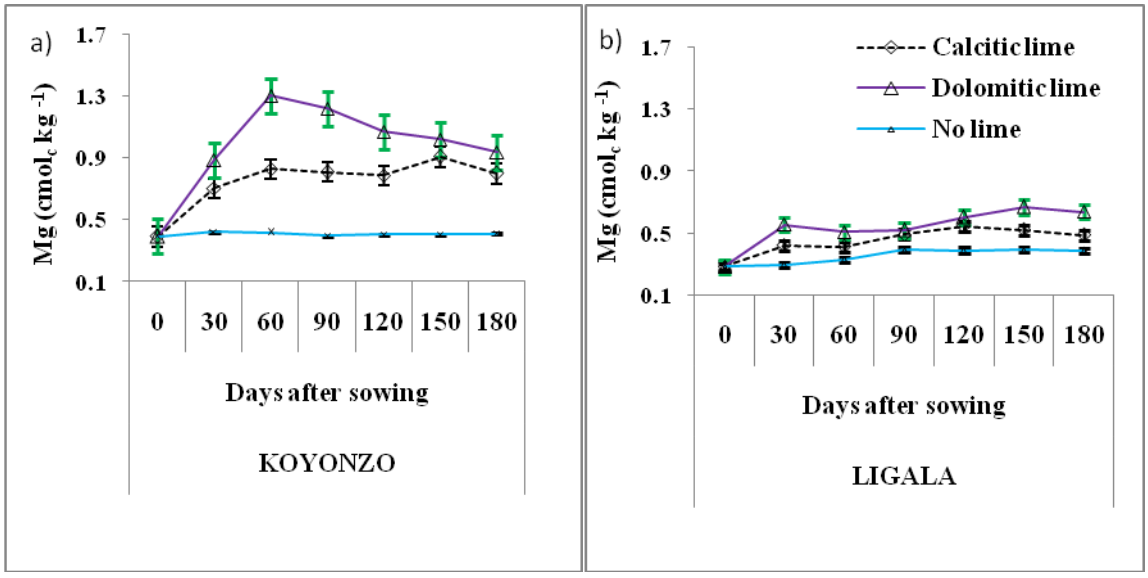

Figure 6: **Changes in soil magnesiumat a) Koyonzo and b) Ligala during two rainy seasons of 2011 following amendment with calcitic or dolomitic limes.**

Lime was only applied before the onset of the 2011LR and time 0-21 are days before lime application. Error bars show standard error of the differrence (SED).

4.3 Effect of lime and inoculants on groundnut nodule number and weight

Site significantly affected the number of nodules produced per groundnut plant across seasons (Table 4). There was higher nodule number per plant at Koyonzo than at Ligala. Both liming and inoculation significantly ($p<0.05$) increased the nodule number per plant in both the sites and seasons. There was significant interaction between lime and inoculants at $p<0.05$. Different sub-plot treatments gave different nodule numbers for the two liming materials. For instance, inoculant A6w gave the highest number of nodules per plant when plots were limed using dolomitic lime (Table 4). Contrast analysis showed significant differences among different sub-plot treatments at $p<0.05$ (Appendix I and II). However some treatment pairs like; A6w and V2w, biofix (the commercial strain) and W1w, Control and N were not statistically different. The lime contrasts indicated no

significant differences between dolomitic lime and calcitic lime but the two lime treatments were statistically different from the treatments without lime.

Nodule dry weight varied with site (Table 4). Nodules at Koyonzo had more dry weight compared to the nodules at Ligala ($p<0.05$). Liming and inoculation significantly increased the nodule weight ($p<0.05$). Liming effects on nodule dry weight cut across all the sites and seasons while different inoculants performed differently in the two sites. From contrast analysis (Appendix III and IV), there were no significant differences between dolomitic lime and calcitic lime in influencing nodule dry weight. However, there were significant differences between lime application and no lime application ($p<0.05$). Similarly for the dry weight, sub-plot treatments differed significantly from each other at $p< 0.05$ although some pairs like A6w and V2w, biofix and W1w, Control and N were not statistically different.

Table 4: Groundnut nodule number and weight as influenced by lime and rhizobia strain treatments at Koyonzo and Ligala sites during two rainy seasons of 2011

		2011 LRs				2011 SRs			
		KOYONZO		LIGALA		KOYONZO		LIGALA	
LIME	**STRAIN treatment**	**Nodule plant^{-1}**	**Dry Wt (mg)**	**Nodule plant^{-1}**	**Dry Wt (mg)**	**Nodule plant^{-1}**	**Dry Wt (mg)**	**Nodule plant^{-1}**	**Dry Wt (mg)**
Calcitic	V2w	14	12.00	11	10.10	18	12.87	16	11.43
	A6w	16	11.83	12	10.67	20	13.43	18	10.63
	W1w	11	5.07	8	3.10	13	5.97	10	5.07
	Biofix	10	6.30	6	4.87	12	7.10	9	6.53
	N	5	2.33	3	1.63	5	2.97	3	2.73
	Control	3	1.77	2	1.00	3	1.97	2	1.87
	MEAN	**10**	**6.55**	**7**	**5.23**	**12**	**7.38**	**10**	**6.37**
Dolomitic	V2w	19	12.77	15	9.67	23	14.17	19	12.63
	A6w	23	14.17	19	12.53	26	12.87	23	11.63
	W1w	12	6.73	10	5.00	13	7.30	11	6.20
	Biofix	12	5.70	9	4.07	13	5.63	10	5.10
	N	4	2.50	3	1.73	4	3.10	3	2.63
	Control	3	1.43	2	1.30	2	1.53	2	14.70
	MEAN	**12**	**7.22**	**10**	**5.72**	**14**	**7.43**	**11**	**6.61**
No lime	V2w	7	5.53	4	4.10	8	6.50	6	5.10
	A6w	9	6.40	6	5.53	10	7.40	8	6.20
	W1w	6	3.37	3	2.30	7	4.40	4	3.77
	Biofix	7	3.97	5	2.40	6	4.97	4	4.67
	N	5	1.93	2	1.60	4	2.63	2	2.40
	Control	2	1.13	2	1.10	3	1.60	2	1.60
	MEAN	**6**	**3.72**	**4**	**2.84**	**6**	**4.58**	**4**	**3.86**
OVERAL MEAN		**9**	**5.83**	**7**	**4.59**	**11**	**6.47**	**8**	**5.61**
SED S		0.15***	0.14***						
SED Sn		0.15***	0.14***						
SED L		0.18***	0.17***						
SED I			0.24***						
SED S* L		0.26***	ns						
SED		ns	ns						
Sn*L		0.26***	ns						
SED S *I		0.36***	ns						
SEDSn *I		0.36***	0.41***						
SED L *I		0.45***	18.2						
CV%		12.6							

V2w, A6w, W1w, and Biofix are rhizobia strain treatments (inoculants), N-represents the treatment where nitrogen was applied but without inoculation, ***- significance at p<0.001, ns-not significant, LR-Long Rain, SR-Short Rain SED – Standard error of the difference, S-Site, Sn-Seasons , L-Lime, I- Inoculant.

Overall, A6w and V2w gave the highest nodule biomass, with the best results obtained in limed treatments.

4.4 Effect of rhizobia inoculation and liming on biological nitrogen fixation during 2011 long rain.

Nitrogen fixation efficiency varied significantly between sites, as evident from % Ndfa values (Table 5). The inoculants tested showed differences in their effect on % Ndfa. Groundnuts inoculated with rhizobia strain A6w derived 47.42 % nitrogen from the air, which was the highest percentage compared to the other strains. However, from contrast analysis biofix and W1w did not differ significantly ($p<0.05$). Generally lime nearly doubled the % Ndfa, which differed significantly between the sites (Table 5). However, contrast analysis showed that there were no significant differences among the lime treatments at $p< 0.05$ (Appendix V).

Table 5: Parameters showing Nitrogen fixation by groundnuts inoculated with 3 indigenous strains as compared to a commercial strain (biofix), control and application of nitrogen under different liming materials at Koyonzo and Ligala during the 2011 long rain

