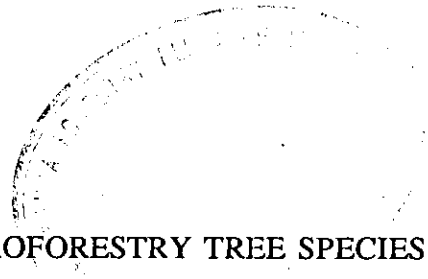

INOCULATION OF SELECTED AGROFORESTRY TREE SPECIES WITH *RHIZOBIUM* AND SUBSEQUENT CROP AND SOIL NITROGEN RESPONSES

A Thesis

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ABSTRACT

INOCULATION OF SELECTED AGROFORESTRY TREE SPECIES WITH *RHIZOBIUM* AND SUBSEQUENT CROP AND SOIL NITROGEN RESPONSES

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University of Guelph, 1995

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This study was conducted to test the effect of *Rhizobium* inoculation on 4 tree legumes: *Leucaena leucocephala, Calliandra calothyrsus, Sesbania sesban* (commonly used in intercropping systems in Kenya) and *Robinia pseudoacacia* (a temperate tree legume). Early growth patterns, intercropping interactions, decomposition and mulching characteristics were investigated with respect to soil and plant nitrogen (N) nutrition. It was found that *Rhizobium* inoculation increased: shoot N concentration in all species (72%, 86%, 68% and 34% respectively), shoot total N content in *L. leucocephala* and *C. calothyrsus*, nodule number and nodule weight in *C. calothyrsus* and nitrate in soils in which *R. pseudoacacia* was grown. In barley intercropped with *C. calothyrsus* and *S. sesban*, barley ear nitrogen concentration, total N content, grain yield, N concentration and N content were also increased. Inoculation also increased decomposition of *L. leucocephala* and *R. pseudoacacia* leaves and barley ear N concentration and N content in soil mulched with mixed leaves of *L. leucocephala*.

ACKNOWLEDGEMENTS

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Finally I thank all my family members for their love and prayers.

I would like to dedicate this thesis to my heavenly father, God Almighty, to my loved ones Mutua and Kioko and to mom and dad.

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cm	centimetres
g	grams
$g.plant^{-1}$	grams per plant
kg	kilograms
$kg.ha^{-1}y^{-1}$	kilograms per hectare per year
L	litres
mg	milligrams
ml	millilitres

GENERAL INTRODUCTION

The element nitrogen (N) is a constituent of proteins and nucleic acids, forms part of every living cell (Postgate, 1978; Marschner, 1986) and is therefore a key element required for plant growth (Danso, 1992). Major roles of N in plant nutrition include: (1) component of chlorophyll; (2) component of amino acids, the building blocks of proteins; (3) essential for carbohydrate utilization; (4) component of enzymes, vitamins and hormones; (5) stimulant of root development and activity; and (6) support to uptake of other nutrients (Stevenson, 1986). Abundance of N leads to green succulent growth in plants, while its deficiency causes loss of photosynthetic colour, reduction in protein production, enhanced senescence of older leaves, stagnant growth, poor yields and crop failures (FitzPatrick, 1986; Marschner,1986; Danso, 1992).

Nitrogen occurs in various forms: (a) as a pure gas (N_2), as nitrous oxide (N_2O), and as nitric oxide (NO) and ammonia (NH_3), (b) in the ionic form as nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+), and (c) in the organic form such as urea ($CO(NH_2)_2$) and organic matter (Sprent, 1987; Bockman *et al.*, 1990; Stevenson, 1986). Higher plants utilize the ionic forms of N (NO_3^- and NH_4^+) (Marschner, 1986).

Nitrogen gas comprises about 79% of the atmosphere and is the ultimate source of N. However, it has low chemical reactivity under most natural conditions and only some bacteria (e.g *Rhizobium* spp.) can utilize it (Sprent, 1987, Bockman *et al.*, 1990). Therefore to be usable by most organisms, N must be converted to nitrate or ammonium and this conversion is called N fixation. Nitrogen can be fixed through biochemical processes in some bacteria (biological N fixation), through the industrial synthesis of

ammonia for conversion into fertilizers, by heating air to high temperatures as in combustion (engines, fires) and by lightning (Bockman *et al.*, 1990).

In agricultural systems crop yields are often increased by applying N fertilizers (Danso, 1992) such as calcium ammonium nitrate, ammonium nitrate, ammonium sulphate, liquid ammonia, urea, nitrophosphate and calcium nitrate (Brady, 1974; Simpson, 1986). However, the recent scarcity of N fertilizers, the high energy required for their manufacture (200-800 atmospheres and 300-600°C), their increased selling prices especially in developing countries, their potential to pollute ground water with unused nitrate, denitrification effects on the ozone layer, transportation, storage, and the application costs have led to a tremendous interest in the search for alternative technologies to fertilizer N for the provision of fixed N for crops (Hardy and Havelka, 1975, Hauk, 1973). The desired technology must provide fixed N at a lower economic cost than fertilizer N and in adequate amounts to support high crop yields.

One of the cheap alternative sources of fixed N that could improve crop yields and sustain a healthy environment is biological N fixation supplied through legume-*Rhizobium* symbioses (Hardy and Havelka, 1975, Ackello *et al.*, 1985). Symbiotic N fixation provides legumes with a mechanism for obtaining N from the air. The symbiotic N fixation process utilizes rhizobia bacteria which invade the legume root system and form nodules in which N fixation occurs. In many situations, the establishment of effectively nodulated legumes requires the addition of specific effective rhizobia through legume-*Rhizobium* inoculation (Smith, 1987).

There are two broad classes of bacteria (based on growth rate) which form N

fixing nodules: (1) fast and (2) slow-growing types. The term *Rhizobium* refers to the fast-growing types while the term *Bradyrhizobium* is used for the slow-growing types (Date and Halliday, 1987).

It is generally estimated that biological N fixation accounts for about 175 million tonnes (t) of fixed N per year compared with about 80 million t of ammonia produced annually through the Haber Bosch industrial process, 75% of which is available for fertilizer use (Elkan, 1992). Furthermore, it is estimated that legume-*Rhizobium* symbioses in tropical soils can fix up to almost 600 $kg.ha^{-1}.y^{-1}$ N (Gibson *et al.*, 1982).

In this study three tropical tree legumes namely *Leucaena leucocephala* (Lam.) De Wit, *Calliandra calothyrsus* Meissner and *Sesbania sesban* (L.) Merr and (one temperate tree legume) *Robinia pseudoacacia* L. (Allen and Allen, 1981; Nair, 1993) were used in four different experiments, documented in chapters one to four. *L. leucocephala*, *C. calotthyrsus* and *S. sesban* are leguminous trees commonly used in intercropping systems in Kenya while *R. pseudoacacia* is a temperate legume with a potential of being used in agroforestry systems. The origin, present distribution and main uses of these four species is as follows (from Nair (1993): *L. leucocephala*, Leguminosae, Mimosoideae, native to central America and Mexico, introduced to South and Southeast Asia, Africa, South America and the Caribbean, uses: fuelwood, fodder, construction, pulpwood, food (pods, seeds, leaves) and alley cropping; *C. calothyrsus*, Leguminosae, Mimosoideae, native to central and south America, introduced to Indonesia, the Philippines, parts of Africa and the Caribbean, uses: fodder, green manure and honey production; *S. sesban*, Leguminosae, Papilionoideae, native to Egypt,

introduced in tropical Africa and Asia, uses: fuelwood, fodder, wood, fibre, green manure, ornamental, erosion control and windbreak and *R. pseudoacacia*, Leguminosae, Papilionoideae, native to northeastern United States, introduced to European temperate and Mediterranean regions, India and Thailand, uses: fuelwood, erosion control, fodder, windbreak, ornamental and honey production.

Chapter one documents the effect of *Rhizobium* inoculation on the early growth of these legumes and their subsequent effect on soil carbon and nitrate levels. In chapter two, the effects of *Rhizobium*-inoculated tree legumes on barley growth and grain yield under intercropping conditions were investigated. In chapter three, the decomposition rate of leaves from inoculated *L. leucocephala* and *R. pseudoacacia* was investigated, and finally, in chapter four, the effect of *Rhizobium* inoculation and point of mulch placement were investigated using *L. leucocephala* and *R. pseudoacacia* as test species. Chapter five (synthesis) summarizes the research findings of this study and presents the overall conclusions.

In chapters two and four, barley (*Hordeum vulgare* L.) (Shewry, 1992) was used as the test crop. Barley grain is principally used as a feed for animals, brewing malt and food for human consumption while barley straw is used for animal bedding. Immature barley plants may be harvested for forage by grazing or by cutting for hay or silage (Poehlman, 1985). In Canada, barley is the second ranking cereal after wheat, and in eastern Canada spring barley is an important cereal and is used mainly as feed grain for swine and poultry (Poehlman, 1985; Bulman and Smith, 1993). In Kenya, barley is grown in the Kenya highlands, 1,800 m above sea level, and is used to feed swine and

for the production of malting samples (Odingo, 1971).

CHAPTER ONE

Early growth patterns in *L. leucocephala, C. calothyrsus, S. sesban* and *R. pseudoacacia* After *Rhizobium* Inoculation

1.1. Introduction

Nitrogen (N) is a key element required for plant growth and development and in most agricultural systems N fertilizers are used to supply N to crops (Danso, 1992). However, in many developed countries the use of N fertilizers has caused health problems (e.g stomach cancer and infant methaemoglobinaemia) due to unused nitrate from agricultural fields leaching into drinking water (Bockman *et al.*, 1990). On the other hand, in tropical countries, N is the most common limiting nutrient for crop production because of low N levels in most soils, high cost (Schroder, 1992) and limited availability of N fertilizers especially to small scale farmers (Wadisirisuk *et al.*, 1988). In the farming systems of these countries symbiotic nitrogen-fixing legumes offer a cheap source of N for crops especially in small scale farming systems (Bohlool, 1988) and consequently leaves of legumes have been used as green manure for many centuries (Alazard *et al.*, 1988). Recently, there has been increasing interest in the use of leguminous trees and shrubs as a source of green manure. Leguminous trees and shrubs have an added advantage over herbaceous legumes because their leaves can be used by farmers over long periods of time.

Legumes establish a symbiotic relationship with bacteria of the genus *Rhizobium* such that the *Rhizobium*-legume symbiosis results in the formation of N fixing nodules

(Lara *et al.*, 1988). During the process of N fixation, N gas is reduced to ammonium (NH_4^+) (Evans *et al.*, 1988) which can be used by higher plants. *Rhizobium* inoculation (the addition of pure culture of *Rhizobium* to legume seeds or seedlings), can enhance and improve growth and yield of the legumes through biological N fixation (Burris, 1988; Dommergues, 1992). The total N content of plants is used to quantify N fixation while the increase in total N provides a direct estimate of N fixation (Lepo and Ferrenbatch, 1987).

Studies on the effect of *Rhizobium* inoculation on both woody and crop legumes are common. Turk *et al.*, (1993) observed that *Rhizobium* inoculation increased shoot N and dry weight, and nodule dry weight in *R. pseudoacacia* in Hawaii. Mulongoy and Owoaje (1992) observed that *Rhizobium* inoculation increased N fixation, dry matter yield and N in *L. leucocephala* in south-western Nigeria. Raj and Patel (1991) observed that *Rhizobium* inoculation increased grain dry forage yields in cowpea in India. Patel and Patel (1989) observed that *Rhizobium* inoculation increased number of nodules per plant in greengram varieties in India. Hadad *et al.*, (1986) observed that *Rhizobium* inoculation increased nodule mass in groundnuts (peanuts) (*Arachis hypogaea*) in Sudan. Further, in Kenya, Ssali (1988) observed that *Rhizobium* inoculation alone had no effect on dry matter yield of beans (*Phaseolus vulgaris*) while *Rhizobium* inoculation coupled with phosphorus increased dry matter yield of beans over control.

Most of the *Rhizobium* inoculation studies reported in the literature were only done at certain age of legume growth and not over an extended period of time. Therefore, the purpose of this study was to monitor dry matter yield (Shoot to root

ratio), total N, and N content of both shoots and roots of inoculated legumes over an eight week period of time and to assess nodule numbers and nodule dry weights in conjunction with changes in soil carbon and nitrate over the course of the experiment.

1.2. Materials and Methods

L. leucocephala, C. calothyrsus and *S. sesban* seeds were obtained from the Kenya Forestry Research Institute (KEFRI), Seed Centre, Muguga, Kenya. *R. pseudoacacia* seeds were obtained from Forestry Canada, Petawawa National Forestry Institute. Clean, undamaged seeds were sorted by hand, swirled for two minutes in 75% ethanol and rinsed in five changes of distilled deionized water. The seeds were then soaked in warm distilled deionized water overnight. *C. calothyrsus* and *L. leucocephala* were nicked with a razor blade before soaking to enhance water uptake into the seeds. The seeds were pregerminated in vermiculite in germination trays for 48 hours, in a greenhouse maintained at a temperature range of 19 to 24°C. Two seedlings were planted in each of the 1 L plastic pots, filled with approximately 2 kg of soil. The soil used (sandy loam) was obtained from the Cambridge Research Station of the University of Guelph, Ontario, Canada and contained 0.329% N, 2.686% total carbon, 1.709% organic carbon and had a pH of 7.35. The soil used had almost similar total N (%), total C (%) and pH compared to Kenyan soils obtained from two study sites selected for "The Effectiveness of Nitrogen Fixation Symbioses Under Arid Conditions of Kenya Project". These sites were Bura Riverine (0.3% N, 2.7% total carbon and a pH 7.6) and Kibwezi Mainland (0.3% N, 2.2% total carbon and a pH of 7.6) (Odee, 1993).

Pots were watered slowly to eliminate differences due to soil moisture content and allowed to equilibrate for 24 hours (Yobterik *et. al.*, 1994) prior to the tree seedling planting. Enough seedlings were planed to allow destructive sampling for every week, from week two to week eight, in three replications. After the seedlings were three days old, the pots were randomly divided into two sets. One set was inoculated with *Rhizobium* (Lipha Tech Inc., Milwaukee, USA) while the other set was left as a control. *L. leucocephala* and *C. calothyrsus* were inoculated with strain LX1271 (*L. leucocephala Rhizobium* strain), *R. pseudoacacia* with strain LX1285 (*R. pseudoacacia Rhizobium* strain) and *S. sesban* with strain LX1328 (*S. grandiflora Rhizobium* strain). 20 ml of *Rhizobium* slurry were applied around the seedlings, in a hole about 5 cm below the soil surface (Hadad *et al.*, 1986). The soil was kept moist throughout the experiment which was carried out in the greenhouse. The greenhouse temperature was maintained between 19 and 24°C and at 16 hours day light, supplemented by high-intensity sodium vapour lamps yielding a quantum flux density at pot level of 6 $\mu mol^{-2}s^{-1}$.

At each sampling time, shoot and root (without nodules) dry weights were determined after the plants were dried at 75°C for 48 hours. Shoot to root ratio was calculated from these dry weights. From the fourth week on, the dry shoots and roots were ground in a Wiley mill to pass through a 810 μm, No. 20 mesh sieve and total Kjedhal N was determined using a Technicon™ AutoAnalyzer™ II. Plant N contents were determined by multiplying the plant total N by its dry weight. At the eighth week, the plants along with the soil were gently removed from the pots and some soil was sampled and frozen (-22°C) for nitrate and carbon analysis; the rest of the soil on the root

was washed off in water. Nodules on each of the plants were removed by hand, counted and dried at 75°C for 48 hours and the dry weights were recorded. Soils for total and organic C determination were air dried at laboratory temperature and sieved to pass through a 250 μm, No. 60 mesh sieve. Soils for organic C determination were burnt in a muffle furnace (SB Linderg) at 475°C for 15 hours and then analysed for inorganic C using a Leco Carbon Determinator CR-12. The inorganic C values obtained were subtracted from total C to get organic C. Total C was also determined using a Leco Carbon Determinator CR-12.

Soil nitrate was extracted by shaking soil with 2 N potassium chloride (KCl) and analysed on the Technicon™ AutoAnalyzer™ II. The nitrate concentration was then calculated and expressed on a dry weight basis.

The data were analyzed statistically as a completely randomised block design using Statistical Analysis System (SAS), (1990). Least significant difference (LSD) and simple 't' tests ($p < 0.05$) were used to separate treatment means.

1.3. Results

Figure 1.1. shows that *Rhizobium* inoculation significantly increased shoot dry weights in *L. leucocephala* ($p<0.05$) at weeks 6 (1.5) and 8 (1.6 times), but decreased it in *S. sesban* ($p<0.05$) at weeks 3 (3 times), 4 (2 times), 5 (2 times), 6 (3 times) 7 (1.6 times) and 8 (1.5 times) and in *R. pseudoacacia* at week 7 (2 times). *Rhizobium* inoculation had no effect on shoot weights of *C. calothyrsus*. Means taken over time (appendix 1) showed that *Rhizobium* inoculation significantly decreased shoot dry weight of *S. sesban* (2 times) but had no effect on dry weights of other species.

Figure 1.2. shows that *Rhizobium* inoculation significantly increased root weights ($p<0.05$) in *L. leucocephala* at week 8 (1.3 times) but decreased in *S. sesban* ($p<0.05$) at weeks 5 (4 times), 6 (4 times), 7 (2 times) and 8 (2 times) and in *R. pseudoacacia* ($p<0.05$) at weeks 4 (2 times), 7 (1.7 times) and 8 (1.6 times). Means taken over time (appendix 1) showed that *Rhizobium* inoculation significantly decreased root weight ($p<0.05$) in *S. sesban* (2.5 times) and had no effect on root weight of other species.

Figure 1.3. shows shoot to root ratios recorded over an 8-week period. *Rhizobium* inoculation significantly increased shoot to root ratios in *L. leucocephala* at week 5 ($p<0.05$) (1.4 times), *S.sesban* ($p<0.05$) at weeks 5 (2 times) and 8 (1.3 times) and *R. pseudoacacia* ($p<0.05$) at week 4 (2 times) but decreased shoot to root ratio in *S. sesban* at weeks 2 ($p<0.05$) (2 times) and 3 ($p<0.01$) (6 times). However, means taken over time (Appendix 2) showed that *Rhizobium* inoculation significantly reduced ($p<0.05$) shoot to root ratio in *S. sesban* (52%) and had no effect on shoot to root ratios of other species. Shoot to root ratios of the inoculated plants were persistently higher after week

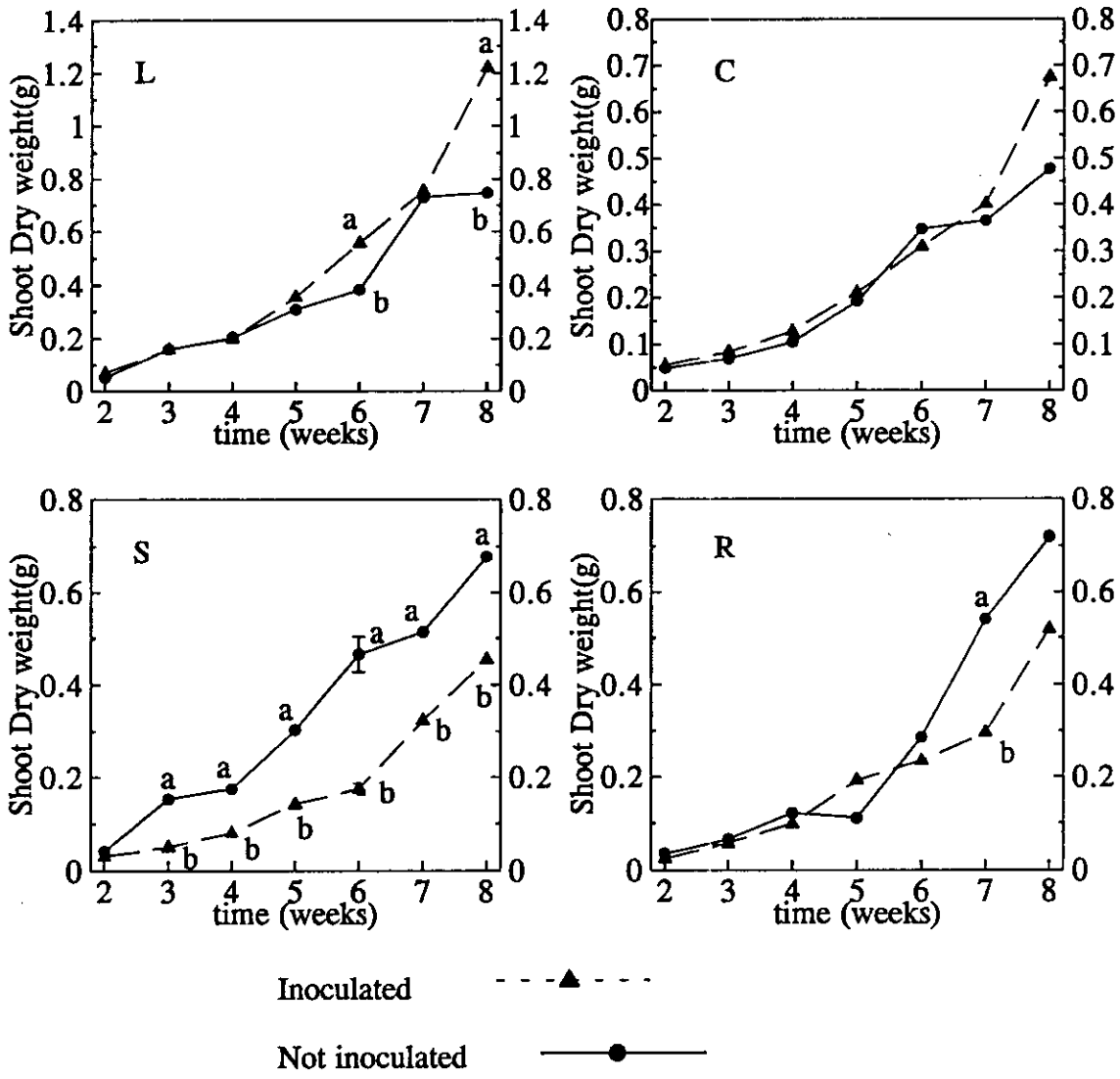

Figure 1.1. Shoot dry weights for (L) *L. leucocephala*, (C) *C. calothyrsus*, (S) *S. sesban* and (R) *R. pseudoacacia* seedlings with and without *Rhizobium* inoculation. a and b at same time beside data points indicates significant difference at $p < 0.05$.

