KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

FACULTY OF RENEWABLE NATURAL RESOURCES

DEPARTMENT OF AGROFORESTRY

INITIAL GROWTH OF *TETRAPLUERA TETRAPTERA* (SCHUM AND THONN.) AS INFLUENCED BY SOILS FROM DIFFERENT LAND USE SYSTEMS

BY

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B.ED. AGRICULTURE

FEBUARY, 2015

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, KWAME NKRUMA UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, IN PARTIAL FULFILMENTOF THE REQUIREMENTS FOR THE AWARD OF MSc. AGROFORESTRY DEGREE

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DECLARATION

I hereby declare that except for the references of other people's work which has been duly cited, the content of this research work submitted as a thesis to the School of Graduate Studies, Kwame Nkrumah University of Science and Technology, Kumasi for the award of MSc Agroforestry degree is my own investigation.

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DEDICATION

I dedicate this thesis to:

The Almighty God for the gift of life, knowledge and resilience

My fiancée Yayra Gbeze and our future children

ACKNOWLEDGEMENT

My first gratitude goes to my supervisor Dr. Kwame Twum-Ampofo for the patience, advice, constructive criticism and research concept without which this research would not have been possible. I also acknowledge the immense intellectual support and spiritual guidance of Dr. Emmanuel Ebanyenle.

My sincere gratitude goes to Mr. Kofi Attakora, the farm technician and Messrs Abugri Adongo and Francis Hackman, the farm labourers for their field assistance. I will also like to thank Frans Mensah and Emmanuel Sarpong of Forest Research Institute of Ghana (FORIG) library who made me have access to important books and literature that made this work possible. My heartfelt appreciation goes to Dr. Nana Ewusi-Mensah and Ayamah Azumah who helped in the staining and assessment of arbuscular mycorrhiza in the roots of seedlings. The help of Mr. Samuel Joe Acquah of the soil laboratory of Faculty of Agriculture, KNUST is deeply appreciated.

My heartfelt gratitude goes to my parents Charles Agbotui and Beatrice Kpodo for their financial support, encouragement and prayers when times were tough. To my senior brothers ProdeoAgbotui, Cyril Boateng and Selorm Tagbor, thanks a lot for the financial support, encouragement and setting the pace for me to follow keenly. My special appreciation goes to Theodora Agbotui and Gladys Asobila-Boateng for the care and pampering during the course of my study. I really appreciate the company of Fred Ajabeng, Kennedy Abagye, Maxwell Mensah, Courage Agudze, Robinson Gbeze, Solomon Gbeze, Mawufemor Tagbor and Sefakor Dzereke for putting smiles on my face and making me have lots of fun during the course of my study.

ABSTRACT

Seedling remains the most vulnerable stage of a tree life cycle. This study aimed at assessing the growth performance of *Tetrapluera tetraptera* seedlings in soils from different land use systems. The experiment was laid in Randomized Complete Block Design with four replicates. The treatments were soils collected from forest reserve, surface mine, farm and teak plantation. Growth parameters that that were measured were height, diameter, sturdiness quotient, relative growth rate (height and diameter) and plant dry weight. The soils used differed in their effectiveness in promoting the growth of seedlings. Seedlings in reserve and farm soil had statistically greater height and relative height growth rate than those in teak and mined soil. Diameter and relative diameter growth rate of seedlings in farm and reserve soils were significantly higher ($P < 0.001$) than those in teak and mined soils. The soils used had a significant effect ($P < 0.001$) on shoot, root and total seedling dry weights. Farm and reserve soil produced seedlings whose dry weights were significantly higher than the other soils. Seedlings from mined, reserve and farm soils had significantly higher nitrogen concentration in all plant parts. Reserve soil produced seedlings with statistically higher plant parts nitrogen uptake and the least was recorded in mined soil. The soils used had a significant effect on plant phosphorus uptake with the higher values been recorded in farm soil and the least in the mined soil. Seedlings in farm and reserve soils had statistically ($P < 0.01$) higher nitrogen and phosphorus use efficiency than those in teak and mined soils. Percentage mycorrhiza colonization was positively correlated with plant total nitrogen concentration ($r = 0.800$, $P < 0.05$). The study has shown that *Tetrapluera tetraptera* seedlings growing in farm and reserve soils exhibit fast growth rate with efficient nutrient acquisition and utilization.

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CHAPTER ONE

INTRODUCTION

Ghana has one of the highest rate of deforestation in West Africa (Benhin and Barbier, 2001) and the major causes have been associated with population growth, shifting cultivation, unsustainable logging, mining, wildfires, fuelwood and charcoal production and plantation establishment (Appiah *et al*., 2009; Cudjoe and Dzanku, 2009). The major identified problems associated with deforestation are soil erosion, climate change, flooding, drought, sedimentation of water bodies, soil erosion and loss of biodiversity (Boahene, 1998).

Soil erosion the most common form of land degradation due to continuing reduce forest cover is threathening sustainable food production and poverty reduction in Ghana (Folly, 1997; Diao and Sarpong, 2007). Estimates of Diao and Sarpong (2007) suggest that soil erosion reduces agricultural income in Ghana by a total of US $ 4.2 billion equivalent to approximately 5% of the total agricultural gross domestic product over the period 2006 - 2015 and also contributes to 5.4% increase in poverty rate in the year 2015.

In the wake of climate change and land degradation, agroforestry systems have been recommended as a sustainable land use methoddue to their ability to supply woody products, conserve biodiversity, sequester carbon and conserve soil (Young, 1989; Piotto *et al.*, 2003; Montagnini, 2005). The fight against climate change in Ghana is important because, the country's economy is highly dependent on agriculture. Since majority of our food production systems are rain fed, directly climate change may influence agriculture by increasing water and heat stress and outbreak of pests and diseases (De Pinto *et al*., 2012). Aside the production of food, fuelwood and medicines, agroforestry offers the environmental service of carbon

sequestration. The inclusion of trees on agricultural landscapes makes it possible for such trees to store more carbon from the atmosphere into their living biomass and the soil (Soto-Pinto *et al*., 2010; Tumwebaze *et al*., 2012).

The success of agroforestry mostly depends on the use of leguminous trees due to their ability to fix atmospheric nitrogen, symbiosis with mycorrhiza and survive in extreme soil conditions (Young, 1989). This attribute improves the nutrient cycling of cropping systems thereby reducing the demand for fertiliser (Young, 1989), making agroforestry the best option for resource poor farmers on fragile and marginal lands in Ghana. However, the widely known, used and studied leguminous trees are exotic with *Leucaena leucocephala*, *Gliricidia sepium* and *Cassia spp*. taking the forefront. The dangers with the use of these exotics is their high potential of becoming invasive species, changing soil physical, biological and chemical soil conditions and supplying farmers with limited non-timber products (Senbeta *et al*., 2002). They also require high financial investments through increase dependency on external seed sources and foreign technologies (Plath *et al*., 2011).

One indigenous leguminous tree, *Tetrapluera tetraptera* found mostly in traditional agroforestry systems and in the wild (Anglaaere, 2005; Omokhua and Ukoimah, 2008) holds alot of promise to be used as a replacement or in combination with this exotics. Observation of nodulation in mature trees in native forests (Diabate *et al*., 2005) has made it to be regarded as having a nitrogen fixing potential (Darwin Initiative, 2000). The fruit is used as spice for preparing soup and flavouring locally manufactured soap and palm wine (Irvine, 1961; Orwa *et al*., 2009). The wood is used for buildings, carvings, boat and manufacture of plywoods (Irvine, 1961; Oteng-Amoako *et al*., 2000). Most importantly, the leaves, fruit and barks are used for the treatment of various ailments such as bilharzia, gonorrhoea, asthma, head and somach aches (Noamesi *et al*.,

1994; Aladesanmi, 2007; Sonibare and Gbile, 2008; Lekana-Douki *et al*., 2011). The deep rooting system and wide spreading canopy has made it to be used in cocoa agroforests (Anglaaere, 2005). The tree has been idenfied to be fast growing (Addo-Danso, 2010), producing lots of litter.

The success of *T. tetrapluera* to be used deliberately in cropping and restoration systems relies on the survival of seedlings in the planted sites. However, the problem that exists is that seedlings remain the most vulnerable during the plant life cycle (Holl, 1998). Blay (1997) observed that the saplings of *T. tetraptera* are frequently destroyed by farmers to prevent casting shade on crops. Deforested soils are not only low in nitrogen, phosphorus and bases such as magnessium, calcium and potassium but also have low pH (Setiadi, 2000). If a particular nutrient is deficient, seedlings may compensate to some extent by increasing capacity to take up the deficient ion but with the deficiency of several necessary nutrients then growth of seedlings is retarded leading to failure of agroforestry systems (Hossain, 2012).

Aside nutrient availability, other important soil factors that influence growth of leguminous seedlings in tropical soils are pH, mycorrhiza and rhizobia (Masutha *et al*., 1997; Binkley and Giardina, 1997; Twumasi, 2005). Most trees in the tropics have been identified to have arbuscular mycorrhiza fungi (AMF) association (Le Tacon *et al*., 1987). AMF improves the growth of seedlings in degraded soils by increasing the absorption of highly immobile nutrients especially phosphorus which is very important for the nodulation of leguminous trees (Twumasi, 2005; Cardoso and Kuyper, 2006; Twum-Ampofo, 2008).

Encouraging the use of *T. tetraptera* as an alternative to commonly used exotic leguminous trees must be preceded by research in order to identify seedlings response in terms of growth, AMF

association and nodule development in soils from different land use systems in order to prevent or reduce their failure when incorporated in cropping and restoration schemes.

1.1 Objectives

The objectives of the study were to;

(i) determine the growth of *T. tetraptera* seedlings in degraded forest soils.

(ii) evaluate the nutrients uptake and use efficiency of *T. tetraptera* in degraded soils.

(iii) establish the correlation between root mycorrhiza association and both seedling morphological and physiological characteristics.

CHAPTER TWO

LITERAURE REVIEW

2.1 Leguminosae

With about 750 genera and close to 20,000 species in terms of importance to man, this family is second only to the Gramineae (Allen and Allen, 1981). A common characteristic of members within this family is the production of pods. The family is believed to have evolved 59 million years ago (Lavin *et al.*, 2005). It is divided into three sub families: Caesalpinioideae,Mimosoideae and Papilionoideae (Franco and Faria, 1997; Sprent and Parsons, 2000).

Comprising of 12,215 – 13,792 species, most of the nodulating species are found in the Papilionoideae (Franco and Faria, 1997). It is only the tribe Dipterygeae whichhas no nodulating species (Franco and Faria, 1997). Two important genera found in this group are the Dalbergia, with about 100 species and Erythrina with 112 species (Sprent and Parsons, 2000). The genus Gliricidia also belongs to these family but has only small number of species. Mimosoideae has 2,506 – 2,920 species with approximately 90% nodulating (Franco and Faria, 1997) with exceptions such as the genus Adenanthera which does not nodulate (Sprent and Parsons, 2000). This sub-family include the genus Acacia and *Leucaena leucocephala* which are very important in most agroforestry systems.

The sub-family Caesalpinioideae has 2,716 − 2,816 species with only 23% nodulating and fixing nitrogen with rhizobia (Franco and Faria, 1997).

2.2 Description of *Tetrapluera tetraptera* (schum and thonn.) Taub

Tetrapluera tetraptera belongs to the family mimosaceae. The generic name originates from a Greek word meaning "four ribs" referring to the ribbed fruits (Orwa *et. al*, 2009).

2.2.1 Distribution and Local Names

The tree falls within the Guineo-Congolian phytoecological region, therefore stretching from Senegal to Democratic Republic of Congo to Uganda and Sudan (Irvine, 1961; Hall and Swaine, 1981; Oteng-Amoako *et al*., 2000). In Ghana, it is found in the moist evergreen, moist and dry semi-deciduous forests (Oteng-Amoako *et al*., 2000), growing and producing fruits very well in the Lophira-Triplochiton association (Taylor, 1960). Figure 2.1 shows the natural distribution of *T. teraptera* in the High Forest Zone of Ghana.

Because it is widespread across West Africa, various ethnic groups have got various local names for this plant. In Nigeria, the Yoruba refer to the plant as Aridan whereas the Igbo's call it Oshosho (Omokhua and Ukoimah, 2008). In Ghana, the Nzema's call it Epelekese, the Fanti's Esem and the Akans call it Prekese. Prekese is the most popular name for the plant in Ghana and according to Taylor (1960), the name Prekese originates from the sound of the seeds when the fruit is shaken.

2.2.2 Botany

In Ghana based on height, two forms of *T. teraptera* exist. The short type (regarded by local people as female) reaches a height of 20 m with a girth of 1 m and the tall type (regarded by the local people as male) reaches a height of 35 m and a girth of 2 m (Oteng-Amoako *et al*., 2000).

In the forest, the crown is small and rounded becoming flat when the tree grows, however in the open the crown spreads (Taylor, 1960; Orwa *et. al*., 2009). The bark of the tree is smooth, thin

and silvery grey to reddish. The leaves of are bipinnate with 12 pairs of alternating leaflets on each pinnae. The leaflet is oblong rounded apex and the base is slightly marginated.

