RICE YIELDS AS INFLUENCED BY NITROGEN FROM *AZOLLA-ANABAENA* ASSOCIATION SUPPLEMENTED WITH INORGANIC FERTILISER NITROGEN IN THE LOWER MOSHI IRRIGATION PROJECT, TANZANIA

BY

NDIMUBANDI EGIDIUS MVUKIYE

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT FOR A
DEGREE OF MASTER OF SCIENCE (SOIL SCIENCE AND LAND
MANAGEMENT) OF SOKOINE UNIVERSITY OF AGRICULTURE

1999

ABSTRACT

A field study was conducted in the Lower Moshi Irrigation Project, Tanzania, to quantify the green-manurial potential of *Azolla* grown as a dual crop with irrigated rice. Parallel studies on the *in situ* decomposition rate of *Azolla* and the effect of the rice canopy on *Azolla* N accumulation were also undertaken.

Azolla alone and in combination with 40 and 80 kg N/ha as urea (45% N) was compared with untreated control or, 160 kg N/ha as urea. These treatments were tested on *Wahiwahi* (indigenous) and IR54 (improved) rice varieties in a split-plot design with 3 replications. Each plot received a basal phosphate dose of 20 kg P/ha as TSP. Rice leaf chlorophyll content, plant height, tiller number, flowering and maturation time, panicle excertion, panicle number per unit area, grain number per panicle, panicle fertility, grain weight and the ultimate grain yield were recorded.

An inoculation rate of 3 t/ha yielded 19.63 t/ha of fresh *Azolla* in 15 days and contained about 26 kg N/ha. The *Azolla* decomposed very fast, loosing 90% of its weight in 3 weeks of incorporation into soil.

Azolla alone significantly (P = 0.05) improved rice leaf chlorophyll and most of the other parameters cited above, the improvement being comparable to that obtained with *Azolla* supplemented with successive amounts of urea or, 160 kg N/ha as urea alone. Despite its low N contribution, *Azolla* demonstrated considerable potential in promoting rice performance under conditions of the Lower Moshi Irrigation Project. Given the low amounts of N contributed by *Azolla*, further studies to identify other possible rice growth-promoting factors in *Azolla* are suggested at the study site and in other rice growing areas in Tanzania.

DECLARATION

I, **Ndimubandi Egidius Mvukiye**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and it neither has been submitted nor is concurrently being submitted for a degree in any other University.

Date........26/11/1999........ Signature....................

COPYRIGHT

ACKNOWLEDGEMENTS

This study could never have been accomplished without the support and encouragement of the following, to whom I owe heavy debts of gratitude:

Professor Iwao Watanabe, whose unselfish discussion on *Azolla* utilisation greatly aroused and solidified my interest in *Azolla* as a biofertiliser in rice production.

The Norwegian Agency for Development Co-operation (NORAD) and all the staff of the Department of Soil Science at Sokoine University of Agriculture, for the financial support and cooperation,respectively.

My dedicated research supervisor Dr G.P. Msumali for his constructive criticism and irreproachable guidance throughout the research work and report writing.

All my fellow workers (casual and permanent) at the KATC and the LMIP, for creating a splendid social environment before and during my study.

DEDICATION

This dissertation is dedicated to my revered parents who made me aware, from the early days of my childhood, that education is endless.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

ARA = Acetylene Reduction Activity
CEC = Cation Exchange Capacity
CV = Coefficient of variation
DM = Dry matter
DNMRT = Duncan's New Multiple Range Test
DTPA = Diethylene Triamine Pentaacetic Acid
EC = Electrical conductivity
FW = Fresh weight
KATC = Kilimanjaro Agricultural Training Centre
LMIP = Lower Moshi Irrigation Project
LSD = Least significant difference
MC = Moisture content
NAFCO = National Agriculture and Food Corporation
NORAD = Norwegian Agency for Development Cooperation
OC = Organic carbon
PI = Panicle initiation
RDM = Residual dry matter
RH = Relative humidity
ROC = Residual organic carbon
ROP = Residual organic phosphorus
RTN = Residual total nitrogen
TSP = Triple superphosphate

CHAPTER ONE

INTRODUCTION

1.1 General

The yield of rice in Tanzania is still very low, currently estimated at 1.5 - 2.5 tonnes per hectare (Kanyeka *et al.*, 1994). A number of factors account for the low rice yields. These (among others) include poor weed management, insect pests and diseases, unavailability of high-yielding varieties and poor/low fertiliser use by farmers (Kihupi, 1997). According to Horibata (1992), the major factor associated with the yield decline in rice yields has been inadequate supply of nitrogen.

It has not been possible to stabilise rice yields even in irrigation schemes where improved agronomic packages for rice production have been disseminated and adopted. In the Lower Moshi Irrigation Project (LMIP) for example, rice yields had improved from 2 t/ha in the early 1980s to an average of about 7 t/ha in 1985 (Horibata, 1992). However, the yields per unit area have continuously been declining over the years to the current 5 t/ha. With regard to the issue of fertilisers as cited above, high rates of N (150 kg/ha) were recommended in order to achieve the high levels of rice yields. This rate of N fertiliser is too high for many farmers to be able to maintain, regardless of the source of N (i.e. industrial fertilisers or the various types of natural fertilisers or manures).

As for the inorganic/industrial fertilisers, their ever-escalating costs have made them unaffordable to the majority of farmers. This has been aggravated by the removal by government, of subsidy on fertilisers. On the other hand, the few farmers who are able to purchase inorganic nitrogenous fertilisers could use them excessively in their effort to achieve the highest possible yields. Excessive use of industrial fertilisers, on the other hand, could be pollutive to the environment.

The use of natural fertilisers (biofertilisers), which constitute a renewable, non-polluting, and generally inexpensive resource could effectively reduce the above constraints.

Farmyard manure, although available in the LMIP area, is not sufficient for all rice growers. It is also bulky to carry from the homestead to the farm, especially since some farmers live as far as 20 kilometres away from their farms. The use of rice straws, on the other hand, is hindered by the difficulties of their incorporation into the soil; their low N content and their slow rate of decomposition especially under flooded conditions. Moreover, in the LMIP and surrounding areas, rice straws are mainly fed to ruminant animals. Green-manuring, with plants that can easily be grown on farm and which decompose easily in flooded conditions, could therefore be the best alternative.

Plants of the genus *Azolla* have been in use in many countries particularly China, Vietnam and India (Watanabe, 1996) and are considered the appropriate source of green manure. The appropriateness of *Azolla* is mainly due to its ability to grow concurrently with rice in flooded soils. It can therefore be intercropped with rice so that no transport costs are incurred as is the case with farmyard manure. In association with the appropriate algal microsymbiont, *Azolla* also has the ability to fix atmospheric nitrogen (Singh, 1979a). Consequently it has a high N content ranging between 3 and 5% (Watanabe, 1996). Mineralization of *Azolla* and transfer of N to rice is also quite rapid (App *et al*., 1980), especially when the *Azolla* is incorporated into the soil. Therefore, the Azolla-N is readily-available to the rice crop in the concurrent season (Singh, 1979a).

Both indigenous and introduced species of *Azolla* have been identified in some areas in Tanzania, and they are, in fact, abundant in the LMIP. *Azolla* can, therefore, be effectively utilised as green manure for rice in the area.

Despite its stated potential, *Azolla* alone, as is the case with other green manure plants, cannot always provide all the nitrogen required for optimum rice yields, particularly the high-yielding varieties. The amount of N contributed by *Azolla* depends the *Azolla* ecotype, soil properties, agroclimatic conditions and *Azolla* management in the field (Watanabe, 1996). Many researchers working on *Azolla* as a biofertiliser to supply N recommend the addition of some supplementary inorganic nitrogen for optimum production of lowland rice when *Azolla* is used. However the levels of the inorganic fertilisers that could effectively be combined with *Azolla* for the optimum yield of a particular rice variety vary, among other things, with the field environment and the *Azolla* ecotype.

1.2 Problem analysis and objectives

Although the potential of *Azolla* as a biofertiliser has, for a long time, been recognised in other countries, its use in both Zanzibar and Tanzania mainland has so far been limited. In many areas, farmers still consider it as a weed and usually they incur additional production cost in removing it from their fields. This is because very little research and information dissemination have been done in Tanzania, to popularise the full potential of *Azolla* as a biofertiliser under the various rice growing environments (Alli, 1996; Wagner, 1996a). Only preliminary studies in the form of unreplicated field trials on the potentials of some exotic species of *Azolla* have so far been done in Tanzania (Alli, 1996; Wagner, 1996a). In those studies, the beneficial effects of *Azolla* on rice crop performance were qualitatively assessed whereby, the rice plants grown with *Azolla* looked comparatively greener and stronger than those without *Azolla*. No systematic studies of *Azolla* appear to have been conducted in Tanzania, in particular, to quantitatively evaluate the N contribution to, and subsequent effect on, rice growth and yield. This study was carried out at the LMIP with the general objective of quantifying the potential of *Azolla* as green manure when intercropped with rice, alone or in combination with inorganic nitrogenous fertilisers. The specific objectives were:

1. To determine the *Azolla* biomass and nitrogen accumulation when grown as a dual crop with rice.

2. To determine the *Azolla* decomposition rate under field conditions.

3. To determine the growth and yield performance of two rice varieties planted with *Azolla* alone and in combination with inorganic fertiliser nitrogen.

CHAPTER TWO

LITERATURE REVIEW

2.1 General morphology of *Azolla* and its microsymbiont *Anabaena azollae*

The genus *Azolla* includes a group of aquatic ferns that occur widely in both temperate and tropical fresh water ecosystems (Becking, 1979). The *Azolla* plant, often called a frond (van Hove, 1989), has a branched, short stem (rhizome) that bears alternately arranged, slightly overlapping, deeply bilobed leaves and adventitious, thin roots. When floating on water it forms a mat that usually covers the water surface when optimal growth conditions exist. In the dorsal (chlorophyllous) lobes of the leaves, reside the nitrogen-fixing blue-green alga *Anabaena azollae Stras.*(Becking, 1979).

Anabaena azollae is a prokaryotic organism which occurs as unbranched filaments formed by two types of cells, the smaller but numerous vegetative ones which are capable of photosynthesising, and the slightly larger ones (heterocysts) that synthesise the enzyme complex (nitrogenase) that enables them to produce ammonium (nitrogen compounds) from atmospheric nitrogen.

The relation between *Azolla* and the alga is, naturally, one of permanent symbiosis because the two organisms are associated in all stages of the life cycle of the fern (Becking, 1979 ; van Hove, 1989) and is automatically transmitted from one generation of *Azolla* to the other (van Hove, 1989). The fern provides various nutrients (photosynthesised carbon compounds) and protection to the alga (microsymbiont). Through its nitrogen-fixing activity, the microsymbiont in turn, provides the amount of nitrogen which, in most cases, is sufficient for itself and the fern (Peters, 1976, 1978; Becking, 1979; van Hove, 1989).

2.2 Occurrence of *Azolla* in Tanzania

Both indigenous and introduced species of *Azolla* occur in Tanzania (Watanabe, 1996). Indigenous species such as *Azolla nilotica* is abundant in the LMIP at Upper Mabogini; Mandaka-mnono village; areas in and around Kahe-NAFCO farms and in Mkomazi valley and Kwemazandu smallholder irrigation scheme in Korogwe district. According to Alli (1996), introduced species occur in some areas of Unguja (Zanzibar) Island (Mtwango, Mwera, Bumbwisudi) and in Pemba Island (Kinyakuzi, Mangwena). In Tanzania mainland, introduced *Azolla* species occur in the Lower Moshi Irrigation Project (LMIP), Kwemazandu (Korogwe) and in the Mkindo smallholder irrigation scheme (Morogoro). The list may not be exhaustive because only limited survey has so far been done to establish with certainty the extent of the occurrence of different *Azolla* species in many areas where rice is grown in Tanzania.