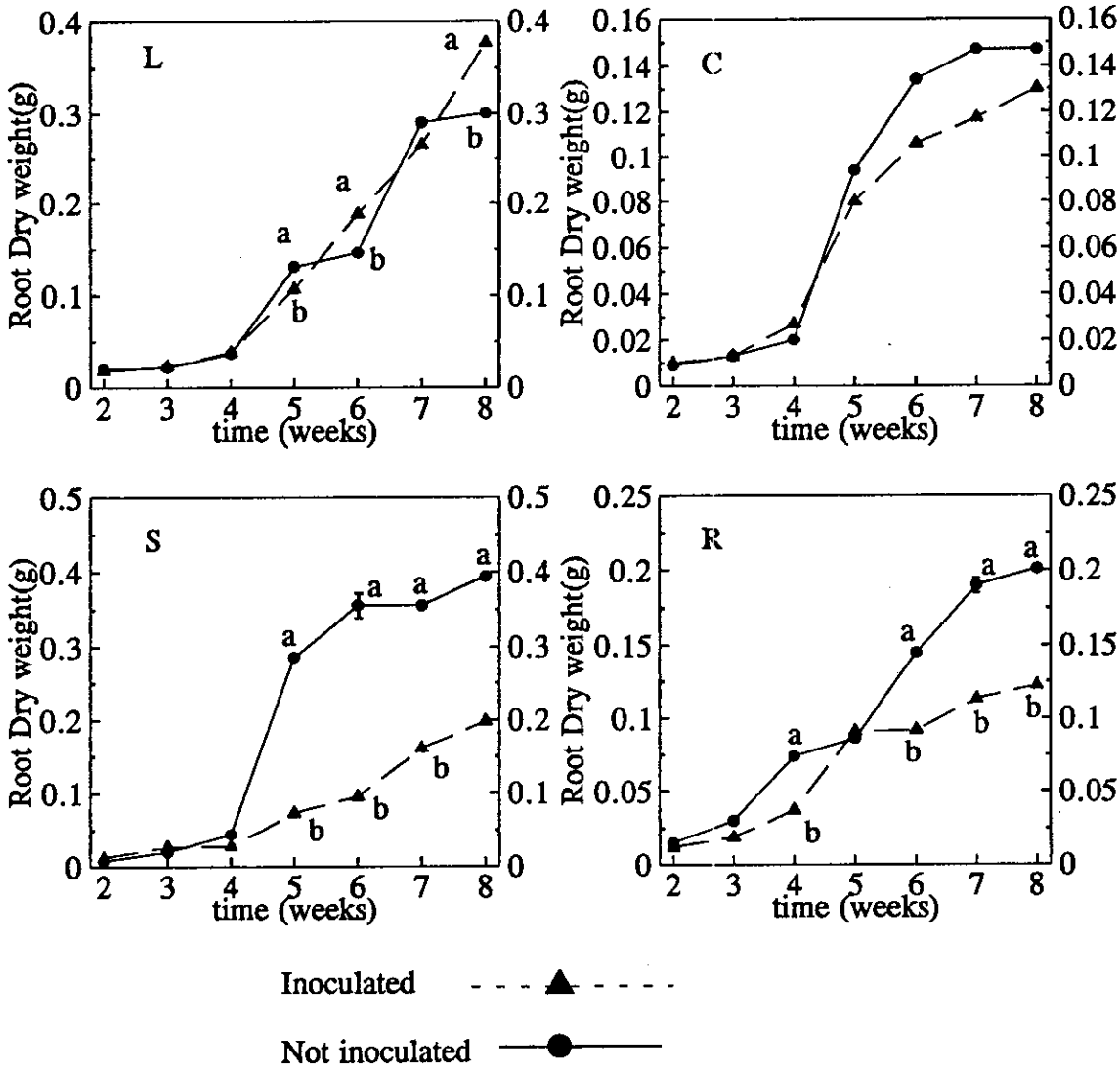

Figure 1.2. Root dry weights for (L) *L. leucocephala*, (C) *C. calothyrsus*, (S) *S. sesban* and (R) *R. pseudoacacia* seedlings with and without *Rhizobium* inoculation. a and b at same time beside data points indicates significant difference at $p < 0.05$.

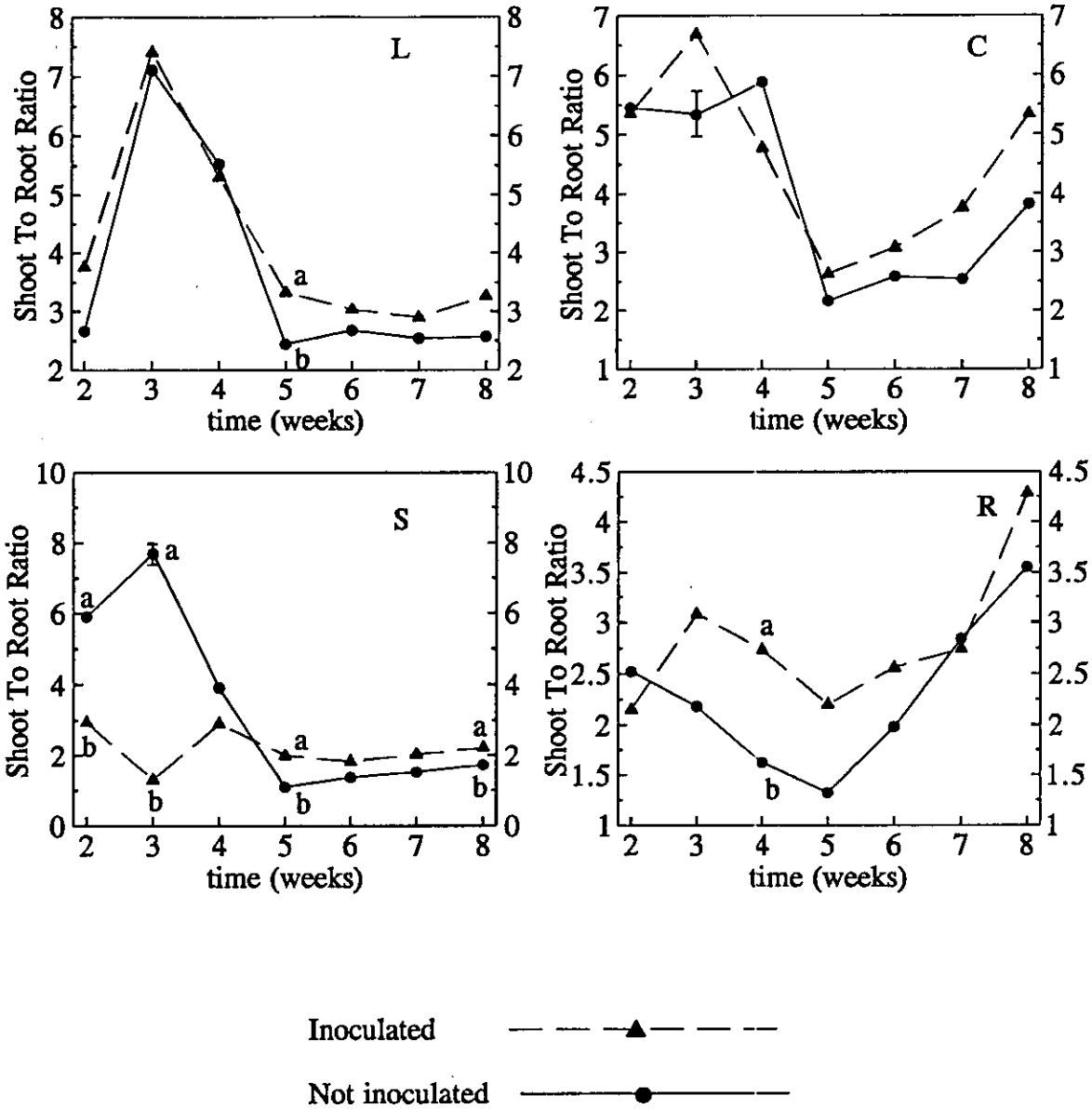

Figure 1.3. Shoot to root ratios for (L) *L. leucocephala*, (C) *C. calothyrsus*, (S) *S. sesban* and (R) *R. pseudoacacia* seedlings with and without *Rhizobium* inoculation. a and b beside data points at the same time indicates significant difference at $p < 0.05$.

4 indicating that shoots of the *Rhizobium* inoculated plants grew faster relative to the roots.

Figures 1.4, 1.5, 1.6 and 1.7 shows shoot total N, shoot total N content, root total N and root total N content respectively recorded between weeks 4 and 8. Figure 1.4. shows that *Rhizobium* inoculation significantly increased shoot total N in *L. leucocephala* ($p<0.05$) all weeks (4, 2, 2, 1.4 and 2 times respectively), *C. calothyrsus* at weeks 5 ($p<0.01$) (11 times), 7 ($P<0.05$) (1.4 times) and 8 ($p<0.05$) (3 times), *S.sesban* ($p<0.05$) at weeks 5 (3 times) and 8 (2 times) and *R. pseudoacacia* ($p<0.05$) at week 6 (2 times). Means taken over time (Appendix 2) showed significant inoculation increases in shoot total N in *L. leucocephala* ($p<0.01$) (72%), *C. calothyrsus* ($p<0.01$) (86%), *S. sesban* ($p<0.01$) (68%) and a ($p<0.05$) increase in *R. pseudoacacia* ($p<0.05$) (34%).

Rhizobium inoculation increased shoot total N content (Figure 1.5) in *L. leucocephala* ($p<0.05$) for all weeks (of 4, 2, 2.4, 2, and 3 times respectively) and in *C. calothyrsus* at weeks 5 ($p<0.01$) (12 times) and 8 ($p<0.05$) (4 times) and a ($p<0.05$) increase in *R. pseudoacacia* ($p<0.05$) at week 5 (2 times) but inoculation significantly reduced ($p<0.05$) shoot total N content in *S. sesban* (2.5 times) at week 6. However, means taken over time (Appendix 2) showed significant shoot N content increases ($p<0.05$) only in *L. leucocephala* (2 times) and *C. calothyrsus* (2.3 times).

Figure 1.6. shows that *Rhizobium* inoculation increased root total N in *L. leucocephala* at week 8 ($p<0.05$) (1.5 times), in *C. calothyrsus* at week 4 ($p<0.01$) (18 times) and in *S. sesban* ($p<0.05$) at weeks 5 (2 times) and 6 (2 times) but decreased it

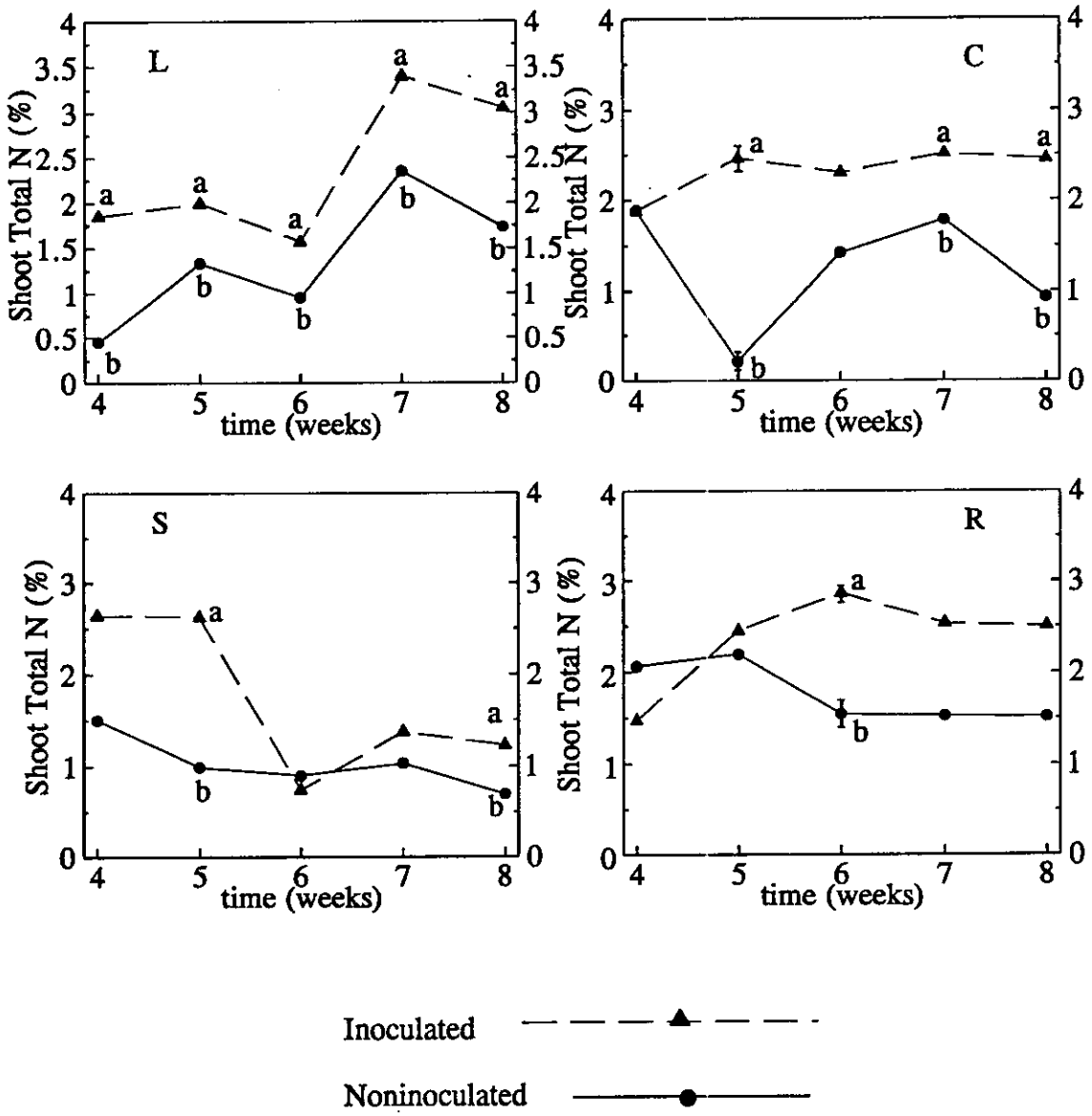

Figure. 1.4. Shoot total N for (L) *L. leucocephala*, (C) *C. calothyrsus*, (S) *S. sesban* and (R) *R. pseudoacacia* seedlings with and without *Rhizobium* inoculation. a and b beside data points at the same time indicates significant difference at $p < 0.05$.

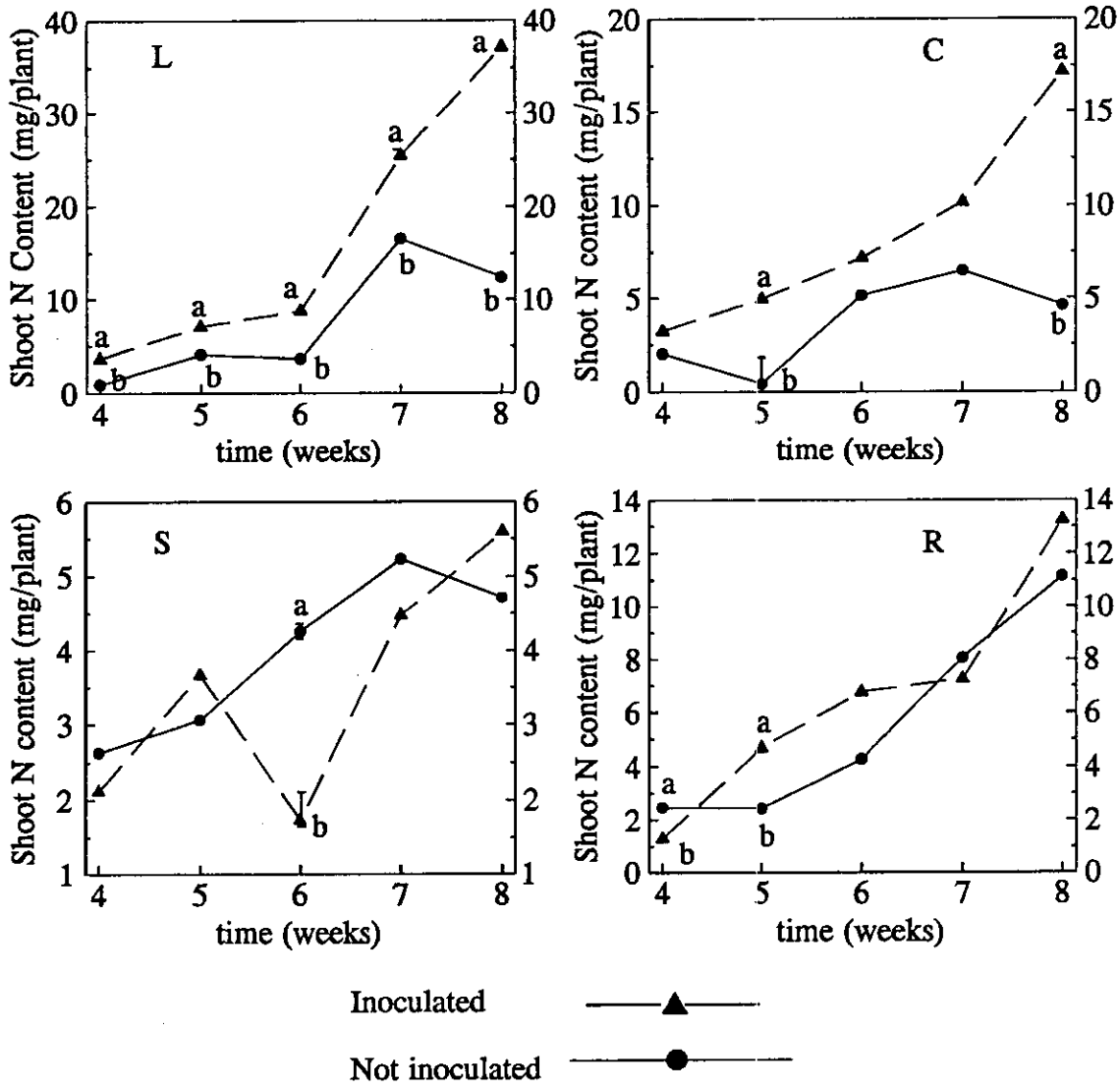

Figure 1.5. Shoot total N content for (L) *L. leucocephala*, (C) *C. calothyrsus*, (S) *S. sesban* and (R) *R. pseudoacacia* seedlings with and without *Rhizobium* inoculation. a and b beside data points at the same time indicates significant difference at $p < 0.05$. Note scale differences.