Flowers are pinkish-cream turning to orange and are either solitary or paired in upper leaf axils or terminal racemens. The tree bears shiny dark brown to black indehiscent fruits hanging at the ends of branches on short and stout stalks. The fruits are characterised distinctively by four longitudinal wing-like ridges which are perpendicular to each other. The fruits harbour small black, flat and hard seeds which rattle in the fruits when shaken (Oteng-Amoako *et al*., 2000).

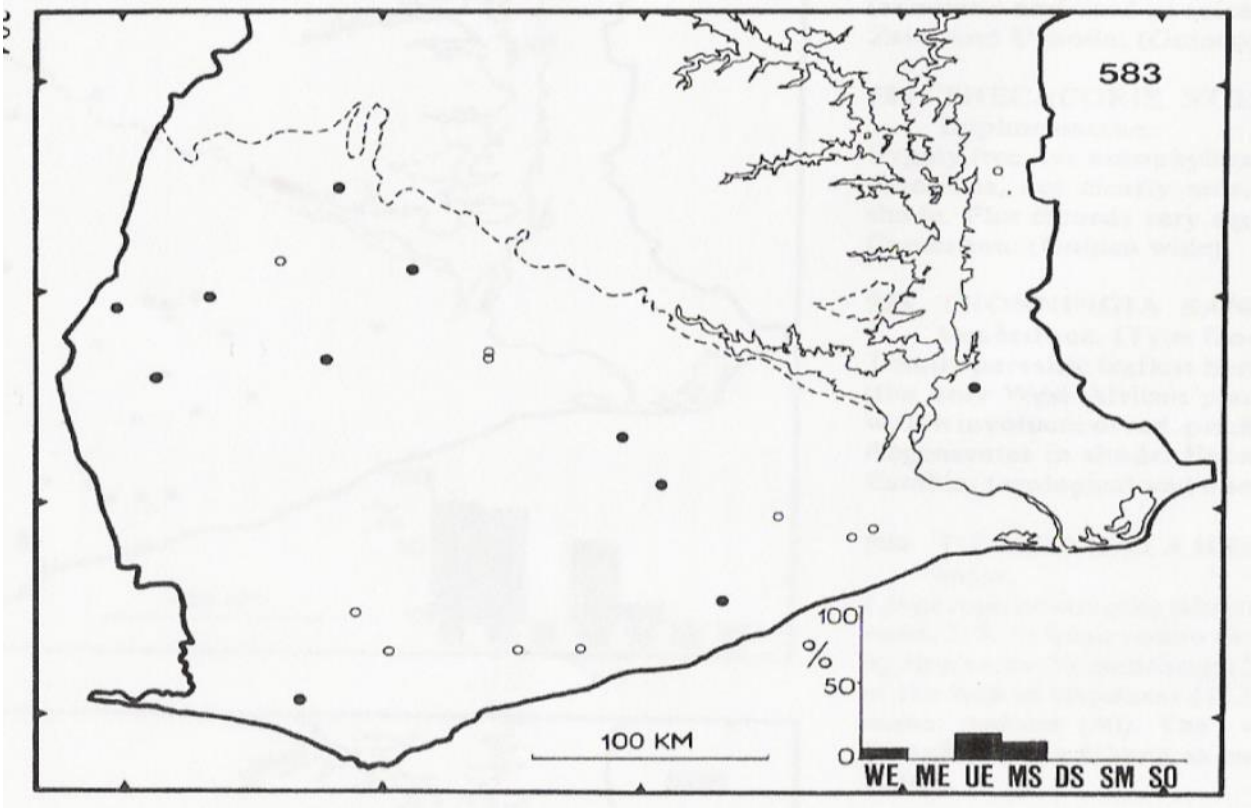

Figure 2.1 Distribution of *Tetrapluera tetraptera* in the High Forest Zone (HFZ) of Ghana.

Legend : ○ and ● both signify locations within the HFZ where *T. tetraptera* can be located however ○ locations originates from Herbarium and Flora records and ● locations originates from the survey of Hall and Swaine, 1976. Source : Hall and Swaine, (1981).

2.2.3 Phenology

According to Taylor (1960), the fruit is deciduous in December. Flowering begins in early March reaching its peak in May and ceasing completely in August (Anglaaere, 2005). Fruit initiation is from March to April reaching its peak from September to October (Anglaaere, 2005). Addo-Danso (2010), observed first fruiting of *T. tetraptera* after 48 months of planting in a mixed species plantation in Ghana.

2.2.4 Propagation

Tetrapluera tetraptera can be propagated both by seeds and by stem cuttings. With sexual propagation, the seeds undergo epigeal germination (Taylor, 1960). However, seeds are dormant due to the presence of hard seed coat which is impervious to air and water. Dormancy is broken by the use of both chemical and mechanical scarification. When scarified by these means, the seeds become permeable leading to germination of up to 90% after 6 days (Ibiang *et al*., 2012). Chemical scarification is mostly done by soaking the seeds in concentrated sulphuric acid for about 15 - 20 minutes followed by rinsing the seeds with tap water. Anglaaere (2005) proposed the use of acid from *Citrus jambhiri Lush*., however his reasearch proved that soaking of seeds in undiluted lemon juice for 12 hours performed weakly when compared to soaking in concentrated sulphuric acid for some minutes.

In mechanical scarification, Ibiang *et al*. (2012) recorded a mean germination percentage of 85% by rubbing of seeds on rough cement wall. Anglaaere (2005) reported a germination percentage of 29.4% by pounding seeds in a 1:1 seed and sand mixture and a germination percentage of 5.4% when seeds where rubbed between sheets of rough sand paper. Vegetatively, the use of indole butyric acid concentrations ranging from 0.2 - 0.8% whilst retaining leaves halved to their

original size increases the rooting percentage of *T. tetraptera* juvenile stem cuttings (Anglaaere, 2005).

2.2.5 Uses

Tetrapluera tetraptera has got numerous medicinal and economic benefits in Ghana. Its wood is moderately hard, durable and easily workable for which reason it is used for carvings, common furniture and cabinet works, boat construction, domestic floorings, stairs and steps, house posts, door and window frames, veneer and plywood and poles (Irvine, 1961; Oteng- Amoako *et al*., 2000). Carbonizing *T. terapluera* fruit waste at 500^{o} C produces high yielding charcoal with high caloric value with low moisture content which is comparable to charcoal from high density and moderately high density wood species of *Cylicodiscus gabonensis* and *Acacia nilotica* respectively (Derkyi *et al*., 2014). The flowers and fruits are used as perfume in locally manufactured pomades and palm wine (Irvine, 1961; Orwa *et al*., 2009). The tree is used as shade in traditional agroforests of cocoa in Ghana (Anglaaere, 2005) and coffee in Uganda (Irvine, 1961). People also use the plant around food crops to protect them against pests (Lekana-Douki *et al*., 2011). As a components of feed, when milled pods is added to the feeds of broilers, it improves the growth performance, reduces cost of production, improves blood components and controls microbial load in broiler chickens (Nweze *et al*., 2011).

In Ghana, the fruit is used as seasoning in the preparation of soup and porridge as well as used in jam preparation (Darkwa, 2013). According to Nwawu and Akali (1986) and Aladesanmi (2007) the fruit is especially used to prepare soup from the first date of birth to prevent post partum contraction. This attribute has been associated to the high concentration of iron in the dry fruit to regenerate lost blood (Abii and Amarachi, 2007; Uyoh *et al*., 2013). The Akans use the fruit in the treatment of hypertension and diabetes in folklore medicine (Caroline and Busia, 2005) and

its potential in the treatment of this diseases has been supported by the extraction of three different flavoids from mature fruits using ethanolic extract (Fleischer *et al*., 2006). Aside these, the supplements of dry fruit in diets play essential role in the reduction of the excessive levels of total cholesterol, LDL-cholesterol, triglycerides and as well decrease the LDL/HDL ratio in the body and hence protect against cardiovascular diseases as well as protection against kidney disorders by decreasing urea and creatinine levels (Ajayi *et al*., 2011). Together with other local herbs, the fruits of *Tetrapluera tetraptera* are used in the treatment of asthma (Sonibare and Gbile, 2008). Irondi *et al*. (2013) have established that at the ripe brown stage, the pod is very effective for the management of oxidative stress and postprandial hyperglycemia in type 2 diabetes.

The plant is used as a mosquito repellent, purgative, an emetic and is a potent plant molluscicides to avoid the transmission of bilharzia (Aladesanmi, 2007). It is also used in the management of leprosy, convulsion, inflammation and rheumatism pains (Aladesanmi, 2007). In Ghana and Nigeria, infusion of the whole fruit is bathed by malaria patients to get relief from feverish conditions (Irvine, 1961; Aladesanmi, 2007). Traditional healers in Ghana, use dried powdery barks of the tree to inhibit stomach ulcerations (Noamesi *et al*., 1994). Furthermore, people living in Haut-Ogooué in Gabon use decoction of *Tetrapleura tetraptera* bark to treat stomach ache and vomiting, fever, headache and deworming (Lekana-Douki *et al.*, 2011). Both the water and ethanol extracts of leaves, barks and roots have demonstrated to exhibit inhibitory effects against disease causing bacteria such *as Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae* (Okoronkwo and Echeme, 2012). The stem bark extract has also been found to be very potent in the treatment of gonorrhoea (Irvine, 1961; Okochi *et al*., 1999).

2.3 Growth Assessment in the Seedlings of Woody Legumes

The importance of growth performance in seedlings is to forecast seedlings that will survive, grow and develop vigrously in the field (Haase, 2008), especially in small holder tropical agroforestry sites where fertiliser and irrigation are not used (Jaenicke, 1999). Growth in seedlings is assessed either morphorlogically or physiologically. However, the two are not considered as been mutually exclusive since a seedling's morphological characteristics is a reflection of it physiological activities (Haase, 2008).

Nonetheless morphological features are popularly used due to the ease with which they can be measured (Thompson, 1985). In assessing growth morphologically, one single characteristic is not sufficient and therefore requires a combination of which the commonly used ones are height, shoot diameter, shoot dry weight, root dry weight and root size (Thompson, 1985; Jaenicke, 1999; Haase, 2007; 2008). Height is an estimate of the photosynthetic capacity and transpirational area of the seedling. Tall seedlings therefore are at an advantage to compete with weeds, but greater transpirational area makes them lose a lot of moisture especially in drier environment and are also susceptible to wind damage (Haase, 2007; 2008). Stem diameter is regarded as a reliable estimate of growth and survivability than height (Thompson, 1985; Haase, 2008) with higher values signifying higher stem volume and root system. When the height is divided by the diameter the result is the sturdiness quotient (Thompson, 1985; Haase, 2007; 2008). A smaller ratio indicates a stocky plant with a higher chance of survival in windy and dry areas (Thompson, 1985; Jaenicke, 1999; Haase, 2007; 2008). Seedlings with sturdiness quotient greater than 6 are undesirable (Jaenicke, 1999). Shoot dry weight signify photosynthetic capacity with higher values signifying growth. Seedlings with greater root dry weight grow and survive than those with smaller ones. These two dry weights are used to determine shoot to root ratio.

Aside anchorage, this ratio reflects the capacityof the root to support above ground biomass in terms of nutrient and water absorption from the soil (Takoutsing *et al*., 2013). In drier areas, a lower shoot to root ratio is required inorder to absorb more water and reduce water loss by transpiration. A lot of disagreements surrounds the correct value of shoot to root ratio (Thompson, 1985), however 2:1 or 1:1 has been proposed for container seedlings (Jaenicke, 1999; Haase, 2007; 2008) and 3:1 for bare root seedlings (Haase, 2007; 2008). Although this dry weights are good estimates of growth they are destructive and time consuming.

Relative growth rate (RGR) is defined as the increase in size per unit time per unit size (Hunt, 2003). Higher RGR has been strongly linked to survivorship due to it been a good measure of the plant ability to grow efficiently and competitively especially when resources become limited (Guan *et al*., 2008). RGR is normally assessed using plant dry weight (Hunt, 2003; Ruiz-Robleto and Villar, 2005; Offiong *et al*., 2010), however other growth parameters especially height and diameter has been used (Guan, 2008; Addo-Danso, 2010; Agyemang *et al.*, 2010). Assessment of roots to mycorrhiza and rhizobia inoculation also are important because they help plants to absorb more nutrients from the soil (McHargue, 1999; Twumasi, 2005; Diouf *et al*., 2008; Twum-Ampofo, 2008). A higher inoculation suggests high growth potential of the plant (McHargue, 1999; Twumasi, 2005; Diouf *et al*., 2008; Twum-Ampofo, 2008).

Morphology alone is not able to explain growth for which reason physiological parameters are also taken with plant nutrient content especially in the tropics. Nutrients in plants govern lots of metabolic processes in the seedling. Seedlings with higher nutrient uptake and use efficiency has been observed to correlate positively with improvement in morphological parameters (Lambert, 1995).

2.4 Growth, Nutrient Uptake and Efficiency of Tree Seedlings in Different Soils

Due to varying physical and chemical properties, same tree species growing under different soil conditions have different growth rates. Singh and Singh (2006) reported *Albizia lebbeck* to produce a biomass of 2.52 g/plant in mine spoil, 1.75 g/plant in mine spoil and full NPK, 2.13 g/plant in mine spoil and half dose NPK, 1.43 g/plant in mine spoil combined with forest soil and 6.35 g/plant in forest soil alone. Under low soil fertility, fast growing trees demonstrate slow growth whereas they increase their growth rate when soil fertility improves. *Sesbania grandifolia* and *Leucaena diversifolia* has been reported by Lambert (1995) to increase growth rate as soil P increased and vice versa. Ecologically, fast growth rate in fertile soil is advantageous as it leads to rapid production of leaves and roots exposing the tree to more light, water and nutrients (Chapin, 1980).