2.3 Environmental requirements of the *Azolla-Anabaena* association for optimum productivity

As any other green plant, *Azolla* grows better where there is adequate supply of air, light, water and essential mineral nutrients (Becking, 1979). Details of these requirements are reviewed in the sections below:

2.3.1 Water

Azolla multiplies best and reaches maximum productivity in terms of biomass per unit time and N_2 accumulation when floating on shallow fresh water, preferably 2.5-5.0 cm deep (Chung Chu, 1987; Lumpkin, 1987b; van Hove, 1989; Ventura and Watanabe, 1991). The shallow depth of water enables *Azolla* roots to come near to, or in contact with the soil and absorb essential plant nutrients (van Hove, 1989; Wagner, 1996) especially at the early stage of its growth (Singh, 1979a). When rooted in only humid soil, the fronds are unable to fragment and disperse hence, they pile (overpopulate) in spots. As a result, their productivity is lowered (van Hove, 1989). Deep water levels, which do not allow firm anchorage of *Azolla* roots to soil, tend to amplify the sweeping effect of winds on *Azolla*, causing it to accumulate on one side of the plot and thus, creating a premature overcrowded condition (van Hove, 1989). In addition, high winds cause turbulence which increases dispersion and fragmentation of *Azolla* fronds. All these factors lower *Azolla* growth and N_2 fixation (Ashton, 1974).

2.3.2 Temperature

The response of various strains of *Azolla* to temperature is very complex (van Hove, 1989). However, it is generally known that high (35-40^{0}C) and low (below 10^{0}C) temperatures do adversely affect *Azolla* to varying degrees, depending on the ecotype (Meelu *et al.*, 1994). Generally, the optimum temperature for *Azolla* growth ranges between 20 and 35^{0}C (Pande, 1978; Lumpkin, 1987b;

van Hove, 1989). The *Azolla* ecotypes are divided into 3 major groups according to their tolerance to adverse temperature ranges (Pande, 1978; Lumpkin, 1987b; van Hove, 1989). Some species such as *A. filiculoides* and *A. rubra* can grow, though slowly, at low, but cannot withstand higher temperatures. Others like *A. mexicana*, *A. nilotica* and many ecotypes of *A. pinnata* hardly survive at very low, but withstand higher temperatures. Yet some other species for example, *A. caroliniana* and *A. microphylla* are not affected by extremes of temperature. Thus, they can grow comparatively faster even at very low, and are quite tolerant of higher temperatures.

2.3.3 Light intensity and duration

In *Azolla*, light affects photosynthesis and appears to regulate nitrogenase activity of the heterocysts of *Anabaena azollae*, independently of CO_2 fixation (Bar *et al.*, 1991).

According to Talley and Rains (1980b) and van Hove (1989), *Azolla* reaches its maximum growth rate at 25-50% of full sunlight, that is, it grows best in less than full sunlight. Both growth of *Azolla* and nitrogenase activity of the microsymbiont were reported by Ashton (1974) in South Africa, to be highest at 50% of full sunlight.

Low light intensities, such as those caused by dense rice canopy, reduce the rate of growth and N_2 fixation of *Azolla*. In South Africa, Ashton (1974) noted that in one-eighth of full sunlight (about 12,000 lx) and in full sunlight, the nitrogenase activity was low (0.5 µmol C_2H_2/g fresh weight/hour). According to Lumpkin (1987a), the rice canopy will start influencing growth, at 2-3 weeks after transplanting, and will stop growth in most *Azolla* species at 45 days after transplanting, depending on crop and environmental characteristics. Moreover, Lumpkin and Bartholomew (1986) reported positive correlation of *Azolla* growth rate with day-length. They also reported that the optimum photoperiod for *Azolla* growth is 20 hours.

Generally, *Azolla* prefers an environment with a certain degree of shading. In rice fields, depending of plant density and growth stage of the rice plant, a shading of 50-75% of the actual sunlight may occur without interfering with the rate of photosynthesis and N_2 fixation in *Azolla* (Becking, 1979).

2.3.4 Minerals

Phosphorus, potassium, calcium and magnesium are important macronutrients for *Azolla* growth (Becking, 1979). Micronutrients are also required by the *Azolla* plants in the usual concentrations required for optimum growth of other green plants but cobalt and molybdenum are specially required in higher amounts by the microsymbiont for N_2 fixation (Lumpkin, 1987b). Azolla also thrives well when supplied with enough (ferrous) iron (Becking, 1979).

When testing the behaviour of *Azolla pinnata* to the deficiency of various macronutrients using fresh weight(FW), total N content and N_2 fixation rate (acetylene reduction assay - ARA) as indicators, Watanabe *et al.* (1977) observed that phosphorus deficiency reduced FW production, total N content and nitrogenase activity (ARA) by 22, 16 and 3.5%, respectively, relative to the control (plants receiving a complete nutrient solution).

When potassium was deficient, FW production and total N content were reduced to 32 and 24% of the control, respectively, but ARA was increased 2.4 times compared to the control.

With calcium deficiency, the plants produced only 9% FW and had 5% total N of the control. Moreover, nitrogenase activity could not be detected, in fact the fronds lost the microsymbiont altogether, under such conditions of Calcium deficiency.

Magnesium deficiency reduced FW production and total N content to 22 and 77% of the control, respectively, but increased the nitrogenase activity, 1.3 times that of the control.

From the observations made by Watanabe *et al.* (1977), it is obvious that the balance of nutrients is important not only for *Azolla* growth (dry matter production) but also, and more importantly, for nitrogen fixation. Nitrogen fixation was increased by the presence of some nutrient elements (P,Ca,Co,Mo) but reduced by others (K,Mg) [Watanabe *et al.*, 1977].

The symptoms of deficiency of some elements on *Azolla*, have visually been ascertained by many researchers. For instance, phosphorus-deficient plants are smaller and fragile; are less vigorous; have a pink to red colour and develop very long roots (Yatazawa *et al.*, 1980). The dorsal lobes of the calcium-deficient plants are intensely red; fronds become easily fragmented (Watanabe, 1982) and roots are short, thin and light in colour (Malavolta *et al.*, 1981). Potassium-deficient plants have dark-brown and stunted roots (Malavolta *et al.*, 1981) and their fronds are yellowish-brown in colour (Watanabe, 1982). Iron-deficient plants, on the other hand, become yellow, due to depleted chlorophyll, and their roots are thin and whitish (Malavolta *et al.*, 1981).

Some levels of various mineral nutrients required by *Azolla* have been arbitrarily suggested by some researchers. In a laboratory experiment by Yatazawa *et al.* (1980), *Azolla* neither grew nor exhibited full nitrogenase activity in a nutrient solution whose macronutrient (P, K, Mg and Ca) concentration was below 0.03, 0.40, 0.40 and 0.50 mmol l^{-1}, respectively. The *Azolla* nitrogenase activity was not fully realised when the concentrations of the above-mentioned respective macronutrients were less than 0.03, 0.60, 0.50 and 0.50 mmol l^{-1}. The minimum concentrations of micronutrients (Fe, Mn, Mo and B) which *Azolla* required for growth were 50, 20, 0.3 and 30 $\mu g l^{-1}$, respectively. In another study by Becking (1979), *Azolla* thrived well when supplied with enough (ferrous) iron, preferably in a concentration of 9.1 kgFe/ha within a pH range of 5.5-6.6.

Under natural soil conditions however, phosphorus is the most limiting nutrient element for *Azolla* growth and N_2 fixation (Watanabe *et al.*, 1980; Subudhi and Watanabe, 1981). However, the requirements vary with *Azolla* species/ecotypes. According to Pande (1978), the application of 1.75 to 3.50 kg P/ha is necessary for proper growth of most strains of *Azolla* in the field. Kondo *et al.* (1989), observed that laboratory-grown *Azolla pinnata* (an isolate from Niger) showed higher growth and N content at a P level of 3.1 ppm. Likewise, Cary and Weerts (1992), in a greenhouse experiment, observed that *A. pinnata* and *A. filiculoides* produced maximum biomass at a concentration of 5 and 20 $mgPl^{-1}$, respectively, in the nutrient solution. According to van Hove (1989), the critical concentration for the onset of P deficiency symptoms is about 0.03 ppm and a deficiency of P [<0.22% of dry matter (DM)] is accompanied by a reduction of nitrogenase activity and nitrogen content in *Azolla*. However, Sikander and Watanabe (1987) reported threshold values for P deficiency to be 0.1% of the *Azolla* DM. When enough P is available, *Azolla* can accumulate N from 1 up to 5.1% of its DM, depending on species and ecotypes (Sikander and Watanabe, 1987; van Hove, 1989).

2.3.5 Relative Humidity

Both low and high humidities have an adverse effect on *Azolla*. At relative humidities (RH) less than 60%, *Azolla* becomes fragile and may dry up (Hamdi, 1982). At higher values of RH, especially those coupled with high temperatures, the growth of insect pests and fungi, which attack *Azolla*, is stimulated (Watanabe, 1982). The optimum RH for *Azolla* as reported by Watanabe (1982) lies between 85 and 90%.

2.3.6 Soil characteristics

The soil characteristics of much importance to *Azolla* growth (other than nutrient content) are pH and salinity. The optimum pH for *Azolla* is in the range of 4.5 - 7.0. However, *Azolla* can survive and fix N_2 over a wider range of pH (3.5 to 10) [Ashton, 1974; Watanabe *et al.*, 1977; Lumpkin, 1987b; Lumpkin and Plucknett, 1980;]. At higher pH values, the indirect adverse effects such as insolubility of certain essential elements and proliferation of cynobacteria, reduce *Azolla* growth (van Hove, 1989).

Various species and ecotypes of *Azolla* exhibit varying degrees of tolerance to salinity but, according to van Hove(1989), soils having an electrical conductivity (EC) beyond 3 $mhos.cm^{-1}$ are generally considered unfavourable to *Azolla* growth.

2.4 Some essential indicators of *Azolla* growth

Azolla growth is indicated by an increase in biomass and productivity (van Hove, 1989). These indicators are reviewed as follows:

2.4.1 Biomass accumulation

According to van Hove (1989), an increase in *Azolla* biomass per unit area, under favourable environmental conditions is represented by a sigmoid curve, partitioned into five phases in which, each two successive phases overlap.

The first phase (exponential growth) starts just after *Azolla* fronds are introduced into the field at a specified inoculation density (fresh weight of *Azolla* per unit area). Individual fronds start multiplying at a slow (but increasing) rate, until they touch each other end to end, but each frond still being able to float freely. At this point, *Azolla* growth enters the second phase (linear growth) whereby *Azolla* fronds expand and interweave rapidly, but at a constant rate, until they form a single-layer thick mat, covering all the available water surface on which it floats. Overcrowding of fronds then starts (third phase). Some fronds either start coming (progressively) on top of one another or start developing an erect bearing. Later on, they start to interweave. These phenomena, later slow down (an indication of decelerating growth). At the end of the third phase, maximum interweaving of *Azolla* fronds is reached and they stop multiplying. At this point, *Azolla* would have attained maximum growth and hence maximum biomass. The maximum biomass is maintained (fourth phase) for some time. At the end of the fourth phase, a large part of *Azolla* fronds will be shielded from light by those above them, hence reducing their photosynthetic activity (Rains and Talley, 1978b) and the overall growth of *Azolla* (Pande, 1978). Finally (fifth phase), the shielded layers (fronds) start degenerating.