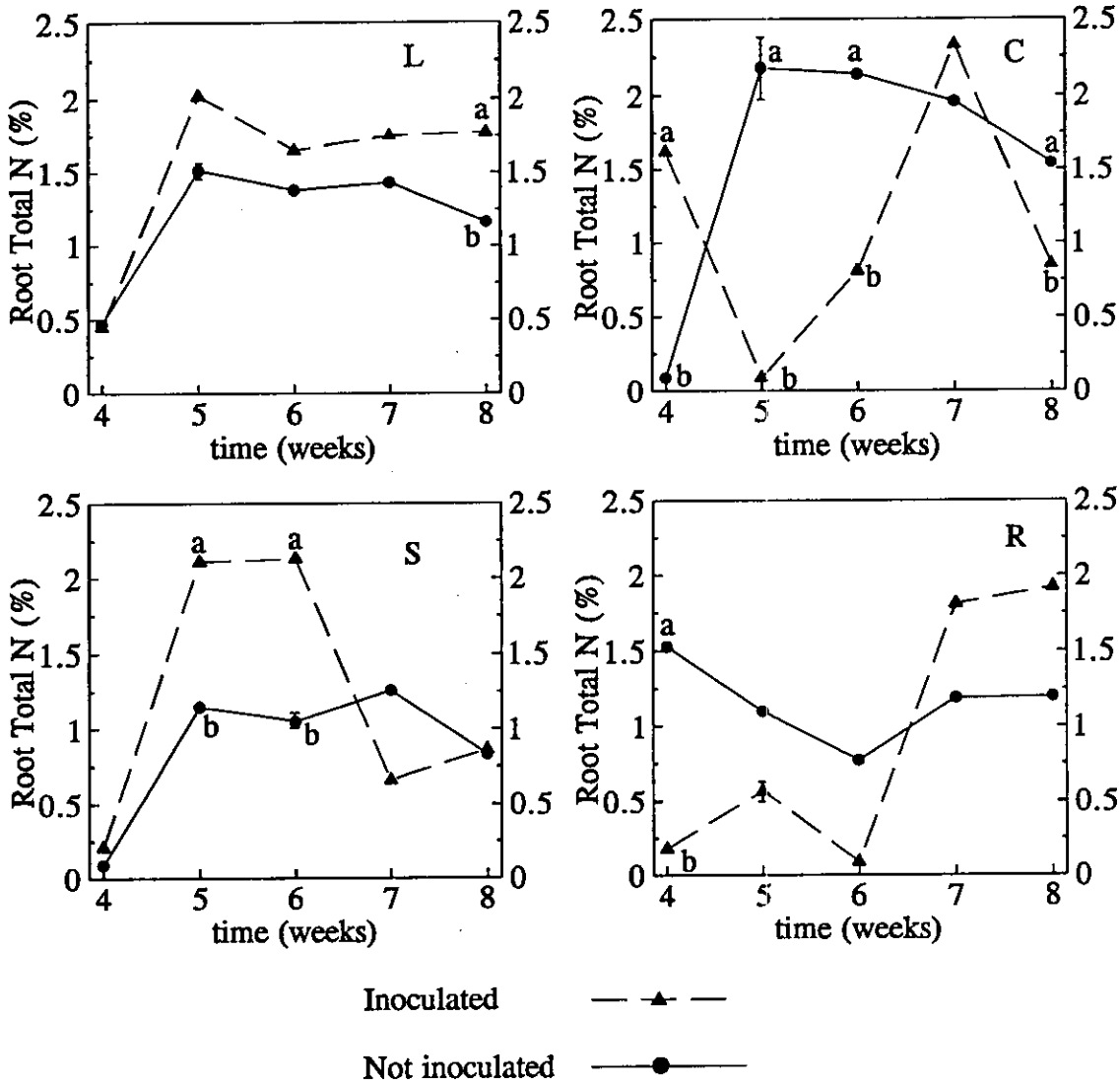

Figure 1.6. Root total N for (L) *L. leucocephala*, (C) *C. calothyrsus*, (S) *S. sesban* and (R) *R. pseudoacacia* seedlings with and without *Rhizobium* inoculation. a and b beside data points at the same time indicates significant difference at $p < 0.05$.

in *C. calothyrsus* at weeks 5 ($p<0.01$) (24 times), 6 ($p<0.05$) (2.6 times) and 8 ($p<0.05$) (2 times) and in *R. pseudoacacia* at week 4 ($p<0.01$) (8.5 times). However, means taken over time (Appendix 2) showed that *Rhizobium* inoculation had no effect on root total N of all species.

Figure 1.7. shows that *Rhizobium* inoculation increased root total N content in *L. leucocephala* ($p<0.05$) at weeks 6 (2 times) and 8 (2 times) and in *C. calothyrsus* ($p<0.01$) at week 2 (29 times) but decreased it in *C. calothyrsus* at weeks 5 ($p<0.001$) (27 times) and 6 ($p<0.05$) (4 times) in *S. sesban* at week 7 ($p<0.05$) (4 times) and in *R. pseudoacaia* at week 4 (17 times). However, means taken over time (Appendix 2) showed that *Rhizobium* inoculation significantly increased ($p<0.05$) root total N content only in *L. leucocephala* (48%) and significantly reduced it in *S. sesban* ($p<0.05$) (57%).

Table 1.1. shows nodule numbers and nodule dry weight data. *Rhizobium* inoculation significantly increased nodule number ($p<0.01$) only in *C. calothyrsus* (16 times) and caused insignificant nodule formation in *S. sesban*. As well, inoculation caused a significant increase in nodule weight ($p<0.01$) in *C. calothyrsus* (10 times) and a significant decrease ($p<0.05$) in *R. pseudoacacia* (2 times).

Table 1.2 shows soil total and organic carbon data. *Rhizobium* inoculation caused significant increases in total C ($p<0.05$) in soils in which *R. pseudoacacia* (4%) and *S. sesban* (6%) were grown, and significant increases in organic C ($p<0.05$) in soils in which *C. calothyrsus* (17%) and *S. sesban* (24%) were grown but caused a significant reduction ($p<0.05$) in organic C in soils in which *R. pseudoacacia* (6%) was grown.

Table 1.3 shows soil nitrate data. Inoculation significantly increased soil

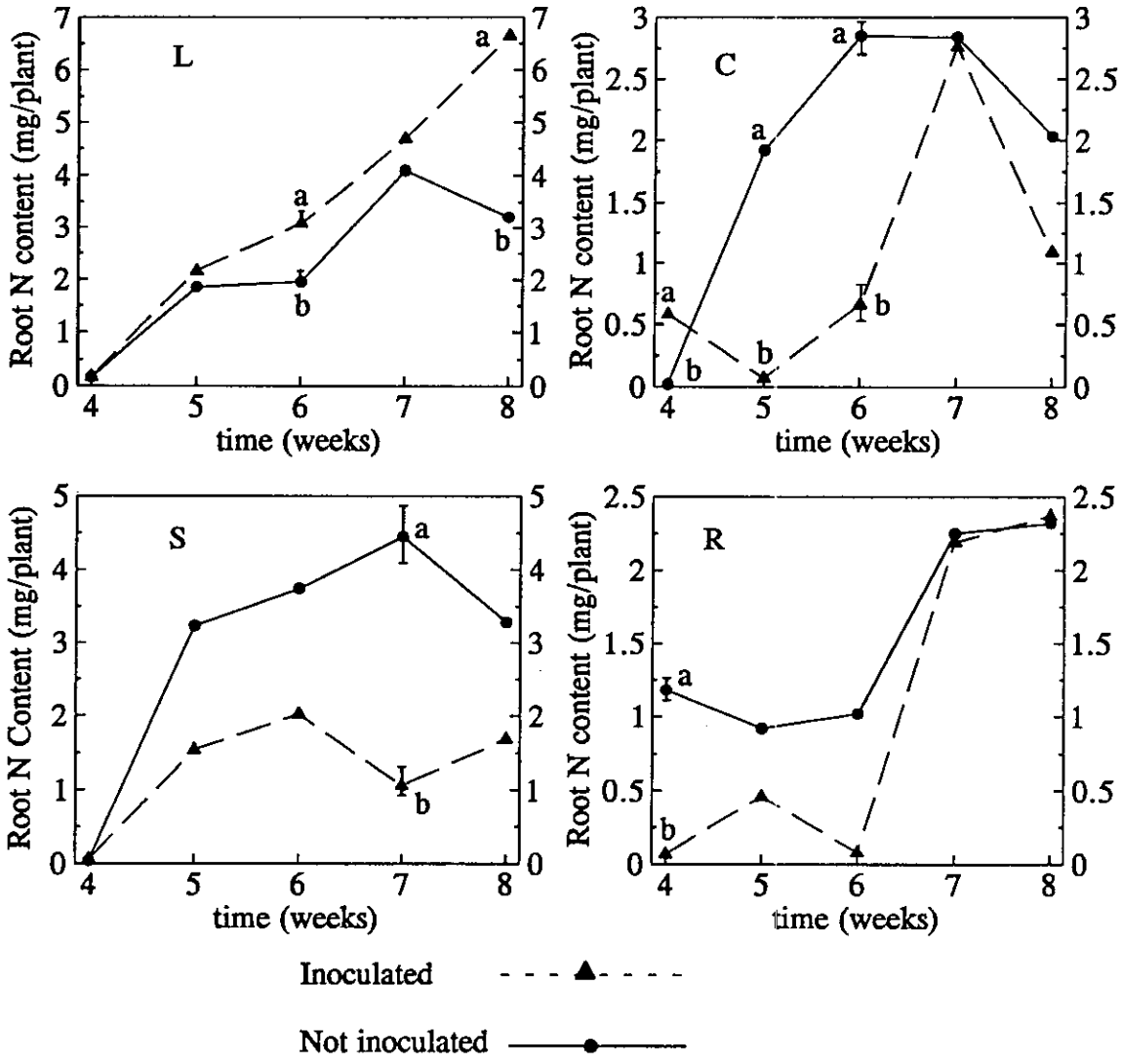

Figure 1.7. Root total N content for (A) *L. leucocephala*, (B) *C. calothyrsus*, (C) *S. sesban* and (D) *R. pseudoacacia* seedlings with and without *Rhizobium* inoculation. a and b beside data points at the same time indicates significant difference at $p<0.05$. Note scale differences.

nitrate ($p<0.01$) only in soils in which *R. pseudoacacia* was grown (3 times) but significantly reduced it in ($p<0.01$) soils in which *S. sesban* were grown (4 times).

Table 1.1. Effect of *Rhizobium* inoculation on nodule numbers and nodule dry weights.

	Nodule Number		Nodule weight (g)	
Species	Inoculated	Control	Inoculated	Control
L. leucocephala	24	11	0.050	0.036
C. calothyrsus	96**	6**	0.061**	0.006**
S. sesban	4	0	0.005	0
R. pseudoacacia	20	37	0.043*	0.087*

** and * indicates significance difference between inoculated and control treatments at $p<0.01$ and $p<0.05$ respectively. Each observation is a mean of six plants.

Table 1.2 Effect of *Rhizobium* inoculation on soil total and organic carbon.

	Total carbon (%)		Organic carbon (%)	
Species	Inoculated	control	Inoculated	Control
L. leucocephala	2.82	2.77	2.15	2.08
C. calothyrsus	2.79	2.74	2.23**	1.90**
S. sesban	2.81*	2.64*	2.39**	1.94**
R. pseudoacacia	2.63*	2.52*	2.30*	2.44*

** and * indicates significant difference between inoculated and control treatments at $p < 0.01$ and 0.05 respectively.

Table 1.3. Effect of *Rhizobium* inoculation on soil nitrate (μg/100g soil).

	Inoculation	
Species	Inoculated	Control
L. leucocephala	569.81	588.86
C. calothyrsus	488.13	520.06
S. sesban	102.57**	423.58**
R. pseudoacacia	182.03**	62.27**

** indicates significant difference between inoculated and control treatments at $p < 0.01$.

Discussion

Success of *Rhizobium* inoculation on shoot and root growth, nitrogen concentration and total nitrogen content varied with time. Data obtained on shoot and root growth showed that *Rhizobium* inoculation reduced shoot and root growth in *S. sesban* over time indicating that inoculation of *S. sesban* seedling with *S. grandiflora* inoculum had a negative effect on *S. sesban* seedling growth. This observation implies that *Sesbania grandiflora* inoculum may not be a suitable inoculum for *Sesbania sesban* as was previously thought. Shoot dry weight studies after *Rhizobium* inoculation (Odee, 1989) showed that a *Rhizobium* inoculum that improved shoot growth in *S. grandiflora* reduced shoot growth in *S. sesban* and resulted in the formation of ineffective nodules in *S. sesban*.

Shoot to root ratio increased in the *Rhizobium* inoculated plants prominently after week 5 indicating that *Rhizobium* inoculation increased shoot growth relative to root growth after the first month after planting. This was probably was due to adequate supply of nitrogen to the inoculated plants through biological nitrogen fixation and therefore allocation of more N to the shoots which resulted in increased shoot growth relative to the root growth (Marschner, 1986). However, this increase was dependent on species and time. *Rhizobium* inoculation also increased shoot total N in all species with the magnitude of increase varying with species and time. This might have been partly due to increase in nodule numbers particularly in *C. calothyrsus* and partly due soil nitrate uptake in *L. leucocephala, C. calothyrsus* (although nitrate uptake was not significant in both species) and *S. sesban* but it was not clear what caused total N increase in *R. pseudoacacia.*

Changes in total N were accompanied by increases in shoot N contents of *L. leucocephala* and *C. calothyrsus* in all weeks, *S. sesban* at weeks 5 and 8, and *R. pseudoacacia* at weeks 5, 6 and 8 (Figure 1.5). These differences in shoot N contents can be attributed to differences in shoot dry weights and shoot total N. It is important to note that the control *S. sesban* seedlings did not form nodules most probably because the soil lacked *Rhizobium* strains specific for *S. sesban* nodule formation. These findings are similar to those of Luyindula and Hague (1992) who found that *Rhizobium* inoculation increased growth, plant N content and nodule numbers in *L. leucocephala* while control *S. sesban* did not form nodules.

Root total N varied greatly with time in all species indicating variation in N allocation in the roots of these species. However, root N content of the inoculated plants was lower in most of the species due to reduction in root growth by the *Rhizobium* inoculation (see shoot:root ratios) and this may indicate that the reduction in root growth after *Rhizobium* inoculation may be species-specific and time-dependent.

Rhizobium inoculation increased nodule numbers and nodule weights in *C. calothyrsus* possibly because there were low rhizobia populations in the soil specific for nodule formation in this species and therefore *Rhizobium* inoculation was necessary to enhance and increase nodule formation. Similar results with respect to nodule formation after *Rhizobium* inoculation were obtained in Egypt by Ahmed and Phelps (1990) who observed an increase in nodule dry weight and total N in *Phaseolus vulgaris* L. after *Rhizobium* inoculation. Low nodule weight observed in *R. pseudoacacia* indicate that there may have been large rhizobia populations in the soil specific for nodule formation

which would have competed for nodule initiation in *R. pseudoacacia.* A similar observation was reported in India by Vadavia *et al.*, (1991) who found that *Rhizobium* inoculation did not increase yield and dry weight of root nodules in chickpea (*Cicer arietinum* L.). They predicted that their observations might have been caused by high populations of indigenous rhizobia in the soil.

Rhizobium inoculation increased total C and organic C in the soil partly due to peat which was used as a carrier of rhizobia in the inocula (Hadad *et al.*, 1986) could

Rhizobium inoculation had no effect on soil nitrate except in *R. pseudoacacia*, probably because there was a high uptake of nitrate by the highly nodulated *Rhizobium* inoculated plants. The observations on nitrate decrease in the soil may be partly explained by the observations of Deane-Drummond and Chaffey (1985) on nitrate uptake on pea (*Pisum sativum L.* cv. Feltham First) seedlings. They observed that following seedling inoculation with *Rhizobium leguminosarum* there was a two-fold increase in net nitrate uptake. They also observed that in inoculated seedlings there was a decrease in number and length of lateral roots. The latter may explain the observations made above on shoot to root ratio, where inoculated plants showed increased ratios. This also explains reduction in root N content observed in the inoculated plants.

1.5. Conclusions

Rhizobium inoculation reduced root growth of companion trees, implying that inoculation may be one way of reducing root growth and therefore reducing competition between crop roots and tree roots in intercropping systems. The increase in shoot total

N and shoot N content (especially in young shoots of *L. leucocephala* and *C. calothyrsus*) is promising to small scale farmers in the tropics who commonly use leaves of *L. leucocephala* and *C. calothyrsus* as the main source of fixed N for crops. The same observation implies that the foliage and shoots from young *L. leucocephala* and *C. calothyrsus* have a high potential for incorporation into the soil in order to increase soil N for crop growth.

The observation that inoculated *L. leucocephala, C. calothyrsus* and *S. sesban* decreased soil nitrate levels (though not significant) implies that intercropping with these trees can result to competition between crops and trees for nitrate. This would be true especially during the early growth of these trees in soils low in nitrates. Competition for nitrate may not be a problem in developed countries, the soils of which have generally high levels of nitrate. With respect to inoculated *R. pseudoacacia,* there may be no need to inoculate it when used in soils with high nitrate levels.

This study shows that N fixation varies with species and time and that therefore there is need to carry out *Rhizobium* inoculation studies over longer periods in order to determine the effect of inoculation on certain legumes. Thus a one time determination of N fixation in a legume may give misleading results and conclusions. This study also indicates that there is need to screen various tree species used in intercropping systems for their N fixing abilities at various ages.

CHAPTER TWO

Benefits of legume intercropping after *Rhizobium* inoculation

2.1. Introduction

Intercropping is a term used to describe the practice of growing annuals or other short duration crops in interspaces between rows of woody perennials (Suresh and Rai, 1990). Intercropping is commonly known as alley cropping or hedgerow intercropping and was developed to reduce or eliminate the long fallow periods characteristic of traditional shifting cultivation (Larbi *et al.*, 1992). It evolved in response to growing populations especially in developing countries where high population growth in some regions resulted in continuous cropping of farmlands, reduction of fallow periods, land degradation, and lower yields and production of food crops (Atta-Krah, 1990).

Leguminous trees play a major role in alley farming or hedgerow intercropping by providing and recycling N and organic matter to annual crops (Ladha *et al.*, 1993). So, leguminous trees are preferred for many types of intercropping (hedgerows) as they can fix atmospheric N into the soil system (Mulongoy and Vander Meersch, 1988). In intercropping systems, it is assumed that the trees draw most of their water and nutrients from depths below those at which the annual crops root systems extend and that competition for water and nutrients is reduced in the upper layers of the soil profile during the main growing season (Rochie, 1983). The principal effects of hedgerows are assumed to be the maintenance of soil organic matter levels and the fixation of atmospheric N, both of which are considered responsible for improved soil fertility and increased crop yields in the alleys (Balasubramanian and Sekayange, 1991).