		KOYONZO				LIGALA			
Lime treatment	**rhizobia treatment**	**N content (g kg^{-1}DM)**	**N yield kg ha^{-1}**	**Ndfa (%)**	**Amount of N (kg ha^{-1})**	**N content (g kg^{-1} DM)**	**N yield kg ha^{-1}**	**Ndfa (%)**	**Amount of N (kg ha^{-1})**
Calcitic	V2w	23.40	28.19	33.86	8.60	23.35	25.62	24.71	6.34
	A6w	23.90	34.70	**42.21**	**13.37**	23.30	32.45	**33.35**	**10.83**
	W1w	23.08	21.66	13.73	2.99	21.94	18.40	6.84	1.29
	Biofix	22.93	18.69	17.95	3.36	21.83	13.89	10.05	1.59
	N	22.65	22.73	5.80	1.33	22.80	20.41	4.43	0.91
	Control	15.90	11.95	2.02	0.24	15.67	10.25	2.60	0.28
	MEAN	**21.98**	**22.99**	**19.26**	**4.98**	**21.48**	**20.17**	**13.66**	**3.54**
Dolomitic	V2w	22.50	30.27	36.30	9.56	22.46	27.64	30.94	8.20
	A6w	22.46	37.12	**47.42**	**14.67**	22.29	35.94	**37.17**	**11.37**
	W1w	22.42	22.01	15.92	3.53	22.36	19.87	15.92	1.51
	Biofix	22.28	19.39	14.82	2.87	22.08	16.94	8.69	1.50
	N	22.15	24.49	4.62	1.13	22.68	22.47	3.24	0.73
	Control	15.75	12.40	3.97	0.49	11.35	7.78	2.57	0.20
	MEAN	**21.26**	**24.28**	**20.51**	**5.38**	**20.54**	**21.77**	**16.42**	**3.92**
No lime	V2w	21.96	21.93	21.72	4.81	22.21	19.84	18.22	3.62
	A6w	21.94	24.51	**27.03**	**6.63**	22.25	22.38	22.23	4.99
	W1w	21.87	17.16	10.93	1.88	21.84	14.87	4.49	0.67
	Biofix	21.71	23.50	6.42	1.51	21.72	21.31	2.71	0.58
	N	21.83	16.36	8.71	1.42	21.43	13.89	1.90	0.26
	Control	10.85	7.71	3.49	0.27	10.33	6.22	1.87	0.12
	MEAN	**20.03**	**18.53**	**13.05**	**2.75**	**19.96**	**16.42**	**8.57**	**1.71**
	OVERAL MEAN	**21.09**	**21.93**	**17.61**	**4.37**	**20.66**	**19.45**	**12.88**	**3.06**
	SED S	Ns	0.271***	0.395***	0.158***				
	SED L	0.408***	0.332***	0.484***	0.193***				
	SED I	0.577***	0.469***	0.685***	0.273***				
	SED S*L	Ns	Ns	ns	Ns				
	SED S*I	Ns	Ns	0.968***	0.387***				
	SED L*I	Ns	0.813***	1.183***	0.474***				

V2w, A6w, W1w, and Biofix are rhizobia strain treatments (inoculants), N-represents the treatment where nitrogen was applied but without inoculation, ***- significance at p<0.001, ns-not significant, LR -Long Rain, SR-Short Rain, SED – Standard error of the difference, S-Site, Sn-Seasons , L-Lime, I- Inoculant.

4.5 Effect of inoculation and liming on yields of groundnuts and maize during two rainy seasons of 2011

Groundnut yields were dependent on site (Table 6 and 7). Koyonzo had significantly ($P<0.05$) higher yields compared to Ligala (Appendix 6 and 7). Inoculation generally increased groundnut yields. At Koyonzo, Inoculants A6w and V2w gave 96.8 % and 75 % yield increase over the control respectively. The N treatment gave 33.9 % higher yields than the control. There was 15 % yield increase in the 2011 SR compared to 2011 LR. Rhizobia inoculation led to the formation of high number of kernels (Plate 1). Fewer kernels were obtained from un-inoculated plots (Plate 2). There was an interaction between lime and inoculants at $p< 0.05$ (Appendix 6 and 7). Inoculant A6w and dolomitic lime interaction gave the highest groundnut yield of 1.1 t ha^{-1} compared to inoculant W1w and dolomitic lime interaction which gave 0.7 t ha^{-1}. This best treatment combination gave 3 % groundnut yields higher at Koyonzo than Ligala. Contrast analysis indicated that there were significant differences between different sub plot treatments (Appendix VI and VII). However there were no significant differences between Biofix and W1w, N and V2w in their effects on groundnut yields ($p<0.05$).

Maize yield differed significantly ($p<0.05$) between sites. Better maize yields were obtained at Koyonzo compared to Ligala and was highest in treatments where groundnut inoculation was carried out (Table 6 and 7). There was an interaction between lime and inoculants in their effects on maize yields (Appendix VIII and IX). From contrast analysis there were significance differences among the rhizobia treatments at $p<0.05$, however biofix and W1w, V2w and N were not statistically different from each other. Similar to rhizobia inoculation, lime increased maize yields. There were significantly

higher yields of maize grain where lime was applied as compared to plots where lime was not applied during both rainy seasons of 2011. Highest maize yields were realized in plots where dolomitic lime was applied and inoculant A6w used. This was translated to 33.2 % maize yield above the control. Generally high maize yields were realized during 2011 SR compared to 2011 LR. For instance the best treatment combination of A6w and dolomitic lime gave 30.8 % higher maize yields in 2011 SR compared to 2011 LR. A6w and dolomitic lime treatment combination gave 35.6 % higher maize yields at Koyonzo compared to Ligala. There was high vigour of maize plants in early stages of growth on limed plots compared to un-limed plots (Plates 3 and 4). From the contrast analysis, there were no significant differences between dolomitic and calcitic lime. However, the two limes were statistically different from the treatment where no lime was applied (Appendix VIII and IX).

Table 6: Performance of different rhizobia inoculants on yield of groundnuts and maize in Kg ha^{-1} at Koyonzo and Ligala during the two rainy seasons of 2011.

	2011 LRs				2011 SRs			
Strain treatment	GROUNDNUT YIELDS (kg ha^{-1})		MAIZE YIELDS (kg ha^{-1})		GROUNDNUT YIELDS (kg ha^{-1})		MAIZE YIELDS (kg ha^{-1})	
	Koyonzo	Ligala	Koyonzo	Ligala	Koyonzo	Ligala	Koyonzo	Ligala
V2w	705.2	585.9	1264.0	1173.0	883.8	852.7	1966.3	1861.0
A6w	778.9	661.8	1363.0	1271.2	958.0	923.9	2064.9	1957.1
W1w	516.7	397.8	782.7	694.2	696.9	666.8	1483.3	1377.8
Biofix	522.9	404.2	747.8	657.9	702.3	600.9	1445.9	1341.8
N	715.0	596.6	1196.3	1109.0	894.0	859.1	1897.9	1792.1
Control	411.9	301.2	543.4	513.6	412.5	320.1	540.8	537.7
MEAN	**608.4**	**491.3**	**982.9**	**903.2**	**757.9**	**703.9**	**1566.5**	**1477.9**
SED S	5.9***	2.7***						
SED Sn	5.6***	2.7***						
SED I	9.7***	4.7***						
SED S*I	ns	6.6*						
SED Sn* I	ns	6.6***						
CV%	6.3	5.6						

Where, V2w, A6w, N, Control, Biofix and W1w are sub plot (rhizobia inoculant) treatments, ***-Significance at p<0.001, SED-Standard error of the difference, *-Significance at 95%, CV%-Coefficient of variation, LRs-Long rains and SRs- Short rains, S-Site, Sn-Seasons ,L-Lime, I- Inoculant.

Table 7: Effects of liming on yields of groundnut and maize in Kg ha^{-1} at Koyonzo and Ligala in two rainy seasons of 2011.

	2011 Rs				2011SRs			
	GROUNDNUT YIELDS (kg ha^{-1})		MAIZE YIELDS (kg ha^{-1})		GROUNDNUT YIELDS (kg ha^{-1})		MAIZE YIELDS (kg ha^{-1})	
Lime trt.	Koyonzo	Ligala	Koyonzo	Ligala	Koyonzo	Ligala	Koyonzo	Ligala
Calcitic	616.5	497.8	1057.2	971.3	796.7	731.3	1656.3	1550.4
Dolomitic	643.3	527.1	1060.3	980.9	821.8	787.9	1667.4	1561.3
No lime	565.4	448.8	831.1	757.5	568.2	450.4	869.7	762.1
MEAN	**608.4**	**491.2**	**982.9**	**903.2**	**728.9**	**656.5**	**1397.8**	**1291.3**
SED S	5.6***	2.7***						
SED Sn	5.6***	2.7****						
SED L	6.9***	3.3***						
SED S *L	ns	ns						
SED Sn *L	ns	ns						
CV%	6.3	5.6						

Where, ***-Significance at p<0.001, *-Significance at p<0.05, SED-Standard error of the difference, CV%-Coefficient of variation, Lime trt.-Lime treatment, LRs-Long rains and SRs- Short rains, S-Site, Sn-Seasons ,L-Lime, I- Inoculant.

Plate 1: Groundnut kernels from inoculated plot where dolomitic lime was applied. Where L1-dolomitic lime, R3-Strain A6w

Plate 2: Groundnut kernels from un-inoculated plot where dolomitic lime was applied. Where L1-dolomitic lime, R0-no rhizobia inoculation.

(Source: Ogega, 2014)

Plate 2: A limed plot showing high vigor of maize and groundnuts during 2011 LR at Kovonzo

Plate 1: Un-limed plot showing yellowing and stunted growth of maize and groundnuts during 2011 LR at

(Source: Ogega, 2014)

4.6 Correlation between crop yields and soil available phosphorous or nitrogen

Correlation analyses were conducted between crop (maize and groundnut) yields and soil available P and soil N at different sampling dates during the growth period. The results are given in Tables 8 and 9 and Figures 7 to 10. Groundnut yield showed a significant ($r > 0.50$) positive linear correlation with soil nitrogen as shown in Figures 11 to 14 for both the sites at the fourth sampling time (90 days after sowing). Also groundnut yields showed a positive correlation with soil available P at 90 DAS at Koyonzo and at 180 DAS at Ligala. There was a poor correlation between soil available P and groundnut yields at 180 DAS at Koyonzo and at 90 DAS at Ligala as shown in Figures 9 and 10. There was a positive correlation between maize yields and both P and N at all sampling times at both sites.