The amount of biomass that *Azolla* can accumulate has been reported by van Hove (1989) and Meelu *et al.* (1994) to range between 12 and 120 t/ha, with an average of

20 - 40 t/ha, depending on field management and environmental conditions.

2.4.2 *Azolla* productivity

Azolla productivity indicates how much biomass is accumulated per unit area over a given time interval i.e., time taken for the *Azolla* mat to completely cover a given area. The time the *Azolla* mat takes to develop and cover a unit area will depend, among other factors, on the interaction between *Azolla* inoculation density (biomass per unit area), time of inoculation, cultural practices, environmental conditions and the *Azolla* ecotypes. With high inoculation rates, complete cover of *Azolla* mat is generally achieved earlier than with low rates of inoculation. At an inoculation rate of 200-300 g (fresh weight) m^{-2}, Ventura and Watanabe (1991) observed that the *Azolla* mat covered an area of one hectare in 10 to 15 days. Singh (1979a) reported that *Azolla* inoculated as an intercrop at 100 g m^{-2} covered a hectare in bunded, flooded fields after rice transplanting in 20 to 30 days. An inoculation density of 250-500 g (fresh weight) m^{-2} in many environmental conditions, was recommended by van Hove (1989), regardless of whether or not *Azolla* is grown as a monocrop or as an intercrop. At this rate, the *Azolla* mat will develop fast enough to be incorporated for timely availability, of its accumulated nutrients to the concurrent rice crop. This would enable the mat to develop earlier and offer a side benefit of controlling weeds, apart from increasing rice yields (van Hove, 1989). Satapathy (1993) recommended *Azolla* inoculation within 5 days after rice transplanting.

It has been reported by Watanabe *et al.*(1977), Pande (1978) and Kulasooriya *et al.*(1987) that, under the best field conditions such as in wetland fields with adequate phosphorus, *Azolla* can double its weight every 3 to 6 days. The maximum productivity that many *Azolla* ecotypes can achieve is 3 t ha^{-1} d^{-1} (van Hove, 1989). This is the peak period of growth and N_2 fixation (i.e., the middle stage of the linear phase of growth).

2.5 *Azolla* N accumulation

Azolla is able to accumulate large amounts of N by absorbing some mineral forms of N from the growth medium and through fixation of atmospheric nitrogen in association with the microsymbiont alga. It is generally accepted that a major portion of the nitrogen fixed by the algal symbiont is passed to the *Azolla* plant (Peters *et al.*, 1979). According to Rains and Talley (1978a,b), Meelu *et al.* (1994) and Watanabe (1996), a fully-grown *Azolla* mat covering an area of one hectare can accumulate 8-121 kg of nitrogen, depending on management. Of this amount of nitrogen, 50 to 90% is contributed by the atmospheric N_2 fixation (Lowendorf, 1982; Watanabe and Liu, 1992; Roger and Ladha, 1992; Watanabe, 1996).

When *Azolla* is intercropped with rice plants (as one aspect of *Azolla* management in the field), complete cover is never reached due to competition for space and light (Becking, 1979). Consequently, the amount of nitrogen accumulated by one crop of *Azolla* intercropped with rice can range between 8 and 75 kg/ha (Rains and Talley, 1978a,b; Watanabe, 1996) but is generally, between 25 and 30 kg/ha (Singh, 1979a).

2.6 Effect of combined (mineral) N in the environment on the growth of, and N_2 fixation by, *Azolla*

The effect of combined N in the environment on the growth of *Azolla* is controversial (van Hove, 1989). Although *Azolla* is capable of growing in N-free media due to the nitrogen-fixing ability of the microsymbiont *Anabaena*, it has been established that the presence of some levels of combined N in the medium increases *Azolla* growth rate. The levels, however, depend on the *Azolla* ecotype. For instance, Cary and Weerts (1992), observed maximum growth of *A. pinnata* and *A. filiculoides* at a nitrogen (NH_4^+ and NO_3^-) level of 1 and 10 mgl^{-1}, respectively. On the other hand, the presence of combined N has also been found to reduce *Azolla* growth. Becking (1979) observed a 34% reduction in *Azolla* FW production when combined N (NO_3^--N) was introduced in the growth medium relative to the FW produced in the N-free medium. The effect however was reported to have been indirect as the NO_3^--N caused the proliferation of green algae which competed with *Azolla* for growth factors.

The nature of the effect of combined N on *Azolla* growth is a function of the interaction of many factors such as the form of combined N, the species of *Azolla* and the prevailing environmental conditions. For instance, Chung-Chu (1987) in China, reported that when the average day temperature was about 17^{0}C, N in the nitrate form was still effective in increasing *Azolla* growth, while N in the NH_4^+ form was ineffective. At the same temperature however, urea suppressed *Azolla* growth.

The presence of combined N in the medium in other instances, is however known to be inhibitive to N_2

fixation by *Azolla*. Combined N adversely affects *Azolla* N_2 fixation mainly by reducing the heterocyst (site for N_2 fixation) frequency in the microsymbiont (Singh and Singh, 1989), although the degree of the reduction is still not well understood. According to Becking (1976, 1979), Peters (1978) and Lumpkin and Plucknett (1980), the reduction in *Azolla* nitrogenase activity in the presence of combined N in the medium is not so pronounced. However, in the study by Singh *et al.* (1992a), NO_3^- levels of 5, 10, 15 and 20 mM caused significant inhibition of N_2-fixation [as measured by the Acetylene Reduction Activity (ARA)], compared to the N-free controls. The reduction occurred at 16, 12, 8 and 4 days after NO_3^- application, respectively.

The above controversy arises from the fact that the nitrogenase in the microsymbiont seems to be protected, the protection being more or less complete depending on the species and the ecotypes (van Hove, 1989). The species whose nitrogenase is more protected will therefore fix substantial amounts of atmospheric N_2, even in the presence of high levels of combined N. The mechanism by which the protection is achieved was however, not indicated.

Generally however, the levels of combined N that cause significant reduction in *Azolla* N_2 fixation, are considered to be substantially high (Becking, 1979). The *Azolla-Anabaena* symbiosis exhibits an important attribute in that it will continue to fix nitrogen even in the presence of some levels of combined nitrogen that are known to be inhibitory to most other N_2-fixing systems (van Hove, 1989). In the work by Manna and Singh (1989), inhibition of the process of nitrogen fixation, as determined by the ARA, was not detected up to a fertilizer rate of 60 kg N/ha in the rice fields. According to Becking (1979) this attribute is possible because both combined N and fixed atmospheric N_2 can be assimilated simultaneously by the roots of the fern and the microsymbiont enclosed in the aerial parts of the bilobed leaves, respectively.

2.7 Techniques of *Azolla* utilisation in rice production

Azolla may either be grown in the rice fields as a monocrop prior to rice transplanting; as an intercrop (dual crop) between rice plants after transplanting; a combination of mono- and inter-crop; or it can be produced in some plots away from the rice fields and later brought and applied in the rice fields as green manure (either as fresh matter or after it has been composted). In the rice fields, *Azolla* may be incorporated (ploughed in or trampled) into the soil or just be allowed to die and decompose on the soil surface (Singh, 1979a; Lumpkin, 1987a). Among these techniques, intercropping, with one or more subsequent incorporations into the soil, has been found to be the most cost-effective, especially with transplanted rice culture (Singh, 1979b; Lumpkin, 1987a; Satapathy, 1993). Intercropping is also a water-saving practice in the sense that the water is shared between *Azolla* and rice as compared to monocropping which requires some considerable amount of water to pre-establish only *Azolla*. More water is then required to establish the subsequent rice crop.

Timely incorporation of *Azolla* maximises its fertilising effect, which is comparable to a split application of inorganic fertiliser N (van Hove, 1989; Rosenani and Chulan, 1992). Early incorporation of *Azolla* into the soil is essential because, in that case, the *Azolla* will decompose faster and supply N (and other nutrients) at the time they are most needed by the rice plants (Singh, 1979a; Tung and Shen, 1985). Moreover, if it is not incorporated early enough (i.e., 15 - 21 days after inoculation), the *Azolla* mat becomes so thick as to suffocate young rice seedlings, or impair rice tillering (Tung and Shen, 1985). The suggested period of incorporation as stated above, is that which allows the *Azolla* to accumulate sufficient nutrients and maximum biomass of 2 to 4 $kg.m^{-2}$. This is the period when the C:N ratio will not have increased beyond 10:1 (Singh, 1979b), that is, when the *Azolla* growth phase is still linear. The essence of timely incorporation is to synchronise the release and availability of Azolla-N with the period when rice plants need it most.

Prior to incorporation, the field should thoroughly be drained for successful *Azolla* incorporation. This is achieved when 80% or more of the *Azolla* biomass remains underground even after re-flooding (van Hove, 1989).

2.8 Release of *Azolla*-N and its availability to the rice crop

Mineralization of *Azolla* and transfer of N to rice is reported to be quite rapid especially when the *Azolla* is incorporated into the soil (App *et al.*, 1980). Singh (1979a) observed that it took between 3 and 6 weeks for *Azolla*, incubated at room temperature, to release 62 to 80% of its N. The Azolla-N was reported to have been available to the concurrent rice crop (Singh, 1979b; Ito and Watanabe, 1985) and its uptake by rice plants was comparable with that of inorganic-N (Rains and Talley, 1978a,b; Rosenani and Chulan, 1992; Watanabe, 1996). It has been reported that *Azolla* excretes into the medium some of its fixed N, in the ammonium (NH_4^+) form, during its growth (Silvester, 1972; National Academy of Sciences, 1979; Chung-Chu, 1984; Watanabe, 1984). However most of the Azolla-N becomes available to rice after the *Azolla* has decomposed. Tung and Shen (1985) noted that *Azolla* could release 26 - 28% of its total N content in the ammonium form, after decomposition.

Mineralization of *Azolla* after incorporation into the soil differs with species. Tung and Shen(1985) found that *Azolla* species which have high lignin content (e.g *Azolla filiculoides*) decomposed more slowly and often showed incomplete mineralisation compared to those with low lignin (e.g *Azolla microphylla*). However, *Azolla* fronds with high N content were generally found to decompose faster than those with low N content, regardless of species (Ventura *et al.*, 1992)

2.9 Response of rice plants to *Azolla*-N

Numerous reports on *Azolla* research conducted in various countries generally indicate beneficial effects of *Azolla* on rice growth and yield (Meelu *et al.*, 1994). Due to its fast mineralization as already stated, Azolla-N benefits the concurrent crop. It has been observed that *Azolla* inoculation increases rice plant height, leaf area index, nitrogen uptake and dry matter (Singh and Singh, 1988). It also increases the number of tillers per hill, number of panicles per square meter, and the 1000-grain weight (Subramani and Kannaiyan, 1987).

The yield response however, depends upon many factors, including the species and quantity of *Azolla* applied, its N content, the cultural method of growing and applying *Azolla*, time and frequency of application, initial soil fertility, geographical location and prevailing weather conditions (Singh, 1979a; Pande, 1978; Rains and Talley, 1978a,b; Kannaiyan *et al.*, 1983; Lumpkin, 1987b). For instance, at Davis, California, Peters (1978) reported that *Azolla*, applied both as a monocrop and an intercrop, increased rice yields by 216% while in China, Lay *et al.* (1989) reported a yield increase of only 30.6%, over the unfertilized controls.