Intercropping trees with crops is currently being studied in many tropical countries as an alternative source of N fertilizers for annual crops especially in small scale farming systems. Studies on intercropping trees with barley using potted plants have been conducted by Thevathasan and Gordon (1994) who investigated the effect of moisture on barley yield when barley was intercropped with fertilized poplar trees. As well, Ntayombya and Gordon (1994) used fertilized *R. pseudoacacia* L. in a pot experiment to investigate N nutrition of barley under various management regimes of soil, fertilizer and cropping system. None of these studies focused on the effect of *Rhizobium* inoculation of intercrop tree and subsequent barley growth and grain yield variation. Therefore, the purpose of this study was to investigate the benefits of intercropping barley with *Rhizobium*-inoculated *L. leucocephala, C. calothyrsus, S. sesban* and *R. pseudoacacia* with respect to barley growth (straw, ear and root biomass) and grain yield as well as total N and total N content of straw, ear, root and grain fractionation in a pot experiment in the greenhouse.

2.2. Materials and Methods

The tree species used, seed source, preparation, pregermination and *Rhizobium* inoculation procedures are similar to those aspects described in chapter one (section 1.2). Two seedlings were planted in soil in plastic pots. The pots measured 36 cm in diameter at the top and were 35 cm high. Soil (2.119% total and 1.988% organic carbon) in the pots weighed approximately 25 kg (air dry weight). The pots were kept moist but no leaching of water from the pots was allowed. Barley (*Hordeum vulgare* L., variety ahadi)

was obtained from the Kenya Seed Company, Kenya, and was planted around the young trees (3 months old) at a depth of about 5 cm (Hadad *et al.*, 1986) at a rate of between 25 to 30 barley plants per pot (Thevathasan and Gordon, 1994). Barley without trees (sole barley) was used to compare barley performance in the absence of intercropping. Barley was sampled twice at 2.5 and 4 months. The harvested barley was divided into roots, straw, ears and grain (grain only at 4 months). Barley components were dried at 75°C for 48 hours, weighed and ground in a Wiley mill to pass through a 810 μm, No. 20 mesh sieve. The ground material was analyzed for Kjeldhal total N using a Technicon™ AutoAnalyzer™ II.

Tree heights were recorded at 3 (barley planting), 5.5 (first barley sampling) and 7 months (barley harvest). To reduce competition for light between barley and the trees, leaves on the lower third of the trees were removed after the barley was one-month old and after it was two and a half months old.

The data were statistically analyzed as a completely randomised block design using Statistical Analysis System (SAS) (1990). Least significant difference (LSD) and simple 't' tests ($p<0.05$) were used to separate treatment means.

2.3. Results

The data in Table 2.1. show that *Rhizobium* inoculation did not have any effect on barley straw, ear or root biomass, regardless of tree species or time sampled. An exception was noted in barley grown with *S. sesban* where conflicting significant differences were noted. Sole barley had significantly higher straw ($p<0.01$), ear

Table 2.1. Barley straw, ear and root dry weights (g.plant^{-1}) after barley was intercropped with both *Rhizobium* inoculated and uninoculated *L. leucocephala, C. calothyrsus, S. sesban* and *R. pseudoacacia.*

	2.5 months			4 months		
Species and inoculation treatment	straw	ears	roots	straw	ears	roots
L. leucocephala not inoculated	0.138	0.042	0.011	0.206	0.036	0.013
L. leucocephala inoculated	0.117	0.043	0.011	0.281	0.052	0.017
C. calothyrsus not inoculated	0.109	0.040	0.010	0.152	0.051	0.011
C. calothyrsus inoculated	0.081	0.032	0.007	0.213	0.031	0.015
S. sesban not inoculated	0.064	0.019	0.009a	0.128a	0.011	0.014
S. sesban inoculated	0.066	0.039	0.013b	0.084b	0.016	0.014
R. pseudoacacia not inoculated	0.189	0.132	0.018	0.371	0.082	0.030
R. pseudoacacia inoculated	0.140	0.117	0.013	0.261	0.067	0.017
Sole barley	0.353	0.235	0.018	0.875	0.146	0.039

a, b indicates a significant difference between treatments, by species, at $p<0.05$.
Sole barley had significantly higher straw ($p<0.01$), ear ($p<0.05$) and root ($p<0.05$) weights compared to intercropped barleys (not indicated in the table).

($p < 0.05$) and root ($p < 0.05$) mass compared to all intercropped barleys.

Table 2.2. shows that for barley straw, ear and root total N, *Rhizobium* inoculation did not have any effect regardless of tree species or time sampled, except that inoculation increased ear total N ($P < 0.05$) in barley grown with *C. calothyrsus* at both sampling times (by 22 and 2 times respectively) and barley grown with *S. sesban* (by 2 times) at 2.5 months, and root total N ($p < 0.05$) in barley grown with *L. leucocephala* and *R. pseudoacacia* (by 1.2 and 2 times respectively) at month 4. There was no significant difference in straw, ear or root total N between the intercropped barleys and the sole barley.

Table 2.3 shows straw, ear and root total N content. The data indicate that *Rhizobium* inoculation did not have an effect on straw, ear or root total N content irrespective of species or sampling time. Exceptions were noted in barley grown with C. *calothyrsus* and *S. sesban* where inoculation increased ($P < 0.05$) ear total N content at 2.5 months by 21 and 4 respectively. Sole barley's straw and ear total N contents were significantly higher ($p < 0.05$) than those of the intercropped barleys.

The data in Table 2.4. shows that *Rhizobium* inoculation increased grain yield and grain N content only in barley grown with *C. calothyrsus* ($p < 0.01$) and *S. sesban* ($p < 0.01$), by about 15 and 3 times (of grain mass) and 15 and 3 times (grain N content) respectively. Sole barley had a significantly higher ($p < 0.01$) grain yield, no significant difference in total N, and a higher ($p < 0.01$) grain total N content compared to the intercropped barleys.

Figure 2.1. shows tree heights recorded at 3, 5.5 and 7 months. Inoculation

Table 2.2. Barley straw, ear and root total N (%) after barley was intercropped with both *Rhizobium* inoculated and uninoculated *L. leucocephala, C. calothyrsus, S. sesban* and *R. pseudoacacia.*

	2.5 months			4 months		
Species and inoculation treatments	straw	ears	roots	straw	ears	roots
L. leucocephala not inoculated	1.314	1.903	0.063	0.745	0.514	0.980a
L. leucocephala inoculated	1.165	0.988	0.594	0.991	0.620	1.190b
C. calothyrsus not inoculated	1.148	0.060a	1.291	0.507	1.090a	0.884
C. calothyrsus inoculated	0.966	1.320b	1.515	0.771	2.340b	1.255
S. sesban not inoculated	0.911	0.830a	0.063	0.799	0.849	0.466
S. sesban inoculated	0.892	1.650b	0.062	1.080	0.610	0.062
R. pseudoacacia not inoculated	1.670a	0.899	1.140a	0.791	1.502	1.030a
R. pseudoacacia inoculated	1.010b	1.402	0.580b	0.903	1.664	2.150b
Sole barley	1.244	1.726	0.764	0.634	1.730	1.075

a, b indicates a significant difference between treatments, by species, at $p < 0.05$. There was no significant difference in straw, ear and root total N between the intercropped barleys and sole barley.

Table 2.3. Barley straw, ear and root N content (mg.plant^{-1}) after barley was intercropped with both *Rhizobium* inoculated and uninoculated *L. leucocephala, C. calothyrsus, S. sesban* and *R. pseudoacacia*.

	2.5 months			4 months		
Species and inoculation treatment	straw	ears	roots	straw	ears	roots
L. leucocephala not inoculated	1.81	0.80	0.01	1.53	0.19	0.13
L. leucocephala inoculated	1.36	0.42	0.07	2.79	0.32	0.20
C. calothyrsus not inoculated	1.25	0.02a	0.13	0.78	0.56	0.10
C. calothyrsus inoculated	0.78	0.42b	0.11	1.64	0.73	0.19
S. sesban not inoculated	0.58	0.16a	0.01	1.02	0.09	0.07
S. sesban inoculated	0.59	0.64b	0.01	0.91	0.10	0.01
R. pseudoacacia not inoculated	3.16a	1.19	0.21a	2.93	1.23	0.31
R. pseudoacacia inoculated	1.41b	1.64	0.08b	2.36	1.11	0.37
Sole barley	4.39	4.06	0.14	5.55	2.53	0.42

a, b indicates a significant difference between treatments, by species, at $p < 0.05$

Sole barley's straw, ear and root total N content were significantly higher ($p < 0.05$) than that of the intercropped barleys (not shown in the table).

Table 2.4. Grain yield, total N and N content of barley after barley was intercropped with both *Rhizobium* inoculated and uninoculated *L. leucocephala, C. calothyrsus, S. sesban* and *R. pseudoacacia.*

Species and inoculation treatments	Grain yield ($g.plant^{-1}$)	Grain Total N (%)	total N Content ($mg.plant^{-1}$)
L. leucocephala not inoculated	0.107	1.055	1.13
L. leucocephala inoculated	0.128	1.766	2.26
C. calothyrsus not inoculated	0.004a	1.976	0.08a
C. calothyrsus inoculated	0.060b	1.988	1.19b
S. sesban not inoculated	0.015a	1.504	0.23a
S. sesban inoculated	0.048b	1.241	0.60b
R. pseudoacacia not inoculated	0.308	1.704	5.25
R. pseudoacacia inoculated	0.240	1.985	4.76
Barley	0.756	1.821	13.77

a, b indicates a significant difference between treatments, by species, at $p<0.05$. Sole barley had significantly ($p<0.01$) higher grain mass and total N content compared to all itercropped barleys (not shown in the table).

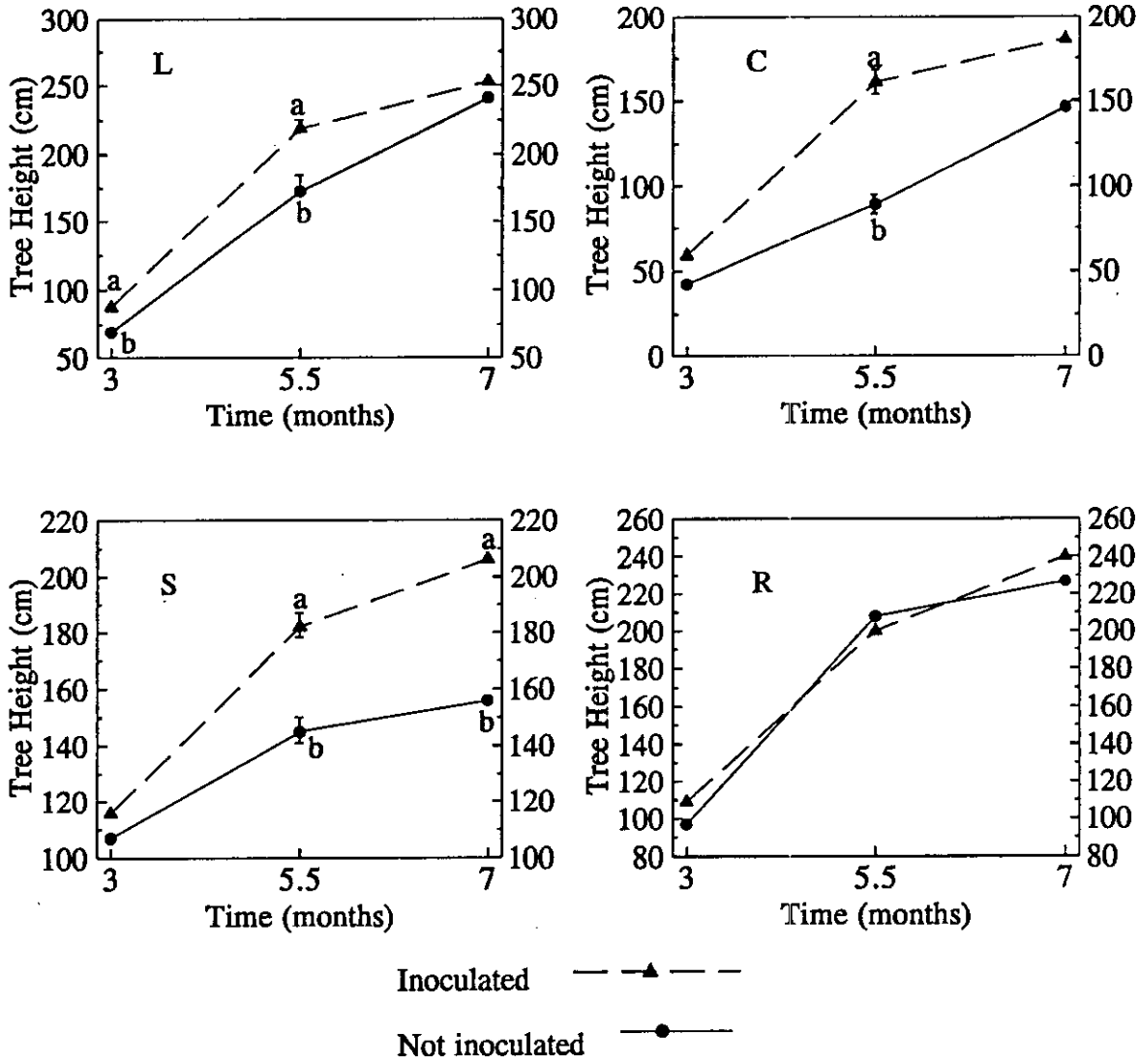

Figure 2.1. Heights of (L) *L. leucocephala*, (C) *C. calothyrsus*, (S) *S. sesban* and (R) *R. pseudoacacia* trees with and without *Rhizobium* inoculation. a and b beside data points at the same time indicates significant difference at $p < 0.05$. Note scale differences.

increased height of *L. leucocephala* at months 3 ($p<0.01$) and 5.5 ($p<0.01$) (by 1.3 each), *C. calothyrsus* at month 5.5 ($p<0.01$) (2 times) and *S. sesban* at months 5.5 ($p<0.05$) and 7 ($p<0.01$) (by 1.3 times each). However, means taken over time (Appendix 3) showed that *Rhizobium* inoculation had no effect on tree heights of all species. *S. sesban* showed highest response (45% height increase) while *R. pseudoacacia* showed lowest response (19% height increase) to *Rhizobium* inoculation.

2.4. Discussion

The effect of *Rhizobium*-inoculated trees on barley biomass, total N and total N contents in straw, ear, root and grain yield varied with species and time. Inoculation of *L. leucocephala* and *R. pseudoacacia* did not have any effect on barley biomass, total N or total N content of both barley vegetative and grain components. However, inoculation increased ear total N, ear total N content, grain yield and grain total N content in *C. calothyrsus* and *S. sesban* implying that inoculation of these two species may be important to increase barley grain production in intercropping systems. The observation that increases in ear total N and total N content in barley grown with *C. calothyrsus* and *S. sesban* corresponded to increase in grain yield and grain total N content indicates that ear total N and total N content may potentially be used as an indication of grain yield and grain N content in barley.

Barley biomass, biomass total N content, grain yield and grain total N content were reduced by intercropping, probably because intercropping resulted in competition between trees and barley for light, nutrients or both (the plants were always watered).

The intercropped trees attained heights of up to 2 m and although the trees were pruned twice some shading may have resulted since the trees and barley were grown in the same pots. Atta-Krah (1989) noted low phosphorus levels in the alleys and grazing plots compared to conventional cropping plots. Balasubramanian and Sekeyange (1991) also noted phosphorus deficiency in the alleys which they attributed to poor maize yields. Becker *et al.*, (1988) suggested that green manure legumes that occupy the field for more than six weeks may compete with crops. Reduction in barley growth and grain yield might have also been caused by an allelopathic effect of the trees on barley as noted by Bhatt and Todaria (1990) who suggested that allelopathic interactions in tree-crop associations in agroforestry can have a significant effect on crop production. The observation that all intercropped barleys had lower biomass production and biomass N contents, lower grain production and grain total N content compared to sole barley portrays what may actually happen at the tree-crop interface (trees and barley were in intimate association in the pots) in the alleys (in alley cropping systems spacing between trees and crops may be as little as 30 cm). For example Balasubramanian and Sekayange (1991) observed a decrease in tuber yield of sweet potato (*Ipomoea batatas* (L.) Lam.) when planted in alleys and a 14% to 32% yield fall off at the tree/crop interface. Increases in tree heights noted after *Rhizobium* inoculation of *L. leucocephala*, *C. calothyrsus* and *S. sesban* at certain months suggested that inoculation of these tree species may be a potential method to increase leaf biomass production for mulching in small scale farming systems of the tropics.

2.5. Conclusions

Results from this study show that the effects of *Rhizobium* inoculation in intercropping systems varies with species and time and that inoculation may be important to increase barley grain yield and grain total N content when *C. calothyrsus* and *S. sesban* are used as intercrops. As well, results from this study showed that intercropping trees with barley did not increase barley grain yield and grain total N content compared to sole barley. This suggests to farmers that crop yields can be reduced at the tree-crop interface and that there is a need to select trees which will allow for intensive cultivation. This also suggests to small scale farmers that the use of leguminous leaf mulches has an advantage over intercropping in that no cultivated land reduction occurs when leaf mulches are used and that more research on mulch management may be of great importance to maximize crop yields per unit area of land. Increases in tree heights noted in *L. leucocephala, C. calothyrsus* and *S. sesban* at certain months after *Rhizobium* inoculation suggests that inoculation has an effect on tree biomass production which varies with time and species and that *Rhizobium* inoculation may be important in leaf biomass production for mulching purposes.

CHAPTER THREE

Effect of *Rhizobium* inoculation on decomposition of leaves of *Leucaena leucocephala* and *Robinia pseudoacacia* in litter bags under greenhouse conditions

3.1. Introduction

Leaf decomposition is the breakdown of chemical bonds formed during the formation of leaf tissue and is enhanced by the physical process of leaching by precipitation or by the action of enzymes produced by microbes. The end-products of decomposition are carbon dioxide, nitrogen (ammonium and nitrate) and other associated elements such as phosphorus, sulphur and micronutrients such as iron (Stevenson, 1986). The rate at which plant leaves (litter) decompose depends on the chemical composition of the leaves and overall environmental conditions (Berg and Staaf, 1981; Stevenson 1986). First, significant weight loss can occur solely due to the physical process of leaching by precipitation as it washes over the litter; low-molecular-weight sugars, polyphenols and amino acids can be lost in this way (Aber and Melillo, 1991; Mason, 1976). The total weight loss by leaching in this manner will depend on the amount of water-soluble material in the litter and the amount of water passing over it. Second, carbon (C) compounds that are not water-soluble but that can be degraded rapidly are preferentially attacked by the microbes (Stevenson, 1986).

Three general characteristics determine the quality of the litter materials relative to microbial decay: (1) the type of chemical bonds present and the amount of energy released by their decay, (2) the size and three-dimensional complexity of the molecules

in which these bonds are found and (3) the nutrient content. The type of C bonds present and the energy they yield constitute the C quality of the material; the nutrient content and the ease with which nutrients are made available constitutes the nutrient quality (Aber and Melillo, 1991). Decomposition generally occurs more rapidly in plant residues with high N concentration and therefore low C to N ratios (Mason, 1976; Bockman *et al.*, 1990). Low N concentrations in decomposing plant residues reduce the rate at which the microorganisms synthesize their proteins and therefore reduce both the microbial population and the rate at which C for microbial energy is processed (Aber and Melillo, 1991). The decomposition rate also varies with plant species but even species with the same C to N ratio often decompose at different rates (Harris and Riha, 1991). With respect to N, the end-product of decomposition process is ammonium, most of which remains in the soil. However, it may be rapidly removed from the soil solution in one of the following pathways: uptake by plant roots, uptake by microorganisms, adsorption on the surface of soil colloids and by chemical binding to organic substances (Tamm, 1991).

Litter bags have been extensively used in leaf decomposition studies (Tian *et al.*, 1992; Sandhu *et al.*, 1990; and White *et al.*, 1988;). Tian *et al.*, (1992) observed that the decomposition rates of prunings of three woody agroforestry plant species, *Acioa barteri, Gliricidia sepium* and *Leucaena leucocephala*, and maize (*Zea mays*) stover and rice (*Oryza sativa*) straw were correlated with C to N ratios, initial N concentration and plant species. Sandhu *et al.*, (1990) observed that the weight loss of decomposing leaves of *L. leucocephala* was correlated with moisture content, mean maximum temperature

and C to N ratio. Further, White *et al.*, (1988) found that total N in decomposing *Robinia pseudoacacia* (black locust) litter increased concurrently with significant decreases in the C to N ratio over time.