Table 8: Correlation between crop yields (t ha^{-1}) and soil nitrogen (%) at different sampling times for Koyonzo and Ligala sites during the two rainy seasons of 2011

SITE		0 DAS	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS
	Groundnut yields	0	0.61	0.82	0.61	0.42	0.8	0.8
	Maize yields	0	0.7	0.79	0.66	0.56	0.78	0.78
	Groundnut yields	0	0.62	0.57	0.66	0.58	0.65	0.76
	Maize yields	0	0.58	0.51	0.61	0.54	0.59	0.7

Where 0-180 DAS are the sampling dates. Values greater than 0.50 show high correlation

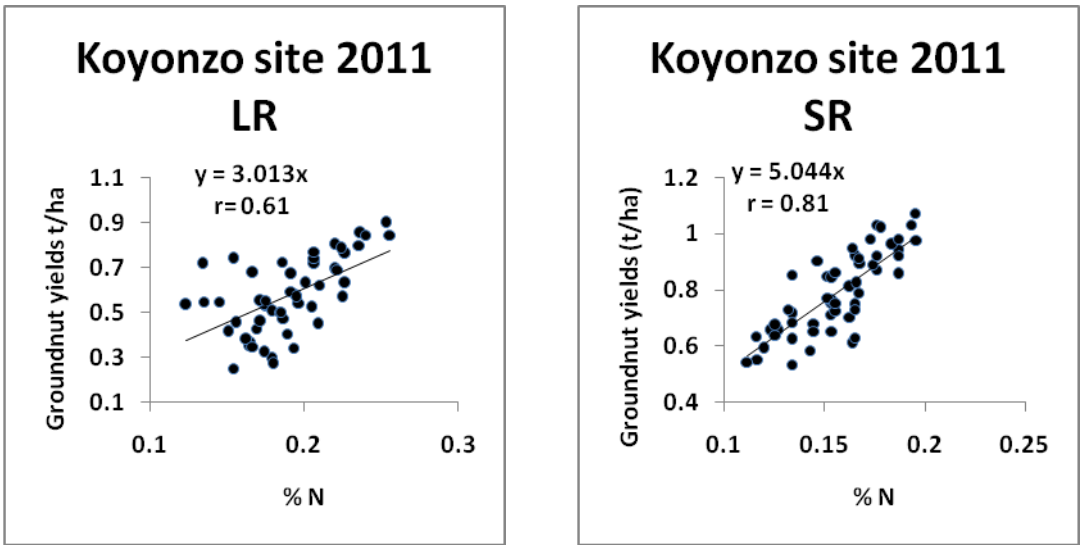

Figure 7: Relationship between soil nitrogen (%) and groundnut yields (tha^{-1}) as observed at fourth sampling (90 days after planting) during the two rainy seasons of 2011 at Koyonzo site.

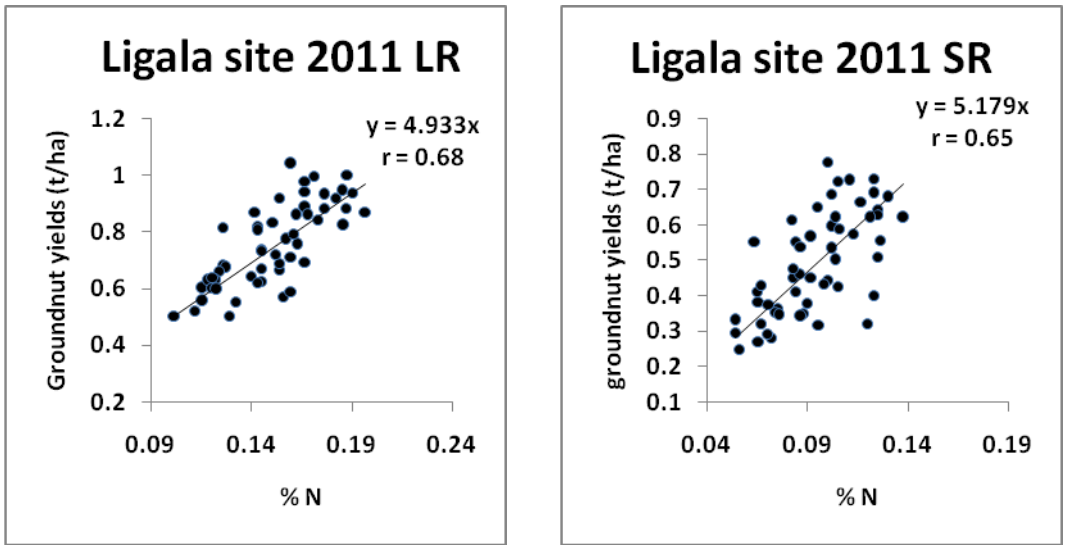

Figure 8: Relationship between soil nitrogen (%) and groundnut yields (t ha^{-1}) as observed at fourth sampling (90 days after planting) during the two rainy seasons of 2011 at Ligala site.

Table 9: Correlation between crop yields (t ha^{-1}) and soil available P (mg kg^{-1}) at different sampling times for Koyonzo and Ligala sites during the two rainy seasons of 2011

SITE		0 DAS	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS
	Groundnut yields	0	0.55	0.43	0.54	0.52	0.48	0.48
	Maize yields	0	0.73	0.61	0.72	0.7	0.66	0.66
	Groundnut yields	0	0.59	0.6	0.59	0.69	0.67	0.68
	Maize yields	0	0.67	0.7	0.69	0.76	0.73	0.73

Where 0-180 DAS are the sampling dates. Values greater than 0.50 show high correlation.

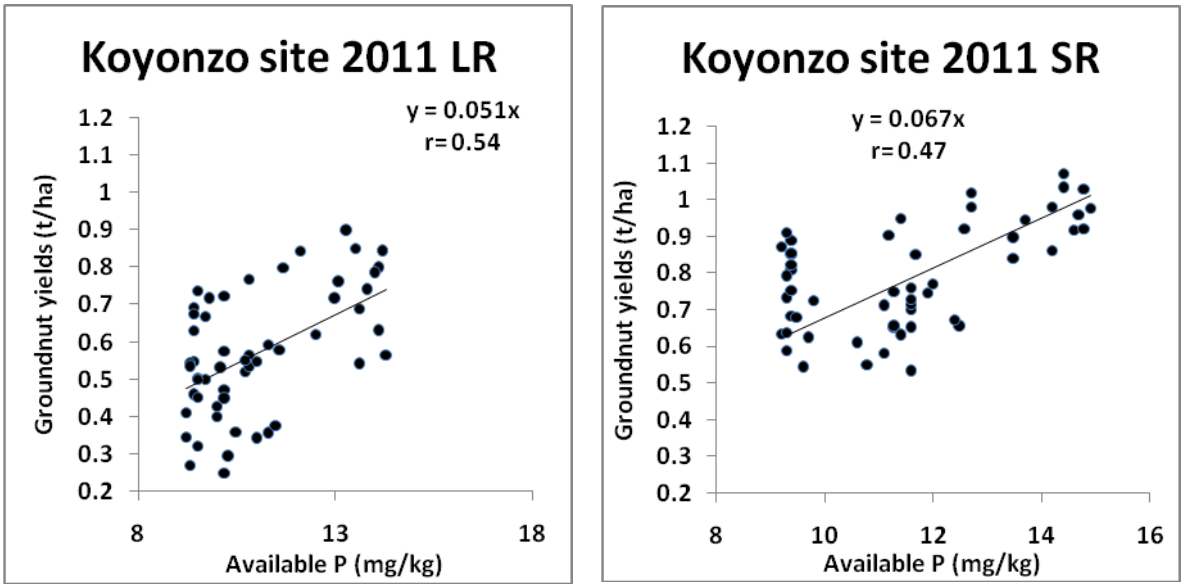

Figure 9: Relationship between soil available P and groundnut yields (tha^{-1}) as observed at fourth sampling (90 days after planting) during the two rainy seasons of 2011 at Koyonzo site.

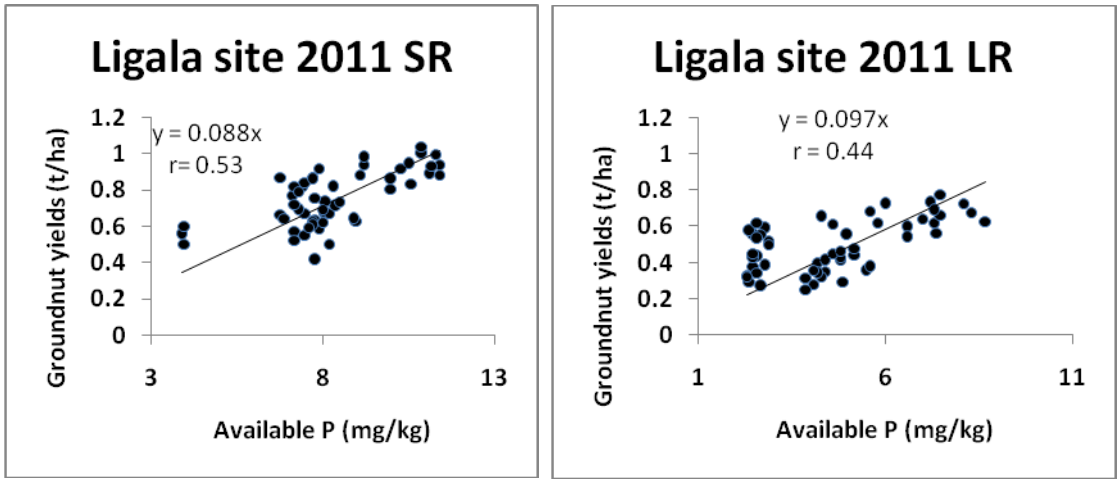

Figure 10: Relationship between soil available P and groundnut yields (t ha^{-1}) as observed at fourth sampling (90 days after planting) during the two rainy seasons of 2011 at Ligala site.