The yield-increasing effect of *Azolla* is more pronounced when it is used in combination with inorganic sources of nitrogen. In Pakistan, Alam (1989) reported results of a pot study in which the combination of *Azolla pinnata* (750 mg fresh weight) with 30 mg N (urea)/kg soil resulted in the highest grain yield (12.92 g) per pot of the rice cultivar IR8-5 as compared to 10.68 g obtained when *Azolla* was used as the sole source of nitrogen. The yield was also greater than the 11.92 g obtained when urea (60 mg N/kg soil) was used as a sole source of nitrogen. In this experiment, the yield (8.24 g/pot) obtained in the control treatment (no *Azolla*, no inorganic fertiliser N) was the lowest.

The effect of the amount of *Azolla* incorporated was clearly shown by Alexander (1988) in his report on a two-year (1980 - 1981) study he had conducted at Pattambi, India. In this study, combining 5, 7.5 and 10 t of *Azolla* with 90 kg N/ha as NPK fertiliser resulted in 3.7, 4.2 and 3.7 t/ha of grain yield, respectively. Similar results were obtained even when 5, 7.5 and 10 t of *Azolla* were combined with 45 kg N/ha as NPK, the yields being 3.4, 3.7 and 3.2 t/ha, respectively. In that study, 7.5 t was considered the optimum amount of *Azolla* for rice production.

Regarding the effect of prevailing weather conditions and the timing of *Azolla* application, Singh and Singh (1988) observed that, in the wet season, the application of 500 kg fresh weight of *Azolla pinnata*/ha, one week after planting resulted in higher yield than applying the same amount of *Azolla* 0, 3 or 4 weeks after planting. Moreover, the yield obtained from applying the same amount of *Azolla* 3 weeks after planting in the wet season was comparable to that obtained by applying the same amount of *Azolla* 4 weeks after planting in the dry season. This indicated that earlier application of *Azolla* was better in the wet season while the *Azolla* applied too early or too late in the season had the same effect of not increasing yield.

Sisworo *et al.* (1990) also reported that, when applied at transplanting and at the maximum tillering stages of rice growth, both *Azolla* and inorganic fertiliser at an equivalent rate of 30 kg N/ha resulted in the same rice yield.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the experimental site

The experimental field was located at the Kilimanjaro Agricultural Training Centre (KATC) trial farm in the Lower Moshi Irrigation Project (LMIP), 17 km south-east of Moshi town. The weather of the site for the previous year and at the time of the experiment, as recorded at the KATC weather station, is presented in Table 1.

3.2 Characterisation of the soils of the experimental site

A composite soil sample was taken at 15 cm depth (plough layer) in the experimental field and analysed in the laboratory for texture using hydrometer method (Gee and Bauder, 1986), pH (in water and in 0.01 M Kcl according to the method described by Mclean (1982), organic carbon (wet oxidation method) as described by Nelson and Sommers (1982), total nitrogen (micro-Kjeldahl method as described by Okalebo *et al.* (1993), DTPA-available zinc as described by Lindsay and Norvel (1978), extractable phosphorous (ascorbic acid method as described by Okalebo *et al.*(1993), cation exchange capacity (C.E.C) by the ammonium acetate method and exchangeable bases (Ca, Mg, K, Na) by atomic absorption spectrophotometry as described by Thomas (1982). The results of the above analyses are presented in Table 2.

3.3 Experimental design and layout

The following treatments were tested, each on two rice varieties namely IR54 (improved, medium-maturing) and *Wahiwahi* (indigenous, early-maturing).

1. Control (no *Azolla*, no inorganic fertiliser N)
2. *Azolla* alone (no inorganic fertiliser N
3. *Azolla* + 40 kg N/ha
4. *Azolla* + 80 kg N/ha
5. 160 kg N/ha (without *Azolla*)

Urea (45% N was used as the inorganic fertiliser referred to in the above treatments.

Table 1. Some meteorological data recorded at the KATC weather station (daily averages) from 1st January 1997 to 31st March, 1998

Month	Relative humidity (%)	Rainfall (mm)	Air temperature (°C)*	Evaporation (mm)
January	63	0.1	22.4	8.0
February	66	0.0	22.4	8.4
March	68	1.2	24.4	7.5
April	84	10.7	25.1	4.6
May	85	5.7	22.5	1.7
June	84	0.8	21.8	2.9
July	83	0.5	21.4	4.4
August	77	4.3	21.8	nr$^{\Psi}$
September	79	0.0	24.2	5.5
October	92	1.9	24.1	4.5
November	90	2.5	24.8	4.2
December	92	3.3	25.3	4.4
January	92	8.2	25.5	4.0
February	92	2.8	25.5	4.0
March	92	1.3	26.7	5.5

Source: Meteorological data sheet, KATC station

* Average of mean daily maximum and minimum, compiled by the author

$^{\Psi}$nr = not recorded

The experiment was a 2 x 5 factorial, arranged in a split-plot design in which the two rice varieties formed the main plot treatments and the five N sources were the sub-plot treatments. Each of the above treatments was replicated 3 times giving a total number of 30 plots, each with an area of 18 m^2 (3 m x 6 m).

The rate of inorganic N used in Treatment 5 was almost equal to the rate (150 kg N/ha) currently recommended at the LMIP (Horibata, 1992).

3.4 Nursery establishment

The nursery plot was mechanically puddled, to make a smooth and wet seedbed, a day before sowing the pre-sprouted rice seeds of each of the two varieties. Nitrogenous fertiliser (urea), at the rate of 57.5 kg N/ha, was applied to the nursery, 14 days after sowing (DAS). This fertiliser rate in the nursery is that

Table 2. Some physico-chemical properties of the soil at the experimental site

Characteristic and Unit	Value
pH (H_2O): 1:2.5 w/v)	7.2
pH (KCl): 1:2.5 w/v)	6.2
Organic carbon (%)	0.66
Total nitrogen (%)	0.07
Extractable phosphorus (mgkg^{-1})	28.05
Exchangeable bases:	
Ca (cmol$_c$kg^{-1})	6.8
Mg (cmol$_c$kg^{-1})	5.9
K (cmol$_c$kg^{-1})	0.16
Na (cmol$_c$kg^{-1})	0.45
Available zinc (mgkg^{-1})	0.93
C.E.C. (cmol$_c$kg^{-1})	13.1
Particle size analysis:	
Clay (%)	43
Silt (%)	33
Sand (%)	24
Textural class : Clay	

recommended for the LMIP (Horibata, 1992). The nursery was well-maintained in terms of proper water control and protection against insect and disease pests for the entire period of 25 days. This was done to ensure strong, healthy and uniform rice seedlings.

3.5 Preparation and management of the experimental field

3.5.1 Land preparation

Puddling and demarcation of the experimental plots was done a day before transplanting. Each plot was bound by an impervious polythene sheet (Plate 1) inserted to a depth of 15 cm in the soil. Raised bunds (Plate 2) were constructed to prevent undesired lateral movement and/or spill-over of water, fertiliser and *Azolla* from one plot to another. In addition, some openings were made through the raised bunds, at 10 cm-height from the plot surface, and shielded by wire mesh. The openings served as safety valves against overtopping of the bunds because they provided spillways for the excessive rain water. The wire mesh was intended to protect *Azolla* from being washed out under conditions of excessive rainwater. Relatively deep ditches were also provided between plots,

Plate 1. Impervious plastic sheets (blue coloured) being inserted around each plot

Plate 2. The stage (15 days after inoculation) at which the *Azolla* mat had completely covered the available space in the plot where the author is standing.

(a)

(b)

Plates 3 (a), (b). A mat of Azolla being incorporated into the soil by hand-pushing and foot-trampling and were always filled with water to saturate the outer sides of the plot bunds hence minimising lateral percolation of water (and fertiliser) from one plot to the other. Whenever the depth of water above the soil surface decreased below 2-3 cm, water was replenished in each plot directly from the irrigation channel. All these measures were taken to minimize chances of treatment mix-up that could occur were water movement allowed across the plots.

Basal phosphatic and initial nitrogenous fertilisers were incorporated in the soil just before puddling was completed. Phosphatic fertiliser (TSP), at the rate of 20 kg P/ha, was incorporated in all plots to supply the 17 kg P/ha recommended for optimum rice production in the LMIP (Horibata, 1992) and the 3 kg P/ha necessary to sustain a better growth of *Azolla* (Pande, 1978). One-half of each of the prescribed inorganic nitrogenous fertiliser rate was incorporated in those plots which were intended to receive supplementary fertiliser N (see section 3.3 above).

After the plots were prepared, water was allowed into the plots. About 3 cm-deep layer of water was allowed to stand in the plots for convenient transplanting of the rice seedlings. This shallow depth of water was maintained for a few days after transplanting to allow for sufficient water, heat and air exchange which are required for proper establishment of the rice seedlings and *Azolla* (van Hove, 1989; Horibata, 1992; Tsuboi, 1992).

3.5.2 Seedling establishment

Twenty five (25) day-old seedlings were transplanted in the experimental plots at a spacing of 20 cm x 20 cm (commonly-used in the LMIP) and at a depth of about 3 cm in the soil, recommended for proper rooting and optimum tillering of rice plants (Tsuboi, 1992). Three seedlings were planted per hill to minimise chances of establishment failure in each hill. Gap-filling of the unavoidable missing hills was done within 10 days after transplanting.

3.5.3 Planting and maintenance of *Azolla*

A strain of *Azolla,* whose species is tentatively considered to be *Azolla caroliniana* (Watanabe, I., personal communication, 1996), and one which is abundant in the LMIP, was broadcast in the respective plots, at the rate of 5.4 kg fresh weight (FW)/plot i.e., 300g FW/m^{2}, just after rice transplanting. The *Azolla* was allowed to grow and multiply in the plots until it formed a mat which completely covered all the available space between the rice plants, in a single layer.

About 3 cm of standing water was maintained in each plot for five days (including the day of transplanting) and thereafter, the depth of water was gradually increased to, and maintained at about 10 cm in the entire duration of the crop. However, two weeks before harvesting, the plots were drained to allow for proper maturity and harvesting of the rice. A metre-rule was used to measure the required depth of water in the plots. Additional irrigation was done when the need arose.

3.6 Determination of *Azolla* growth and analysis of its chemical composition

The quantity and quality of *Azolla* was determined before its incorporation into the soil. This was done after the *Azolla* mat was mature i.e., after the mat had completely covered the available space in the plots (Plate 2). On the average, it took 15 days for the inoculated *Azolla* to mature.

The *Azolla* biomass (in terms of fresh weight) was determined in the field at the appropriate time as stated above. The chemical composition was determined by laboratory analysis of a representative sample drawn from the lot that was left in the field for incorporation into the soil. The sampling procedures are as detailed below.

Two representative samples were collected from two areas within each plot. The sampling area (0.25 m^2) was demarcated by a square frame (50 cm x 50 cm), the ends of which were vertically pushed into the soil to a shallow depth (few centimetres). Using a perforated plastic bowl the entire mat of *Azolla* in the sampling area was quantitatively recovered. Each *Azolla* sample was thoroughly rinsed in water to remove any adhering soil and other contaminants. The cleaned sample was allowed to drain thoroughly. Then the sample was weighed on a sensitive balance (accurate to 10 g) to determine its fresh weight. Two such determinations were made and averaged for each replicate plot in the relevant treatments. The total *Azolla* biomass in the entire plot was calculated while the amount per hectare was obtained by extrapolation. The two samples obtained as explained above were then mixed to get a composite lot. One-half of the composite sample was returned to the plots for subsequent incorporation into the soil while the other was taken to the laboratory for its N, P and organic carbon (OC) content determination, following the procedures described in section 3.2. Results of the *Azolla* chemical analysis are presented in Table 3.