Most leaf decomposition studies of agroforestry woody legume species have focused on legumes not inoculated with *Rhizobium*. In addition, little attention has been paid to the water content of the various leaves of woody legumes used in litter bag decomposition studies. Therefore, the objective of this study was to determine the effect of *Rhizobium* inoculation on weight loss (percent weight remaining), water content, N concentration and content, total C and C to N ratio in decomposing leaves of *Leucaena leucocephala* and *Robinia pseudoacacia* over a short period of time using litter bags.

3.2. Materials and Methods

Leaves of both inoculated and uninoculated *L. leucocephala* and *R. pseudoacacia* were obtained from seven month-old trees. The trees were grown in potted soil in the greenhouse maintained at a temperature of between 19 and 24°C. The leaves were dried at 75°C for 48 hours. Exactly 3 g of the dry leaves were weighed into 10x10 cm bags (White *et al.*, 1987) made of 100% polyester, (Pepcap^R, mesh size 500 μm, mesh No. 32) (Yamoah *et al.*, 1986). The litter bags were sealed with a stapler. All litter bags were randomly placed flat on moist soil in germination trays and the trays were randomly placed on a greenhouse bench. About 100 ml of tap water was poured through each individual litter bag daily except on the sampling day. The germination trays were randomised twice weekly. The experiment was carried out in a greenhouse maintained

at a temperature of between 19 and 24°C.

Four litter bags of each leaf treatment were randomly collected from the trays after one, two, four, six and eight weeks. Fresh weights of each bag's contents were taken and the leaves oven dried at 65°C for 72 hours. The dry leaves were weighed and ground in a Wiley Mill to pass trough a 810 μm, No. 20 mesh sieve. The leaves were analyzed for total N and total C using a Technicon™ autoAnalyzer™ and Leco Carbon Determinator CR-12 respectively. Water content (%), C to N ratio and N content of each leaf type were also determined.

The data were analyzed statistically as a randomised complete block design using Statistical Analysis System (SAS) (1990). Least significant difference (LSD) and simple 't' tests ($p<0.05$) were used to separate treatment means. A log (natural log) transformation was done to normalise C to N ratio (C:N).

3.3. Results

Figure 3.1 shows change in dry weight and water content in leaves of *L. leucocephala* and *R. pseudoacacia* recorded over a 8-week period. *Rhizobium* inoculation enhanced decomposition in both leaves of *L. leucocephala* and *R. pseudoacacia* with a significant inoculation increase in *L. leucocephala* ($p<0.05$) at week 8 (16%). However, means taken over time (Appendix 4) showed that inoculation did not have any effect on leaf decomposition of both species. *Rhizobium* inoculation significantly reduced leaf water content ($p<0.05$) in *L. leucocephala* at weeks 1 (37%), and 4 (26%), and in *R. pseudoacacia* at week 2 (40%), but increased it ($p<0.05$) in *R. pseudoacacia* at week

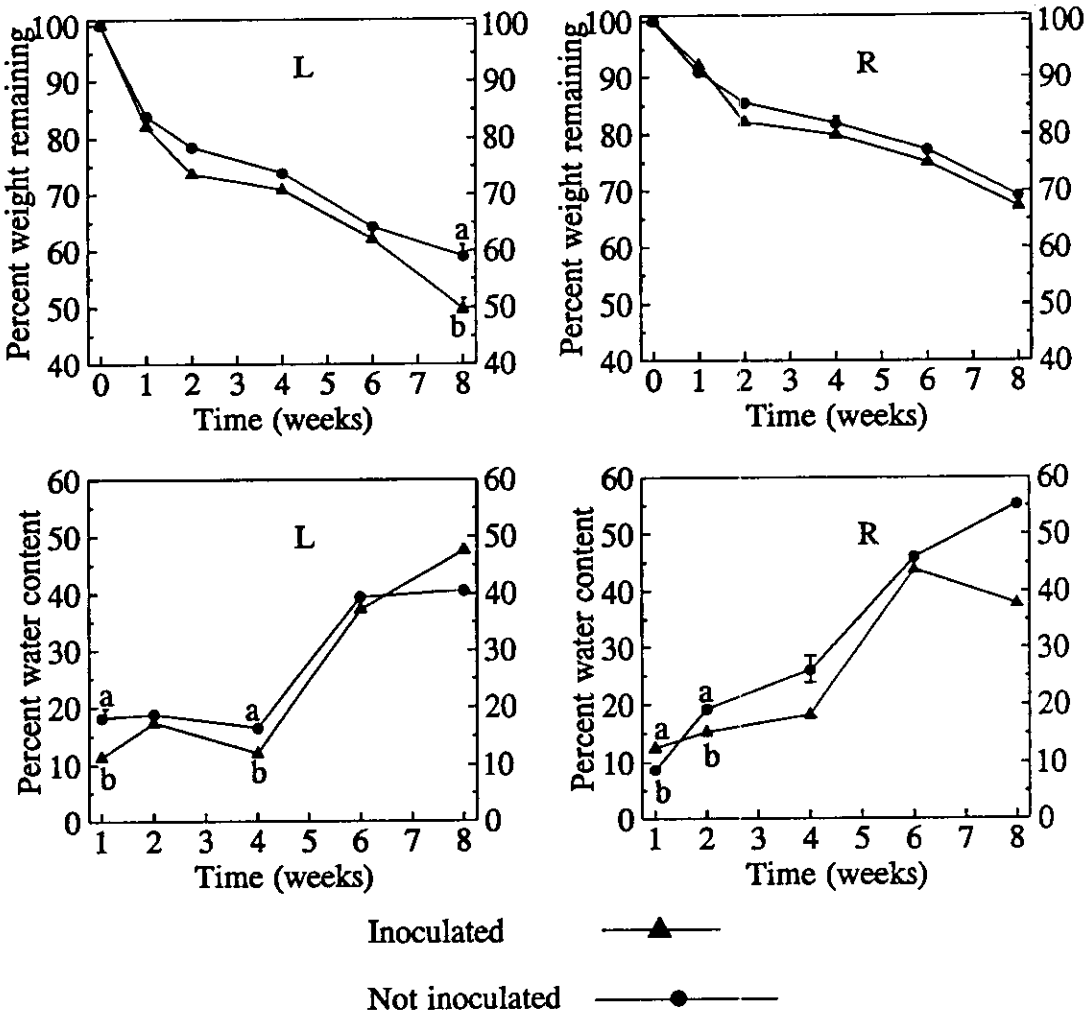

Figure 3.1. Percent weight remaining and percent water content in decomposing leaves of (L) *L. leucocephala* and (R) *R. pseudoacacia.* a and b beside data points at the same time indicates significant difference at $p < 0.05$.

1 (43%). However, means taken over time (Appendix 4) showed that inoculation did not have any effect on leaf water content of both species.

Figure 3.2 shows leaf N concentration and leaf total N content taken over an 8-week period. *Rhizobium* inoculation had no effect on leaf N concentration of both species in all 8 weeks; further means taken over time (Appendix 3) showed no inoculation effect on N concentration of both species. However, *Rhizobium* inoculation reduced leaf total N content in *L. leucocephala* ($p<0.05$) at week 2 (10%) and means taken over time (Appendix 4) showed that inoculation significantly reduced ($p<0.05$) leaf total N content in *R. pseudoacacia* (19%)

Figure 3.3 shows leaf total C and C to N ratio recorded over 8 weeks. *Rhizobium* inoculation increased ($p<0.05$) total C (7%) and C to N ratio (11%) in *L. leucocephala* at week 2. However, means taken over time (Appendix 4) showed that inoculation had no effect on total C of both species but significantly increased C to N ($p<0.05$) ratio in *R. pseudoacacia* (31%).

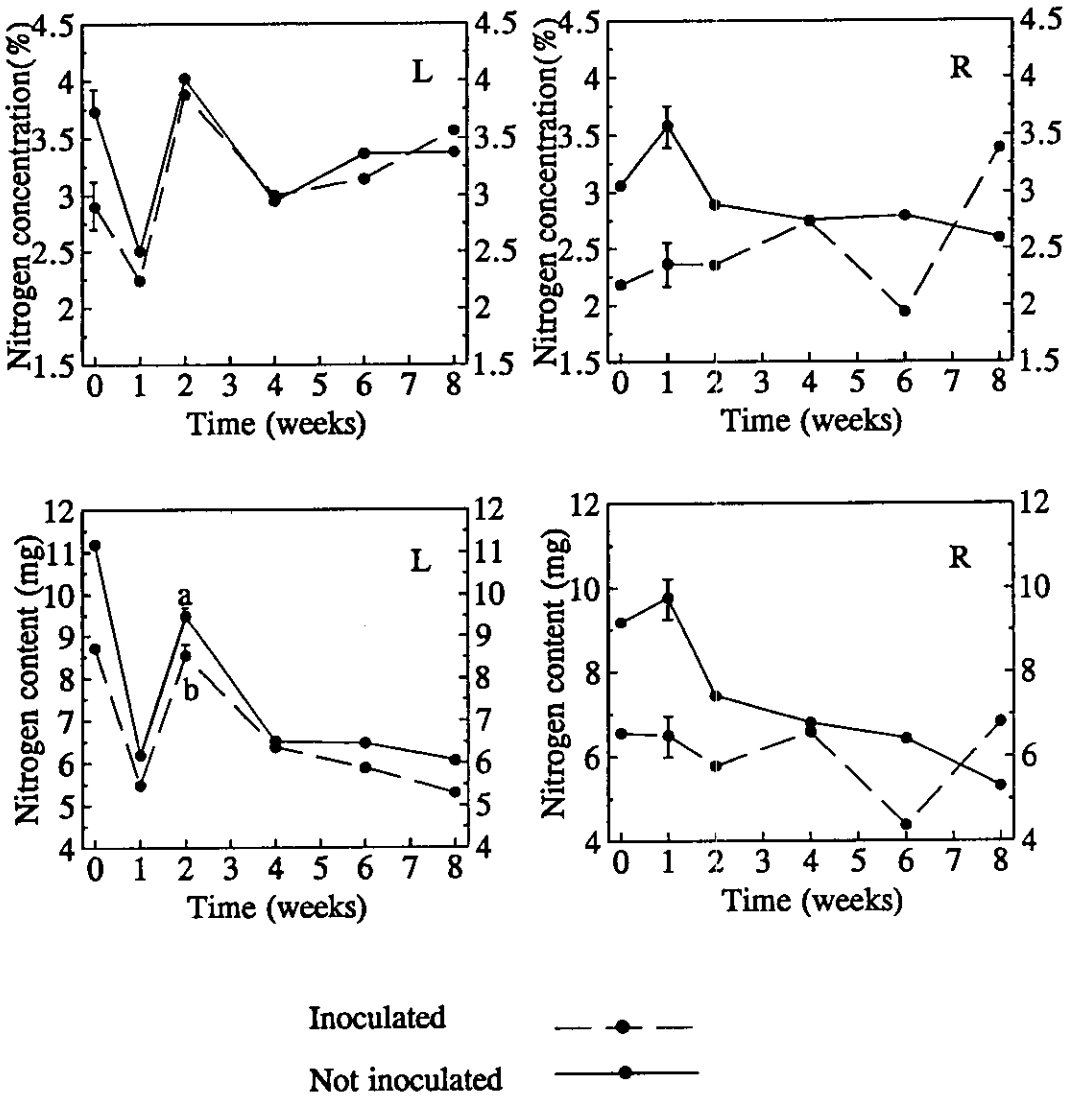

Figure 3.2. Total N (%) and N content (mg) in decomposing leaves of (L) *L. leucocephala* and (R) *R. pseudoacacia*. a and beside data points at the same time indicates significant difference at $p < 0.05$.

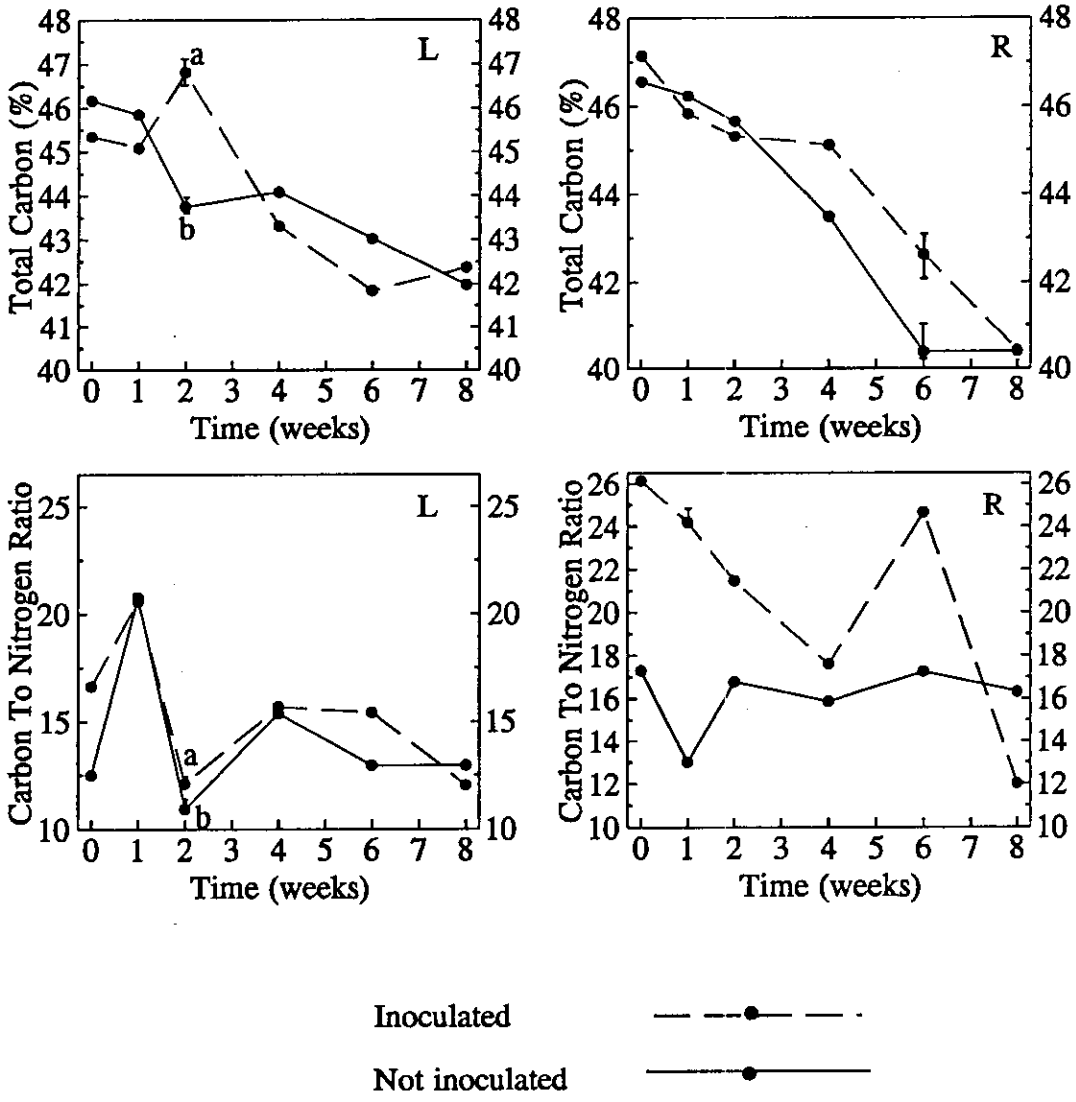

Figure 3.3. Total C (%) and C to N ratio in decomposing leaves of (L) *L. leucocephala* and (R) *R. pseudoacacia*. a and b beside data points at the same time indicates significant difference at $p < 0.05$.

3.4. Discussion

Rhizobium inoculation decreased percent weight remaining in both *L. leucocephala* and *R. pseudoacacia* indicating that *Rhizobium* inoculation probably results in the formation of rapidly degradable, high energy C compounds and readily available nutrient content for the microbes (Mason, 1976; Aber and Melillo (1991) in the leaves of inoculated plants. However, this relationship was only statistically significant at week 8 for *L. leucocephala.* Further, the observed weight decreases in the leaves of inoculated plants appears not to be controlled by initial total N, N content and C to N ratio but rather by species type and *Rhizobium* inoculation. These results are similar to the observations made by White *et al.*, (1988) who found that initial N content and C to N ratio were not related to percent weight remaining in control *R. pseudoacacia.* Species differences in percent weight remaining might have partly been due to preferential attack of *L. leucocephala* leaves by soil microbes (bacteria and fungi) which also contributed to the high total N observed in *L. leucocephala* leaves between weeks 2 and 8, and probably highly leached low-molecular-weight compounds in the leaves of *L. leucocephala* before week 2 (Figure 3.2).

Water content in the decomposing leaves varied with species and *Rhizobium* inoculation and this difference in leaf water content may have been influenced by leaf size, probably explaining why *R. pseudoacacia* (large leaved) had higher leaf water content (Figure 3.1) than *L. leucocephala.* Water content in the leaves from inoculated trees was lower than that from control plants (at several sampling periods) probably because *Rhizobium* inoculation increased growth (see intercropping experiment: tree

heights) and lowered leaf size of the inoculated plants relative to the control plants.

Rhizobium inoculation decreased initial total N in both *L.leucocephala* and *R. pseudoacacia* (Figure 3.1) probably due to the high growth rate of the *Rhizobium*-inoculated plants (see intercropping experiment:tree heights) which lowered leaf total N of the inoculated plants. Further, it was observed that uninoculated plants shed more leaves than the inoculated plants which may have resulted in more N allocation to the remaining leaves of control plants resulting in the high total N observed in these plants. These findings are in contrast to what was observed earlier in young *L. leucocephala* and *R. pseudoacacia* in which inoculated plants had higher total N (see chapter one) implying that total N in leaves varies with age of plants and whether plants are inoculated or not.

3.5. Conclusions

Although *Rhizobium* inoculation did not statistically increase percent leaf weight loss (decomposition), results from this experiment showed a consistent trend which implies that inoculation may have a potential to increase decomposition of leaves of *L. leucocephala* and *R. pseudoacacia*. In addition, although decreases in leaf water content, N concentration and total N content (total N content in *L. leucocephala*) were not statistically significant the consistent trends observed from this study calls for more studies to be done to verify the implications of these observations in terms of leaf water content and soil N and other nutrients which may be supplied by decomposing leaves from *Rhizobium* inoculated *L. leucocephala* and *R. pseudoacacia*.

CHAPTER FOUR

Effect of *Rhizobium* inoculation and mulch placement on barley growth, nitrogen uptake, and soil nitrate changes

4.1. Introduction

Many tropical soils are low in both total and plant-available N and yields are often limited by N supply (Ladha *et al.*, 1993). Therefore, in the smallholder farming systems of the tropics and subtropics increasing use is being made of the leaves of leguminous trees as a source of nutrients (particularly N) for crop growth (Gutteridge, 1992).

In alley cropping systems, annual crops are grown between rows of trees and branches are pruned regularly to provide leaf mulch for the interplanted crops (Atta-Krah, 1990; Balasubramanian and Sekayange, 1991). Sometimes the legume leaves used as mulch are ploughed into the soil (Catchpoole and Blair, 1990) to facilitate decay and release of nutrients to the soil for crops. Benefits of mulching include the reduction of erosion hazards, better infiltration of rain water and less evaporation, lower soil temperature, supply of organic matter and nutrients, higher biological activity, better root growth and suppression of weeds (Schroth *et al.*, 1992).

Studies on leaf mulching are common: Zoysa *et al.*, (1990) for example, observed that use of *L. leucocephala* as a green manure increased rice N uptake and grain yield in Sri Lanka. In Australia, Gutteridge (1992) observed that *S. sesban, L. leucocephala* and *Gliricidia sepium* mulches increased yield of maize stover. In Canada Yobterik *et al.*, (1994) observed that incorporation of mulches of *L. leucocephala* and *C. siamea*

increased N uptake and biomass production in corn. In western Nigeria, Larbi *et al.*, 1992 observed that maize (*Zea mays* L.) grain yields increased as *L. leucocephala* and *Gliricidia sepium* mulches were increased and in Rwanda Barasubranian and Sekayange (1991) obrserved that tree mulches increased soil nutrients (carbon, nitrogen, magnesium, phosphorus, calcium and potassium) and grain yields in beans (*Phaseolus vulgaris* L.) and sorghum (*Sorghum bicolor* L) but noted poor mulch response in maize. These studies concentrated on mulches that were not from *Rhizobium* inoculated trees and did not pay much attention to the point of mulch placement. Therefore, the purpose of this study was to investigate the effect of leaf mulches from *Rhizobium*-inoculated trees and their point of placement on barley early growth heights and biomass production (straw dry weight), total N and N content; soil pH and nitrate changes.