CHAPTER FIVE

DISCUSSION

5.1 Initial soil physical and chemical characteristics of the study sites

The two study sites had low soil pH. According to Okalebo et al. (2002), soils with pH below 5.0 are strongly acidic while those with pH between 5.1-6.0 are moderately acidic. Low soil pH constrains crop productivity by limiting availability of some essential plant nutrients and increasing that of the soil solution's toxic elements such as aluminium and manganese (Brady and Weil, 2002). These are regarded as the major causes of poor crop performance in acid soils (Brady and Weil, 2002). Aluminium may be concentrated enough to limit or stop root development and as a result plants cannot absorb water and nutrients. The plants in turn become stunted and exhibit nutrient deficiencies especially those of phosphorus (Coyne, 1999). Toxic levels of manganese interfere with normal growth processes in the aerial plant part, which stunts the plant, discolours it and causes poor yields (Kennedy, 1992).

There are numerous causes of soil acidity that may be associated with low soil pH on the study sites. Parent rock material can give rise to acidic soils after weathering. Soils that originate from granite rocks are likely to become more acidic than those developed from shale or limestone (Jodie and Pete, 2000). Basic parent materials contain relatively much Fe and Al in easily weatherable minerals and little silica. Soils at both sites were of volcanic origin, mainly basalt, which are well drained and vary from dark red Nitisols, Ferralsols to dark brown Acrisols (GOK, 1997). These soils are generated from

ferralitilization process whereby desilication and build-up of high levels of sesquioxides (Al and Fe oxides) takes place (Rossuim et al., 1993).

Excessive rainfall leaches the soil basic elements such as calcium, magnesium, sodium and potassium leaving the soils concentrated with H^+ ions hence soil acidity. As well during organic matter decomposition hydrogen ions are produced which are a major cause of soil acidity. This contribution of soil acidity is significant for a short period (Nuhu, 2012). Harvesting of high yielding crops can also increase soil acidity. During growth, crops absorb basic elements such as calcium, magnesium and potassium to satisfy their nutritional requirements. As the crop yield increases more of these nutrients are removed from the soil (Nuhu, 2012). The use of ammonium based fertilizers contributes to soil acidity. This is through the nitrification of ammonia whereby H^+ ions are liberated (Kennedy, 1992).

Liming is a major way of adding basic materials to neutralize the acid present in the soil. Agricultural limestones are commonly used as liming materials. As lime dissolves in the soil, calcium moves to the surface of the soil particles replacing the H^+ ions. These ions react with the carbonate to form carbon dioxide and water. The resultant product is a soil that is less acidic (Natale et al., 2004).

Phosphorus levels at both sites were low because their levels of available P were below the critical value of 10 mg Kg^{-1} (Okalebo et al., 2002). Phosphorus is a component of the nucleic acid and so it plays a vital role in plant reproduction in which grain production is an important result (Plaster, 2003). It is also critical in biological energy transfer process that is vital for life and growth. Low availability of P in the soil is attributed to its slow

diffusion and fixation in soils. Apatites, strengite and variscite are the primary sources of mineral phosphorus. They are very stable and the release of available P from these minerals by weathering is generally too slow to meet the crop demands (Obura et al., 2003). Most of phosphorus is absorbed by plants in the form of primary orthophosphate (HPO_4^{2-}) and secondary orthophosphate ($H_2PO_4^-$). P is not low in the atmosphere and rarely does it leach beyond the reach of roots. It is immobile in the soil and its availability is related to soil pH (Ligeyo, 2007). In very acidic soils, Al and Fe are available to form insoluble phosphate compounds making phosphate less available.

Both Koyonzo and Ligala sites had very low nitrogen in the soil as their levels were below 0.2 %. Nitrogen can be lost from the soil surface through leaching where soluble NO_3^- move with soil water below the root zone. Nitrogen is also lost through volatilization as ammonia gas. And mainly occurs in manures and fertilizer products containing urea (Ahn, 1993). Nitrates can be converted back to nitrogen gas through denitrification process during nitrogen cycle. This process is amplified when soils are saturated with water for 2-3 days. Soil erosion and surface run off also contribute to removal of nitrogen in the soil system although not to a large extent. Practice of conservation tillage can minimize such losses (Dobermann, 2005). Removal of crop residue is another large contributor of N losses from the soil. Inadequate nitrogen in the soil leads to less uptake of N by plants and in turn essential processes like nucleic acid and DNA formation are interfered with. Less nitrogen in the plants slows down the rate of photosynthesis and therefore low crop yields.

Both study sites had low carbon content in the soil as each site recorded a value less than the critical level of 1.7 %. Some human practices can contribute to low carbon in the soil.

For example, burning of crop residues, which removes soil cover and leads to immediate and continuing losses of soil organic carbon. Overgrazing is another practice that eliminates organic carbon from the soils. These practices were common in the two study sites. The lower carbon content at Ligala compared to Koyonzo was attributed to presence of termites in that region which feed on maize stalks hence compromising on the carbon content of those soils (Waigwa, 2002).

Calcium in the two sites was very low as the values were below the critical value of 1.0 $Cmol_c\ Kg^{-1}$. Calcium is an important constituent of plant cell wall and can only be supplied in the xylem sap. It is also important in soil amendment and helps to maintain chemical balance in the soil. Calcium is not a leachable nutrient and deficiency symptoms can be seen on crops grown in soils that contain the insoluble forms of calcium such as calcium carbonate. High levels of other cations such magnesium, aluminium and potassium will reduce calcium uptake in some crops (Lippert, 2000)

Levels of magnesium at the study sites were moderate as they attained levels above the critical value of 0.3 $Cmol_c\ Kg^{-1}$ according to Okalebo et al. (2002). Magnesium is the central core of the chlorophyll molecule in plant tissues. It is also a co-factor vital in the function of specific enzyme systems (Branch, 2007). Magnesium is naturally obtained from parent rock material but the application of dolomitic limestone is the most cost effective method of supplying magnesium in the soil system (Ranjit et al., 2007).

5.2 Effect of lime on soil pH

Application of lime significantly increased soil pH above the control in the two sites. However, dolomitic and calcitic lime did not differ significantly in their effect on soil pH.

The soil pH rose a few weeks after lime application but was raised more during 2011 SR compared to 2011 LR. This can be attributed to the liming effect which with time releases Ca^{2+} and Mg^{2+} to replace the H^{+} , Al^{3+} and Mn^{+} ions by mass action hence raising the base saturation (Plaster, 2003). The pH increased gradually until the end of 2011 LR. After harvesting the first season crop, the pH dropped slightly then started rising at the beginning of the second season probably due to tillage operations that lead to the disturbance of soil particles, bringing them in contact with lime for further reaction. The finding on pH increase upon lime application agrees with those of Hunter et al. (1997) and Opala (2011). It has also been documentated that Ca^{2+} and Mg^{2+} alleviate soil acidity (Kochian, 1995). At Koyonzo site, the soil pH rose to 6.6 and hence the acidity was effectively corrected in that field. The soil pH at Ligala was raised up to 5.6, a level which is considered as moderately acidic. In this region liming did not completely eradicate soil acidity. This calls for more interventions either in terms of increasing quantity of lime used or application of alternative liming material that may be more effective such as quicklime and hydrated lime that have relatively higher neutralising strength (http://www.ctahr.hawaii.edu/mauisoil/c_acidity.aspx) compared to the limestones used in the present study.

5.3 Effects of lime on available phosphorus and soil nitrogen

Although P was uniformaly supplied as Triple Super Phosphate (TSP) to all experimanntal plots at the beginning of every season, available soil P increased significantly at the two sites as a result of lime application. This explains why there was a peak in available P in the soil at 60 DAS and 150 DAS. At this sampling period most of the fixed P had been released into the soil solution for plant uptake. In both seasons, at

harvesting, the levels of available P reduced because some of the P had been taken up by the plants. This result agrees with work done by Gentili and Huss-Danell, 2003. Phosphorus is one of the most limiting nutrient elements in SSA soil system and therefore any available phosphorus is readily taken up by the plants (Otinga, 2007).

There was gradual increase in soil N at the beginning of every season for both sites. Effective nodulation for most legumes such as groundnuts, soy beans, common beans and alfalfa starts at 42 DAS (Bottomley, 1995) and thereafter, the concentration of NO_3^- in the soil increases for plant uptake. In this study, the highest % of N in the soil was recorded at 60 DAS during the 2011 LR and 150 DAS during the 2011 SR. This was attributed to the fact that at this period there is maximum fixation of atmospheric nitrogen taking place. Towards the end of each cropping season, there was a sharp decline in the soil nitrogen because most of the nitrates had been taken up by plants. Moreover, nitrates are highly leached (Mupangwa and Tagwira, 2005), which could also explain the observed decline in the nitrogen content of the soils.