3.7 Incorporation of *Azolla* into the soil

Having determined the amount of *Azolla* in each plot, the level of water in the plots was allowed to subside to let the mat of *Azolla* touch the soil surface in order to facilitate its incorporation. Then the mat, together with hand-pulled weeds, were incorporated into the soil by hand-pushing and foot-trampling (Plates 3a and 3b). To determine the quantity of *Azolla* that was incorporated, the plots were re-flooded a day after each incorporation to allow the unincorporated *Azolla* to float. Then, the amount of unincorporated *Azolla* was determined using the procedures explained in section 3.6. The amount of *Azolla* incorporated was then calculated as the difference between the amount before incorporation and that which was floating, less that which was taken for N, P and OC determinations. With these methods of incorporation, 60 to 90% (mean of 80%) of the *Azolla* that was intended for incorporation could be incorporated. Two incorporations were achieved before the rice plant canopy reached a state where further incorporations without causing substantial physical damage to the rice plants was impossible. The second incorporation was done 30 days after transplanting (DAT).

The disturbance (mechanical pressure) caused by trampling in the plots with *Azolla* was also applied to the other plots by weeding the *Azolla*-free plots at the same time as those with *Azolla*. Weeds were also pushed or trampled into the soil in the same way the *Azolla* mat was treated.

3.8 Determination of *Azolla* decomposition rate

The major interest in performing this subsidiary experiment was to determine the decomposition pattern of *Azolla* in that particular field, since its rate of decomposition is likely to be related to the nutrient released from it. The best green manure is the one which would release the nutrients at the rate which would enable their proper and timely utilisation by the rice plant with minimum losses.

Table 3. Biomass accumulation and chemical composition of the *Azolla* ecotype used as a green manure

Characteristics and units		Value
Fresh weight	(t/ha)	19.63
Dry weight	(t/ha)	1.01
Total nitrogen	(%)	2.57
Total phosphorous	(%)	0.27
Organic carbon	(%)	37.80
C:N	(ratio)	15:1

The procedure, in principle, was that based on the litter-bag technique as described by Anderson and Ingram (1993). Some modifications were made to suit the environment and the equipment that were available. The *Azolla* material (500g FW) was loosely packed in onion meshed (1 mm)-bags, in such a way as to mimic the thickness of the *Azolla* single layer (about 1 cm) in the field before incorporation. The bags were used to facilitate easy retrieval of the undecomposed material from the soil at each sampling.

The bags were buried at about 15 cm depth in the soil and left to incubate *in situ*. The same level of water was maintained at the site of incubation as it was in the main experimental plots. Sampling was done on the 1st, 2nd and 3rd weeks after incorporation. Three bags were retrieved at each sampling time. The retrieved bags and their contents were thoroughly washed under the tap and over a series of meshes with varying sizes, arranged from the largest (4 mm) down to the smallest (0.6 mm). All the material that passed through the 0.6 mm-mesh was considered to have been decomposed. This smallest mesh size had conveniently been used by Wangari (1995).

At each sampling time, the remaining manure material was oven-dried at 60^0C to constant weight. It was then ground to a fine dust using the laboratory **CYCLOTEC 1093** sample mill. The following parameters were then determined on it:

(i) Residual (i.e., undecomposed) dry matter
(ii) Residual organic carbon
(iii) Residual phosphorus
(iv) Residual total nitrogen

The quantities of DM, N, P and OC that were released on decomposition, at any sampling time, were implied from their residual amounts.

The above parameters were monitored because in a previous study by Nikokwe (1992), they were found to be fairly consistent in describing the decomposition pattern of

Leucaena and *Clotalaria* green manures. It was desired to see if this consistency also applies for *Azolla* geen manure.

The methods used for the chemical analysis for the residual N, P and OC were the same as those used in characterising the *Azolla* (see Section 3.2). The residual DM was obtained by weighing the undecomposed *Azolla* material on a sensitive **METTLER P162** balance (accurate to 0.01 g). The results of residual DM, N, P and OC are indicated in Figures 1, 2, 3 and 4, respectively.

3.9 Measurement/determination of the response of the rice crop to the two sources of N, in their varying combinations

Response of the rice crop to each treatment was determined by observing the general growth performance. The parameters recorded were: leaf chlorophyll content, plant height, number of tillers per unit area, date of heading, and date of earliest possible harvesting (maturity), panicle excertion, number of panicles, number of grains on a panicle, percentage of ripened grains(fertility), weight of grains and ultimately, the grain yield per unit area.

Details of the determinations of these parameters, are presented as follows:

3.9.1 Measurement of leaf chlorophyll content

The amount of chlorophyll present in leaves can serve as an indicator of the overall condition of the plant, particularly the N content. Plants become greener (i.e., have more chlorophyll) as their nitrogen content increases, and *vice versa*. Greener leaves will show higher chlorophyll meter reading (SPAD values) than chlorotic leaves.

The procedure first involved visual assessment of the overall colour of the rice plants in all replicate treatments (i.e., all plots), then the degree/intensity of the greenish colour (chlorophyll content) was determined using a **Minolta chlorophyll meter (SPAD-502)** that indicates the relative amount of chlorophyll present in plant leaves as SPAD values. For simplicity, the chlorophyll content of 10 newly-expanded leaves per plot (one leaf per hill) was determined at the booting growth stage. The booting stage is indicated by the bulging of the flag-leaf sheath as a result of the expansion of the rice panicle which, at this stage, is still enclosed in the sheath. The SPAD values of the 10 leaves was taken as the average plot value. These values are presented in Table 4.

3.9.2 Plant height and number of tillers

The sample size for the above determinations was eight(8) hills per plot, excluding border rows (two rows adjacent to plot bunds, on all sides of the plot) which showed border effect (e.g. unusually better growth). The random plot sampling technique was used as recommended by Gomez and Gomez (1984).

Plant height was measured at maturity (the rice growth stage explained in section 3.9.3). For plant height, eight single-hill sampling units were used. In this case, the height of the tallest plant in each of the eight selected hills was recorded (Tsuboi, 1992). The number of tillers per hill was counted at the stage of panicle initiation for both varieties (Tsuboi, 1992). Panicle initiation stage is a rice growth stage whereby the panicle starts to form at the apex of the topmost node of the rice stem. The stage marks the beginning of the reproductive stage of the rice plant.

Tillers were counted at two points (sampling units) per plot. Each unit comprised four adjacent hills that formed a unit square (each hill being located at the corner of the square). The results of plant height and number of tillers are presented in Tables 5 and 6, respectively.

3.9.3 Heading and earliest possible harvesting (maturity) time

Tsuboi (1992) defined the heading and earliest possible harvesting (maturity) dates as the dates at which 50-60% of the total panicles are headed and at which 80% of the grains on a panicle turn yellow, respectively. Although heading and maturity dates are characteristic of a variety, they can serve as indicators of the nitrogen status of the plants because, at the same level of P, plants deficient in nitrogen normally tend to head and mature a bit earlier than those with enough or excessive nitrogen (Tsuboi, 1992). Rice plants supplied with excessive N grow luxuriously. If much of the N is supplied in the early stage of plant growth, the onset of the reproductive phase is delayed. On the other hand, plant maturity may be prolonged if much of the N is supplied late after the reproductive stage has started (Tsuboi, 1992).

The procedure to determine heading and maturity involved selecting 10 hills per plot for which the panicle numbers were recorded everyday, starting on the day the first plant headed until normal heading was complete, then the date corresponding to the time when 50% of all plants in the ten hills had headed, was taken as the heading date. For the maturity date, the same 10 hills as above, were used but only the oldest panicle in each hill was observed daily for maturity (yellowing) of its grains, which normally starts from the tip of the panicle and progresses towards the base. The panicle was conveniently divided into five equal parts, each representing 20% of the panicle. The maturity date was recorded when four parts of each panicle (80% of the grains on each of the panicles) had yellowed. The results of heading and earliest possible harvesting time are respectively presented in Tables 7 and 8.

3.9.4 Panicle excertion

Panicle excertion eventually exposes the spikelets to adequate fertilisation, short of which they will be either completely infertile or the kernels will not develop to full size, the overall effect of which is a severe reduction in the final yield.

Sheath rot caused by a fungus *Acrocylindrium oryzae* occurred at the booting stage of plant growth and interfered with the normal heading of many plants in the experiment, causing poor panicle excertion and many unripe grains (poor panicle fertility).

To determine whether the effect of the disease was treatment-related, 10 hills per plot were used. Conventional rating of the disease incidence and severity was, however, not done because the appropriate disease rating scales relevant for the sheath rot disease were not at the author's disposal at the time. Therefore, the effect of the disease was arbitrarily estimated by rice panicle excertion. The decision to use the number of fully excerted panicles to estimate the effect of the disease was arrived at after observing no disease symptoms on fully-excerted panicles. Moreover, the size of the panicle excerted was inversely related to the seriousness of the disease symptoms on the sheath that was enclosing the particular panicle. It was also reported by Horibata (1992) that sheath rot disease causes poor panicle excertion. The number of fully excerted panicles, expressed as a percentage of the total number of panicles in the 10 hills was used as the indicator of the extent of the effect of the disease in each treatment. The results of this parameter are presented in Table 9.

3.9.5 Measurement of yield determinants and the grain yield

At maturity, water was drained out of the plots to facilitate final sampling and harvesting, which were done seven days after the plot drainage.

3.9.5.1 Yield determinants

The yield determinants as defined by Matsushima (1967) are: number of panicles, number of grains, percentage of ripe grains (panicle fertility) and grain weight. At harvest, the yield determinants as defined above were recorded, using a sample of 10 hills per plot. The number of panicles per square metre (Pm^{-2}) was determined as follows. First, the number of panicles in the ten hills (P_{10}) was counted and divided by 10 to get the average number for a single hill. The average for a single hill was multiplied by the number of hills per square metre (Hm^{-2}), obtained by dividing the area by the plant spacing. Thus,

$$Pm^{-2} = P_{10}/10 \times Hm^{-2} \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The results of this parameter are presented in Table 10. For the number of grains and grain weight, fertile grains were first separated from infertile ones. The following procedure was followed. All grains (both fertile and infertile) on the plants from the 10 hills were threshed and then put in water. Fertile grains sunk while infertile ones floated. After this separation, all sinkers (S) and floaters (F) were sun-dried.

To get the total number of grains per panicle (S + F), floaters were first counted, using a counting machine (**Everwell Corporation's MULTI-AUTO COUNTER, Model OC IUR 80701**). As ripen grains (sinkers) were comparatively many in all treatments, counting them directly by a counting machine would have been time-consuming so their number was indirectly obtained first by weighing them on a sensitive balance [**Everwell Corporation's KAMOSHITA**] (accurate to 0.05 g) to get their total weight (Ws_t). Then, the weight was multiplied by 1000 and the product divided by the weight of 1000 grains (W_{1000}) to obtain the number of ripen grains (sinkers) in all panicles (S_t). Thus,

$$S_t = Ws_t/W_{1000} \times 1000 \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The total number of grains per panicle (G_p) was then calculated as the sum of floaters and sinkers divided by the total number of panicles in the 10 hills (P_{10}), thus:

$$G_p = (S_t + F)/P_{10} \ldots\ldots\ldots\ldots\ldots\ldots(3)$$

The results of this parameter are presented in Table 11.