According to Chapin (1980), the advantages of slow growth rate in infertile soil are:

(i) Less nutrients are absorbed during slow growth and hence trees are less likely to exhaust available soil nutrients.

(ii) Slow growth is for survival since the tree adjust it physiological functioning to be in tandem with slow nutrient supply.

(iii) During slow growth, nutrients are absorbed in excess of immediate growth requirement (luxury consumption). These nutrients are used for growth when available soil nutrients become exhausted.

Increase in resource acquisition occurs in luxury accumulation because as a particular nutrient becomes deficient, the tree increases its capacity to absorb that nutrient at the expense of other

nutrients (Aerts and Chapin, 2000). This leads to increase in nutrient concentration in plant biomass without any increase in biomass.

Absorbed nutrients are used in the production of biomass. The ability of a tree to absorb nutrients from the soil and use that nutrients in the production of shoots and roots is known as nutrient use efficiency (Wang *et al*., 1991; Kumar *et al*., 1998; Shujauddin and Kumar, 2003). This implies that the amount of nutrient absorbed and carbon fixed determines whether a particular tree can grow in a paticular soil or not (Aerts and Chapin, 2000). Nutrient use efficiency provides a good measure to evaluate differences in nutrient cost of biomass production (Kumar *et al*., 1998; Shujauddin and Kumar, 2003).

Nutrient use efficiency is highly dependent on plant nutrient uptake (Aerts and Chapin, 2000; Baligar *et al*., 2001). Nutrient availability and uptake is seriously affected by soil physical and chemical conditions (Baligar *et al*., 2001; Malik and Rengal, 2013). Adverse physical conditions such as poor structure and texture, high bulk density, high or low water holding capacity and poor aeration changes root distribution and architecture. In effect, this reduces the roots ability to explore large volume of the soil to pick up nutrients and reduces nutrient uptake and ultimately nutrient use efficiency (Baligar *et al*., 2001; Malik and Rengal, 2013). The excess or deficiency of essential nutrients have also been observed to influence root morphological parameters such as length, thickness, root hairs and growth expressed as dry weight and/or root : shoot (Marschner, 1995; Indieka and Odee, 2005). Soil organic matter improves soil structure, reduces leaching and improves water holding capacity and hence improves nutrient uptake and nutrient use efficiency (Baligar *et al*., 2001).

According to Wang *et al*. (1991), a good tree suited for a particular site should:

(i) Achieve rapid growth.

(ii) When harvested they must take less nutrients from sites (ie. High nutrient efficiency).

(iii) Be better suited to poor sites where growth may be limited by the rate at which nutrients are made available.

2.5 Soil Factors and Initial Growth of Woody Legumes

The growth of seedlings of woody legumes is influence by many soil factors of which nutrient availability, pH, mycorrhiza and rhizobia play very important roles.

2.5.1 Nutrient Availability

Nutrient availability has been observed to influence the initial growth of legumes by impacting on dry matter distribution, nutrient acquisition and nutrient use efficiency (Cassman *et al*., 1980; Lambert, 1995). When plants grow under deficient nutrient conditions, more biomass production and nutrients is invested in the roots at the expense of the shoot (Cassman *et al*., 1980; Fredeen *et al*.,1989; Lambert, 1995; Twum-Ampofo, 2008), leading to an increase in root to shoot ratio. Brouwer (1962) explained this phenomenon by hypothesing that because roots are close to nutrients, they take a large chunk of the deficient nutrients and as a result they grow more than the shoot, but they begin to exhibit reduced growth rate when carbohydrate from shoot reduces considerably. High investment of resources into root growth may also be result in root growth in the direction of zones where there is high concentration of the deficient nutrients (Chapin, 1980). High biomass allocation to roots is high in nitrogen deficiency than that of phosphorus (Andrews *et al*., 1999), resulting in plants responding in such a way that plants with low N:P to allocating

more biomass to roots than high N:P growing at the same rate (Güsewell and Bollens, 2003). As a response to shoot nutrient deficiency, roots also increase nutrient absorption efficiency when growing in infertile soils (Chapin, 1980; Lambert, 1995). Nutrient absorption efficiency may not necessarily be linked to a larger root system (Lambert, 1995), but association of roots to mycorrhiza in infertile soils increases surface area for nutrient absorption (Habte and Turk, 1991; Twum-Ampofo, 2008). Another growth response of plants in infertile soil is the efficeint use of absorbed nutrients. *Gliricidia sepium* has been identified to produce lots of biomass under both high or low phosphorus conditions due to it high nutrient use efficiency (Habte and Turk, 1991; Lambert, 1995). The combined compensatory effects of high root : shoot, high nutrient absorption efficiency and efficient nutrient utilization may not fully compensate for reduced nutrient availability, resulting in serious hampered growth with reduced nutrient availability. Reduced growth rate seems to be the final plant response to soil infertility. N and P are mostly deficient in tropical soils and this inhibit the growth of leguminous seedlings (Lambert, 1995; Marques *et al*., 2001; Twumasi, 2005).

2.5.1.1 Nitrogen

Heavy rainfall, high temperature and leaching have made most tropical soils to be deficient in nitrogen. Most legumes are able to utilise nitrogen both in the soil and the one fixed by rhizobia. Nitrogen deficiency in the form of stunted growth and chlorosis has been reported in legumes grown under low nitrogen concentration but not in nitrogen fertilised legumes (Ribert and Drevon, 1996; Nosheen *et al*., 2004; Indieka and Odee, 2005; Weber *et al*., 2007). Nitrogen therefore is a primary limiting factor of leguminous seedlings growth (McHargue, 1999). Due to their ability to fix nitrogen, legumes can grow in soil deficient in nitrogen, however for seedlings the levels of N in the soil must be sufficient to support vigrous vegetative growth and support

nodulation before nitrogen fixation commences (Cassman *et al*., 1980; Minchin *et al*., 1981). Cassman *et al*. (1980) reported an increase in the initial growth response of soyabean when they were supplied with nitrogen. However, application of higher levels of mineralised N in the form of nitrate or ammonium stimulates vegetative growth of leguminous seedlings but inhibits nodule formation and nitrogen fixation (Cassman, 1980; Nosheen *et al*., 2004; Indieka and Odee, 2005; Weber *et al*., 2007). This is because investments in carbon and energy cost involved in absorbing and assimilating N from NO_3^- or NH_4^+ is less compared to nitrogen fixation (Thomas *et al*., 2006). But this has been demonstrated not to always be the case. Based on the amount of NO_3^- applied, Graham (1984) reported that assimilatory cost of NO_3^- may be less or more to nitrogen fixation. To stimulate the initial growth of leguminous seedlings in nitrogen deficient soils, nitrogen must be supplied in small quantities until nitrogen fixation starts.

2.5.1.2 Phosphorus

Tropical soils are highly deficient in P due to high acidity and weathering (Plassard and Dell, 2010). In acidic soils, P deficiency is caused by the complexing of $H_2PO_4^-$ by aluminium and iron hydroxides, converting large proportions of total P into forms unavailable to plants (Cardoso and Kuyper, 2006). Legumes require high amount of P inorder to make nitrogen fixation possible(Magadlela, 2013). Its been estimated that 20% of total plant P is transferred to the nodules during nitrogen fixation (Magadlela, 2013). As a result phosphorus content per unit dry weight of nodules is higher than in shoots and roots (Adu-Gyamfi and Fujika, 1989). Under deficient P conditions, nitrogen fixing legumes prioritize the partioning of dry matter between roots and nodules than between shoots and underground structures (Cassman *et al*.,1980). Phosphorus fertilisation tend to stimulate legume growth rate, nodule function and increase nitrogen content in biomass (Ribert and Drevon, 1996; Tsvetkova and Georgiev, 2003; Isaac *et*

al., 2011; Magadlela, 2013). Higher P concentration has been reported to decrease nodule number and their nitrogen fixation (Tsvetkova and Georgiev, 2003; Magadlela, 2013). Legumes have developed various mechanisms to survive in P deficient soils. One of such mechanisms is their associations with mycorrhiza which are better at scavenging the soil for P. This makes mycorrhiza inoculated seedlings to harbour more P in their biomass (Twumasi, 2005; Twum-Ampofo, 2008; Diouf *et al*., 2008). Other mechanisms include reduction in growth rate inorder to limit N requirements, improving nitrogen fixation efficiency of nodules and obtaining N from other external sources (Magadlela, 2013). Another key strategy employed by legumes in deficient P soils is phosphorus use efficiency as well as higher root efficiency for the uptake of P from the soil (Lambert, 1995).

2.5.2 pH

The tropics is dominated by highly acidic soils which contain Al and or Mn in toxic levels to legumes (Young, 1989; Sanchez and Logan, 1992). Aluminium damages root growth and hence reduces nutrient uptake and translocation within the plant (Matsumoto, 2000). Damage of plant roots and low nutrient content restricts nodulation in legumes. Strictly speaking, acidity alone may not limit legume growth and nodulation, but when it induces aluminium and manganese toxicity, then thisis highly likely to occur (Freire, 1984). Aside this toxicities, growth in these soils is inhibited by high P fixation by aluminium and manganese. About 38% of soils in the humid tropics demonstrates high P fixation in them (Sanchez and Logan, 1992).

Some woody legumes are very tolerant to acidic conditions. Under a pH of 4.3, *Acacia seyal, Albizia lebbeck, Dalbergia sisoo, Acacia galpinii, Acacia auriculiformis and Acacia erioloba* has been recorded to sufficiently grow, nodulate and fix nitrogen (Masutha *et al*., 1997). The adaptability of these trees seedlings in such acidic soils is based on their P intake and use

efficiency. Growing in P deficient acidic soil, Lambert (1995) observed that *Acacia auriculiformis* produced more biomass with equal distribution of biomass between leaves and stem and a little investment in biomass, P and N to below ground structures.

Soil acidity influences legume root association with beneficial micro-organisms. Rhizobia response is more sensitive to soil acidity, but their response varies with species (Morón *et al*., 2005). Strains adapted to soil acidity are related to their ability to regulate their internal pH (Morón *et al*., 2005). According to the authors, nodulation is made possible by the production of more Nod factor by rhizobia which makes the production of nodules at a pH of 4.5, although the number of nodule formed are lesser than those formed at higher pH. Between the pH of 5.5 - 7.5, Wang *et al*. (1993) observed that colonization by mycorrhiza was sightly affected with the greatest colonization been observed at pH of 5 - 6. Liming has been observed to increase P availability in acidic soils leading to the production of greater biomass and nodule formation in *Leucaena leucocephala* (Kisinyo *et al*., 2005).

2.5.3 Rhizobia

These bacteria live in close association with roots of plants and are housed in specially made sacks called symbiosomes which are enclosed in nodules (Franche *et al.*, 2009; Sprent *et al*., 2013). Nodule formation by rhizobia promotes the growth of seedlings by making available inexhaustible nitrogen to them (Lambert, 1995; Diouf *et al*., 2008). Nonetheless, this comes at a cost in terms of high carbon and energy required in nitrogen fixation, nodule formation and maintenance (McHargue, 1999). Burris and Roberts (1993) states that one molecule of nitrogen fixed requires 20 - 30 molecules of adenosine triphosphate (ATP). Its been reported that about 12.8 - 28.2% of the total carbon fixed by photosynthesis by the plant is utilized for nodule function (Graham, 1984). As a result of this high requirements, growth of leguminous seedlings

is hampered slightly until nodules are fully formed and functioning to compensate for those loses. Hacin *et al*. (1997) observed in soyabeans that before nodule formation, more carbon was invested into nodule initiation leading to reduced root growth in addition to nitrogen deficiency until nitrogen fixation started. Other authors have observed similar growth trends in seedlings of woody legumes too. An initial nitrogen deficiency wasobserved in *Sesbania sesban* until nodules where able to fix enough nitrogen to support vigrous shoot growth (Indieka and Odee, 2005). Ribert and Drevon (1996) reported nitrogen deficiency in nodulated seedlings of *Acacia mangium* but not for urea fertilised counter parts. The short term reduced growth rate of leguminous seedlings is an investments into the future for rapid growth and survival when nitrogen fixation commences.

2.5.4 Mycorrhiza

The important role of mycorrhizae in the growth of leguminous seedlings is the acquisition of nutrients especially those that diffuse slowly such as phosphorus (Lambert, 1995, Twumasi, 2005 and Twum-Ampofo, 2008). AM fungi role is very imporant especially during the seedlings stage when roots are not fully developed. The ability for AM fungi to absorb nutrients is due to:

(i) Extension of extraradical hyphae of mycorrhizal fungi into large volume of the soil increasesing the surface area for nutrient uptake (Tawaraya *et al*., 2001).

(ii) Kinectic uptake of P is higher in hyphae than root hairs (Sanders and Tinker, 1973).

(iii) Interaction between plant root and mycorrhiza modify rhizosphere environment in which P solubilization and availability are strengthened (Cardoso and Kuyper, 2006; Xie *et al*., 2014).