5.4 Effects of lime on calcium and magnesium in the soil

Application of Calcitic lime led to significantly high amounts of Ca in the soil compared to the other lime treatments. This is because calcitic lime contains a higher percentage of calcium ions (40 %) than dolomitic lime (22 %) (Sorenson and Butts). Both calcitic and dolomitic limes are pulverized limes that are applied to the soil to neutralize acidity (Whiteny et al., 1993). The major difference between the two limestones is their chemical composition where the degree of calcium carbonate and magnesium carbonate differ. Calcitic lime contains more than 90 % calcium carbonate and less than 10 % magnesium carbonate. Dolomitic lime contains 50-90 % calcium carbonate and 10-50 % magnesium

carbonate (Mwangi et al., 1999). The highest increase in the level of magnesium in the soil was realized in treatments where dolomitic lime was applied. Dolomitic lime has a higher percentage of magnesium (11 %) compared to calcitic lime (4.5 %). Hence the observed difference is attributed to the individual contributions from each of the liming materials. Some work done by Guo et al. (2010) reported similar results of increase in calcium content after soils were limed using calcitic lime while an increase in magnesium content when soils were limed using dolomitic lime.

5.5 Effects of experimental treatments on nodule number and weight

Generally lime application increased nodule number per plant. This could be explained by the pH raising effect of lime, creating a more conducive environment for nodualtion. The higher pH was favourable for root colonisation by rhizobia, which resulted in increased nodulation of the groundnut roots. These results support earlier work done by Fatima et al. (2006) and Hussain et al. (2008). These findings of increased nodulation after lime application and inoculation has also been reported by Guo et al. (2010) whose work was on lurcene. From lime contrast analysis, there were no significant differences between dolomitic and calcitic lime in influencing nodule number and weight. This means that in terms of providing a good pH for nodule formation and subsequent nitrogen fixation, both materials can be used interchangeably depending on their cost. Consequently, nodule number was directly proportional to nodule weight per plant whereby an increase in nodule number led to an increase in nodule weight per plant.

Inoculation significantly increased nodule number and weight. Usually, inoculation increases the population of rhizobia in the soil. With a large population of rhizobia in the soil, there are higher chances of root infection and colonisation hence more nodule

formation. Rhizobia A6w significantly gave high nodule number and weight. This is attributed to its high intrinsic ability to compete for nodule occupancy. It also had a higher acid and aluminum tolerance compared to the other rhizobia and hence made it to compete favorably. The indigenous rhizobia inoculants, A6w and V2w, perfomed better than the commercial inoculant (Biofix) in terms of nodule number while W1w did not differ significantly from biofix in nodulation effectiveness. Apparently the two outstanding indigenous rhizobia are better adapted to the environment of western Kenya, making them to have higher competitive ability to occupy nodules. Recent studies indicate close relationship between rhizobia and legume yields (Yakubu et al., 2010 and Yahui et al., 2011).

There were fewer nodules formed in the N treatment which was not significantly different from the control. These result shows that there was low rhizobia population in plots treated with N, minimizing infection of groundnut roots by rhizobia and subsequently limiting nodulation. Furthermore, high levels of mineral N in the soil hinder nodule formation (Hussain et al., 2008). Mineral N inhibits the rhizobia infection process and nitrogen fixation due to impairment of the recognition mechanism by nitrates (Fatima, 2007).

There were more nodules formed as a result of rhizobia inoculation in the second season (2011 SR) for both sites compared to the first season (2011 LR). This was probably as a result of increase in the population of inoculant rhizobia in the soil arising from the residual effect of inoculation from the first season and further inoculation in the second season. These results agree with work reported by Mahadkar and Saraf (1987).

Generally, Koyonzo had 13 % more nodule number per plant compared to Ligala.. This was attributed to the differences in soil fertility levels at the two sites. Phosphorus, nitrogen, carbon, calcium and magnesium were higher at Koyonzo compared to Ligala. These elements are very important for rhizobia survival (Bottomley, 1995). Further, Ligala site was more acidic compared to Koyonzo. Acidity limits rhizobia population growth and this fact may further explain why there were low numbers of nodules at this site.

5.6 Effect of experimental treatments on nitrogen fixation

Generally rhizobia inoculation led to higher amounts of nitrogen fixed. Approximately 14.67 kg N ha^{-1} was realised from indigenous rhizobia inoculant A6w when dolomitic lime was applied to ameliorate soil acidity. This was equivalent to 47.42 % of Ndfa and gave indication that a farmer can cut down on the cost needed to buy inorganic fertilizers to supply N into the soil almost by half. These results concure with those of Okito et al. (2004), although in their case more nitrogen (55 % Ndfa) was fixed.The difference is attributed to the fact that in Okito et al. (2004), a different soil type was used. They worked on a Nitisol which is characterised by higher fertility levels compared to the Ferralsols and Acrisols used in the present study.

Results from this study showed that the rhizobia strain A6w was the most superior of all the inoculants tested here for nitrogen fixation efficiency. This strain formed a larger number of effective nodules due to its competitive ability for nodule occupancy. The A6w strain fixed 27.6 % higher amount of nitrogen at Koyonzo than at Ligala and again this can be attributed to the relatively higher fertility status of the Koyonzo soils as

compared to Ligala. There were very low amounts of N fixed in plots where N fertilizer was applied and further the amount of N fixed in this treatment was not significantly different from that fixed in the control. According to Hussain et al. (2008), the presence of combined forms of nitrogen in the soil hinders the BNF process. Such evidence was also reported by Vanlauwe et al. (2000), Vern der Krift et al. (2001) and Wagner (2012).

5.7 Effect of experimental treatments on groundnut yields

Significantly higher yields were realised in plots where rhizobia inoculation was done. Inoculation increased rhizobia population which in turn occupied root nodules in high numbers and enhanced N fixation. Nitrogen is vital in chlorophyll formation. Improved N supply through BNF most likely contributed to more chlorophyll synthesis and an increased rate of photosythesis in groundnuts generating food reserves that were translocated to the kernels and added up to their dry matter content (Alam et al., 2005). There were higher groundnut yields due to higher N fixation at Koyonzo than at Ligala that translated to more DM of the kernels. At both sites groundnut yields were higher during 2011 SR than in the 2011 LR. This could be due to the residual effect of rhizobia inoculation which resulted in higher nitrogen fixation in the second season. This results concur with Rifat et al. (2008) who conducted a research on BNF of summer legumes and their residual effects on subsequent rainfed wheat yields. Numerous publications have indicated the necessity of legume inoculation with effective and efficient rhizobia strains especially when the soil is void of the specific rhizobia agents (Jensen and Hauggaard, 2003 and Verma et al., 2005).

The positive yield response of groundnuts to lime application can be attributed to P availability after liming. P is important in ATP formation and is very crucial for the

nitrogenase enzyme that facilitated the nitrogen fixation process (Coyne, 1999). Results also showed that dolomitic lime gave the highest groundnut yields compared to other lime treatments. At Koyonzo it gave 17.11% yield increase above the control while at Ligala it gave 13.01 % above the control. Yield response of groundnuts to calcitic lime was as a result of calcium being available within the 10 cm of top soil where most pods were concentrated. According to Sorenson and Butts, calcuim supply in the podding zone is critical for the production of quality kernels.

5.8 Effect of experimental treatments on maize yields

Both lime and rhizobia inoculation increased maize yields on both sites. Maize has extensive fibrous roots which tapped the nitrates into its root hairs for uptake. The nitrogen taken up was important for grain filling and chlorophyll formation which eventually translated to high DM content in maize grain. Enhanced nitrogen fixation by groundnut due to improved soil conditions via treatment application yielded nitrates that were also available for uptake by intercropped maize. However, maize yields in this experiment were lower than those reported by Thuita, (2007) who worked in Bungoma site in Western Kenya. This is because, in the present study, there was no external source of nitrogen for maize use but it entirely depended on N fixed by the groundnuts which was low compared to the FURP recommendations of 78 kg N ha $^{-1}$ for optimum maize yields. This finding on cereals benefiting from nitrogen fixed by legumes is related to work done by Trannin et al., 2000. Available evidence indicates that N could be transferred from legumes to the associated cereal plants in an intercropping system via pathways of roots and nodular tissue decay (Ta and Faris, 1988; Trannin et al., 2000), via

exuded compounds from legume plant roots (Tom et al., 1994) or via transfer from mycorrhizal fungus (Hamel and

Smith, 1991). Usually cereals have a stronger ability to absorb soil nutrients (Rynne et al., 1994) than legumes. Lime recorded yield increase of 131. 32 % and 98.76 % were recorded at Koyonzo and Ligala respectively. Rhizobia inoculation accounted for 80.96 % and 47.09 % yield increase at Koyonzo and Ligala respectively. These results of increased maize yields agree with those of Arnold and Wayne (2006).

5.9 Correlation between crop yields and soil nitrogen

There was a positve correlation between soil nitrogen and groundnut yields at the two sites for both seasons during the fourth sampling period (90 DAS). Nitrogen is a vital component of amino acids which are the building blocks of all proteins including enzymes that catalyze virtually all the biological processes. Nitrogen is critical for chlorophyll formation and its availability in the soil facilitates its uptake that later is translated into dry matter content and hence high yields.