The panicle fertility (Pf), i.e., the percentage of ripe grains was calculated by dividing the number of ripe grains per panicle ($S_t/10$) by the total number of grains per panicle (Gp) and then multiply the quotient by 100. Thus,

$$Pf = S_t/10G_p \times 100 \ldots\ldots\ldots\ldots\ldots\ldots(4)$$

The results of this parameter are presented in Table 12.

To get the weight of 1000 grains, two batches, each of 1000 fertile grains, were taken and weighed to get their weights (Ws_1 and Ws_2). The average of their weights was taken as the 1000-grain weight (W_{1000}), thus:

$$W_{1000} = (Ws_1 + Ws_2)/2 \ldots\ldots\ldots\ldots\ldots\ldots(5)$$

The results of this parameter are presented in Table 13.

3.9.5.2 Grain yield

The grain yield was determined by the *reaping test* at maturity (Tsuboi, 1992). The sampling area per plot was 6 m^2 in accordance with the minimum (5 m^2) recommended sampling area for rice yield determination (Mughogho, personal communication, 1997). The sample plants from each plot were cut (reaped), collected in respective onion bags, threshed and winnowed to obtain the grains which were then weighed. Using a grain moisture tester, (**RICETER J301**), the grain's moisture content on the fresh weight basis(MC_{fw}) at harvest was determined. The final grain yield was then expressed in t/ha at 14% Mc_{fw}, thus:

$$GWt_y = Gwt_h \times [100 - x/(100 - 14)] \ldots\ldots (6)$$

where: Gwt_y = Grain yield at 14% MC

Gwt_h = Grain weight at harvest

x = Grain moisture content (%) at harvest

The results of this parameter are presented in Table 14.

3.10 Statistical analysis

Statistical analysis of all the data was done using the MSTAT programme. Analysis of variance as outlined by Gomez and Gomez (1984) was used to assess treatment effects on rice yields and its determinants. Means of the significant treatments were separated by the Duncan's New Multiple Range Test (DNMRT) or the *t*-test as described by Gomez and Gomez (1984).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 *Azolla* nitrogen accumulation

The amount of nitrogen accumulated by the fully-grown *Azolla* mat was calculated as the product of the *Azolla* dry weight and the percent total nitrogen (Table 3). On the average, one crop of *Azolla* accumulated 26 kg N/ha. That means that the two crops of *Azolla* accumulated 52 kg N/ha before the canopy of the rice plants prevented further quantification of biomass and N accumulation. This nitrogen was a sum total of the fixed N_2 from the air and that which was accumulated from the soil and water. However, in the absence of N tracers such as the ^{15}N isotope, it was not possible to determine the proportion of the total N that was fixed from the air. As *Azolla* was allowed to remain in the plots throughout the growing period of the rice crop, the total amount of biomass and N accumulated and released by the *Azolla* for the whole growing period of the rice crop could have been more than the above estimate (of 52 kg/ha). Nevertheless, the amount of biomass and N accumulated by the *Azolla* after full development of rice crop canopy was expected to be small due to the negative effect of the dense rice canopy on the overall growth and N_2 fixation by *Azolla*. Even when complete mineralisation of the Azolla-N is assumed to have taken place, it can safely be speculated that the amount of N from *Azolla* that was available to the rice plants was still insufficient to support a reasonable yield of the crop. This speculation would substantiate the observations by earlier researchers that *Azolla* alone may not supply N in quantities enough for optimum rice yields. The need for supplementary inorganic (fertiliser) N is then clearly justified, as has been the case in the present study.

It is clear that the amount of N accumulated by the *Azolla* in an intercropped situation as was the case in this study does not represent the full potential of the fern to accumulate N. It is expected that more N could have been accumulated had the *Azolla* been grown as a monocrop because, in such a situation, the *Azolla* would have been free of the effect of excessive shading (therefore, low light intensities) by the canopies of the rice crop. Excessively low light intensities have previously been reported by many researchers (Ashton, 1974; Lumpkin, 1987a and van Hove, 1989, among others) to inhibit both growth of, and N_2 fixation by *Azolla*. This phenomenon was observed in a separate (ancillary) experiment to study the effect of rice canopy on *Azolla* growth and N accumulation. In this study, the *Azolla* (not supplemented with inorganic fertiliser) accumulated a maximum of 38 kg N/ha under the canopy of the indigenous variety as opposed to a maximum of 25 kg N/ha accumulated by the *Azolla* grown under the canopy of the improved variety whose growth was more profuse and hence caused higher shading.

4.2 Decomposition rate of *Azolla* and estimates of *Azolla*-N available to the associated rice crop

The decomposition rate of *Azolla* is an important factor determining its effectiveness as a fertiliser on rice growth because it is only after *Azolla* has decomposed that most of its accumulated nutrients are released into the growth medium for uptake by the current or subsequent crop (Tung and Shen, 1985). The efficient use of *Azolla* requires knowledge of its pattern of decomposition in the field environment, a view that is held by previous workers (Wen *et al.*, 1987).

The decomposition pattern of *Azolla*, as determined by the residual: dry matter (RDM), organic nitrogen (RON), organic phosphorus (ROP) and organic carbon (ROC) is presented in Figure 1. The decomposition of *Azolla* occurred most rapidly in the first week and then slowed down in subsequent weeks. It was observed that 85, 49, 20 and 28% of *Azolla* DM, N, P and OC, respectively, had already been mineralised by the end of the first week of incubation. At the end of the third week, the respective values were 90, 72, 45 and 32%. It is evident from Fig. 1 that the indices (parameters) used in evaluating the decomposition of *Azolla* in the present study, were not entirely consistent. Results based on residual dry matter would indicate a very fast decomposition of *Azolla*. However the same picture (of rapid decomposition of *Azolla*) is only slightly depicted when the residual total N is used as the parameter of evaluating decomposition.

The impression of rapid decomposition of *Azolla* is very poorly reflected when residual organic P or C are used as indices of decomposition (Figure 1). It is evident from these results that residual organic P and C cannot be used as reliable parameters in monitoring the

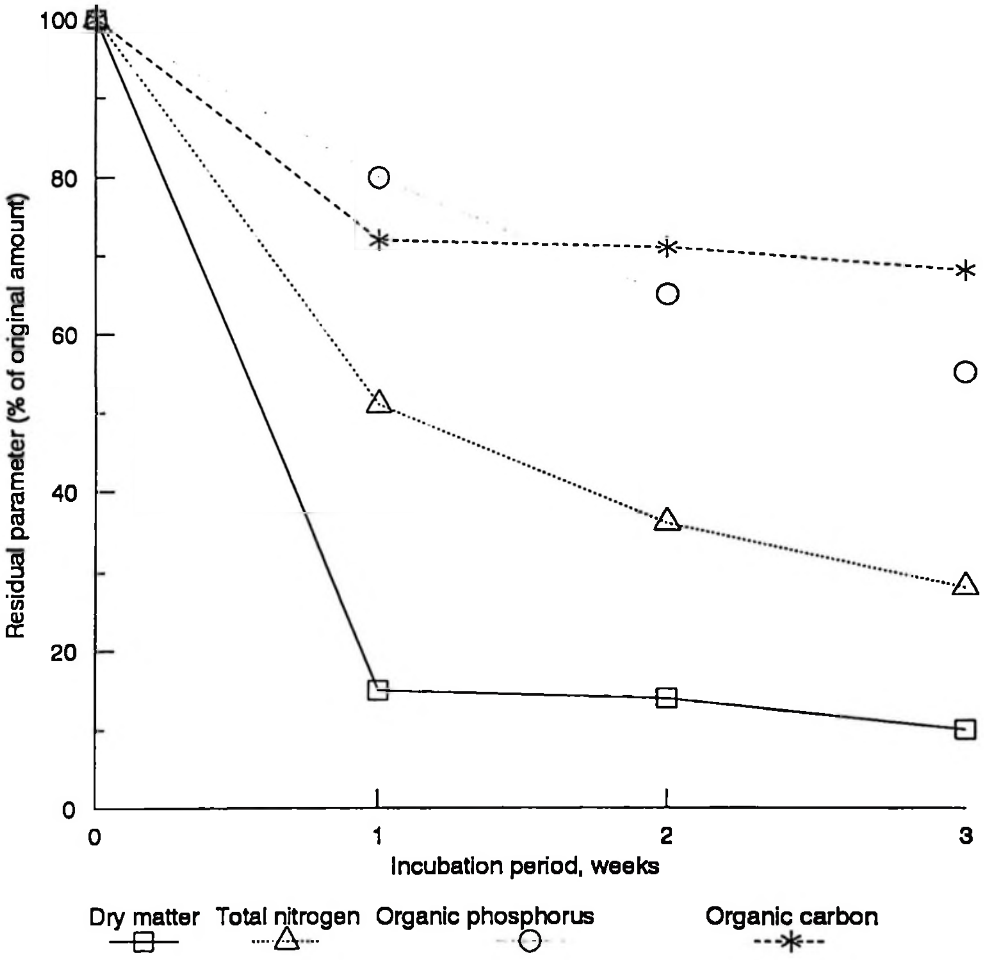

Figure 1. Changes in dry matter, total nitrogen, organic phosphorus and organic carbon of Azolla green manure with time

decomposition of *Azolla*. It is however, interesting to note that in a previous study, Nikokwe (1992) found a very consistent relationship between (residual) DM, total nitrogen, organic phosphorus and organic carbon in describing the decomposition pattern of *Leucaena* and *Crotalaria*.

It must be noted that all the decomposition indices were calculated based on the dry matter. It would therefore, be expected that the reduction in DM of *Azolla* due to decomposition would have resulted in concomitant reduction in the other indices at any one sampling time.

That this was not the case, could be due to some degree of adsorption of some tiny particles of organic N, P and C on the remaining (retrievable) fraction of *Azolla*. These fractions, which would otherwise be regarded as having been completely mineralised according to the procedure used, would be added on to the residual DM and appear erroneously in the analysis as high levels of residual total N, organic P and C. It is important to note that lack of consistence between RDM, RTN, ROP and ROC was also reported by Wangari (1995) in a study to determine the decomposition pattern of the wild sage (*Lantana camara*).

Based on the above argument, RDM was considered to be the most reliable criterion of judging the decomposition pattern of *Azolla*. The decomposition trend, as depicted by the RDM, would indicate that the decomposition of *Azolla* was very fast and hence a large part of its N and P content was (by implication) mineralised in the first three weeks after its incorporation into the soil. This observation is consistent with earlier findings by Wang *et al.*(1987) who reported that the highest mineralisation of *Azolla* in the flooded environment was recorded in the third week after fresh *Azolla* was added to the soil.

The decomposition of a large proportion of *Azolla* in such a short period would imply that rice crop could make use of the early release of the Azolla-N. This was actually thought to have been the case in this study as plants were seen to have changed in colour (became greener) and grew vigorously only 2-3 weeks after *Azolla* incorporation compared to those which were grown without *Azolla* (see Section 4.3.1).

Although the actual amount of the Azolla-N that the rice crop recovered was not determined, You *et al.* (1987) reported that the rice crop is able to recover only 40-

60% of the Azolla-N. This would imply that the amount of Azolla-N that the rice plants recovered was even smaller than the estimated 52 kg/ha as indicated above. This necessitates additional supply of N from other sources e.g. soil inorganic N reserves and/or fertiliser N. This observation further underscores the rationale of supplementing *Azolla* with inorganic fertilizer N.