Jansa *et al*. (2003) reported that 27% and 9% of added ^{33}P and ^{65}Zn respectively were transported to maize by AM fungi at a distance of 5 cm from roots within 25 days. Several authors have presented results demonstrating higher nutrient intake and higher growth rate of seedlings of woody legumes inoculated by AM fungi (McHargue, 1999; Rao and Tak, 2001; Twumasi, 2005; Twum-Ampofo, 2008). It has been reported that AM fungi inoculated *Pithecellobium rufescens* had greater height, leaves, dry weight and nutrient content than uninoculated seedlings (McHargue, 1999). Rao and Tak (2001) also observed the influence in the increased in height, dry weight and nitrogen, phosphorus, calcium and magnessium in the growth of *Acacia ampliceps*, *Acacia eriopoda*, *Albizia lebbeck*, *Azadirachta indica* and *Colophospermum mopane*. Root colonisation of leguminous woody seedlings is enhanced when nutrients especially phosphorus is deficient in the soil (Twumasi, 2005; Diouf *et al*., 2008).

2.6 Tripartite Symbiotic Association and Growth of Legumes

The ecological advantage of legumes to grow in N and P deficient soils hinges on tripartite symbiotic association (legume: rhizobia: mycorrhiza) (Marques *et al*., 2001; Twumasi, 2005; Twum-Ampofo, 2008). It is well established that legumes require high amount of phosphorus inorder to maintain nodules and nitrogen fixation. Mycorrhiza absorbtive capacity of phosphorus is able to supply P for plants to tranfer to rhizobia in nodules to make nitrogen fixation possible. As a result even under severephosphorus deficient soils, legumes tend to have increased growth, nodule number and dry weight with little addition of P fertilizer (Diouf *et al*., 2008). Else in the absence of mycorrhiza, legume growth will have to be sustained by higher P fertilizer (Diouf *et al.*, 2008). Inoculation of legumes with mycorrhiza generally positively correlate with nodulation by rhizobia (Abd-Alla *et al*., 2014; Meghvansi and Mahna, 2009) except in a few cases where rhizobia was observed to reduce colonization by certain strains of mycorrhiza (Twum-Ampofo,

2008). This has been speculated to be caused by competition for colonization sites (Chalk *et al*., 2006).

Availability of N and P from microsymbionts stimulates legumes growth and in return, legumes transport photosynthates in the form of carbon for their survival. The symbionts in the association therefore act as sinks and sources of C, N and P. The roots of legumes, rhizobia and mycorrhiza competite for photosynthates from the shoot. Of the total carbon fixed daily 13%, 12% and 17% are translocated respectively to the root, nodule and mycorrhiza for their maintenance (Paul and Clark, 1989). When the legume is very efficient at uptaking P on its own, then association with mycorrhiza may tend to negatively impact on the growth of legumes. Habte and Turk (1991) reports that high carbohydrate costs by *Cassia recticulata* to maintain mycorrhiza association led to 50% in the reduction of root mass.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of Experimental Site

The experiment was conducted at the Faculty of Renewable Natural Resouces (FRNR) experimental farm within the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. It is located on latitude 06^{o} 43^{o} N and longitude 01^{o} 36^{o} W with an altitude of 287.1 m above sea level. The area lies within the moist semi deciduous forest in Ghana which has an average of 1488 mm rainfall annually and an annual temperature of of 26.6^{o} C (Twumasi, 2005).

3.2 Experimental Design and Treatments

The experiment was laid in a Radomised Complete Block Design (RCBD) with four treatments (soils from different land use systems) replicated in four blocks.Each treatment had a total of 80 plants. The soils under the various land use systems were; forest reserve, surface mine, slash and burn agriculture (farm) and teak plantation. The forest reserve soil which is an acrisol (FAO / UNESCO, 1990) was collected under the canopy of *Tetrapluera tetrapera* in the arboretum of Bobiri Forest Reserve. The soil is reddish in colour and heavily leached. The soils subjected to surface mining and harvested teak plantation were also acrisols (FAO / UNESCO, 1990). However, the mine soil was collected from Owere mines top soil stockpile in Konongo and it is reddish brown, gravelly and moderately heavy to medium texture (Chishonlm and Affleck, 1996). Whereas the soil from harvested teak plantation which has a dark colour and was collectedthree months after seven years old teak was harvested from Duampopo in the Ejisu Juabeng Municipality. Only the soil from slash and burn agriculture was an alisol (FAO / UNESCO, 1990) and it was collected from Kuntenase in the Bosomtwe District. The soil is

yellowish brown and has been used to cultivate plantain and seasonally intercropped with maize and cassava.

The experiment was conducted under an erected shade made of bamboo and palm fronds.

3.3 Soil Collection and Filling of Polybags

Soil surface was cleared with cutlass to remove any vegetation covering it. Top soil was collected from the surface of the soil to a depth of 30 cm into sacks. Stones and other foreign materials were hand picked during filling of the pot. Black polybags which had holes punctured under them were filled with 2 kg of soil. The ground where polybags were placed was linned with plastic carpet to prevent root penetration to the ground.

3.4 Soil Analysis

Air dried soil samples were sieved through a 2 mm nylon sieve and this was used for chemical analysis of the soils.

3.4.1 Soil pH

This was determined using a soil solution ratio of 1:1 (Eckert, 1988). Ten gram of air dried soil was placed into a 100 ml beaker followed by the addition of 10 ml of distilled water. The suspension was stirred vigorously for 20 minutes. Soil–water suspension was left to stand for 30 minutes to allow suspended clay settle out from the suspension. A pH meter (WTW pH / Cond 3400i) was calibrated with blank at a pH of 4 and 7 respectively. The electrode of the pH meter was inserted into the partly settled suspension and readings were recorded.

3.4.2 Organic Carbon and Organic Matter

The Wakler-Black wet combustion method outlined by Nelson and Sommers (1982) was used to determine organic carbon of the soils. Two grams of soil sample was weighed into a 500 ml

Erlenmeyer flask followed by the addition of 10 ml of 1.0 N Potassium dichromate solution and 20 ml of concentrated H_2SO_4. The mixture was swirlled ensuring that the solution was in contact with all the soil particles. The flask and it content were allowed to cool on an asbestos sheet for 30 minutes. After which, 200 ml of distilled water, 10 ml of orthorphosphoric acid and 2.0 ml of diphenylamine indicator was added. The solution was titrated with 10 N of ferrous sulphate solution until the colour changed to blue and then to a green colour. The titre value was recorded as well as that for the blank solution. The soil organic carbon content was calculated as;

$$\% \text{ Organic carbon } = \frac{M \times 0.39 \times mcf\,(V_1 - V_2)}{W}$$

Where M = molarity of ferrous sulphate

V_1 = ml of ferrous sulphate solution required for blank

V_2 = ml of ferrous sulphate solution required for sample

mcf = moisture correcting factor

W = weight of air dry sample in gram

Organic matter was calculated by multiplying the % organic carbon with 1.724 (Van Bemmelen factor).

3.4.3 Total Nitrogen

The macro-kjeldahl method was used for the determination of the total nitrogen(Bremner and Mulvaney, 1982). Ten gram of air dry soil was poured into a 500 ml long – necked kjeldahl flask followed by the addition of 10 ml of distilled water. The mixture was allowed to stand for 10 minutes. One spatula full of kjeldahl catalyst and 20 ml concentrated H_2SO_4 was added to the

mixture. The mixture was digested using Gerhartz digestion block at 350^{o} C for 2 hours until it was clear and colourless. The flask was cooled and fluid was decanted into a 100 ml volumetric flask. Distilled water was used to top the fluid to the mark on the neck of the flask. An aliquot of 10 ml of fluid was transferred into the kjeldahl distillation apparatus followed by the addition of 20 ml of 40% NaOH. The fluid was distilled with 10 ml of 4% boric acid and three drops of mixed indicator in a 500 ml conical flask for 4 minutes. The distillate was titrated with 0.l N HCl till blue colour changed to grey and sudden change to pink. The nitrogen concentration (% N) was assessed by;

$$\% \text{ N} = \frac{14 \times (\text{A} - \text{B}) \times \text{N} \times 100}{1000 \times 1}$$

Where; A = Volume of standard HCl used in the sample titration

B = Volume of standard HCl used in the blank titration

N = Normality of standard HCl

3.4.4 Available Phosphorus

The Bray P1 method was used for the determination of phosphorus (Bray and Kurtz, 1945). Two gram of air dry soil was placed into a 50 ml shaking bottle after which 20 ml of Bray P extracting solution was added. Mixture was shaked vigrously for a minute and filtered into 100 ml conical flask. Ten milliliter (10 ml) of filtrate was pippeted into a 25 ml volumetric flask followed by the addition of 1 ml of molybdate reagent and 1.0 ml of diluted ascorbic acid. The solution was topped to the 25 ml mark. The solution was shaked vigorously and allowed to stand for 15 minutes. Measurement was based on percentagetransmission at 600 nm wavelength on a colorimeter **(**Jenway 6051I **)**. Values of percentage transmittance (T) obtained were converted to

$\log_2 T$. A graph was ploted using phosphorus standard solutions to obtain the actual concentration of phosphorus. The concentration of P in the extract was obtained by comparing the results with a standard curve plotted.

Available phosphorous (P) mg/Kg = (Y/A) ÷ 10

Where; Y = $\log_2 T$ of the sample

A = constant obtained from the graph

3.4.5 Exchangeable Cations (K, Ca and Mg)

For the determination of K, Ca and Mg, 10 g of air dried soil was weighed into an extraction bottle followed by the addition of 100 ml of 1.0 N ammonium acetate (NH_4OAc) solution (Black, 1986). Bottle together with its content was shaked in a mechanical shaker for one hour. Supernatant solution was filtered through a number 42 Whatman filter paper. Aliquots of the filtrate was used for the determination of K, Ca and Mg.

Using 10 ml aliquot from the above solution, potassium was measured on a flame photometer (Jenway PFP 7) after the calibration of the photometer with prepared standards. Using the meter reading of a standard curve, the concentration of potassium in the soil extract was determined.

Potassium (K) Cmol/Kg = (Y/B) ÷ 39.1

Where; Y = Flame photometer reading of the sample

B = Constant value from the curve

39.1 = Atomic weight of K

Calcium was measured by taking 10 ml aliquot from the above solution. Followed by the addition 10 ml of 10% KOH solution and 1 ml of 30% Triethanolamine. Three drops of 10% KCN solution and a few crystals of Cal-red indicator was then added and the solution was shaken vigorously to ensure a uniform mixture.The mixture was titrated with 0.02 N EDTA solution until there was blue colour as endpoint.

Calcium (C) Cmol/kg = Titre value of Ca x 2

Magnessium was measured by taking 10 ml aliquot of the above stock solution, followed by the addition of 5 ml of ammonium chloride-ammonium hydroxide buffer solution and 1 ml of triethanolamine. Three drops of 10% KCN solution and a few drops of EBT indicator was added after which the solution was shaked vigorously. Mixture was titrated with 0.02 N EDTA solution until an endpiont of blue colour was reached.

Magnessium (Mg) Cmol/kg = Titre value for [(Ca+Mg) – Titre value for (Ca)] x 2

3.5 Seeds Treatment Before Sowing

Seeds were obtained from Forest Research Institute of Ghana. The seeds had a very hard testa as a result a small side of the seeds were slightly cut with a nail cutter to make it very permeable to water. They were then soaked in water at room temperature for 24 hours. Four holes each of depth 1 cm was made into the soil after which four seeds were placed into each hole. Seeds were then covered with a thin layer of soil and gently pressed with the fingers to ensure ancorage of roots upon germination.

3.6 Cultural Practices

Watering was carried out every morning unless it rain’s the previous night. After two weeks of germination, thinning was carried out to one plant per plot. Weeds were uprooted from pots

every morning after watering actively. The surrounding area was weeded with cutlass at monthly intervals. Small snails that were found attached to the polybags were handpicked every morning. On the 10th week due to high rainfalls, winds and humidity shade was completely removed.

3.7 Data Collection

3.7.1 Height and Diameter

From the 4th week 20 seedlings per treatment were randomly sampled forheight and diameter measurement every fortnight until the 24th week. Height was measured from the cotyledonary node to the apical bud using a meter rule calibrated in centimeters. The diameter was taken at the cotyledonary node with a digital vernier caliper.

The height and diameter was used for the following calculation;

(i) $SQ = \frac{H}{D}$

(ii) RHGR ($cmcm^{-1}week^{-1}$) = $\frac{LnH_2 - LnH_1}{t_2 - t_1}$

(iii)RDGR ($\mu m\mu m^{-1}week^{-1}$) = $\frac{LnD_2 - LnD_1}{t_2 - t_1}$

Where; SQ = Sturdiness Quotient

H = Height

D = Diameter

Ln = Natural logarithm

RHGR = Relative Height Growth Rate

RDGR = Relative Diameter Growth Rate

The RGRH and RGRD was determined using the slope of a graph.

3.7.2 Biomass

On the 24th week, randomly sampled seedlings were carefully uprooted from the wet soils to prevent damage to the fine roots. The roots were washed with water and partitioned into shoots and roots. They were placed into separate paper envelopes and oven dried at 70^{o} C for 72 hours.