5.10 Correlation between crop yields and soil available phosphorus

There was a positive correlation betweeen soil available P and groundnut yields. However, this was not the case in all the sites and seasons. Phosphorus is important for ATP formation which provides energy for N fixation process. Phosphorus is also required by plants for various life processes namely; energy transfer, constituent of nucleic acid and genetic code (Nyambati, 2000). Nitrogen fixing species require more P supply to sustain the plant related processes as well as the nitrogen fixation (Buresh et al., 1997). Increased available P contributed to enhanced fixation of nitrogen, overall plant growth

and development, culminating in higher yields. However, according to Sanginga et al. (1995) most of the N fixing legumes are able to use their internal P efficiently and may therefore not respond to external P application. The host plant has high P content in form of adenosine nucleotides needed to fuel the energy demanding nitrogenase reaction (Sprent and Raven 1985). This might explain the poor correlation between groundnut yield and soil available P in some cases. Also, soil available P and soil nitrogen showed a positive correlation with maize yields. These elements are critical for grain filling and there their availability at all sampling periods contributed to high maize yields.

Liming is necessary to ameliorate soil acidity. Cosequently inoculation of groundnut seeds with effective rhizobia strains increased nodule number per plant and eventually high levels of atmospheric nitrogen fixation. Liming also had positive effects on some chemical elements in the soil such as calcuim, magnesium phosphorus and organic carbon. Improved soil fertility has great influence on crop yields. High amounts of nitrogen fixed led to increased yields of groundnut and intercropped maize.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

1. Liming significantly led to increased soil pH, P, N, Ca and Mg at both sites. However there were no significant differences between calcitic and dolomitic lime in raising soil pH, P and N. Dolomitic lime significantly led to increased levels of magnesium in the soil as compared to calcitic lime. Similarly calcitic lime significantly led to increased levels of soil calcium than dolomitic lime.
2. Different indigenous rhizobia fixed significantly different amounts of nitrogen. A6w fixed the highest amount of N (14.67 kg N ha-1) at Koyonzo under dolomitic lime soil amendment, whereas, W1w fixed the lowest amount of N among the indigenous rhizobia (3.13 kg N ha-1 and 1.5 kg N ha-1 of Koyonzo and Ligala respectively) . All indigenous rhizobia strains fixed higher amounts of N compared to one commercial strain (Biofix) which fixed 2.87 kg N ha-1 at Koyonzo and 1.50 kg N ha-1 at Ligala.
3. Liming increased nodule number and weight per plant above the control. Liming also led to higher amount of nitrogen fixed however there was no significant difference between dolomitic and calcitic lime in enhancing amount of N fixed.
4. Both liming and inoculation increased both groundnut and maize yields. Significant differences existed among different rhizobia inoculants in enhancing crop yield.

6.2 Recommendations

1. Apply either calcitic or dolomitic lime at least one month prior to planting in soils diagonised as acidic to ameliorate soil acidity.
2. Inoculate groundnuts with indigenous rhizobial strain A6w prior to planting for high nitrogen fixation.
3. In acid soil amendment, it is recommended to use both dolomitic and calcitic lime interchangeably depending on their costs so as to enhance high amounts of nitrogen fixation.
4. The practice of liming acid soils prior to planting and groundnut seed inoculation with rhizobia A6w can be adopted for increased crop yield.

6.3 Areas of further research

1. Molecular characterization of the indigenous rhizobial used in this study
2. The indigenous rhizobial can be tested on their efficiency under low soil phosphorus

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APPENDICIES

Appendix I: ANOVA for nodule number for Koyonzo and Ligala during 2011 long rain.

Variate: Nodule number per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	79.0556	39.5278	46.42	
Block.*Units* stratum					
SITE	1	186.7037	186.7037	219.24	<.001
Lime	2	658.6667	329.3333	386.73	<.001
Inoculant	5	1832.3333	366.4667	430.33	<.001
SITE.Lime	2	2.7407	1.3704	1.61	0.207
SITE.Inoculant	5	32.0741	6.4148	7.53	<.001
Lime.Inoculant	10	472.3333	47.2333	55.47	<.001
SITE.Lime.Inoculant	10	4.4815	0.4481	0.53	0.866
LIME CONTRAST	2	658.67	329.33	13.10	<.001
Contrast Dolomitic lime vs calcitic lime	1	98.00	98.00	3.90	0.051
Contrast Dolomitic lime vs Control	1	242.00	242.00	9.62	0.002
Contrast Calciti lime vs Control	1	648.00	648.00	25.77	<.001
Residual		1032	590.28	25.15	
INOCULANT CONTRAST	5	1832.33	366.47	25.87	<.001
A6w vs Biofix	1	306.25	306.25	21.62	<.001
A6w vs Control	1	1225.00	1225.00	86.47	<.001
A6w vs N	1	950.69	950.69	67.11	<.001
A6w vs V2w	1	40.11	40.11	2.83	0.096
A6w vs W1w	1	266.78	266.78	18.83	<.001
Biofix vs Control	1	306.25	306.25	21.62	<.001
Biofix vs N	1	177.78	177.78	12.55	<.001
Biofix vs V2w	1	124.69	124.69	8.80	0.004
Biofix vs W1w	1	1.36	1.36	0.10	0.757
Control vs N	1	17.36	17.36	1.23	0.271
Control vs V2w	1	821.78	821.78	58.01	<.001
Contrl vs W1w	1	348.44	348.44	24.60	<.001
N vs V2w	1	600.25	600.25	42.37	<.001
N vs W1w	1	210.25	210.25	14.84	<.001
V2w vs W1w	1	100.00	100.00	7.06	0.009
Residual		100	1416.61	14.17	

Appendix II: ANOVA for nodule number for Koyonzo and Ligala during 2011 short rain.

Variate: Nodule number per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	10.436	5.218	4.15	
Block.*Units* stratum					
SITE	1	119.070	119.070	94.78	<.001
Lime	2	940.747	470.374	374.43	<.001
Inoculant	5	3260.580	652.116	519.10	<.001
SITE.Lime	2	0.407	0.203	0.16	0.851
SITE.Inoculant	5	21.083	4.217	3.36	0.009
Lime.Inoculant	10	672.381	67.238	53.52	<.001
SITE.Lime.Inoculant	10	2.500	0.250	0.20	0.996
Residual	70	87.937	1.256		
LIME CONTRAST	2	940.75	470.37	11.64	<.001
Dolomitic lime vs calcitic lime	1	46.08	46.08	1.14	0.288
Dolomitic lime vs control	1	858.36	858.36	21.23	<.001
calcitic lime vs Control	1	506.68	506.68	12.53	<.001
Residual		1034	163.96	40.43	
INOCULANT CONTRAST	5	3260.58	652.12	35.36	<.001
A6w vs Biofix	1	658.78	658.78	35.72	<.001
A6w vs Control	1	2031.00	2031.00	110.13	<.001
A6w vs N	1	1764.00	1764.00	95.66	<.001
A6w vs V2w	1	51.36	51.36	2.79	0.098
A6w vs W1w	1	544.44	544.44	29.52	<.001
Biofix vs control	1	376.36	376.36	20.41	<.001
Biofix vs N	1	266.78	266.78	14.47	<.001
Biofix vs V2w	1	342.25	342.25	18.56	<.001
Biofix vs W1w	1	5.44	5.44	0.30	0.588
Control vs N	1	9.40	9.40	0.51	0.477
Control vs V2w	1	1436.41	1436.41	77.89	<.001
Control vs W1w	1	472.34	472.34	25.61	<.001
N vs V2w	1	1213.36	1213.36	65.80	<.001
N vs W1w	1	348.44	348.44	18.89	<.001
V2w vs W1w	1	261.36	261.36	14.17	<.00
Residual		1001	844.13	18.44	

Appendix III: ANOVA for nodule number dry weight for Koyonzo and Ligala during 2011 long rain.

Variate: Nodule dry wt (mg)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	37.4246	18.7123	45.77	
Block.*Units* stratum					
SITE	1	41.1934	41.1934	100.77	<.001
Lime	2	207.4624	103.7312	253.75	<.001
Inoculant	5	1200.0105	240.0021	587.10	<.001
SITE.Lime	2	1.8135	0.9068	2.22	0.116
SITE.Inoculant	5	10.3727	2.0745	5.07	<.001
Lime.Inoculant	10	172.7065	17.2706	42.25	<.001
SITE.Lime.Inoculant	10	2.1754	0.2175	0.53	0.862
Residual		70	28.6154	0.4088	
LIME CONTRAST					
Dolomitic lime vs Calcitic lime	1	6.01	6.01	0.42	0.516
Dolomitic lime vs No lime	1	122.46	122.46	8.66	0.004
Calcitic lime vs No lime	1	182.72	182.72	12.92	<.001
Residual		103	1456.89	14.14	
INOCULANT CONTRAST	5	1200.010	240.002	51.69	<.001
A6w vs Biofix	1	286.174	286.174	61.63	<.001
A6w vs Control	1	712.890	712.890	153.53	<.001
A6w vs N	1	610.090	610.090	131.39	<.001
A6w vs V2w	1	12.134	12.134	2.61	0.109
A6w vs W1w	1	316.247	316.247	68.11	<.001
Biofix vs Control	1	95.714	95.714	20.61	<.001
Biofix vs N	1	60.580	60.580	13.05	<.001
Biofix vs V2w	1	180.454	180.454	38.86	<.001
Biofix vs W1w	1	0.751	0.751	0.16	0.688
Control vs N	1	4.000	4.000	0.86	0.356
Control vs V2w	1	539.014	539.014	116.08	<.001
Contrl vs W1w	1	79.507	79.507	17.12	<.001
N vs V2w	1	450.147	450.147	96.94	<.001
N vs W1w	1	47.840	47.840	10.30	0.002
V2w vs W1w	1	204.490	204.490	44.04	<.001
Residual		100	464.339	4.643	

Appendix IV: ANOVA for nodule dry weight for Koyonzo and Ligala during 2011 short rain.