4.3 Response of the rice crop to the sources of nitrogen in their varying proportions

4.3.1 Visual appearance and chlorophyll content of rice plants in response to the different N sources

4.3.1.1 Visual appearance of the rice plants

Visually, both of the rice varieties were seen to have responded similarly to the different nitrogen sources (in their varying proportions). Plants that were treated with *Azolla* (alone or with supplementary fertiliser N) and those which received the highest inorganic fertiliser N without *Azolla*, grew more vigorously than those in the control plots (which received neither *Azolla* nor urea). The treated plants also appeared greener than those untreated as can be exemplified by Plates 4 and 5. By the of the second week after the incorporation into soil of the first crop of *Azolla*, the change of colour in the Azolla-treated rice plants was already clearly evident.

As the intensity of green colour is a function of the chlorophyll content, plants grown with *Azolla* (alone or with supplementary fertiliser N) and those grown with the highest level of inorganic fertilizer N (without *Azolla*) could have synthesized more chlorophyll than the untreated ones. This was found to be the case as is explained in the following section in which a quantitative evaluation of chlorophyll content is examined in relation to the experimental treatments.

Plate 4. Effect of unsupplemented *Azolla* on the appearance of the indigenous rice variety at the panicle initiation (PI) stage

Plate 5. Effect of unsupplemented *Azolla* on the appearance of the improved rice variety at the panicle initiation (PI) stage

Plate 4. Effect of unsupplemented *Azolla* on the appearance of the indigenous rice variety at the panicle initiation (PI) stage

Plate 5. Effect of unsupplemented *Azolla* on the appearance of the improved rice variety at the panicle initiation (PI) stage

4.3.1.2 Chlorophyll content and the vigour of the rice plants

Regarding the chlorophyll content (assessed quantitatively), only the effect of the N sources was statistically significant (Table 4), meaning that the N sources affected the rice plant chlorophyll content independently of variety. Accordingly, only the overall effect of the N sources needed, and was accorded, more emphatic examination/discussion as follows:

As seen from Table 4, *Azolla* (alone or with supplementary fertiliser N) and the highest level of inorganic fertilizer N (without *Azolla*) increased the chlorophyll content in rice plants compared to the control treatment (where nothing was added). The higher chlorophyll content was indicated by the significantly ($P = 0.05$) higher SPAD values in the *Azolla*-treated plants and those grown with the highest level of inorganic fertilizer N (without *Azolla*) than those in untreated plants (Table 4).

This was expected because both *Azolla* and the inorganic fertiliser were sources of N therefore, their application in the field was expected to increase plant chlorophyll content over that of the plants in the control (where the N was comparatively low).

The chlorophyll content of the plants grown with supplemented *Azolla* tended to increase with an increase in supplementary fertiliser N. As seen in Table 4, the plants which received *Azolla* with supplementary fertiliser N had in fact, a significantly (P = 0.05) higher chlorophyll content than those that received the highest rate of urea-N while plants which received *Azolla* alone, had the same chlorophyll content as those that were supplied with the highest rate of fertiliser N only. It would appear that these results cannot be explained only by the mere amount of N supplied by *Azolla* to the plants. It can be speculated that the rice plants utilized Azolla-N more efficiently than that from urea. Such a proposition has also been substantiated by earlier workers (Kulasooriya *et al.*, 1987). Such a phenomenon of inefficient utilisation of urea-N by the rice plants could have occurred due to the fact that a substantial amount of the urea-N, in the absence of *Azolla*, may have been lost (albeit its being split-applied) through leaching, denitrification and ammonia volatilisation,

Table 4. Effect of nitrogen sources on leaf-chlorophyll of two rice varieties* (an average of three replications)

Nitrogen source	Leaf chlorophyll content (SPAD values)		
	V1	V2	N source means
1. No *Azolla*, no inorganic fertiliser N	23.5	27.4	25.4 d
2. *Azolla* alone	28.4	30.6	29.5 bc
3. *Azolla* + 40 kg N/ha	29.9	30.5	30.2 ab
4. *Azolla* + 80 kg N/ha	32.3	30.6	31.5 a
5. 160 kg N/ha without *Azolla*	27.0	28.8	28.0 c
Variety means	28.2	29.6	

$LSD_{0.05}$ to compare nitrogen source means	1.8
CV (%) for variety means	12.84
CV (%) for N source means	5.05

*V1,V2 refer to *Wahiwahi* (indigenous) and IR54 (improved) rice varieties, respectively.

In a column, means followed by a common letter are not significantly (P = 0.05) different according to the Duncan's New Multiple Range Test.

although the N lost due to these processes was not quantified. In the flooded condition that was maintained in the experimental rice plots, a substantial amount of the easily soluble urea might have been leached beyond the root zone of the rice plants, making it inaccessible to the rice plants. Some denitrification could have occurred after urea hydrolysis and oxidation of its resultant ammonia to nitrates (NO_3^-), which could have dissolved in the percolating water and moved down to the soil reduced zone where it subsequently got denitrified (Sisworo *et al.*, 1990). Although the soil pH (pH 7.2) as determined before flooding the field is that which is not likely to cause substantial ammonia volatilisation losses from urea (Watanabe and Liu, 1992), the pH of the solution above the soil surface where urea was applied might have been raised, especially during the daytime, by the photosynthetic activities of some species of aquatic plants (algae and others) that were seen growing in the rice fields, although they were not quantified. According to Villegas and San Valentin (1989), the aquatic plants utilise the carbon dioxide (CO_2) gas dissolved in water, in the presence of sunlight, to manufacture their own food (the process is called photosynthesis). As the dissolved CO_2 would form weak Carbonic acid which tends to lower the water pH, its removal by the aquatic plants would tend to increase the water pH, a condition that favours ammonia volatilisation. The photosynthetic activity of aquatic plants has been reported by Watanabe and Liu (1992) to raise the pH of water in rice fields, resulting in accelerated volatilisation losses of ammonia. Ammonia volatilisation was reported by Villegas and San Valentin (1989) to be substantial in aquatic environment (although this contention of substantial ammonia volatilisation in aquatic environment needs re-examination given the fact that the solubility of ammonia in water is substantially high). High urea-N losses due to the obove-mentioned processes at the site of the study are however questionable given the fact that higher yields of rice have been achieved by farmers who apply the current recommended rate of 150 kg of inorganic fertiliser N, as urea.

On the other hand, the proposed higher efficiency of utilisation of Azolla-N by the rice crop could not be the only reason for the better performance of the rice plants which were grown with *Azolla* since the amount of N that was accumulated by, and released from one crop of ***Azolla*** in the present study was estimated to be less than 26 kg N/ha (see Section 4.2). The alternative explanations (which will also apply in cases where ***Azolla*** improved other parameters to be explained later in the following sections) may be that besides the possibility that the rice plants utilized the Azolla-N more efficiently than urea-N, the *Azolla* may also have accumulated large amounts of other mineral elements from the soil, some of which are chlorophyll-enhancers (e.g. iron). The *Azolla* might have released such minerals later, for eventual consumption by the associated rice plants. *Azolla* is known to be efficient in accumulating (scavenging for) various mineral elements from the environment (Watanabe, personal communication, 1996). Furthermore, the *Azolla* may have temporarily retained the N of the supplementary fertiliser (by either absorbing it from the environment when *Azolla* was growing or by adsorbing it after *Azolla* had decayed and become part of the soil organic matter). Such retained-N, may have been protected from loss through leaching. The retained-N could later be taken up by the rice plants. It was reported by Watanabe (1996) that the *Azolla* mat could reduce the amount of N that could be lost through leaching by absorbing and temporarily storing a part of the applied inorganic N , and release it later when the rice roots have extended enough to be able to absorb it as fast as it is released. The *Azolla* mat could also have reduced the loss of applied N that could have occurred through ammonia volatilisation, by intercepting and preventing the sunlight from reaching the aquatic plants, thereby reducing photosynthetic activity of the aquatic plants and their consequence on ammonia volatilisation already explained above. In the study by Villegas and San Valentin (1989), it was observed that the *Azolla* cover could reduce ammonia volatilization by 20 - 50% of that without *Azolla*, in aquatic environments. The overall effect of the *Azolla* (of preventing losses of N) would be to increase the amount of nitrogen available to rice plants. This amount of N, in combination with the other accumulated minerals (particularly iron) as already speculated above, could have caused the rice plants to manufacture much more chlorophyll than the plants not treated with *Azolla*.

As the amount of chlorophyll ultimately determines the ability of plants to trap solar energy necessary for photosynthesis, it can be deduced from the chlorophyll content data that rice plants which received *Azolla* green manure in combination with the supplementary fertiliser N were generally more efficient in photosynthesizing, and, from the results of this study, the plants were even more efficient than those which received the highest level of inorganic fertiliser N, hence the reason for their more vigorous growth. For this contention of efficient photosynthesis to be relevant, it would be expected to be substantiated by other aspects of crop growth response and the ultimate grain yield. This was clearly reflected in many parameters (especially yield determinants and ultimate grain yield) as seen from Appendix Table 1 and as discussed in the relevant sections.

4.3.2 Plant height and tiller number

Plant height at crop maturity, and the number of tillers at maximum tillering stage, would indicate whether or not the optimum conditions prevailed during the growing period because, under optimal conditions such as of adequate nutrition, rice plants will exhibit a large number of generally tall and strong tillers which have the highest probability of bearing panicles. Consequently, high grain yields would be expected from such plants. The height and number of tillers exhibited by a plant are a characteristic of variety, but they can be improved or reduced by agronomic management.

The height and tiller number attained by the two rice varieties in response to the different N sources are presented in Tables 5 and 6. It is worth mentioning here that, in the case of plant height, the interaction of rice variety and N sources was detected to be significant ($P = 0.05$), meaning that the effect of the N sources was dependent on the variety in as far as rice plant height is concerned (Table 5). In the case of tiller number, the interaction effect was not significant but both varietal and N source effects were detected as statistically significant. This means that each of the two factors influenced tiller number independently of each other, warranting separate examination for each of these factors (Table 6).

4.3.2.1 Plant height

As evident from Table 5, plants of the local variety were generally taller than those of the improved variety, at each level of the nitrogen source. This difference was considered to be genetically controlled. When each variety is examined, *Azolla* (supplemented or not supplemented with fertiliser N) and the highest level of fertilizer N (without *Azolla*) significantly (P = 0.05) increased the height of the plants of the local variety relative to the untreated control (no *Azolla*-, no fertiliser-N). This was also true for the improved variety, as expected since *Azolla* and the inorganic fertiliser contain the N that is required for plant growth. *Azolla* and the inorganic fertiliser added more N to that already existing in the natural soil. The additional N caused the rice plants to grow more vigorously hence their greater height than that of the plants in the untreated plots. It can also be seen from

Table 5 that supplementing *Azolla* with inorganic fertiliser N had a tendency towards a progressive increase in the height of plants of the indigenous variety as opposed to those of the improved variety (in which the effect of supplementing *Azolla* with fertiliser N was not revealed). The difference in varietal response to supplementing *Azolla* with fertiliser N may also indicate their genetic difference whereby relatively higher levels of N are required for the plants of the improved variety to show a marked increase in height. Short stature is one of the desirable traits of most of the improved varieties which enable the plants to tolerate some levels of N which could cause lodging of most of the indigenous varieties. The trait (i.e., short stature) enable the plants to carry heavy panicles that

Table 5. Effect of nitrogen sources on plant height of two rice varieties* (an average of three replications)

Nitrogen source	Plant height at maturity (cm)		
	V1	V2	N source means
1. No *Azolla*, no inorganic fertiliser N	95.4 c	70.8 d	83.1
2. *Azolla* alone	111.4 b	82.4 c	96.9
3. *Azolla* + 40 kg N/ha	116.1 ab	82.6 c	99.3
4. *Azolla* + 80 kg N/ha	123.2 a	85.9 c	104.5
5. 160 kg N/ha without *Azolla*	122.9 a	84.7 c	103.8
Variety means	113.8	81.3	

$LSD_{0.05}$ to compare variety means at same N source treatment is 5.7

$LSD_{0.05}$ to compare N source means at same variety treatment is 10.7

CV (%) for variety means 2.44

CV (%) for N sources means 3.11

*V1,V2 refer to *Wahiwahi* (indigenous) and IR54 (improved) rice varieties, respectively.