The dry weight were used for the determination;

(i) Shoot dry weight (SDW) (g/plant)

(ii) Root dry weight (RDW) (g/plant)

(iii) Shoot to root ratio

(iv) Total plant dry weight (TDW)(g/plant)

3.7.3 Determination of Percent Mycorrhiza Colonization

Sampling of roots for staining was carried out on the 24th week. The staining was carried out by following the procedures outlined by Kormanik and McGraw (1982). Roots were sampled randomly from each treatment and stored in 70% alcohol and placed in a refrigerator. Stored roots were cut into lengths of 4 cm and were washed thoroughly several times with water and transferred into labelled mccartney bottle. The roots were then cleared by covering with 10% KOH solution and heated in a water bath at 90^{o} C for 2 hours. The KOH was poured out from the bottles and roots were rinsed several times with tap water to remove the KOH. After which 30% H_2O_2 was used to bleach roots until their dark colour disappeared followed by rinsing of the roots several times with tap water to remove the H_2O_2. The roots were then stained with 0.05% trypan blue dye for 24 hours making sure that the roots were fully immersed in the dye. Trypan blue was poured off from the roots and they were washed several times with tap water. The roots were

then stored in acidic glycerol for 24 hours to remove the remaining stain. Roots were left in the acidic glycerol in the mccartney bottle to await assessment.

Percentage mycorrhiza colonisation was determinated using procedures outlined by McGonigle *et al*. (1990). Ten pieces of root from each treatment were selected and they were laid vertically on a microscopic slide using foreceps (five roots per slide).Slide was observed under a light microscope using magnification between 10X- 40X. The microscope field view was moved to make five complete passes across each root specimen on a slide perpendicular to it short axis to scan for fungal structures (hyphae, vesicles or arbuscles). Presence or absence recording was based on whether the cross hatch in the field view hits a fungal structure or not. Percentage mycorrhiza colonisation (% MC) was determined using the following relationship;

$$\% \text{ MC} = \frac{\text{Number of infected intersections (Presence)}}{\text{Total number of intersections (infected+uninfected)}} \times 100$$

3.7.4 Plant Analysis

Plant material used for dry weight determination was used for nutrient analysis. The plant materials were grinded into fine powder using a laboratory miller.

3.7.4.1 Dry Ashing of Plant Tissue

One gram of finely grinded plant tissue was placed into a porcelain crucible. The crucible was heated at 500^{o} C for four hours, after which the crucible was removed and allowed to cool. The ignited residue was moistened with 2 ml distilled water. Five milliliter (5 ml) of 8 N HCl was carefully added to the mixture. The crucible was covered and placed on a steam water bath for 20 minutes. The mixture was filtered through a Whatman number 42 filter paper with the filtrate been collected in a 100 ml volumetric flask.Distilled water was added to the solution until it

reaches the 100 ml mark. The solution was shaked vigrously to ensure complete mixture. The digest was used for the determination of phosphorus.

3.7.4.2 Determination of Phosphorus Content

Phosphorous was determined colometrically using the vanadium phosphomolybdate method (Motsara and Roy, 2008). Five milliliter of the digest was measured into a 50 ml volumetric flask, followed by the addition of 10 ml of vanadomolybdate reagent. The volume was increased using distilled water after which the solution was shaked vigorously and left to stand. After 30 minutes a yellow colour developed and this was read on a colorimeter (Jenway 6051) at a wavelength of 430 nm . Values for percentage transmittance (% T) obtained were converted to $\log_2 T$. A graph was ploted using phosphorus standard solutions to obtain the actual concentration of phosphorus. The concentration of P in the extract was obtained by comparing the results with a standard curve plotted. The available phosphorus was calculated with the following equation;

Available Phosphorous (P) mg/Kg = (Y/A)÷10

Where Y = $\log_2 T$ of the sample

A = constant obtained from the graph

3.7.4.3 Determination of Nitrogen Content

The total nitrogen was determined using micro Kjeldahl digestion and distillation following the same procedure as section 3.3.3.

3.7.5 Nutrient Uptake and Use Efficiency

Nutrient concentration and plant dry weight data were used for the calculation of N and P nutrient uptake and use efficiencies with the following equations;

Shoot N or P uptake = Shoot N or P conc. (mg / g) × Shoot dry weight (g)

Root N or P uptake = Root N or P conc. (mg / g) × Root dry weight (g)

Total plant N or P uptake = Shoot N or P uptake + Root N or P uptake

$$\text{Nitrogen Use Efficiency (NUE)} = \frac{\text{total plant dry weight (g)}}{\text{N concentration in total plant (mg / g)}}$$

$$\text{Phosphorus Use Efficiency (PUE)} = \frac{\text{total plant dry weight (g)}}{\text{P concentration in total plant (mg / g)}}$$

3.8 Statistical Analysis

Data was analysed using analysis of variance (ANOVA) in genstat twelfth edition. Mean separation was done using least significance difference (LSD) at $\alpha = 0.05$ when significant difference was observed between treatments. Mycorrhiza root colonization (% MC) values did not meet the assumptions of ANOVA even after tranformation as a result they were analysed using friedman's non parametric test. Spearman rank correlation was used to determine the correlation between % MC and seedling morphological and physiological parameters. Pearson correlation coefficient was used for the correlation between root dry weight and other morphological and physiological parameters.

CHAPTER FOUR

RESULTS

4.1 Chemical Properties of Soils

The chemical properties of the soils used for the research are shown in Table 4.1. The pH of the soils were 5.89, 6.89, 6.56 and 6.61 for reserve, mine, farm and teak respectively. The highest total nitrogen of 0.7% was recorded for farm soil and the least of 0.1% was recorded for mine soil. Farm soil recorded the highest available phosphorus of 11.29 mg / Kg and the lowest of 2.7 mg / Kg was recorded for mine soil. The range of values for potassium, magnesium and calcium for the soils were 0.65 Cmol / Kg – 1.94Cmol / Kg, 2.76 Cmol / Kg – 3.92Cmol / Kg and 3.14 Cmol / Kg – 5.5 Cmol / Kg respectively. The soils had relatively high organic matter with highest been recorded in farm soil (4.33%) and lowest in teak soil (2.07%).

Table 4.1 Chemical properties of the soils from different land use systems

CHEMICAL PROPERTIES	**SOILS FROM DIFFERENT LAND USE SYSTEMS**			
	RESERVE	**MINE**	**FARM**	**TEAK**
pH	5.89	6.89	6.56	6.61
N %	0.18	0.1	0.7	0.63
P (mg / Kg)	5.7	2.7	11.29	4.18
K (Cmol / Kg)	0.81	1.37	1.94	0.65
Mg (Cmol / Kg)	3.58	3.92	2.76	3.74
Ca (Cmol / Kg)	5.5	6.52	8.38	3.14
OM %	4.14	2.17	4.33	2.07

4.2 Height and Diameter

The effect of soils from different sites on the height of *T. teraptera* is shown in Fig. 4.1. Highly significant differences ($P<0.001$) were observed between treatment means. The highest growth in height of 13.76 cm was observed from seedlings growing in soil from farm whereas the least growth in height of 8.05 cm was observed in mine soil. Diameter was also significantly ($P < 0.001$) influenced by soil (Fig. 4.2). The highest mean diameter of 3.27 mm was recorded in farm soil whereas the least diameter of 2.14 mm was observed in mine soil. No significant difference in diameter was observed between farm soil and reserve soil.

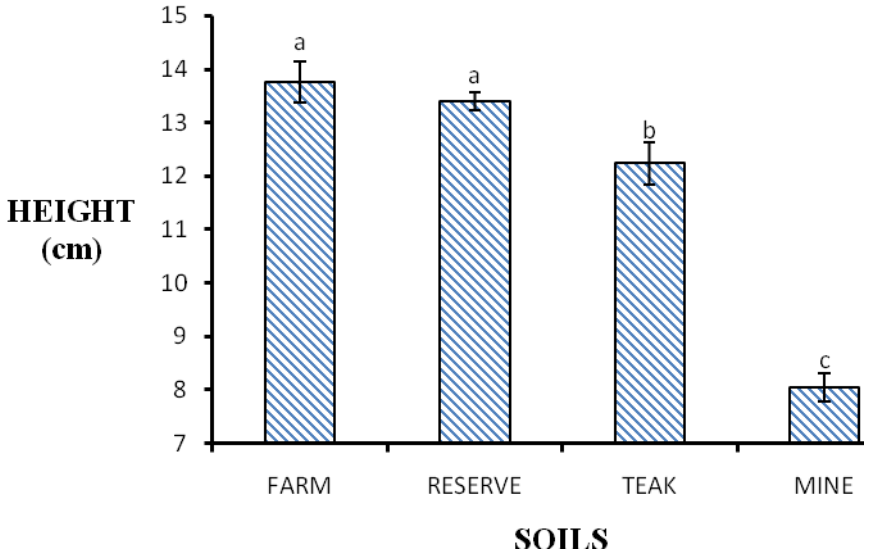

Figure 4.1 Height of *Tetrapluera teraptera* seedlings grown in soils from different sites.
Error bars indicate standard error of mean. Different letters on top of error bars indicate significant difference ($P \leq 0.05$) using Least Significant Difference (LSD).

4.3 Sturdiness Quotient and Relative Growth Rate (Height and Diameter)

After six months of growth, although slight differences were observed in Sturdiness Quotient (SQ) of treatment means, this wasn't statistically significant (Fig. 4.3). Contrary to SQ, both Relative Height Growth Rate (RHGR) and Relative Diameter Growth Rate (RDGR) of treatment means were statistically significant ($P < 0.01$). Mean RHGR was 0.065 $cmcm^{-1}week^{-1}$, 0.064

cmcm^{-1}week^{-1} , 0.048 cmcm^{-1}week^{-1} and 0.036 cmcm^{-1}week^{-1} for farm, reserve, teak and mine soil respectively (Fig. 4.4). Similarly, mean RDGR was highest (0.1024 µmµm^{-1}week^{-1}) and lowest (0.0764 µmµm^{-1}week^{-1}) in farm soil and mine soil respectively. No significant difference was observed between the mean RDGR of farm and reserve (0.0947 µmµm^{-1}week^{-1}) (Fig. 4.5).

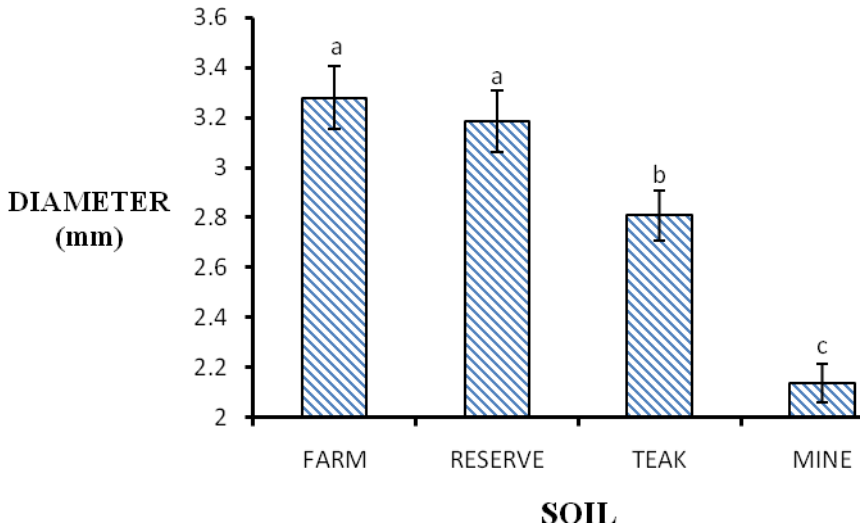

Figure 4.2 Diameter of *Tetrapluera teraptera* seedlings grown in soils from different sites.
Error bars indicate standard error of mean. Different letters on top of error bars indicate significant difference ($P \leq 0.05$) using Least Significant Difference (LSD).

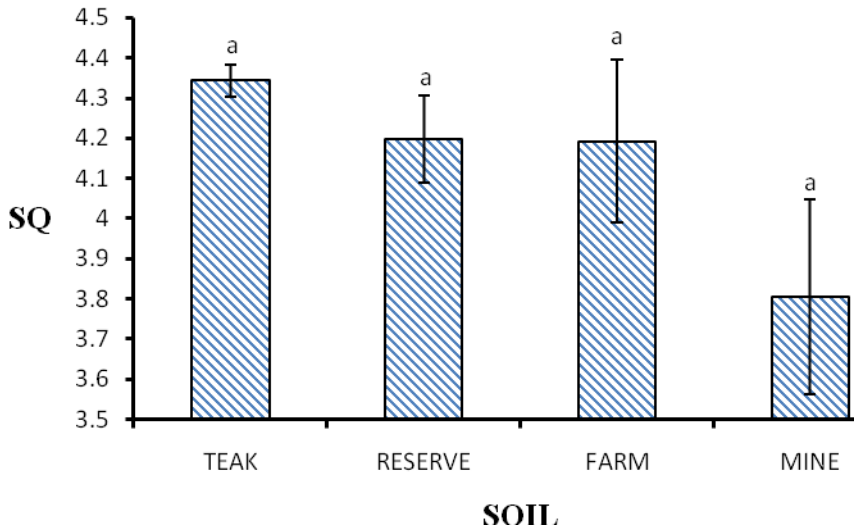

Figure 4.3 Sturdiness Quotient (SQ) of *Tetrapluera teraptera* seedlings grown in soils from different sites.
Error bars indicate standard error of mean. Different letters on top of error bars indicate significant difference ($P \leq 0.05$)using Least Significant Difference (LSD).