4.2. Materials and Methods

Leaves from both *Rhizobium* inoculated and uninoculated *L. leucocephala* and *R. pseudoacacia* were either placed on the surface or mixed up with soil in 3 L pots at a rate of 4.835 g per pot (equivalent to 2.5 tonnes dry matter per hectare, pot surface area) (Gutteridge, 1992). The soils used in each pot weighed about 3.0 kg. Enough barley (*Hordeum vulgare*, variety *ahadi*, obtained from the Kenya Seed Company, Kenya, was planted in all soil treatments (which had been watered slowly to eliminate differences due to soil moisture content and allowed to equilibrate for 24 hours) to allow a destructive sampling of barley straws and soil at three times and with three replications. The pots were randomized in a greenhouse bench and kept moist by gentle watering. The

greenhouse was maintained at temperatures of between 19 and 24°C and 16 hours of day light and 8 hours night. Heights of the tallest two barley plants in each pot in all treatments were taken after every two days for five days starting from the third day after barley germinated (sixth day after planting). After two weeks, barley was thinned to 4 plants per pot. The pots were randomized within the bench twice every week. Barley straws were cut at the soil level and surface soil samples were taken at the upper 5 cm. This was a destructive sampling which was done at two, four and six weeks. The barley straws were dried at 75°C for 48 hours, weighed and ground to pass through a 810 μm, No. 20 mesh sieve. The ground barley was analyzed for total N (%) using a Technicon™ AutoAnalyzer™ II. N content of the barley was determined by multiplication of total N by the dry weight of barley.

Soil pH in water, 1:5 (soil:water) was determined with a pH meter (Cole-Parmers). Soil nitrate was extracted using 2N potassium chloride (KCl) by shaking soil in a 250 ml conical flask for one hour, at a ratio of 1:10 (soil:KCl); nitrate concentration was determined as total N above, and then expressed on an oven dry weight basis (μg/100g soil).

The data were analyzed as a randomised complete block design using the Statistical Analysis System (SAS, 1990). Means were separated by least significant difference (LSD) and simple 't' tests at $p<0.05$.

4.3. Results

In this experiment the mixed mulches represent the standard and conventional method of mulching. So, if surface placed mulches caused a higher response than the mixed mulches the response was regarded as an increase, but if they caused a reduced response, the response was regarded as a decrease. For weekly responses the graphed data are discussed with reference to mulch placement and leaf type. For means taken over time, both leaf type and inoculation by species were compared.

4.3.a) Barley Analyses.

Figure 4.1. shows barley heights recorded between days 6 and 14. Surface placed mulches increased barley heights in all treatments with significant increases in barley mulched with leaves of uninoculated *L. leucocephala* ($p < 0.05$) at days 6 (0.92 times), 12 (1.3 times) and 14 (1.1 times); inoculated *L. leucocephala* ($p < 0.01$) at days 6 (1.4 times) and 12 (1.2 times); uninoculated *R. pseudoacacia* ($p < 0.01$) at days 6 (1.5 times), 8 (1.2 times), 12 (1.3 times) and 10 ($p < 0.05$) (0.90 times) and inoculated *R. pseudoacacia* ($p < 0.01$) at days 6 (1.6 times), 8 (1.2) and 12 (1.2 times). However, means taken over time (Appendix 4) showed that surface placed mulches resulted in significant height increases only in *R. pseudoacacia* ($p < 0.05$) treatments (1.2 times each). Surface placed leaves of inoculated *L. leucocephala* gave the highest mean height among *L. leucocephala* treatments while the highest mean height among *R. pseudoacacia* treatments was observed in uninoculated surface placed leaf treatment.

Figure 4.2 shows barley straw weights recorded between weeks 2 and 6. Surface

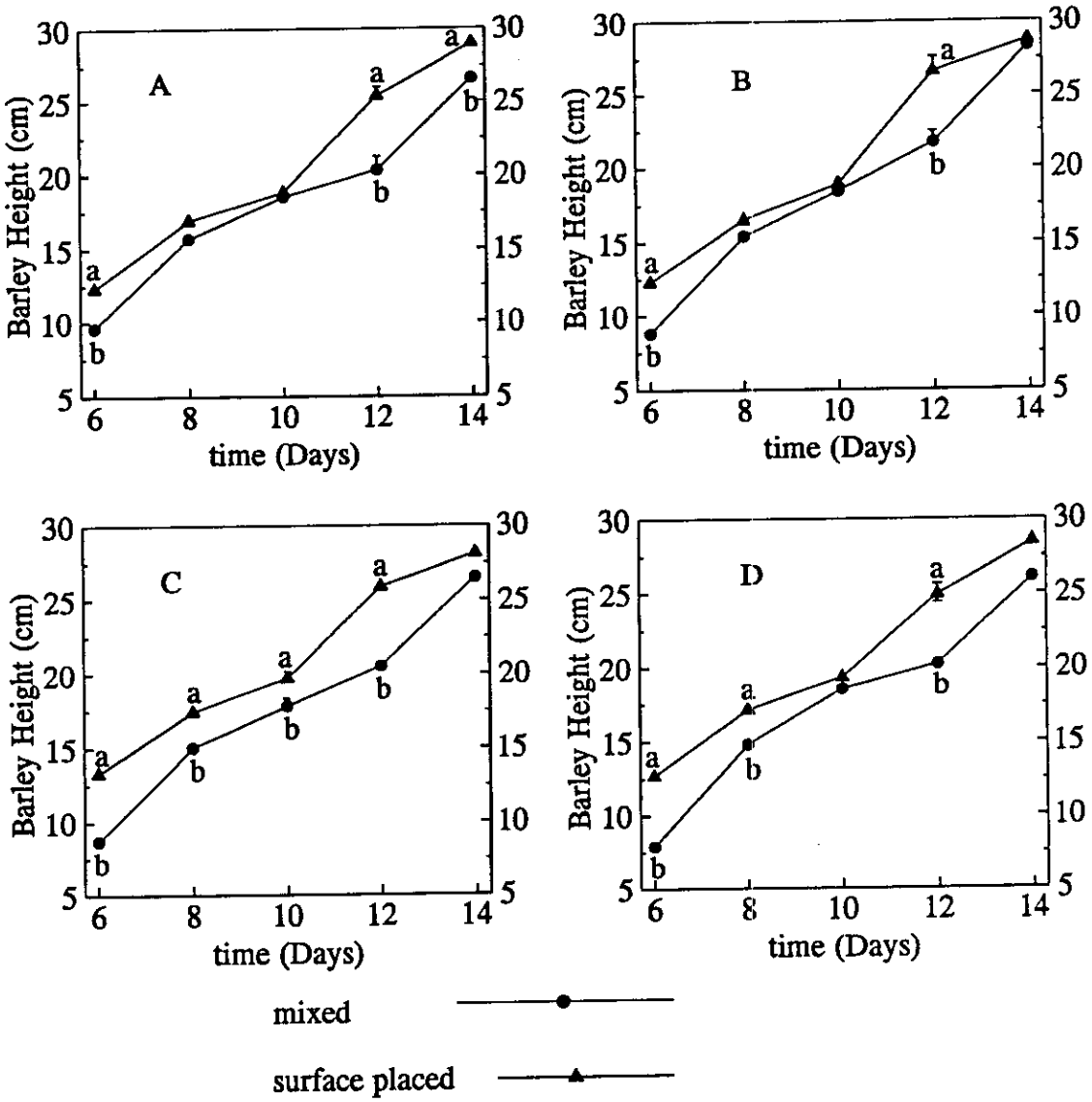

Figure 4.1. Heights of barley after mulching with leaves of (A) uninoculated and (B) inoculated *L. leucocephala*, (C) uninoculated and (D) inoculated *R. pseudoacacia*. a, b beside data points at the same time indicates significant difference at $p<0.05$.

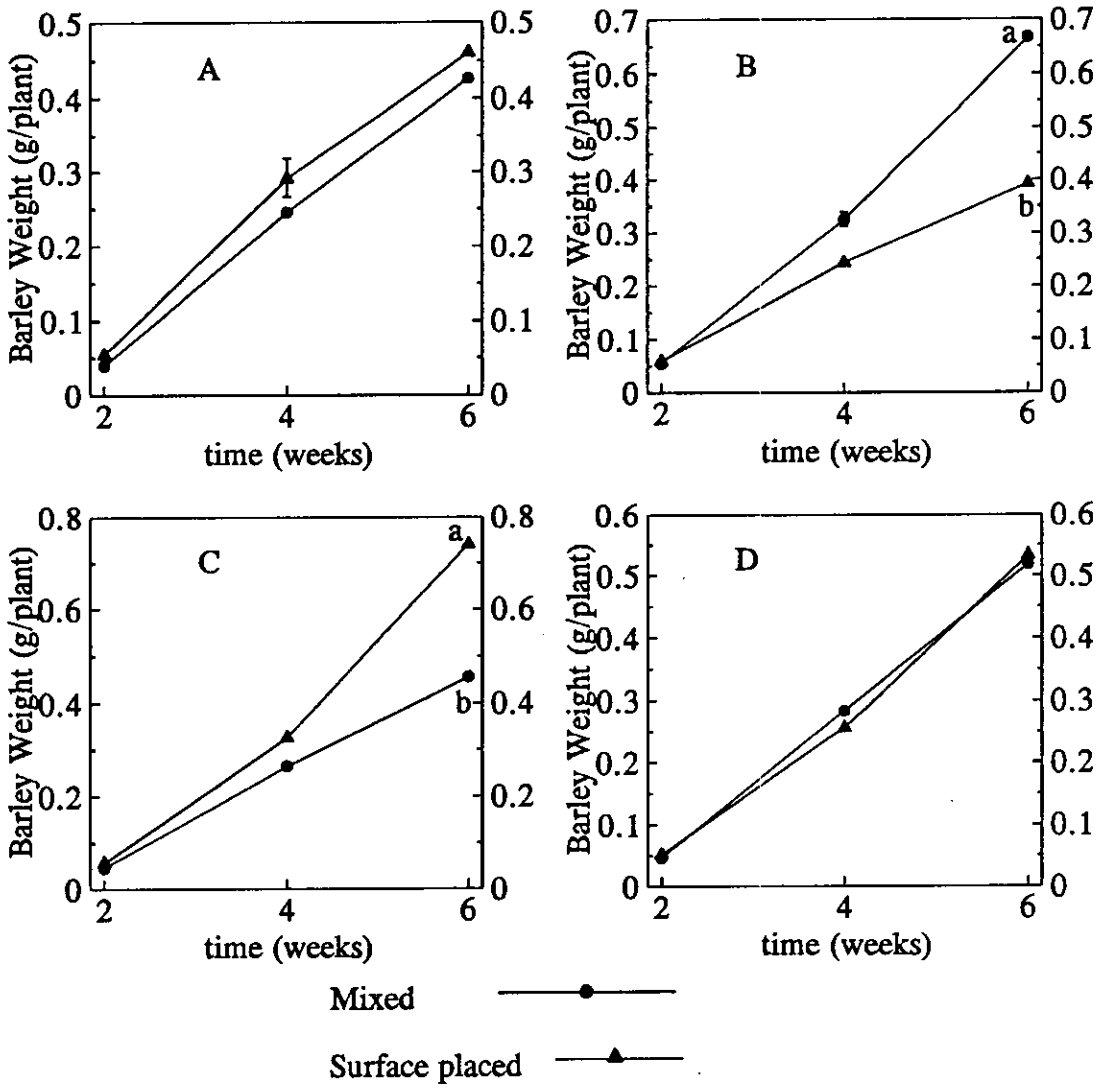

Figure 4.2. Barley straw dry weights (g.plant^{-1}) after mulching with leaves of (A) uninoculated and (B) inoculated *L. leucocephala*, (C) uninoculated and (D) inoculated *R. pseudoacacia*. a, b beside data points at the same time indicates significant difference at $p<0.05$.

placed leaves increased straw weights in barley mulched with leaves of uninoculated *L. leucocephala* and *R. pseudoacacia* with a significant weight increase of barley mulched with leaves of uninoculated *R. pseudoacacia* ($p<0.05$) at week 6 (1.6 times). Placing leaves of inoculated *L. leucocephala* on the surface decreased straw weight in all weeks with a significant decrease at week 6 ($p<0.05$) (2 times). However, means taken over time (Appendix 4) showed no treatment differences. The highest mean straw weight among *L. leucocephala* treatments was noted after using mixed leaves of inoculated *L. leucocephala* while the highest mean straw weight among *R. pseudoacacia* was observed after using surface placed leaves of uninoculated *R. pseudoacacia*.

Figure 4.3 shows straw total N also recorded between weeks 2 and 6. Total N generally decreased over time in all treatments, and barley mulched with surface placed leaves of inoculated *R. pseudoacacia* maintained a consistent but insignificant low total N in all weeks relative to mixed leaves. There was also a significant total N increase in barley mulched with surface placed leaves of uninoculated *L. leucocephala* ($p<0.05$) at week 6 (3 times). However, means taken over time (Appendix 4) showed no treatment differences. The highest total N means among *L. leucocephala* and *R. pseudoacacia* treatments were observed in uninoculated and surface placed leaves.

Figure 4.4 shows straw total N content also taken between weeks 2 and 6. Barley mulched with surface placed leaves of both uninoculated *L. leucocephala* and *R. pseudoacacia* had relatively higher total N content compared to that mulched with mixed leaves, with a significant higher total N content in *L. leucocephala* ($p<0.05$) at week 6 (4 times). However, surface placed leaves of inoculated *R. pseudoacacia* maintained a

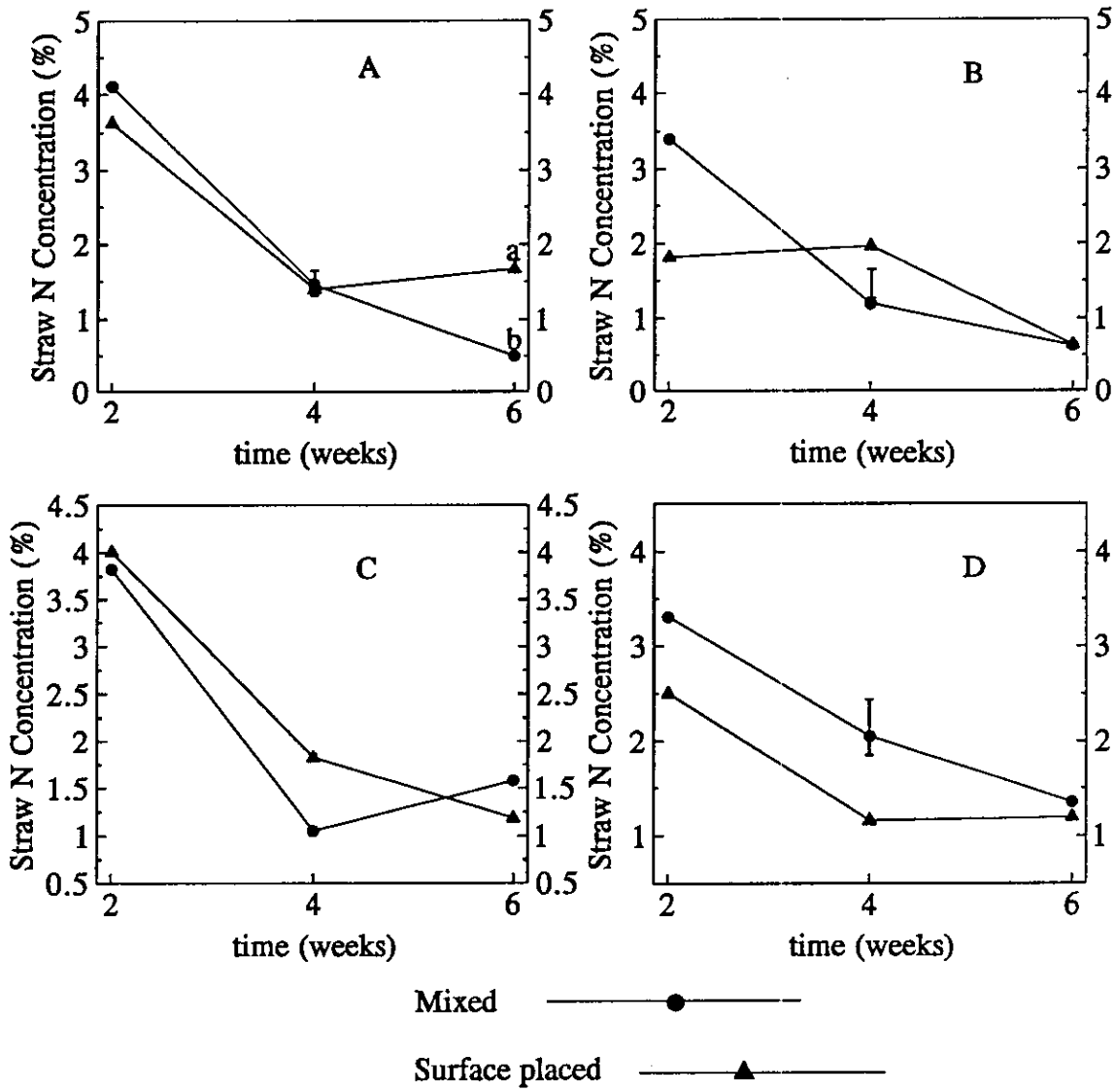

Figure 4.3. Barley straw N concentration (%) after mulching with leaves of (A) uninoculated and (B) inoculated *L. leucocephala*, and (C) uninoculated and (D) inoculated *R. pseudoacaçia*. a, b beside data points at the same time indicates significant difference at $p < 0.05$.