Variate: Nodule dry wt plant (mg)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	34.881	17.440	10.51	
Block.*Units* stratum					
SITE	1	19.593	19.593	11.80	<.001
Lime	2	179.486	89.743	54.07	<.001
Inoculant	5	1235.347	247.069	148.85	<.001
SITE.Lime	2	0.359	0.180	0.11	0.898
SITE.Inoculant	5	9.552	1.910	1.15	0.342
Lime.Inoculant	10	172.718	17.272	10.41	<.001
SITE.Lime.Inoculant	10	2.513	0.251	0.15	0.999
Residual		70	116.193	1.660	
LIME CONTRAST	2	179.49	89.74	5.94	0.004
Dolomitic lime vs calcitic lime	1	0.36	0.36	0.02	0.877
Dolomitic lime vs control	1	141.40	141.40	9.36	0.003
calcitic lime vs Control	1	127.47	127.47	8.44	0.005
Residual		1031	556.27	15.11	
INOCULANT CONTRAST	5	1235.347	247.069	49.37	<.001
A6w vs Biofix	1	206.880	206.880	41.34	<.001
A6w vs Control	1	679.471	679.471	135.78	<.001
A6w vs N	1	522.123	522.123	104.34	<.001
A6w vs V2w	1	0.071	0.071	0.01	0.905
A6w vs W1w	1	217.071	217.071	43.38	<.001
Biofix vs control	1	136.500	136.500	27.28	<.001
Biofix vs N	1	71.684	71.684	14.33	<.001
Biofix vs V2w	1	214.623	214.623	42.89	<.001
Biofix vs W1w	1	0.122	0.122	0.02	0.876
Control vs N	1	10.347	10.347	2.07	0.154
Control vs V2w	1	693.444	693.444	138.57	<.001
Control vs W1w	1	128.444	128.444	25.67	<.001
N vs V2w	1	534.380	534.380	106.79	<.001
N vs W1w	1	65.880	65.880	13.17	<.001
V2w vs W1w	1	225.000	225.000	44.96	<.001
Residual		100	500.413	5.004	

Appendix V: ANOVA for % Ndfa for Koyonzo and Ligala during 2011 long rain.

Variate: % Ndfa

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	1	117.479	117.479	41.79	
Block.*Units* stratum					
SITE	1	513.334	513.334	182.58	<.001
Lime	2	606.285	303.142	107.82	<.001
Inoculant	5	10511.298	2102.260	747.73	<.001
SITE.Lime	2	1.364	0.682	0.24	0.786
SITE.Inoculant	5	132.970	26.594	9.46	<.001
Lime.Inoculant	10	557.292	55.729	19.82	<.001
SITE.Lime.Inoculant	10	35.603	3.560	1.27	0.286
Residual	35	98.403	2.812		
LIME CONTRAST	2	606.3	303.1	1.74	0.183
Calcitic lime vs Dolomitic lime	1	20.7	20.7	0.12	0.732
Calcitic lime vs No lime	1	349.1	349.1	2.00	0.162
Dolomitic lime vs No lime	1	349.1	349.1	2.00	0.162
Residual	681	1850.3	174.30		
INOCULANT CONTRAST	5	10511.30	2102.26	70.25	<.001
A6w vs Biofix	1	3576.07	3576.07	119.49	<.001
A6w vs control	1	6191.45	6191.45	206.89	<.001
A6w vs N	1	5579.98	5579.98	186.45	<.001
A6w vs V2w	1	275.54	275.54	9.21	0.003
A6w vs W1w	1	3741.50	3741.50	125.02	<.001
Biofix vs control	1	356.66	356.66	11.92	<.001
Biofix vs N	1	221.98	221.98	7.42	0.008
Biofix vs V2w	1	1866.31	1866.31	62.36	<.001
Biofix vs W1w	1	1.87	1.87	0.06	0.803
Control vs N	1	15.89	15.89	0.53	0.469
Control vs V2w	1	3854.72	3854.72	128.80	<.001
Control vs W1w	1	306.88	306.88	10.25	0.002
N vs V2w	1	3375.59	3375.59	112.79	<.001
N vs W1w	1	183.10	183.10	6.12	0.016
V2w vs W1w	1	1986.35	1986.35	66.37	<.001
Residual	65	1945.25	29.93		

Appendix VI: ANOVA for groundnut yields for Koyonzo and Ligala during 2011 long rain.

Variate: groundnut yield (kg ha^{-1})

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	162832.06	81416.03	1267.43	
Block.*Units* stratum					
SITE	1	370773.93	370773.93	5771.94	<.001
Lime	2	112628.17	56314.08	876.66	<.001
Inoculant	5	1851015.44	370203.09	5763.05	<.001
SITE.Lime	2	32.57	16.29	0.25	0.777
SITE.Inoculant	5	242.07	48.41	0.75	0.586
Lime.Inoculant	10	78110.06	7811.01	121.60	<.001
SITE.Lime.Inoculant	10	296.09	29.61	0.46	0.909
Residual		70	4496.61	64.24	
LIME CONTRAST	2	112628	56314	2.52	0.086
Dolomitic lime vs Calcitic lime	1	14196	14196	0.63	0.428
Dolomitic lime vs No lime	1	45000	45000	2.01	0.159
Calcitic lime vs No lime	1	109746	109746	4.90	0.029
Residual		103	2304967	22378	
INOCULANT CONTRAST	5	1851015	370203	65.34	<.001
A6w vs Biofix	1	593413	593413	104.74	<.001
A6w vs Control	1	1191008	1191008	210.21	<.001
A6w vs N	1	37507	37507	6.62	0.012
A6w vs V2w	1	50325	50325	8.88	0.004
A6w vs W1w	1	623047	623047	109.97	<.001
Biofix vs Control	1	103041	103041	18.19	<.001
Biofix vs N	1	332544	332544	58.69	<.001
Biofix vs V2w	1	298116	298116	52.62	<.001
Biofix vs W1w	1	361	361	0.06	0.801
Control vs N	1	805805	805805	142.22	<.001
Control vs V2w	1	751689	751689	132.67	<.001
Contrl vs W1w	1	91204	91204	16.10	<.001
N vs V2w	1	940	940	0.17	0.685
N vs W1w	1	354819	354819	62.62	<.001
V2w vs W1w	1	319225	319225	56.34	<.001
Residual		100	566580	5666	

Appendix VII: ANOVA for groundnut yields for Koyonzo and Ligala during 2011 short rain.

Variate: groundnut yield (kg ha^{-1})

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	123065	61532	18.38	
Block.*Units* stratum					
SITE	1	52272	52272	15.61	<.001
Lime	2	105803	52902	15.80	<.001
Inoculant	5	1978300	395660	118.17	<.001
SITE.Lime	2	6151	3075	0.92	0.404
SITE.Inoculant	5	17891	3578	1.07	0.385
Lime.Inoculant	10	116314	11631	3.47	<.001
SITE.Lime.Inoculant	10	31162	3116	0.93	0.511
Residual		70	234382	3348	
LIME CONTRAST	2	105803	52902	2.24	0.112
Dolomitic lime vs calcitic lime	1	30053	30053	1.27	0.262
Dolomitic lime vs control	1	105647	105647	4.47	0.037
calcitic lime vs Control	1	23005	23005	0.97	0.326
Residual		103	2436472	23655	
INOCULANT CONTRAST	5	1978300	395660	70.16	<.001
A6w vs Biofix	1	753424	753424	133.59	<.001
A6w vs Control	1	1204872	1204872	213.64	<.001
A6w vs N	1	37313	37313	6.62	0.012
A6w vs V2w	1	47597	47597	8.44	0.005
A6w vs W1w	1	604247	604247	107.14	<.001
Biofix vs control	1	52747	52747	9.35	0.003
Biofix vs N	1	455400	455400	80.75	<.001
Biofix vs V2w	1	422283	422283	74.88	<.001
Biofix vs W1w	1	8220	8220	1.46	0.230
Control vs N	1	818120	818120	145.06	<.001
Control vs V2w	1	773520	773520	137.15	<.001
Control vs W1w	1	102613	102613	18.19	<.001
N vs V2w	1	625	625	0.11	0.740
N vs W1w	1	341251	341251	60.51	<.001
V2w vs W1w	1	312667	312667	55.44	<.001
Residual		100	563976	5640	

Appendix VIII: ANOVA for maize yields for Koyonzo and Ligala during 2011 long rains