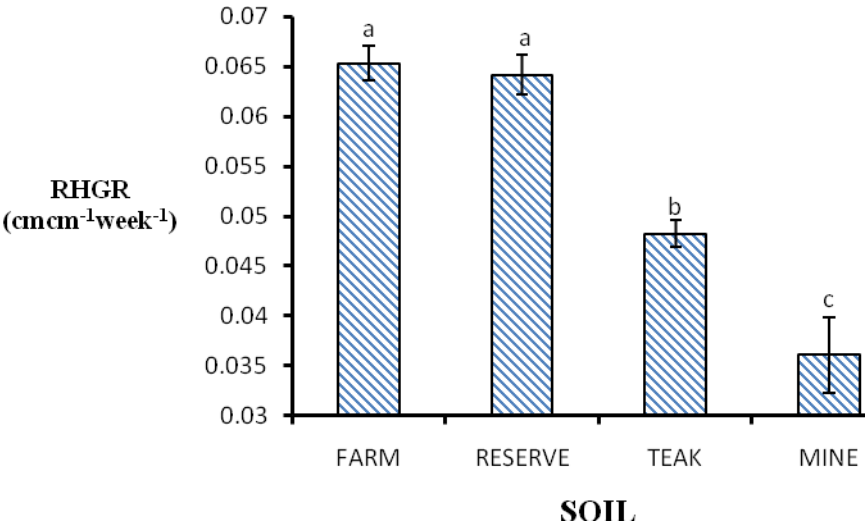

Figure 4.4 Relative Height Growth Rate (RHGR) of *Tetrapluera teraptera* seedlings grown in soils from different sites.
Error bars indicate standard error of mean. Different letters on top of error bars indicate significant difference ($P \leq 0.05$) using Least Significant Difference (LSD.

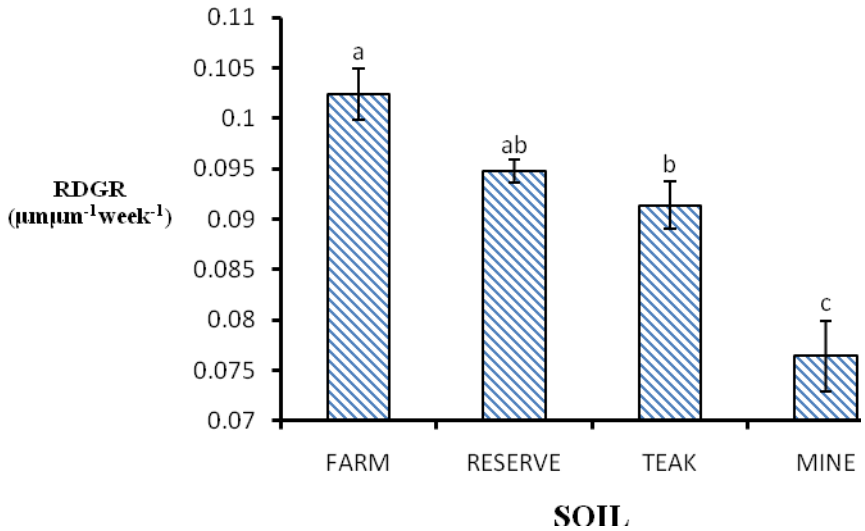

Figure 4.5 Relative Diameter Growth Rate (RDGR) of *Tetrapluera teraptera* seedlings grown in soils from different sites**.**
Error bars indicate standard error of mean. Different letters on top of error bars indicate significant difference ($P \leq 0.05$) using Least Significant Difference (LSD).

4.4 Biomass

Soils had highly significant effect ($P < 0.001$) on Shoot Dry Weight (SDW). The highest SDW of 3.72 g/plant was obtained from farm soil whereas the least of 1.17 g/plant was recorded for mine soil. No significant difference was observed between farm and reserve soil (Fig. 4.6). Similarly, soils had a significant effect ($P < 0.001$) on Root Dry Weight (RDW). Again, farm soil recorded the highest RDW (3.92 g/plant) with the least RDW been recorded for mine soil (1.23g/plant). No significant difference was observed between farm soil and reserve soils (Fig. 4.7). Total dry weight (TDW) followed a similar trend as SDW and RDW ($P < 0.001$). The decreasing trend of mean TDW is farm soil (7.63 g/plant), reserve soil (6.87 g/plant), teak soil (4.51 g/plant) and mine soil (2.40 g/plant) (Fig. 4.8).

Contrary to the above trend, no significant effect ($P > 0.05$) was observed for shoot to root ratio between seedlings grown in the different soils (Fig. 4.8).

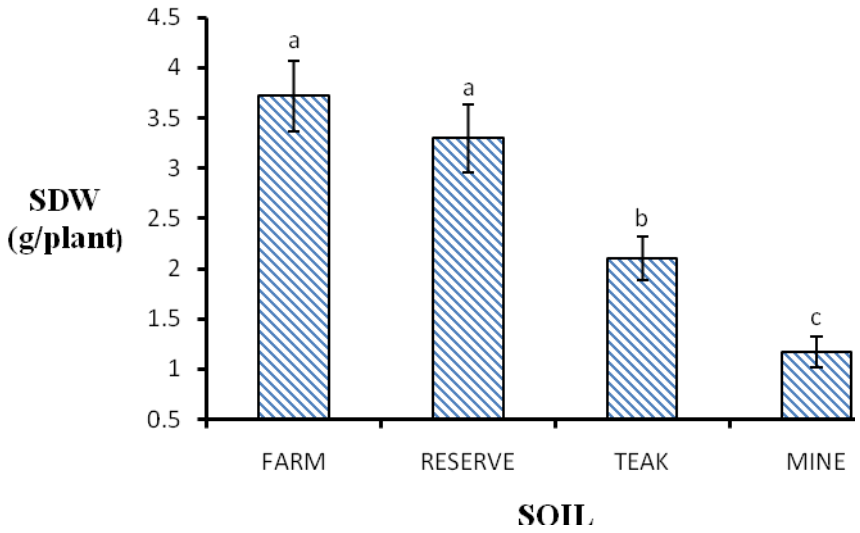

Figure 4.6 Shoot Dry Weight (SDW) of *Tetrapluera teraptera* seedlings grown in soils from different sites.
Error bars indicate standard error of mean. Different letters on top of error bars indicate significant difference ($P \leq 0.05$) using Least Significant Difference (LSD).

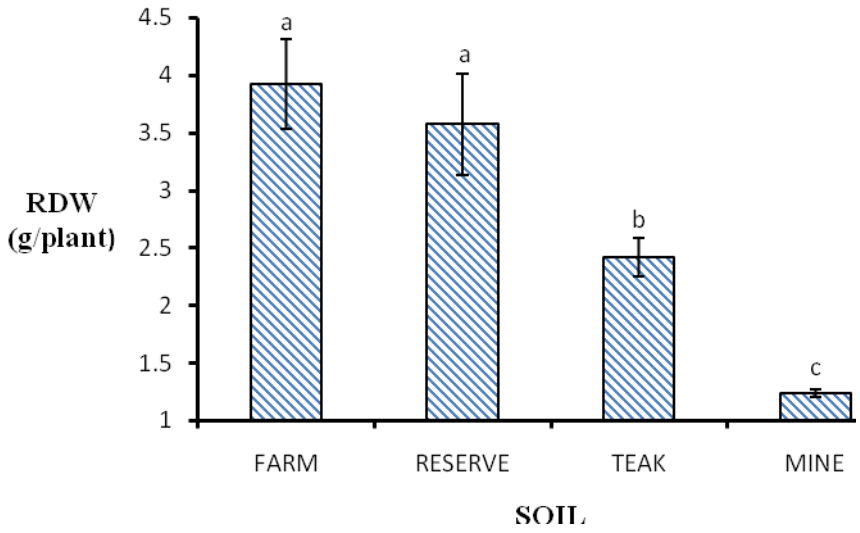

Figure 4.7 Root Dry Weight (RDW) of *Tetrapluera teraptera* seedlings grown in soils from different sites.
Error bars indicate standard error of mean. Different letters on top of error bars indicate significant difference ($P \leq 0.05$) using Least Significant Difference (LSD).

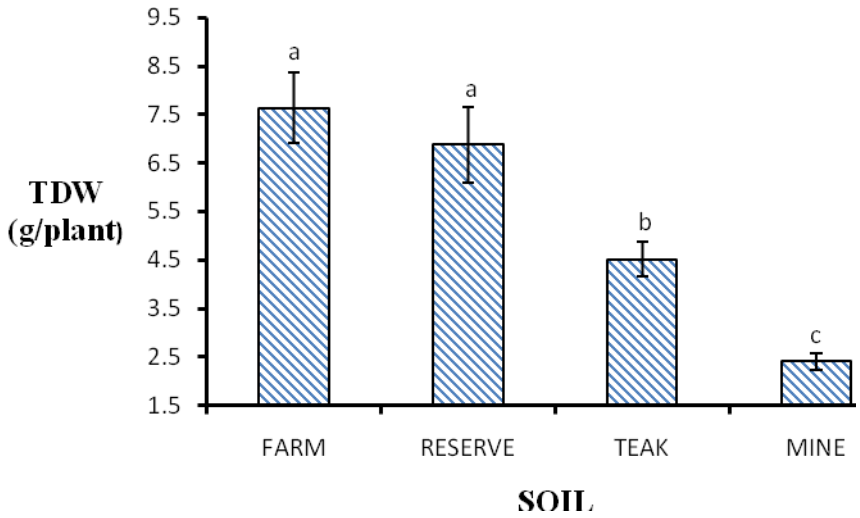

Figure 4.8 Total Dry Weight (TDW) of *Tetrapluera teraptera* seedlings grown in soils from different site.
Error bars indicate standard error of mean. Different letters on top of error bars indicate significant difference ($P \leq 0.05$) using Least Significant Difference (LSD).

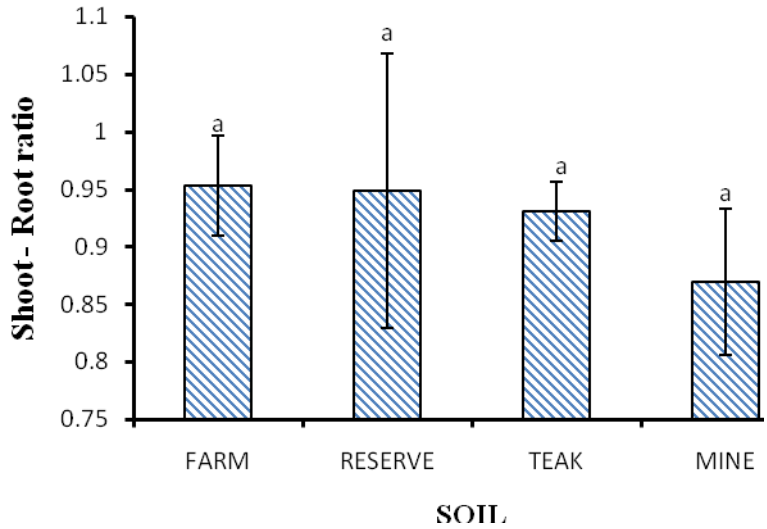

Figure 4.9 Shoot - Root ratio of *Tetrapluera teraptera* seedlings grown in soils from different site.
Error bars indicate standard error of mean. Different letters on top of error bars indicate significant difference ($P \leq 0.05$) using Least Significant Difference (LSD).

4.5 Biomass Nutrient Concentration and Uptake

4.5.1 Nitrogen Concentration (% N) and Uptake (mg)

ANOVA showed significant effect (P = 0.05) of different soils on shoot nitrogen concentration (% N). The highest shoot nitrogen concentration (1.52%) and lowest (1.12%) was observed in reserve and teak soil respectively. No significant difference was observed between the % shoot N of reserve, farm and mine soil (Table 4.2). Soils had a significant effect (P = 0.05) on root nitrogen concentration (Table 4.2). Aside reserve soil which wasn't significantly different from mine soils, all the other soils had a lower % root N to mine soil. Significant effect (P = 0.05) of seedling % total N was observed with the highest of 2.24% recorded in reserve soil and least of 1.71% recorded in teak soil (Table 4.2)

After six months of growth, soils had a significant effect (P < 0.01) on shoot N uptake. The highest shoot N uptake was recorded in reserve soil (50.0 mg/plant) and least of 15.6 mg/plant was recorded in mine soil (Table 4.2). Significant effect (P < 0.01) was observed on root N uptake of seedlings grown in different soils. Root N uptake were 25.50 mg/plant, 24.54 mg/plant, 14.47 mg/plant and mine 10.40 mg/plant for reserve soil, farm soil, teak soil and mine soil respectively. Similarly, soils significantly (P < 0.01) influenced total seedling N uptake. The highest value of 153.2 mg/plant was observed in reserve soil which was statistically similar to farm soil (148.8 mg/plant) but was significantly higher than 76.7 mg/plant and 52.5 mg/plant for teak and mine respectively (Table 4.2)

Table 4.2 Statistical analysis of the effect of soils on nitrogen concentration and content of *Tetrapluera tetraptera* at 24 weeks after planting

SOILS	NITROGEN CONCENTRATION (%)			NITROGEN UPTAKE (mg)		
	SHOOT	**ROOT**	**TOTAL**	**SHOOT**	**ROOT**	**TOTAL**
Farm	1.34[a] (± 0.11)	0.63[b] (± 0.02)	1.97[ab] (± 0.12)	49.0[a] (± 3.20)	24.54[a] (± 2.39)	148.8[a] (± 11.13)
Reserve	1.52[a] (± 0.05)	0.72[ab] (± 0.05)	2.24[a] (± 0.09)	50.0[a] (± 5.06)	25.50[a] (± 2.79)	153.3[a] (± 16.27)
Teak	1.12[b] (± 0.10)	0.60[b] (± 0.04)	1.71[b] (± 0.12)	23.1[b] (± 1.83)	14.47[b] (± 1.51)	76.7[b] (± 5.44)
Mine	1.33[a] (± 0.05)	0.84[a] (± 0.06)	2.17[a] (± 0.08)	15.6[b] (± 2.12)	10.40[b] (± 0.64)	52.5[b] (± 4.64)
P Value	0.013	0.023	0.016	< 0.001	< 0.001	< 0.001

Figures in the same column followed by same superscript letter are not significantly different at $P \leq 0.05$ level using LSD test (n = 5). Numbers in parenthesis are standard error of the means.