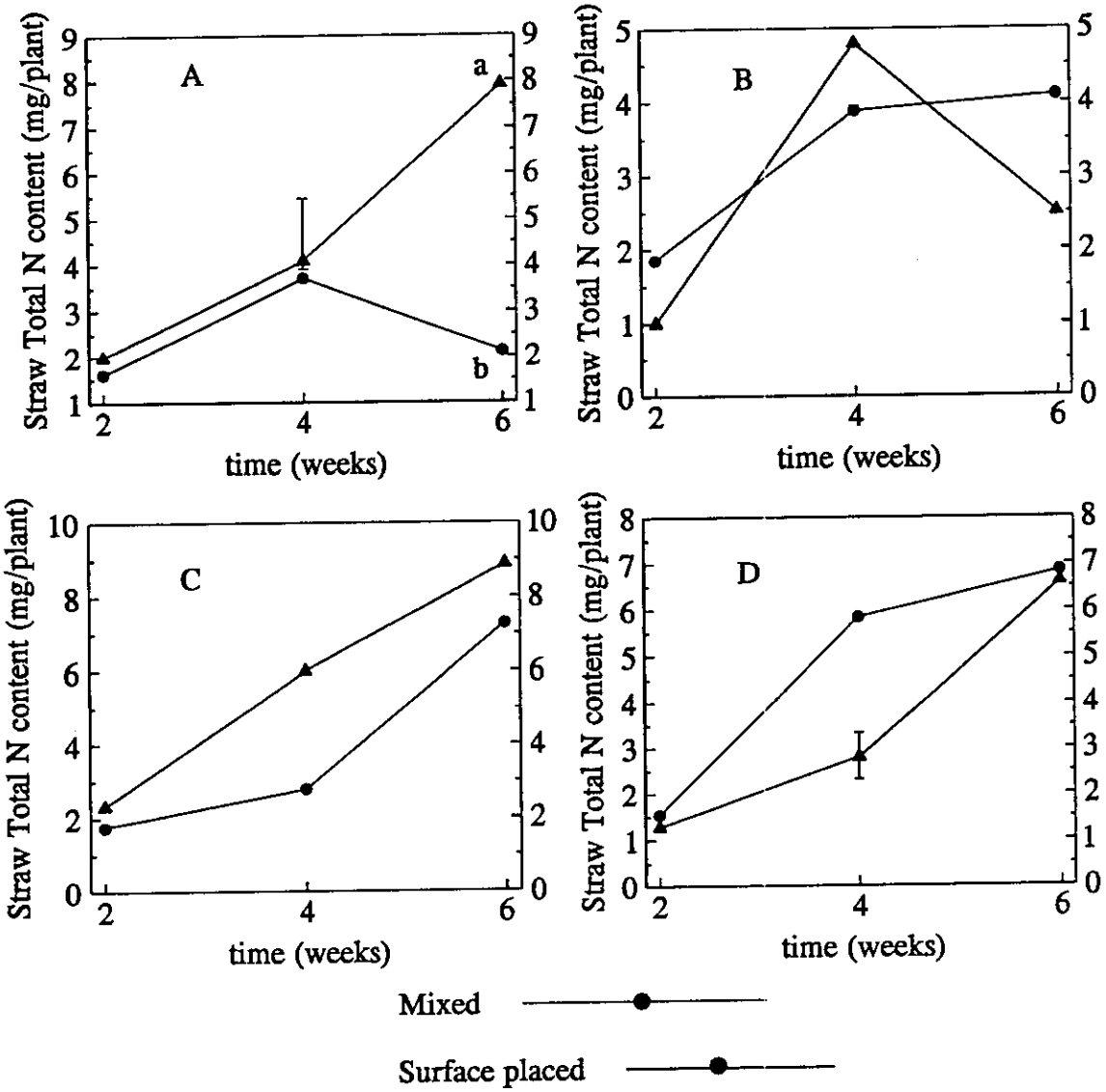

Figure 4.4. Barley straw total N content after mulching with leaves of (A) uninoculated and (B) inoculated *L. leucocephala*, (C) uninoculated and (D) inoculated *R. pseudoacacia*. a, b beside data points at the same time indicates significant difference at $p < 0.05$.

lower but insignificant N content in all weeks. Means taken over time (Appendix 4) showed no significant differences between treatments, although the highest total N content among *L. leucocephala* and *R. pseudoacacia* treatments was recorded in uninoculated and surface placed leaves.

Table 4.1 shows ear dry weights, total N and N content data recorded at week 6. Surface placed leaves of inoculated *L. leucocephala* reduced ($p<0.05$) ear weight (1.6 times) while surface placed leaves of uninoculated *R. pseudoacacia* increased ($p<0.05$) it (1.5 times). The highest ear weight was recorded in mixed leaves of inoculated *L. leucocephala* among *L. leucocephala* treatments and the highest ear weight in surface placed leaves of uninoculated *R. pseudoacacia* among *R. pseudoacacia* treatments. Surface placed leaves significantly ($p<0.05$) reduced ear total N in both inoculated (6 times) and uninoculated (2 times) *L. leucocephala* and uninoculated *R. pseudoacacia* (2 times). The highest total N among *L. leucocephala* and *R. pseudoacacia* treatments was recorded in mixed uninoculated leaves of both species. Placing leaves on the surface decreased ($p<0.01$) ear N content of barley mulched with inoculated *L. leucocephala* (9 times). The highest ear N content among *L. leucocephala* treatments was recorded after using mixed leaves of inoculated *L. leucocephala* while the highest ear N content among *R. pseudoacacia* was recorded in mixed and uninoculated leaves.

Table 4.1. Ear dry weights (g.plant^{-1}), total N (%) and N content (mg.plant^{-1}) after barley was grown in soil mulched with mixed and surface placed leaves of both *Rhizobium* inoculated and uninoculated *L. leucocephala* and *R. pseudoacacia*.

	Dry Weight (g.plant^{-1})		Total N (%)		N Content (mg.plant^{-1})	
species and inoculation	Mixed	surface	Mixed	Surface	Mixed	Surface
L. leucocephala not inoculated	0.26	0.25	1.25a	0.59b	3.30	1.50
L. leucocephala inoculated	0.35a	0.22b	1.06a	0.16b	3.70a	0.40b
R. pseudoacacia not inoculated	0.23a	0.34b	2.07a	1.12b	4.80	3.80
R. pseudoacacia inoculated	0.28	0.23	1.03	1.23	2.90	2.80

a, b indicated a significant difference between treatments, by species, at $p<0.05$.

4.3.b) Soil Analysis

Figure 4.5 shows soil pH recorded between weeks 2 and 6. Soil pH generally decreased at week 4 and increased again at week 6 in all soil treatments; with significant increases ($p<0.05$) in surface placed leaves of both uninoculated *L. leucocephala*, at weeks 2 (1.03 times) and 6 (1.1 times), and *R. pseudoacacia* at week 6 (1.04 times). However, means taken over time (Appendix 4) showed no treatment differences. Surface placed leaves of uninoculated *L. leucocephala* had the highest pH among *L. leucocephala* treatments while inoculated mixed leaves of *R. pseudoacacia* had the highest pH among *R. pseudoacacia* treatments.

Figure 4.6 shows soil nitrate recorded between weeks 2 and 6. Nitrate in mixed leaves peaked at week 4 and dropped at week 6 while nitrate in surface placed leaves gradually increased with time. Surface placed leaves of both uninoculated *L. leucocephala* (1.4 times) and *R. pseudoacacia* (2.5) significantly decreased nitrate ($p<0.05$) at week 4, but significantly increased ($p<0.01$) it in uninoculated leaves of *L. leucocephala* at week 6 (6 times), inoculated *L. leucocephala* at weeks 4 (2.6 times)

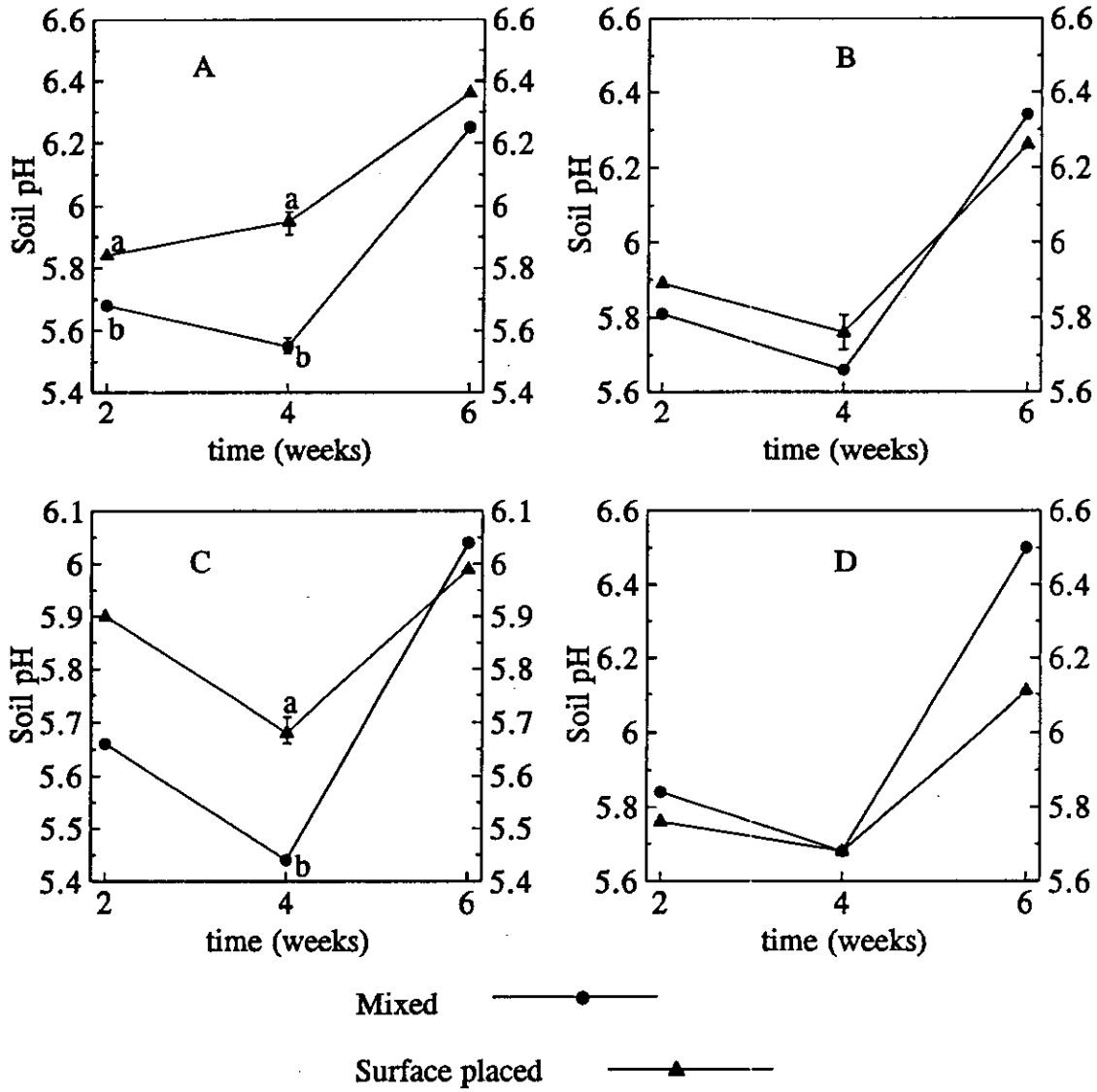

Figure 4.5. Soil pH after mulching with leaves of (A) uninoculated and (B) inoculated *L. leucocephala*, (C) uninoculated and (D) inoculated *R. pseudoacacia*. a, b beside data points at the same time indicates significant difference at $p < 0.05$.

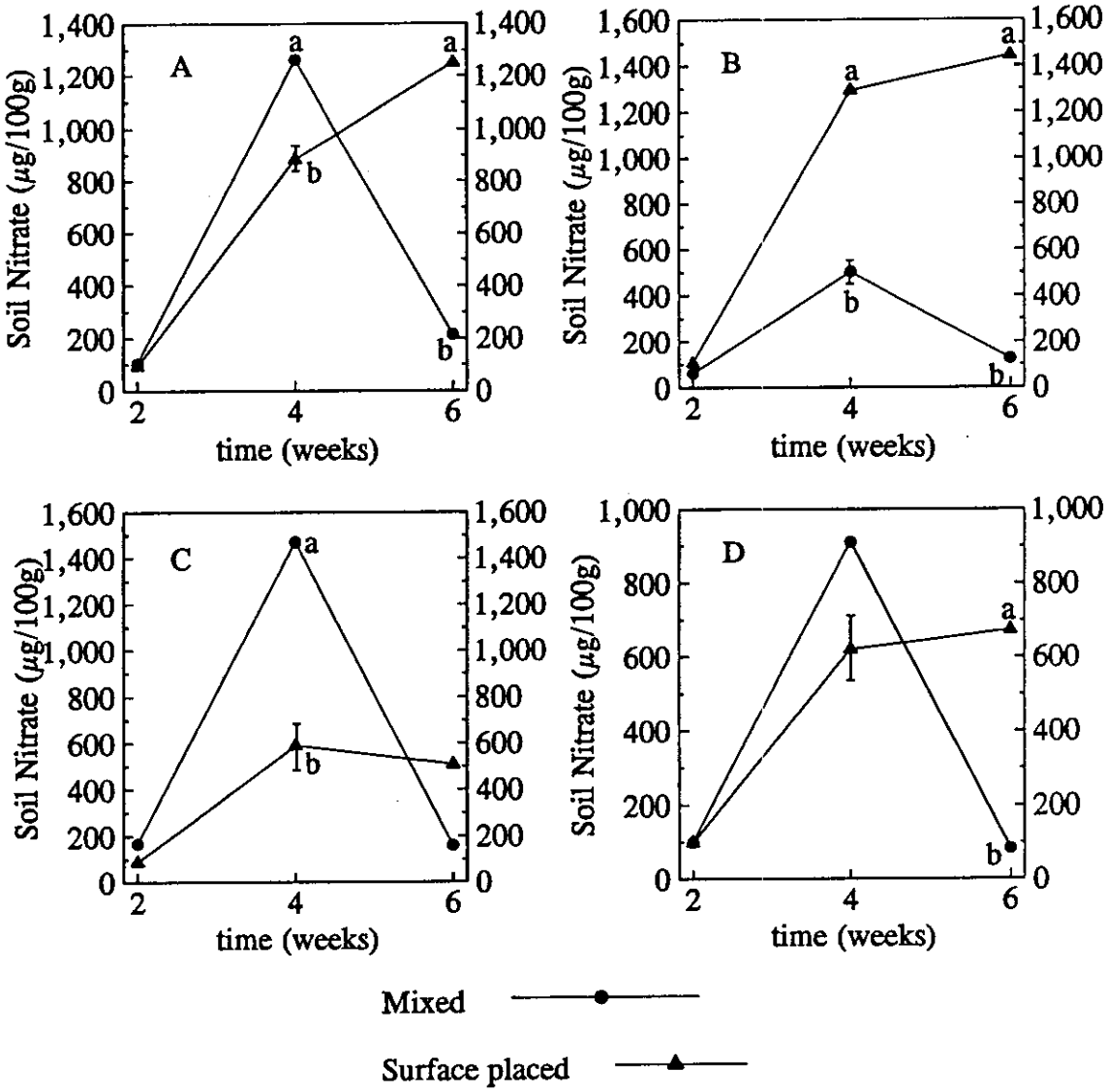

Figure 4.6. Soil nitrate after mulching with leaves of (A) uninoculated and (B) inoculated *L. leucocephala*, and (C) uninoculated and (D) inoculated *R. pseudoacacia*. a, b beside data points at the same time indicates significant difference at $p<0.05$.

and 6 (11.5 times) inoculated *R. pseudoacacia* at week 6 (8 times). However, means taken over time (Appendix 4) showed a significant nitrate increase ($p < 0.05$) only in surface placed leaves of inoculated *L. leucocephala* (4 times). The highest mean soil nitrate was observed in surface placed leaves of inoculated *L. leucocephala* among *L. leucocephala* treatments while the best nitrate among *R. pseudoacacia* treatments was observed in mixed leaves of uninoculated *R. pseudoacacia.*

4.4. Discussion

Higher mean barley heights were recorded when leaf mulches were placed on the soil surface probably because the presence of leaves on the soil surface during barley germination might have increased cell elongation in the plumule in response to limited light supply to the germinating barley. However, the effect of mulch on barley heights varied with tree species from which the leaves were obtained and type of leaves used implying that point of mulch placement and mulch type determined barley heights.

Barley straw dry weights were consistently higher (although insignificant) in surface placed uninoculated leaves of both *L. leucocephala* and *R. pseudoacacia* probably indicating a prolonged growth effect due to lack of light caused by placing leaf mulches in the soil surface during barley germination in these mulch treatments. However, the highest mean straw weight among *L. leucocephala* treatments was noted in mixed leaves from inoculated *L. leucocephala* while the highest weight among *R. pseudoacacia* was noted in surface placed leaves of uninoculated *R. pseudoacacia* both of which corresponded to low nitrate levels. It was not clear whether low nitrate levels were due

to plant uptake or slow mulch decomposition. Varying effects of mulch placement on crop dry weight has also been observed by Gutteridge (1992) who found that *S.sesban* was effective in increasing maize stover dry weights when mixed with soil, whereas *L. leucocephala*, *C. callothyrsus* and *A. cunninghamii* resulted in higher maize stover dry weights when placed on soil surface.

Barley straw N concentration decreased with time probably due to allocation of N in the growing parts of barley (Marschner, 1986) and therefore dilution of N in most growing parts of barley; this N allocation varied with leaf type, point of placement and species. The observed barley straw N contents were influenced by dry weight, total N, mulch type, point of placement and species.

Barley ear results indicated that mulching with mixed leaves of inoculated *L. leucocephala* and mixed leaves of uninoculated *R. pseudoacacia* improved ear total N and N content both of which indicated that these treatments may be important in increasing grain yield and grain N content (based on observations made in chapter two). This observation may then imply that *Rhizobium* inoculation may be necessary to improve mulch quality of *L. leucocephala* but not *R. pseudoacacia* and that mixing leaves with soil is important to improve barley yield.

The pH decrease at week 4 coincided with high nitrate levels mostly in mixed leaves but pH increased after week 4. Brady (1974) suggested that during decomposition of organic matter carbonic acid formed is too weak to cause low pH often detected in most soils. So, he further suggested that inorganic acids such as sulphuric acid and nitric acid (from decomposing matter and sometimes from certain fertilizers such as ammonium

sulphate under microbial activity) are potential suppliers of hydrogen ions in the soil. However, pH rose after week 4 due to reduced fresh organic matter in the soil, especially mixed treatments which slowed decomposition.

Soil nitrate data indicated that mixed leaves were effective in releasing nitrate within a month after which nitrate supply drastically dropped as opposed to surface placed leaves which gradually released nitrate with time. However, based on ear total N and N content observations made after using mixed leaves of *L. leucocephala* and mixed leaves of uninoculated of *R. pseudoacacia* it may be tempting to say that the time at which nitrate is supplied to the plant, leaf type, point of placement and species may commonly affect barley performance.

4.5. Conclusions

This study shows that point of mulch placement, mulch type and mulch species are important aspects to be considered by a farmer contemplating mulching. However, results obtained from barley ear total N and N content implies that mixing leaves with soil is still the most effective way of mulch placement. There is a need to carry out this study over a longer period of time under field conditions in order to get more information on the effectiveness of the use of leguminous leaf mulches as a source of fixed N for crops. As concerns nitrate and pH, more study also needs to be done to assess the effects of pH and nitrate fluctuations (as observed here) on overall crop yields under field conditions.

CHAPTER FIVE

SYNTHESIS

Rhizobium inoculation reduced root growth of inoculated seedlings implying that inoculation may be one way in which root growth can be reduced in intercropping systems where farmers are concerned about competition between trees and crops (Chapter One). The reduction in root growth also indicated that *Rhizobium* inoculation should not be done where elaborate root growth is required (e.g where leguminous trees are grown for soil erosion control as roots are required to hold soil particles together). As well, *Rhizobium* inoculation increased shoot total and N contents at varying amounts (depending on species) indicating variation in N fixation across legumes used in intercropping and mulching practices. Results from this study showed that there is need to screen and select the best leguminous trees in N fixation for intercropping and mulching purposes. *Rhizobium* inoculation also reduced nitrate levels. Based on this observation it may be necessary to carry out studies on soil nitrate responses during tree legume establishment for intercropping purposes in tropical soils which are often low in soil nitrate.

Inoculation of some intercrop tree species increased intercropped barley grain yield, grain total and N contents implying that *Rhizobium* inoculation can be an important practice in intercropping systems (Chapter Two). However, the observation that intercropping as a whole reduced overall grain yield and grain N content indicated that intercropping alone may not be a solution to increasing crop yields especially in small scale farming systems. This means that more emphasis should be placed on the use and

management of leaf mulches from the leguminous trees. The observation that *Rhizobium* inoculation increased tree heights (Chapter Two) implies that there is need to carry out more studies on legume-*Rhizobium* inoculation to investigate the effect of inoculation on leaf biomass production.

In Chapter Three, it was shown that leaves from *Rhizobium* inoculated trees decomposed faster than those from uninoculated trees of the same species indicating that *Rhizobium* inoculation may be one way in which leaf mulch decomposition can be enhanced in farming systems. However, it may be necessary to investigate whether this increase in leaf decomposition is coupled with nutrient release and how these nutrients affect crop growth and yield and how all of these relate to performance of leaf mulches from the uninoculated tree species.

In Chapter four, *Rhizobium* inoculation, point of mulch placement and species affected barley growth, total N concentration and N contents as well as soil pH and nitrate levels at varying magnitudes. These observations indicated that there is need to investigate, under field conditions, the use of mulches with respect to mulch types, species and point of placement, and their consequent effect on crop growth and yields and soil.