Variate: maize yield ($kgha^{-1}$)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	105816.2	52908.1	149.04	
Block.*Units* stratum					
SITE	1	171203.7	171203.7	482.27	<.001
Lime	2	1195550.1	597775.0	1683.89	<.001
Inoculant	5	9563505.3	1912701.1	5387.94	<.001
SITE.Lime	2	679.1	339.6	0.96	0.389
SITE.Inoculant	5	13414.4	2682.9	7.56	<.001
Lime.Inoculant	10	469678.6	46967.9	132.31	<.001
SITE.Lime.Inoculant	10	1998.4	199.8	0.56	0.838
Residual		70	24849.8	355.0	
LIME CONTRAST	2	1195550	597775	6.01	0.003
Dolomitic lime vs Calcitic lime	1	716	716	0.01	0.933
Dolomitic lime vs No lime	1	870980	870980	8.76	0.004
Calcitic lime vs No lime	1	921629	921629	9.27	0.003
Residual		103	10245329.	99469	
INOCULANT CONTRAST	5	9563505	1912701	101.88	<.001
A6w vs Biofix	1	3396035	3396035	180.89	<.001
A6w vs Control	1	5597167	5597167	298.14	<.001
A6w vs N	1	243378	243378	12.96	<.001
A6w vs V2w	1	87025	87025	4.64	0.034
A6w vs W1w	1	3013696	3013696	160.53	<.001
Biofix vs Control	1	273529	273529	14.57	<.001
Biofix vs N	1	1821150	1821150	97.01	<.001
Biofix vs V2w	1	2395788	2395788	127.61	<.001
Biofix vs W1w	1	11413	11413	0.61	0.437
Control vs N	1	3506256	3506256	186.76	<.001
Control vs V2w	1	4288351	4288351	228.42	<.001
Contrl vs W1w	1	396690	396690	21.13	<.001
N vs V2w	1	39336	39336	2.10	0.151
N vs W1w	1	1544220	1544220	82.25	<.001
V2w vs W1w	1	2076481	2076481	110.61	<.001
Residual		100	1877374.	18774	

Appendix IX: ANOVA for maize yields for Koyonzo and Ligala during 2011 short rain

Variate: maize yield (kg ha^{-1})

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	110373.4	55186.7	127.29	
Block.*Units* stratum					
SITE	1	300516.8	300516.8	693.15	<.001
Lime	2	1322256.7	661128.3	1524.91	<.001
Inoculant	5	25020413.6	5004082.7	11542.06	<.001
SITE.Lime	2	13.7	6.9	0.02	0.984
SITE.Inoculant	5	37.5	7.5	0.02	1.000
Lime.Inoculant	10	385536.5	38553.7	88.93	<.001
SITE.Lime.Inoculant	10	81.5	8.1	0.02	1.000
Residual		70	30348.6	433.6	
LIME CONTRAST	2	1322257	661128	2.65	0.076
Dolomitic lime vs calcitic lime	1	2178	2178	0.01	0.926
Dolomitic lime vs control	1	1037040	1037040	4.15	0.044
calcitic lime vs Control	1	944167	944167	3.78	0.055
Residual		103	25736948	249873	
INOCULANT CONTRAST	5	25020414	5004083	245.44	<.001
A6w vs Biofix	1	3428052	3428052	168.14	<.001
A6w vs Control	1	18176011	18176011	891.51	<.001
A6w vs N	1	248004	248004	12.16	<.001
A6w vs V2w	1	85264	85264	4.18	0.043
A6w vs W1w	1	3032242	3032242	148.73	<.001
Biofix vs control	1	5816940	5816940	285.31	<.001
Biofix vs N	1	1831962	1831962	89.86	<.001
Biofix vs V2w	1	2432040	2432040	119.29	<.001
Biofix vs W1w	1	12137	12137	0.60	0.442
Control vs N	1	14177735	14177735	695.40	<.001
Control vs V2w	1	15771488	15771488	773.57	<.001
Control vs W1w	1	6360484	6360484	311.97	<.001
N vs V2w	1	42436	42436	2.08	0.152
N vs W1w	1	1545878	1545878	75.82	<.001
V2w vs W1w	1	2100567	2100567	103.03	<.001
Residual		100	2038791	20388	

Appendix X: Procedures for some selected soil elements

Determination of soil pH

The soil pH was determined by adding 25ml of distilled water to 10g of soil (<2 mm) in a beaker and the suspension stirred for 10 minutes and then stirred again for 2 minutes. The soil pH was then measured using a glass electrode on a pH meter (Okalebo et al, 2002).

Soil particle size analysis

Soil particle analysis was done using the procedure of sedimentation that involves the dispersion of soil particles into constituents using sodium hexametaphosphate (calgon) solution and subsequent sedimentation of particles. Sedimentation allows the particles to settle to the bottom of the cylinder according to size, density and the viscosity of the fluid (Stokes law). After 2 hours 50 g of air-dried soil (<2 mm) was weighed into a 500ml beaker, 10 ml of calgon was added after the soil had been saturated with distilled water and the mixture allowed to stand for ten minutes. The suspension was then quantitatively transferred into a string cup where further dispersion was done using an electric high speed stirrer for two minutes. The suspension was then transferred in to a graduated cylinder and topped with distilled water up to the 1130ml mark. These contents were covered well and inverted ten times and a hydrometer inserted and the first reading taken at 40 seconds (H_1). Then the contents were inverted again ten times and allowed to stand for two hours and the hydrometer left in the cylinder. A second hydrometer reading (H_2)

was taken at 2 hour timing. Temperature reading was taken concurrently with both hydrometer readings.

Calculation

$$\% \text{ sand} = \left(\frac{50.0 - H_1}{50}\right) \times 100$$

$$\% \text{ clay} = \frac{H_2 \times 100}{50}$$

% silt = 100 (% sand + % clay)

A textural triangle was used to assign the textural class of the soil

Available phosphorus

Soil extraction for available P was done using the bicarbonate solution (0.5 M NaHCO3 at pH 8.5) method (Olsen et al, 1954). The bicarbonate extractant decreases the concentration of Ca as CaCO3 in the calcareous, alkaline and neutral soils containing calcium phosphates. The result is an increase of the P concentration in the solution. In acid soils containing Al and Fe phosphates, P concentration in the solution increases as the pH rises. Precipitation reactions in acid and calcareous soils are reduced to a minimum because the concentration of Al, Fe and Ca remain at low levels in this extractant solution. P was then measured calorimetrically using a spectrophotometer after the development of a blue colored phosphomolybdate complex.

Colometric P measurements

The available P was determined by adding 10ml of each P standard solution (0, 0.5, 1, 2.5, 5.0, 7.5, 10.0 and 12.5 ppm P), sample filtrate and reagent blanks into 50 ml volumetric flasks. To suppress the interference of fluorides and sulphates, 5ml of 0.8 M boric acid was added into each flask. 10 ml of ascorbic acid reducing agent was added and the flasks topped using distilled water to the 50ml mark and shaken well. After 1 hour, the absorbance was read at 880 nm (Murphy and Riley, 1962). Concentration of P ppm P in soil = concentration of P in solution x 100.

Digestion procedure for total N and P in plants and soil

The principle involved in the digestion of plant and soil materials is oxidation of the organic material into soluble N and P components (NH_4 and phosphate) in H_2SO_4 /Se/$LiSO_4$/H_2O_2 digestion mixture. Hence, 0.3g of dry ground plant material (20 mesh) or soil was weighed into a dry and labeled digestion tube and 4.4 ml of the digestion mixture was added including two reagent blanks for each batch of samples. The mixture was then digested slowly on a block digester up to a temperature of 360' C for three hours until the solution is clear and allowed to cool. It was then quantitatively transferred into 50ml volumetric flasks and topped to the mark with distilled water and transferred into 75 ml storage bottles. The mixtures were used to determine both total P and N.

Determination of total N from sample digests

It was done using the colometric method. In a clean set of 50 ml volumetric flasks 0, 5, 10, 15, 20 and 25 ml of the standard solution was added. (100 µg NH4 +/ml). 0.2 ml of the sample was pipetted using a micropipette into clearly marked test tubes. 5 ml of the reagents N1 (made by dissolving 34g of sodium salicylate, 25g of sodium citrate and 25g

of sodium citrate in about 750 ml of distilled water). 0.12g of sodium nitroprusside was then added and shaken well and topped to make 1000ml with distilled water and allowed to stand for fifteen minutes. Then five ml of reagent nitrogen (prepared by dissolving 30g of NaOH in 750 ml of distilled water) was added and well shaken. Absorbance was read at 655 nm after standing for one hour for color development.

N% = (C x W) x 0.01

Where C = corrected concentration (µg/ml)

W = weight of sample

Determination of calcium and magnesium in the soil

5 g of air dry soil (< 2 mm) was weighed into a clean plastic bottle with a stopper. 100 ml of 1 M (NH_4OAc) ammonium acetate solution (pH 7) was then added and the contents shaken for 30 minutes and filtered through No. 42 whatman paper. This is the soil extract *A* that was used for calcium and magnesium determinations. For determination of calcium solution A was diluted 10 times. 5 ml of the soil extract solution A was pipetted into a 50 ml volumetric flask. 1 ml of 26.8% lanthanum chloride solution was added and the contents diluted to the mark with 1M NH_4OAc extraction solution. The solutions were sprayed into the atomic absorption spectrophotometer flame for Ca measurement.

For determination of magnesium, soil extract solution *A* was diluted 25-fold. 2 ml of the soil extract solution A was pipetted into a 50 ml volumetric flask. 5 ml of 5000 ppm Sr as $SrCl_2$ was added and filled up to the mark with the 1 M NH_4OAc extracting solution. The solutions were sprayed into the flame of the atomic absorption spectrophotometer.

$$\% \text{ Mg and } \% \text{ Ca} = \frac{(a-b) * v * f * 1000}{}$$

$$1000^{*}\ w$$

Where;

a = concentration of calcium and magnesium in the soil samples

b = concentration in the blank extract

v = volume of extracting solution

w = weight of soil samples

f = dilution factor