4.5.2 Phosphorus Concentration (% P) and UPTAKE (mg)

Although the shoot phosphorus concentration (% P) was 0.23%, 0.20%, 0.19% and 0.19% for farm, mine, reserve and teak respectively, there was no significant difference between soils ($P > 0.05$) (Table 4.3). Similarly, soils had no significant effect ($P > 0.05$) on % root P (Table 4.3). The highest % root P of 0.22% was recorded in mine and the least of 0.14% was recorded in teak soil. The decreasing order of % total P was farm soil (0.42%), mine (0.42%), reserve (0.37%) and teak (0.33%) but no statistical difference ($P > 0.05$) was observed between soils (Table 4.3).

Table 4.3 Statistical analysis of the effect of soils on phosphorus concentration and content of *Tetrapluera tetraptera* at 24 weeks after planting

SOILS	PHOSPHORUS CONCENTRATION (%)			PHOSPHORUS UPTAKE (mg)			
	SHOOT	**ROOT**	**TOTAL**	**SHOOT**	**ROOT**	**TOTAL**	**SHOOT P : N**
Farm	0.23^{a} (± 0.03)	0.19^{a} (± 0.03)	0.42^{a} (± 0.04)	8.68^{a} (± 1.63)	7.58^{a} (± 1.86)	32.5^{a} (± 6.24)	0.18^{a} (± 0.03)
Reserve	0.19^{a} (± 0.03)	0.18^{a} (± 0.01)	0.37^{a} (± 0.03)	6.42^{ab} (± 1.52)	6.48^{ab} (± 0.99)	25.9^{ab} (± 4.96)	0.12^{a} (± 0.12)
Teak	0.19^{a} (± 0.02)	0.14^{a} (± 0.03)	0.33^{a} (± 0.06)	4.00^{bc} (± 0.74)	3.59^{bc} (± 1.02)	15.3^{bc} (± 3.42)	0.17^{a} (± 0.03)
Mine	0.20^{a} (± 0)	0.22^{a} (± 0.03)	0.42^{a} (± 0.03)	2.34^{c} (± 0.31)	2.70^{c} (± 0.37)	10.2^{c} (± 1.23)	0.15^{a} (± 0.01)
P Value	0.615	0.32	0.397	0.023	0.026	0.015	0.352

Figures in the same column followed by same superscript letter are not significantly different at $P \leq 0.05$ level using LSD test (n = 5). Numbers in parenthesis are standard error of the means.

Soils significantly ($P < 0.05$) influenced the shoot P content of seedlings. The highest shoot P uptake of 8.68 mg/plant was observed in farm soil and this was significantly higher than teak (4.00 mg/plant) and mine (2.34 mg/plant). Analysis of variance showed that soils had a significant effect ($P < 0.05$) on root P uptake. Aside reserve soil whose root P uptake (6.48 mg/plant) wasn't significantly different from that of farm soils (7.58 mg/plant), both teak soils (3.59 mg/plant) and mine soils (2.70 mg/plant) had lower root P uptake when compared to farm soil. Significant difference ($P < 0.05$) was observed in seedling total P uptake. The highest total P uptake of 32.5 mg/plant was observed in farm and least of 10.2 mg/plant in mine soil. No

statistical diference was observed between farm and reserve soil (25.9 mg/plant) (Table 4.3). The shoot P and N ratio was not significantly different ($P < 0.05$) for seedlings grown in different soils (Table 4.3).

4.6 Nutrient Use Efficiency

Nitrogen Use Efficiency (NUE) of seedlings was significanly ($P < 0.01$) influenced by soils. The NUE of seedlings in farm soil (0.396 g/mg) was significantly higher than those in teak soil (0.269 g/mg) and mine soil (0.111 g/mg). No significant difference was observed between the NUE of seedlings in farm soil and reserve soil (0.310 g/mg) (Fig. 4.10). Soils had a significant influence ($P < 0.01$) on Phosphorus Use Efficiency (PUE) of seedlings (Fig. 4.11). The highest PUE of 1.865g/mg and lowest of 0.576g/mg was recorded for reserve and mine respectively.

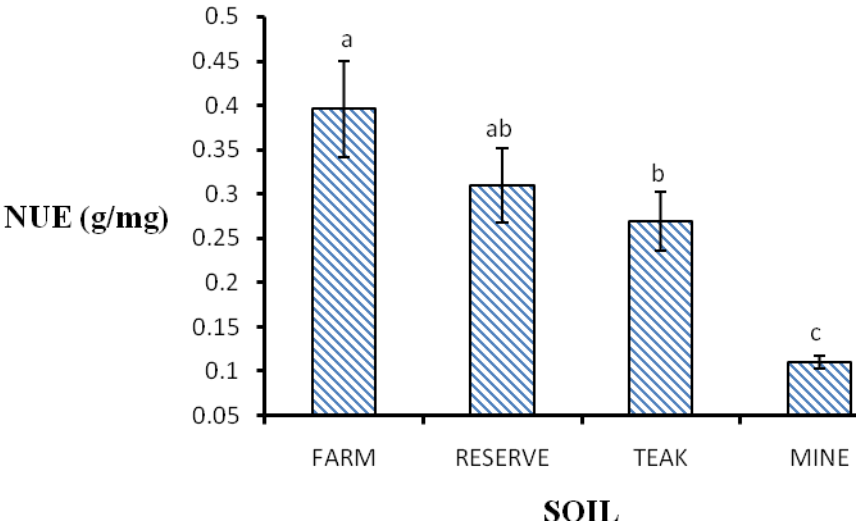

Figure 4.10 Nitrogen Use Efficiency (NUE) of *Tetrapluera teraptera* seedlings grown in soils from different site.
Error bars indicate standard error of mean. Different letters on top of error bars indicate significant difference ($P \leq 0.05$) using Least Significant Difference (LSD).

4.7 Percentage Mycorrhiza Colonization (% MC) and Nodulation

The percentage mycorrhiza colonization (% MC) are 43%, 39.5%, 31% and 25% for reserve, mine, teak and farm soils respectively but no significant difference ($P > 0.05$) was observed between them (Fig. 4.12). No nodules were observed on any of the seedlings growing in the various soils.

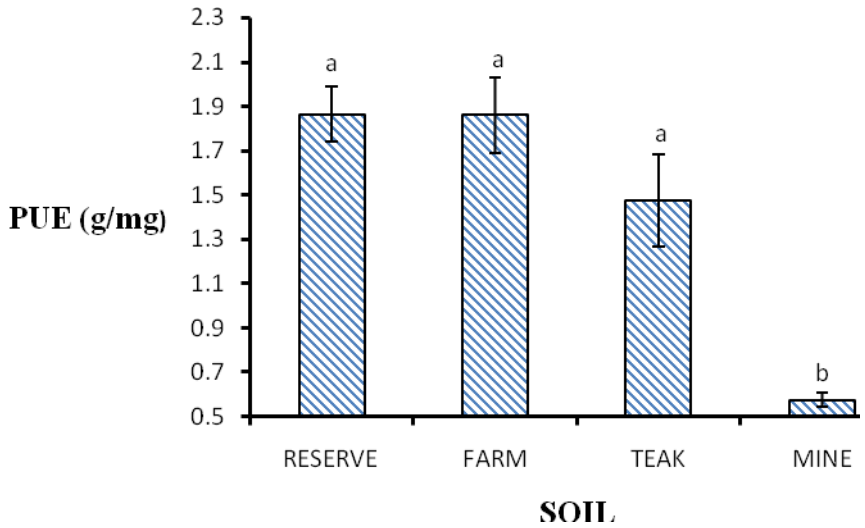

Figure 4.11 Phosphorus Use Efficiency (PUE) of *Tetrapluera teraptera* seedlings grown in soils from different site.
Error bars indicate standard error of mean. Different letters on top of error bars indicate significant difference ($P \leq 0.05$) using Least Significant Difference (LSD).

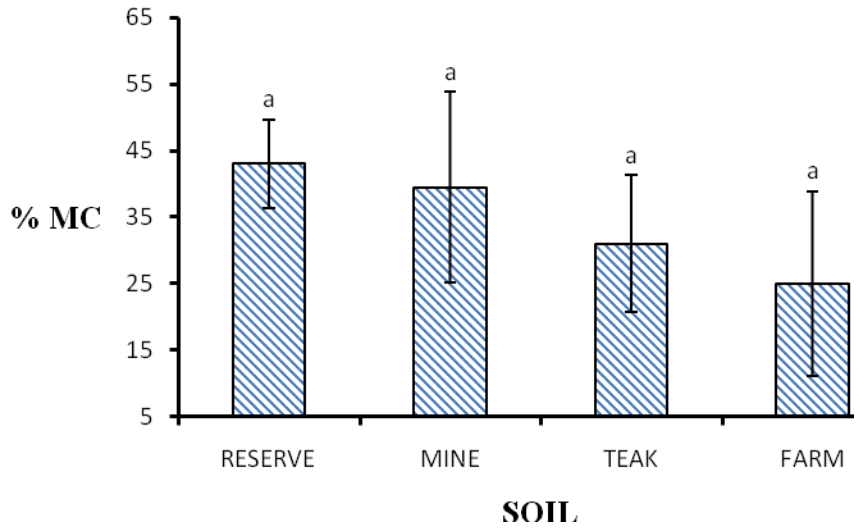

Figure 4.12 PercentageMycorrhiza Colonisation (% MC) of *Tetrapluera teraptera* seedlings grown in soils from different site.
Error bars indicate standard error of mean. Different letters on top of error bars indicate significant difference ($P \leq 0.05$) using Least Significant Difference (LSD).

4.8 Correlation Analysis

The relationship between Percentage Mycorrhiza colonization (% MC) and other seedling morphological and physiological features was determined. Aside total seedling nitrogen concentration which showed a positive correlation with % MC, none of the measured parameters showed any significant correlation (Table 4.4). There was a significant negative correlation between % MC and shoot P to N ratio. Root dry weight showed a positive correlation with seedling total dry weight, shoot N uptake, root N uptake, total N uptake, shoot P uptake, root P uptake and total P uptake (Table 4.4).

Table 4.4 Correlation of Percentage Mycorrhiza colonization and root dry weight with other seedlingmorpological and physiological parameters.

SEEDLING PARAMETERS	**PERCENTAGE MYCORRIZA COLONIZATION**	**ROOT DRY WEIGHT**
Total dry weight	-0.400	0.9993**
Total N concentration	0.800*	-0.0012
Total P concentration	-0.211	-0.0367
Shoot N uptake	0.200	0.9629*
Root N uptake	0.200	0.9688*
Total N uptake	0.200	0.9658*
Shoot P uptake	-0.400	0.9655*
Root P uptake	-0.400	0.9671*
Total P uptake	-0.400	0.9705*
Shoot P : N	-1.00**	0.011

*and ** means significant at 5% and 1% propability levels respectively

CHAPTER FIVE

DISCUSSION

The growth of seedlings is affected by a number of ecological factors of which soil plays a very important role. This research has shown the growth potentials and limitations of *T. teraptera* seedlings in soils of different fertilities.

5.1 Morphological growth

No differences were observed in the morphological growth of seedlings growing in reserve and farm soil. This finding is contrary to Blay (1997) who observed that *T. teraptera* seedlings in farm had higher growth rate than those in reserve. Contrary to Blay's research which was conducted at different locations with varying microclimate conditions, this research was conducted at one site with the treatments having the same microclimate conditions. Therefore, the differences in growth observed by Blay may be related to variation in microclimate conditions such as light intensity and duration, temperature and humidity instead of soil fertility. Seedlings growing in farm and reserve soils demonstrated greater heights than those in teak and mine soils. Height is a good estimator of growth as it determines the photosynthetic capacity of seedlings. However interpretation of greater height as an indication of seedling survival is contradictory as taller seedlings tend to be very susceptible to drought due to greater transpirational area (Haase, 2007; 2008). Meanwhile on weedy sites taller seedlings may have greater competitive advantage (Haase, 2007; 2008). Diameter on the other hand is a good indicator of both growth and survival. Higher stem diameter as in the case of seedlings in farm and reserve soils imply that these seedlings have higher carbohydrate reserve, water, greater root system and resistant to desiccation than those in teak and mine soil (Tsakaldimi *et al*., 2012). Positive correlation has been reported between seedling diameter and other growth

morphological parameters such as height, shoot, root and total dry weight (Ivetić *et al*., 2013). Similarly in this research, seedlings having greater diameter also recorded greater heights, relative growth rates and dry weights. Although there were differences in height and diameter of seedlings in the different soils, irrespective of the soil there was a balance between these two growth morphological parameters. This led to the observation of no statistical difference observed in sturdiness quotient and as such all the seedlings are immune to wind damage.