Results obtained in this study varied greatly with tree age and species (especially with respect to leaf total N and leaf N content) and these observations call for further investigation of tree age-inoculation interactions to determine whether and up to what time *Rhizobium* inoculation improves legume N concentration and N content. As well, more studies on leaf decomposition, mulch application, management and selection of

leguminous trees need to be carefully conducted to assess the importance of *Rhizobium* inoculation.

In summary, this study showed that *Rhizobium* inoculation may be important in:

1) reducing shoot to root ratio;

2) increasing shoot N concentration in *L. leucocephala*, *C. calothyrsus*, *S. sesban* and *R. pseudoacacia* and shoot total N content in *L. leucocephala* and *C. callothyrsus;*

3) increasing nodule numbers and nodule weight in *C. calothyrsus;*

4) increasing soil total carbon (%) in soils in which *R. pseudoacacia* and *S. sesban* are grown and organic C in soils in which *S. sesban* and *C. calothyrsus* are grown;

3) increasing ear N concentration, total N concentration, grain yield, grain N concentration and N content of barley intercropped with *C. calothyrsus* and *S. sesban;*

5) improved tree growth (tree heights) and reduced leaf shedding;

6) enhanced leaf decomposition in *L. leucocephala* and *R. pseudoacacia* and

8) improved barley ear N concentration and total N content of barley if leaves of *L. leucocephala* are mixed with soil.

However, this study is not conclusive and more work needs to be done to confirm these findings. The data obtained in this study may be used in future *Rhizobium* inoculation studies of *L. leucocephala, C. calothyrsus, S. sesban and R. pseudoacacia* with respect to seedling growth, nitrogen concentration and total nitrogen content as well as soil nitrate and carbon levels, tree-crop interaction characteristics, leaf decomposition and mulch application and management.

LITERATURE CITED

Aber, J.D. and J.M. Melillo. 1991. Terrestrial ecosystems. Saunders College Publishing, Philadelphia. pp. 429.

Ackello, O., Q. Paris and W.A. Williams. 1985. A methodology for estimating residual fertilizer nutrients. E. A. Agric. For. J. 49 (1):14-20.

Ahmed, B. and R.H. Phelps. 1990. Nitrogen fixation by *Phaseolus vulgaris* L. in response to inoculation. p. 644. *In*: Gresshoff, P.M., L.E. Roth, G. Stacey and W.E. Newton (eds). Nitrogen fixation: achievements and objectives. Chapman and Hall, New York. pp. 869.

Alazard, D., I. Ndoye and B. Dreyfus. 1988. *Sesbania rostrata* and other stem-nodulated legumes. pp. 765-769. *In*: Bothe, H., F.J. de Bruijn and W.E. Newton (eds). Nitrogen fixation: hundred years after. Proceedings of the 7th International Congress on Nitrogen Fixation. Stuttgart, New York. PP 878.

Allen, O.N. and E.K. Allen. 1981. The leguminosae. University of Wisconsin Press, Madison. pp. 812.

Atta-Krah, A.N. 1990. Alley farming with *Leucaena*: Effect of short-term grazed fallows on soil fertility and crop yields. Expl Agric. 25:1-10.

Balasubramanian, V. and L. Sekayange. 1991. Effects of tree legumes in hedgerows on soil fertility changes and crop performance in the semi-arid highlands of Rwanda. Biol. Agric. Hort. 8:17-32.

Becker, B. J.K. Ladha, I. Watanabe and J.C.G. Ottow. 1988. Seedling vs. vegetative propagation of stem-nodulating green manure *Sesbania rostrata*. Biol Fert Soils.

6:279-281.

Berg, B. and H. Staaf. 1981. Leaching accumulation and release of nitrogen in decomposing forest litter. pp. 33:163-178. *In*: Clark, F.E. and T. Rosswall (Eds) Terrestrial nitrogen cycles. Ecol. Bull. Stolkholm.

Bhatt, B.P. and N.P. Todaria. 1990. Studies on allelopathic effects of some agroforestry tree crops of Garhwal Himalaya. Agrf. systs. 12:251-255.

Bockman C., O. Kaarstad, O.H. Lie and I. Richards. 1990. Agriculture and fertilizers. Agriculture Group, Norsk Hydro a.s, Olso. pp. 245.

Bohlool, B. 1988. *Rhizobium* technology: Applications for international agricultural development in the tropics. pp. 759-764. *In:* Bothe, H., F.J. Brujin and W.E. Newton (eds). Nitrogen fixation: hundred years after. Proceedings of the 7th International Congress on Nitrogen Fixation. Stuttgart, New York. pp. 878.

Brady, N.C. 1974. The nature and properties of soils. 8th edition. Macmillan Publishing Co., Inc. New York. pp. 639.

Bulman, P. and D.L. Smith. 1993. Yield and yield component response of spring barley to fertilizer nitrogen. Agron. J. 85:226-231.

Burris, R.H. 1988. 100 years of discoveries in biological N_2 fixation. pp. 21-30. *In:* Bothe, H., F.J. de Bruijn and W.E. Newton (eds). Nitrogen fixation: hundred years after. Proceedings of the 7th International Congress on Nitrogen Fixation. Stuttgart, New York. pp. 878.

Catchpoole, D.W. and G.J. Blair. 1990. Forage tree legumes. III* release of nitrogen from leaf, faeces and urine derived from *Leucaena* and *Gliricidia* leaf.

Aust.J.Agric.Res. 41:539-547.

Danso, S.K.A. 1992. Biological nitrogen fixation in tropical systems: Twenty years of biological nitrogen fixation research in Africa. pp. 3-13. *In*: Mulongoy, K., M. Gueye and D.S.C. Spencer (eds). Biological nitrogen fixation and sustainability of tropical agriculture. John Wiley and Sons. Chichester. pp. 488.

Deane-Drummond, C.E. and Chaffey, N.J. 1985. Characteristics of nitrate uptake into seedlings of pea (*Pisum sativum* L. cv. Feltham First). Changes in net NO_3^- uptake following inoculation with *Rhizobium* and growth in low nitrate concentrations. Plant Cell and environment. 8:517-523.

Date, R.A. and J. Halliday. 1987. Collection, isolation, cultivation and maintenance of rhizobia. pp. 1-29. *In:* Elkan, G.I. (ed). Symbiotic nitrogen fixation technology. Mercel Dekker, Inc. New York. pp. 440.

Dommergues, Y.R. 1992. Management of the soil microbial populations to optimize tree establishment and growth, with particular reference to nitrogen-fixing bacteria. pp. 113-131. *In*: Mulongoy, K., M. Gueye and D.S.C. Spencer (eds). Biological nitrogen fixation and sustainability of tropical agriculture. John Wiley and Sons. Chichester. pp. 488.

Elkan, G.H. 1992. Biological nitrogen fixation systems in tropical ecosystems: An overview. pp. 27-40. *In*: Mulongoy, K., M. Gueye and D.S.C. Spencer (eds). Biological nitrogen fixation and sustainability of tropical agriculture. John Wiley & Sons. Chichester. pp. 488.

Evans, H.J., S.A. Russel, F.J. Hanns, H. Papen, S.L. Sayavendra, M. Zuber and P.A.

Boursier. 1988. Hydrogenase and nitrogenase relationships in *Rhizobium*: some recent developments. pp. 577-582. *In*: Bothe, H., F.J. de Bruijn and W.E. Newton (eds). Nitrogen fixation: hundred years after. Proceedings of the 7th International Congress on Nitrogen Fixation. Stuttgart, New York. pp. 878.

FitzPatrick, E.A. 1986. An introduction to soil science. Second edition. Longman group (FE) limited, Hong Kong. pp. 255.

Gibson, A.H., B.L. Dreyfus and Y.R. Dormmergues. 1982. Nitrogen fixation by legumes in the tropics. p. 328. *In*: Dommergues, Y.R. and H.G. Diem (eds). Microbiology of tropical soils and plant productivity . Martinus Nijhoff/Dr. W. Junk Publishers, The Hague.

Gutteridge, R.C. 1992. Evaluation of the leaf of a range of tree legumes as a source of nitrogen for crop growth. Expl Agric. 28:195-202.

Hadad, M.A. T.E. Loynachan, M.M. Musa and N.O. Mukhtar. 1986. Inoculation of groundnut (peanut) in sudan.

Hardy, R.W.F. and U.D. Havelka. 1975. Nitrogen fixation research: a key to world food?. Science 188:633-643.

Harris, M.M. and S.J. Riha. (1991). Carbon and nitrogen dynamics in forest floor during short-term laboratory incubations. Soil Biol. Biochem. 23 (11):1035-1041.

Hauk, R.D. 1973. Nitrogen tracers in nitrogen cycle studies: Past use and future needs. J. Environ. quality 2(3):317-327.

Ladha, J.K., M.B. Peoples, D.P. Garrity, V.T. Capuno and P.J. Dart. 1993. Estimating nitrogen fixation of hedgerow vegetation using the nitrogen-15 natural abundance

method. Soil Sci.Soc.Am.J. 57:732-737.

Lara, M., J.L. Ortega, B.E. Olguin and F. Sanchez. 1988. Nodule expression and nitrogen metabolism in *Phaseolus vulgaris* root nodules. pp. 617-622. *In* Nitrogen fixation: hundred years after. Bothe, H., F.J. de Bruij and W.E. Newton (eds). Gustav Fischer, Stuttgart. pp. 878.

Larbi, A., M.A. Jabbar, A.N. Atta-Krah and J. Cobbina. 1992. Effect of taking a fodder crop of maize grain yield and soil chemical properties in *Leucaena* and *Gliricidia* alley farming systems in Western Nigeria. Expl. Agric. 29:317-321.

Lepo, J.E. and S.M. Ferrenbach. 1987. Measurement of nitrogen fixation by direct means. pp. 221-255. *In*: Elkan, G.I. (ed). Symbiotic nitrogen fixation technology. Marcel Dekker, Inc. New York. pp. 440.

Luyindula, N. and I. Hague. 1992. Effect of *Rhizobium* inoculation and phosphorus on growth and nitrogen fixation in tree legumes grown on highland vertisols. pp. 109-112. *In:* Mulongoy, K., M. Gueye and D.S.C. Spencer (eds). Biological nitrogen fixation and sustainability of tropical agriculture. John Wiley and Sons, Chichester. PP. 488.

Marschner, H. 1986. Mineral nutrition of higher plants. Academic press, Harcourt Brace Javanovich, publisher. London. pp. 674.

Mason, C.F. 1976. Decomposition. Edward Arnold (Publishers) limited, London. pp. 58.

Mulongoy, K. and B.T. Owoaje. 1992. Early growth and symbiotic properties of three woody legumes grown on a sandy soil in south western Nigeria. pp. 139-136. *In*:

Mulongoy, K., M. Gueye and D.S.C. Spencer (eds). Biological nitrogen fixation and sustainability of tropical agriculture. John Wiley and Sons, Chichester. pp. 488.

Mulongoy K. and M.K. Van der Meersch. 1988. Nitrogen contribution by *Leucaena (Leucaena leucocephala)* prunings to maize in alley cropping system. Biol. Fert. Soils. 6:282-285.

Nair, P.K.R. 1993. An introduction to agroforestry. Kluwer Academic Publishers, Dordrecht. pp. 499.

Ntayombya, P. and A.M. Gordon. 1994. Effects of black locust on productivity and nitrogen nutrition of intercropped barley. pp. 69-75. *In:* Schultz, R.C. and J.P. Colletti (eds). Opportunities for Agroforestry in the Temperate Zone Worldwide: Proceedings of the Third North American Agroforestry Conference. Iowa State University, Ames, Iowa. pp. 449.

Odee, D.W. 1989. A cross-inoculation study on indigenous rhizobia and selected tree legumes. Kenya Forestry Research Institute Research Note No. 3. pp. 21.

Odee, D.W. 1993. The ecology of nitrogen-fixing symbioses under arid conditions of Kenya. PhD thesis, University of Dundee, Scotland (U.K). pp. 145.

Odingo, R.S. 1971. The Kenya highlands: land use and agricultural development. East African Publishing house, Nairobi. pp. 229.

Patel, F.M. and L.R. Patel (1989). Response of greengram varieties to phosphorus and *Rhizobium* inoculation. Indian J. Agron. 36(2) 295-297.

Poehlman, J.M. 1985. Adaptation and distribution. pp. 1-17. *In*: Barley. Rasmusson,

D.C. (ed). American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Publishers, Madison.

Postgate, J.R. 1978. Nitrogen fixation. Edward Arnold (publishers), London. pp. 67.

Raj, V.C. and R.B. Patel. 1991. Response of summer cowpea to nitrogen, phosphorus and *Rhizobium* inoculation. Indian J. agron. 36(2):285-286.

Rochie, K.O. 1983. Intercropping tree legumes with annual crops. pp. 103-116. *In:* Huxley, P.A. (ed). Plant research and agroforestry. International Council for Research in Agroforestry (ICRAF), Nairobi.

Sandhu, J., M. Sinha and R.S. Ambasht. 1990. Nitrogen release from decomposing litter of *Leucaena leucocephala* in the dry tropics. Soil Biol. Biochem. 22 (6) 859-863.

Schroder, E.C. 1992. Improvement of the *Phaseolus*, *Rhizobium* symbiosis, with particular references to the Caribbean region. pp. 79-95. *In*: Mulongoy, K., M. Gueye and D.S.C. Spencer (eds). Biological nitrogen fixation and sustainability of tropical agriculture. John Wiley and Sons, Chichester. pp. 488.

Schroth, G. W. Zech and G. Heimann. 1992. Mulch decomposition under agroforestry conditions in a sub-humid tropical savanna processes and influence of perennial plants. Plant and soil 147: 1-11.

Simpson, K. 1986. Fertilizers and manures. Longman, London. pp. 254.

Smith, R. S. 1987. Production and quality control of inoculants. pp. 391-411. *In:* Elkan I. (ed). Symbiotic nitrogen fixation technology. Mercel Dekker, Inc. New York. pp. 440.

Sprent, J.I. 1987. The ecology of the nitrogen cycle. Cambridge University Press.

Cambridge. pp. 151.

Stevenson, F. J. 1986. Cycles of soil carbon, nitrogen, phosphorus, sulfur, micronutrients. John Wiley and sons, New York. pp. 380.

Shewry, P.R. (1992). Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology, The Alden Press Ltd, Oxford. pp. 610.

Suresh, K.K. and R.S.V. Rai. 1990. Studies on intercropping with silk cotton (*Ceiba pentandra* (L.) Guertn). Trop. Agric. 68(1):37-40.

Ssali, H. 1988. *Rhizobium phaseoli* inoculation trails on farmers's fields in Kenya. E. Afr. For. J. 53(3) 151-157.

Tamm, O.C. 1991. Nitrogen in terrestrial ecosystems: questions of productivity, vegetational changes and ecosystem stability. Springer-verlag, Berlin. pp. 115.

Tian, G., B.T. Kang and L. Brussaard. 1992. Biological effects of plant residues with contrasting chemical compositions under humid tropical conditions-Decomposition and nutrient release. Soil Biol. Biochem. 24(10):1051-1060.

Thevathasan, N.V. and A.M. Gordon. 1994. Moisture and fertility interactions in a potted-barley intercropping. pp. 257-260. *In:* Schultz, R.C. and J.P. Colletti (eds). Opportunities for Agroforestry in the Temperate Zone Worldwide: Proceedings of the Third North American Agroforestry Conference. pp. 449.

Turk, D., H.H. Keyser and P.N. Singleton. 1993. Response of tree legumes to Rhizobial inoculation in relation to the population density of indigenous rhizobia. Soil Biol. Biochem. 25 (1):75-81.

Vadavia, A.T., K.K. Kalaria, J.C. Patel and N.M. Baldha. 1991. Influence of organic,

inorganic and biofertilizers on growth, yield and nodulation of chickpea. Indian J.Agro. 36(2):263-264.

Wadisirisuk, P., N. Boonkerd, V. Thananusont and A. Nitayajarn. 1988. Rhizobial strains selection for mungbean cv. Kampang Saen 1 and 2. p. 789. *In*: Bothe, H., F.J. de Bruijn, and W.E. Newton (eds). Nitrogen fixation: hundred years after. Stuttgart, New York. pp. 878.

White, D.L., B.L. Haines and L.R. Boring. 1988. Litter decomposition in Southern Appalachian black locust and pine-hardwood stands:litter quality and nitrogen dynamics. Can.J.For.Res. 18:54-63.

Yamoah, C.F. A.A. Agboola and K. Mulongoy. 1986. Decomposition, nitrogen release and weed control by prunings of selected alley cropping shrubs. Agrof. syst. 4: 239-246.

Yobterik, A.G., V.R. Timmer and A.M. Gordon. 1994. Screening agroforestry tree mulches for corn growth: a combined soil test, pot trial and plant analysis approach. Agrof. syst. 25:1-14.

Zoysa, A.K.N., G. Keerthingsinghe and S.H. Upasena. 1990. Effect of *Leucaena leucocephala* (Lam) de Wit. as a green manure on nitrogen uptake and yield of rice. Biology Fertil. Soils. 9:68-70.

APPENDICES

Appendix 1. Means over time for shoot and root dry weight: data analysis Chapter One.

	Shoot Dry weight ($g.plant^{-1}$)		Root Dry weight ($g.plant^{-1}$)	
Species	Inoculated	Control	Inoculated	Control
L. leucocephala	0.473	0.369	0.145	0.135
C. calothyrsus	0.266	0.229	0.069	0.078
S. sesban	0.178a	0.333b	0.085a	0.209b
R. pseudoacacia	0.203	0.268	0.069	0.106

a and b beside data values indicates significant difference at $p < 0.05$.

Appendix 2. Means taken over time for shoot to root ratio, shoot N concentration, shoot N content, root N concentration and root N content: chapter one data analysis.

Species and inoculation	shoot root ratio	shoot N concentration (%)	shoot total N content ($mg.plant^{-1}$)	root N concentration (%)	root total N content ($mg.plant^{-1}$)
L. leucocephala not inoculated	3.644	1.361**	7.52**	1.189	2.26*
L. leucocephala inoculated	4.141	2.371**	16.41**	1.527	3.35*
C. calothyrsus not inoculated	3.967	1.245**	3.73**	1.581	1.93
C. calothyrsus inoculated	4.516	2.321**	8.43**	1.241	1.03
S. sesban not inoculated	3.307*	1.021**	3.98	0.875	2.95*
S. sesban inoculated	2.173*	1.717**	3.51	1.195	1.28*
R. pseudoacacia not inoculated	2.286	1.765*	5.67	1.158	1.54
R. pseudoacacia inoculated	2.819	2.362*	6.64	0.915	1.03

** and * indicates a significance between inoculated and control treatments within species at $p<0.01$ and $p<0.05$ respectively.

Appendix 5a). Means taken over time for barley height (cm), straw weight (g.plant^{-1}), straw total N (%) and straw N content (mg.plant^{-1}): barley data analyses chapter four.

	barley height (cm)		straw weight (g.plant^{-1})		straw total N (%)		straw N content (mg.plant^{-1})	
species and inoculation	mixed	surface	mixed	surface	mixed	surface	mixed	surface
L. leucocephala not inoculated	18.13	20.48	0.24	0.27	2.02	2.23	2.50	4.70
L. leucocephala inoculated	18.50	20.58	0.35	0.23	1.74	1.51	3.30	2.90
R. pseudoacacia not inoculated	17.68a	20.87b	0.26	0.37	2.15	2.30	3.90	5.50
R. pseudoacacia inoculated	17.50a	20.78b	0.28	0.28	2.23	1.50	4.80	2.90

a and b indicates a significant difference between placement treatment within species and inoculation treatment at $p < 0.05$.

Appendix 5b). Means taken over time for soil pH and nitrate: soil data analyses chapter four.

	soil pH		soil nitrate (μg.100 g^{-1} soil)	
Species and inoculation	mixed	surface	mixed	surface
L. leucocephala not inoculated	5.83	6.05	497.90	745.50
L. leucocephala inoculated	5.94	5.97	232.90a	923.90b
R. pseudoacacia not inoculated	5.71	5.86	633.40	471.00
R. pseudoacacia inoculated	6.01	5.85	365.50	572.60

a and b indicates a significant difference between placement treatment, within species and inoculation treatment at $p < 0.05$.