Seedling dry weight is a demonstration of the net gain in photosynthesis, with those having higher dry weights having greater chance of survival (Tsakaldimi *et al*., 2012). Seedlings growing in reserve and farm soils having greater dry weights therefore have higher chance of survival than those in teak and mine soils. Differences observed on the morphological growth of seedlings in the different soils can be attributed to soils influence on root growth (Fig. 4.7) as this was observed to be positively correlated with shoot nitrogen uptake ($P < 0.05$, $r^2 = 0.96$); root nitrogen uptake ($P < 0.03$, $r^2 = 0.97$); total nitrogen uptake ($P < 0.05$, $r^2 = 0.97$); shoot phosphorus uptake ($P < 0.05$, $r^2 = 0.97$); root phosphorus uptake ($P < 0.05$, $r^2 = 0.97$); and total phosphorus uptake ($P < 0.05$, $r^2 = 0.97$) (Table 4.7). Significant differences observed in root growth of seedlings across the different soils are probably due to differences in the soil organic matter (Table 4.1). According to Baligar *et al.* (2001) through the improvement of improvement of soil structure, water holding capacity and leaching reduction, soil organic matter influences root development. This favours roots to explore large volume of the soil to absorb nutrients. Farm and reserve soils having high organic matter of 4.33% and 4.14% respectively produced seedlings with greater root biomass (Fig. 4.7). Biomass allocation ratio as an indication of nutrient stress has been documented by many authors (Chapin, 1980; Lambert, 1995; Twum-Ampofo, 2008). However contrary to these reports, no significant difference was observed in the

shoot to root ratio of the seedlings growing in the different soils (Fig 4.9). This finding affirms the assertion by Aerts and Chapin (2000) who stated that rapidly growing seedlings send more photosynthates to roots for them to absorb more nutrients leading to support growth. Matured *T. teraptera* trees have been reported to have deep root system (Anglaaere, 2005). Therefore shoot to root ratio, as an indication of nutrient stress is a poor indicator in this regard when it comes to *T. teraptera* seedlings.

Although percentage mycorrhiza colonization (% MC) was low, this research confirms that native mycorrhiza is part of *T. teraptera* root system. Similar low % MC by indigenous mycorrhiza has been reported in *Albizia adianthifolia*, *Albizia zygia* and *Albizia ferruginea* in the absence of inoculation and phosphorus fertilizer application (Twumasi, 2005). All these trees including *T. tetrap*tera are all mimosaceae legumes.

No nodules were found on seedlings in all treatments and this contradicts report of Diabete *et al.* (2005). This may result from the fact that *T. teraptera* may not be promiscuous in nodulation and may require inoculation. Furthermore nodulation has been documented to require very high phosphorus cost to seedlings (Magadlela, 2013) and based on Halm (1978) classification of Ghana's soil according to available soil phosphorus ratings all the soils used were low in P except for farm which is slightly moderate. This confirms the report about *Acaciella angustissima* which is also a mimosaceae legume having no nodulation in the absence of inoculation and supplementation with phosphorus fertilizer (Ruiz-Valdiviezo *et al*., 2009).

5.2 Physiological growth

Luxury consumption and slow growth has been reported to be a survival strategy for plants growing in nutrient deficient soils (Chapin, 1980; Lambert, 1995; Aerts and Chapin, 2000) and this was observed in seedlings growing in mine soil. Although seedlings in mine soil had high

nutrient concentrations (Tables 4.2 and 4.3) seedlings growing in it produced the least biomass (Fig. 4.6, 4.7 and 4.8) due to low nutrient use efficiency.

Variations in root growth across the soils contributed to the different nutrient uptake and nutrient use efficiency as was reported by Baligar *et al*. (2001) and Malik and Rengal (2013). Higher nutrient use efficiency in soils from farm (NUE = 0.396 g/mg; PUE = 1.865 g/mg), reserve (NUE = 0.310 g/mg; PUE = 1.865 g/mg) and teak (NUE = 0.269 g/mg; PUE = 1.475 g/mg) led to higher growth rate of seedlings. This is further supported by the higher relative growth rate in these soils which implies how efficiently these seedlings will grow when resources are limited.

Greater fluctuation was observed in N concentration in seedlings (Table 4.2) but same phenomenon was not observed in P concentration (Table 4.3). Therefore, this implies that basal internal P demand has to be met before increase in growth occurs. The supremacy of internal P demand is a reflection of how deficient the soils used are in phosphorus. Farm soil and reserve soil having higher phosphorus levels of 11 mg/Kg and 5.7 mg/Kg respectively produced seedlings with greater biomass (Fig. 4.6, 4.7 and 4.8). Increasing phosphorus level has been identified to increase growth, phosphorus concentration and content in *Albizia adianthifolia*, *Albizia zygia*, *Albizia ferruginea* and *Acacia senegal* (Twumasi, 2005; Isaac *et al*., 2011). Contrary to reports by McHargue (1999), Rao and Tak (2001), Twum-Ampofo (2008) and Twumasi (2005) concentration, no correlation was observed between % MC and other growth parameters measured (Table 4.4) with the exception of total seedling N.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The benefits of trees in agroforestry systems can be fully realized after trees survive the vulnerable seedlings stage and develop to become mature trees.

This research has proved that although *Tetrapluera tetraptera* is an indigenous leguminous tree, its seedling growth is affected by various soil conditions. Overall, the study demonstrated that *T. tetraptera* seedlings can survive and grow in all the studied soils. However, growth of seedlings in mine soil was significantly reduced compared to farm, reserve and teak soils. The reduction in growth rate was a survival strategy adapted by the seedlings in low nitrogen and phosphorus and high water logging conditions. No significant difference was observed in the growth of seedlings in farm and reserve soil. Since these soils had the highest growth rate it implies that under ideal soil conditions *T. tetraptera* seedlings will have fast growth rate. Although growth of seedlings in teak soil was higher than seedlings in mine soil it was lower than farm and reserve soil. This reduced growth rate was probably due to low organic matter in teak soil.

Seedlings growing in reserve and farm soil had greater root biomass that made them acquire nutrients more efficiently. Unlike mine soil which exhibited luxury consumption, seedlings in reserve and farm soil converted absorbed nutrients to biomass production. Low soil phosphorus was reflective in biomass low phosphorus concentration in seedlings growing in the different soils. The study confirm mycorrhiza been part of the root system of *T. tetraptera* seedlings. However aside seedlings total nitrogen concentration, percentage mycorrhiza colonization did not improve other growth parameters of seedlings.

6.2 Recommendations

(i) Growth was higher in farm, reserve and teak soils than mine soil. Since this observation was made under nursery conditions within a short period (6 months), it is recommended that the research is replicated under field conditions for a longer period to see if the same results can be obtained.

(ii) Farm, reserve, teak and mine soils used were low in both nitrogen and phosphorus. This might have influenced the growth of *Tetrapluera tetraptera* seedlings in these soils. As a result, further research could be conducted on the influence of nitrogen and phosphorus fertilizer on the growth of *T. tetraptera* in these soils.

(iii) The absence of nodulation and low mycorrhiza root colonization observed in this study requires a further research on the growth response of *T. teraptera* to inoculation with different strains of arbuscular mycorrhiza fungi and rhizobia.

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APPENDICES

Appendix 1 Analysis of variance test for seedling morphological parameters as affected by different soils

(a) Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	133.50	44.50	2.03	
Block.*Units* stratum					
Treatment	3	4530.70	1510.23	68.86	<.001
Residual	873	19145.72	21.93		
Total	879	23809.92			

d.f. = degree of freedom, s.s. = sum of squares, m.s. = mean sum of square, v.r. = variance ratio, F pr. = F propability

(b) Diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	6.356	2.119	0.83	
Block*Units* stratum					
Treatment	3	176.724	58.908	23.04	<.001
Residual	873	2231.607	2.556		
Total	879	2414.687			

(c) Sturdiness Quotient

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.63559	0.21186	2.60	
Block*Units* stratum					
Treatment	3	0.63937	0.21312	2.62	0.115
Residual	9	0.73323	0.08147		
Total	15	2.00819			

(d) Relative Height Growth Rate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.00001059	0.00000353	0.12	
Block*Units* stratum					
Treatment	3	0.00233537	0.00077846	26.17	<.001
Residual	9	0.00026772	0.00002975		
Total	15	0.00261368			

(e) Relative Diameter Growth Rate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.00002555	0.00000852	0.27	
Block*Units* stratum					
Treatment	3	0.00142257	0.00047419	14.99	<.001
Residual	9	0.00028473	0.00003164		
Total	15	0.00173285			

(f) Shoot Dry Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.8025	0.2675	0.83	
Block*Units* stratum					
Treatment	3	16.0881	5.3627	16.62	<.001
Residual	9	2.9043	0.3227		
Total	15	19.7949			

(g) Root Dry Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	1.6939	0.5646	1.82	
Block*Units* stratum					
Treatment	3	17.8149	5.9383	19.11	<.001
Residual	9	2.7973	0.3108		
Total	15	22.3060			

(h) Total Dry Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	4.391	1.464	1.20	
Block*Units* stratum					
Treatment	3	67.658	22.553	18.44	<.001
Residual	9	11.004	1.223		
Total	15	83.053			

(i) Shoot to Root Ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.12042	0.04014	2.78	
Block*Units* stratum					
Treatment	3	0.01777	0.00592	0.41	0.750
Residual	9	0.12994	0.01444		
Total	15	0.26813			

Appendix 2 Analysis of variance test for seedling physiological parameters as affected by different soils

(a) Shoot Nitrogen Concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.17997	0.05999	3.50	
Block*Units* stratum					
Treatment	3	0.32527	0.10842	6.32	0.013
Residual	9	0.15431	0.01715		
Total	15	0.65954			

(b) Root Nitrogen Concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.004275	0.001425	0.15	
Block*Units* stratum					
Treatment	3	0.149625	0.049875	5.28	0.023
Residual	9	0.085075	0.009453		
Total	15	0.238975			

(c) Total Nitrogen Concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.19207	0.06402	1.69	
Block*Units* stratum					
Treatment	3	0.68122	0.22707	5.99	0.016
Residual	9	0.34131	0.03792		
Total	15	1.21459			

(d) Shoot Nitrogen Uptake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	132.91	44.30	1.02	
Block*Units* stratum					
Treatment	3	3757.86	1252.62	28.82	<.001
Residual	9	391.13	43.46		
Total	15	4281.90			

(e) Root Nitrogen Uptake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	63.69	21.23	1.47	
Block*Units* stratum					
Treatment	3	668.66	222.89	15.41	<.001
Residual	9	130.21	14.47		
Total	15	862.56			

(f) Total Nitrogen Uptake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	1846.7	615.6	1.61	
Block*Units* stratum					
Treatment	3	31097.0	10365.7	27.14	<.001
Residual	9	3437.9	382.0		
Total	15	36381.6			

(g) Shoot Phosphorus Concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.005675	0.001892	0.74	
Block*Units* stratum					
Treatment	3	0.004825	0.001608	0.63	0.615
Residual	9	0.023075	0.002564		
Total	15	0.033575			

(h) Root Phosphorus Concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.009050	0.003017	1.00	
Block*Units* stratum					
Treatment	3	0.012150	0.004050	1.35	0.320
Residual	9	0.027100	0.003011		
Total	15	0.048300			

(i) Total Phosphorus Concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.027725	0.009242	1.36	
Block*Units* stratum					
Treatment	3	0.022425	0.007475	1.10	0.397
Residual	9	0.061025	0.006781		
Total	15	0.111175			

(j) Shoot Phosphorus Uptake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	14.563	4.854	0.83	
Block*Units* stratum					
Treatment	3	92.464	30.821	5.25	0.023
Residual	9	52.846	5.872		
Total	15	159.872			

(k) Root Phosphorus Uptake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	28.627	9.542	2.22	
Block*Units* stratum					
Treatment	3	64.225	21.408	4.98	0.026
Residual	9	38.693	4.299		
Total	15	131.544			

(l) Total Phosphorus Uptake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	327.55	109.18	1.66	
Block*Units* stratum					
Treatment	3	1219.21	406.40	6.17	0.015
Residual	9	592.86	65.87		
Total	15	2139.62			

(l) Nitrogen Use Efficiency

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.020062	0.006687	1.20	
Block*Units* stratum					
Treatment	3	0.171269	0.057090	10.27	0.003
Residual	9	0.050052	0.005561		
Total	15	0.241383			

(m) Phosphorus Use Efficiency

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.0819	0.0273	0.25	
Block*Units* stratum					
Treatment	3	4.4148	1.4716	13.51	0.001
Residual	9	0.9804	0.1089		
Total	15	5.4772			

Appendix 3 Friedman's test of the influence of soils on percentage mycorrhiza colonization of seedlings

(a) Percentage Mycorrhiza Colonisation

Based on 4 blocks of 4 treatments
Friedman's statistic = 5.40
Adjusted for ties = 5.40
P-value using chi-square approximation (3 d.f.) = 0.145
Based on 3 degrees of freedom
Warning: P-value is approximate - check with values below if borderline.
5% point = 7.80
1% point = 9.60