In a row or column, means followed by a common letter are not significantly (P = 0.05) different according to the *t*-test and the Duncan's New Multiple Range Test, respectively.

result from such high levels of N without lodging, and that is one of the reasons why the improved variety are considered to be more efficient than the indigenous varieties in utilising N. Although supplementing *Azolla* with fertiliser N tended to progessively increase plant height in the indigenous variety, it is important to note that even with this (indigenous) variety, plants which received *Azolla* supplemented with only 40 kg N/ha gave plant height which did not differ significantly from the plant height attained with higher levels of supplementation (80 kg N/ha) or with the highest level of fertiliser (160 kg N/ha, without *Azolla*) [Table 5]. The results of supplementing *Azolla* with inorganic fertiliser N were consistent with those observed by Ramasamy *et al.* (1987) who reported that supplementing *Azolla* with 40 kg of inorganic N increased the plant height of a rice variety IR20 by 6% over that attained by *Azolla*-treated plants without supplementation with the inorganic N, at the same levels of P and K.

The above results, i.e, lack of significant difference in the effect of *Azolla* supplementation with 40 and 80 kg N/ha would seem to indicate that the supplementation of *Azolla* with fertiliser N may not have been necessary for the purpose of improving the growth performance of the rice crop. Apparently, the observed height-promoting effect of the combination of *Azolla* with inorganic fertiliser N was mainly contributed by *Azolla* and not by the fertiliser. In fact, this was clearly substantiated in the case of the improved rice variety whereby *Azolla* alone matched even the highest level of fertiliser in increasing the plant height (Table 5). Similar effects of *Azolla* alone in promoting plant growth performance, have been indicated in almost all the other parameters, namely, plant chlorophyll content, flowering/ heading time, maturation time, number of panicles, number of grains per panicle, grain weight and the ultimate grain yield (see Appendix I). On the overall, the results would indicate that there is substantial potential of *Azolla* as a green manure even when used as the sole source of nutrients in promoting rice production but, as is evident from Table 5, particularly with some growth parameters e.g. plant height, differential response to *Azolla* as a sole source of nutrients, must be expected between different rice varieties.

4.3.2.2 Tiller number

As seen from Table 6, plants of the improved variety had, on the overall, significantly ($P = 0.05$) higher tiller number than those of the indigenous variety, a property which is genetically-controlled, as was the case with the plant height.

When the overall effect of the N sources was examined, it can be seen from Table 6 that *Azolla* (supplemented or not supplemented with inorganic fertiliser N) and the highest level of fertiliser N (without *Azolla*), significantly increased the tiller number over the control.

It is important to note that *Azolla* supplementation even with the lowest level of N (40 kg/ha) gave a tillering effect which did not differ significantly (P = 0.05) from that obtained with the highest rate of N (without *Azolla*). The superiority of the supplemented over the unsupplemented *Azolla* in promoting tillering would indicate that there is potential of increasing the efficiency of *Azolla* as a green manure by supplementing it (*Azolla*) with inorganic fertilisers in rice growth performance, although not for all parameters (see the case of grain yield - Section 4.5.5).

Table 6. Effect of nitrogen sources on number of tillers of two rice varieties' (an average of three replications)

Nitrogen source	Tiller count at maximum tillering stage (no./4 hills)*		
	V1	V2	N source means
1. No *Azolla*, no inorganic fertiliser N	49	71	60 c
2. *Azolla* alone	56	88	72 b
3. *Azolla* + 40 kg N/ha	57	91	74 a
4. *Azolla* + 80 kg N/ha	63	94	78 a
5. 160 kg N/ha without *Azolla*	63	105	84 a
Variety means	57 b	90 a	

$LSD_{0.05}$ to compare nitrogen source means	11
$LSD_{0.05}$ to compare variety means	21
CV (%) for N source means	12.56
CV (%) for variety means	18.30

'V1,V2 refer to *Wahiwahi* (indigenous) and IR54 (improved) rice varieties, respectively.

*One sampling unit was composed of four hills (see Section 3).

In a row or column, means followed by a common letter are not significantly (P = 0.05) different according to the *t*-test and the Duncan's New Multiple Range Test, respectively.

Similar cases have already been reported in which the supplementation of *Azolla* with inorganic fertiliser N greatly increased the number of tillers over the unsupplemented *Azolla*. For instance, Ramasamy *et al.* (1987) reported that supplementing *Azolla* with 40 kg N/ha increased the number of tillers of a rice variety IR20 by 21% over those attained by plants treated with *Azolla* alone. The speculations as to why the supplementation of *Azolla* with inorganic fertiliser N would be superior to unsupplemented *Azolla* or inorganic fertiliser alone, have already been given when discussing the effect of *Azolla* on chlorophyll content (see Section 4.3.1.2).

4.3.3 Heading/flowering and maturation

4.3.3.1 Heading/flowering

The interaction between crop variety and N sources was found to be statistically significant, meaning that the effect of the N sources was dependent on crop variety in influencing flowering/heading time of the rice plants.

As presented in Table 7, plants of the local variety headed significantly (P = 0.05) earlier than those of the improved variety, at each level of the N source. This observation was not surprising because the local variety used in the present study is known to be genetically early-maturing. It is logical that it should flower/head earlier than the improved variety.

When the response of each individual variety to the different N sources is considered, the improved variety (V2) headed significantly ($P = 0.05$) earlier in the control (untreated) plots than plants in all the treated plots. This was not true with the indigenous variety (V1) in which all plants headed significantly earlier except those which received the highest rate of fertiliser N alone.

In the case where plants of the improved variety headed significantly earlier in the control plots than plants in all the other treatments, the explanation for this observation could be that, in the untreated plots, the amount of N was low. The improved variety, which require higher amounts of N, probably did not get enough N in this situation. Consequently, it had to cut short the vegetative period and flower early. According to Tsuboi (1992), rice plants in nutritional stress normally

Table 7. Effect of nitrogen sources on flowering/heading time of two rice varieties* (an average of three replications)

Nitrogen source	Flowering/heading time (DAT)$^{◊}$		
	V1	V2	N source means
1. No *Azolla*, no inorganic fertiliser N	64 c	72 b	68
2. *Azolla* alone	63 c	76 a	69
3. *Azolla* + 40 kg N/ha	66 c	77 a	71
4. *Azolla* + 80 kg N/ha	65 c	79 a	72
5. 160 kg N/ha without *Azolla*	70 b	76 a	73
Variety means	66	76	

$LSD_{0.05}$ to compare variety means at same N source treatment is 3

$LSD_{0.05}$ to compare N source means at same variety treatment is 5

CV (%) for variety means	3.87
CV (%) for N source means	2.42

*V1,V2 refer to *Wahiwahi* (indigenous) and IR54 (improved) rice varieties, respectively.

◊DAT denotes days after transplanting.

In a row or column, means followed by a common letter are not significantly (P = 0.05) different according to the *t*-test and the Duncan's New Multiple Range Test, respectively.

exhibit such a phenomenon. In a similar situation (i.e., untreated plots) however, the natural soil N reserves (estimated from the soil analysis as 16 kg/ha) probably provided enough N for the indigenous variety which is known to have lower requirements for N than the improved variety.

In the case where the indigenous variety headed significantly later in the highest N rate, the explanation may be that the first two splits of the highest rate of fertiliser may have supplied N early enough and in such amounts that may have been beyond the requirements of the plants of the indigenous variety, which are not only early-maturing but also are less demanding in their N nutrition. Plants of this variety, may therefore, have enjoyed luxurious levels of N and so, could have spent a longer time in the vegetative growth phase. A phenomenon like this is known to be exhibited by rice (Tsuboi, 1992) and many other annual cereals. The first two splits of fertiliser N however, may not have supplied enough N to cause any noticeable effect on heading/flowering in plants of the improved variety, which, as has already been explained above, requires larger amounts of nitrogen than the indigenous variety.

4.3.3.2 The onset and duration of maturation

In the case of maturation, both varietal and N source effects were found to be statistically significant but their interaction effect was not significant. This means that each of the two factors (variety and N sources) influenced maturation independently of each other.

As seen from Table 8, the indigenous variety matured significantly ($P = 0.05$) earlier than the improved variety. This response was expected since the indigenous variety is early-maturing and in fact flowered/headed significantly earlier than the improved variety, as discussed above. The phenomenon of an early onset of maturity, as is the case of flowering/heading time, is genetically controlled and hence, the varietal differences as seen in this study were not surprising.

When the effect of the N sources is examined across the two varieties (Table 8), *Azolla* supplemented with 80 kg fertiliser N/ha and the highest level of fertiliser N (without *Azolla*), significantly ($P = 0.05$) delayed the rice crop maturity compared to all the other treatments. Prolonging the maturation process (i.e., making the grains on panicles stay longer before they ripened) could

Table 8. Effect of nitrogen sources on maturity time of two rice varieties* (an average of three replications

Nitrogen source	Maturity time (DAS)[φ]		
	V1	V2	N source means
1. No *Azolla*, no inorganic fertiliser N	99	117	108 b
2. *Azolla* alone	98	117	107 b
3. *Azolla* + 40 kg N/ha	99	119	109 b
4. *Azolla* + 80 kg N/ha	102	122	112 a
5. 160 kg N/ha without *Azolla*	102	122	112 a
Variety means	100 b	119 a	

$LSD_{0.05}$ to compare variety means	5
$LSD_{0.05}$ to compare nitrogen source means	2
CV (%) for variety means	1.59
CV (%) for N source means	1.52

*V1,V2 refer to *Wahiwahi* (indigenous) and IR54 (improved) rice varieties, respectively.

[φ] DAS denotes days after sowing.

In a row or column, means followed by a common letter are not significantly (P = 0.05) different according to the *t*-test and the Duncan's New Multiple Range Test, respectively.

only have been achieved through a sustained supply of N to the rice plants, with much of the N made available during the late stage of their growth. According to Tsuboi (1992), large amounts of N supplied late in the reproductive stage may prolong the time required for the grains to ripen (attain maturity). Prolonged maturity may be beneficial as it may result in perfect grain filling (as would be indicated by the weight of the grains) although in the present study, this was not distinct (though studied) [Table 13].

4.4 Panicle excertion as an estimate of the effect of sheath rot disease

The effect of sheath rot disease was indirectly estimated based on the percent of partially excerted panicles, for the reasons already explained in Section 3.9. The results on panicle excertion determination (as described earlier in section 3.9) are presented in Table 9.

The effects of the N sources and of the interaction between the N sources and variety on panicle excertion were not found to be statistically significant. Only the varietal effect was significant.

As seen in Table 9, plants of the indigenous variety exhibited a significantly (P = 0.05) higher percentage of fully excerted panicles than those of the improved variety, indicating that the improved variety was more seriously attacked by the disease than the indigenous one(see Section 3.9). The observation could be explained by two factors, namely, the difference in varietal resistance to the disease attack and the timing of the disease incidence. In the former case, the indigenous variety might have been inherently resistant to the disease. The indigenous variety, which matured earlier, might have escaped the disease attack which occurred late in the season.

Since little is known about the mechanism of inherent resistance of each of the two varieties to sheath rot disease, timing of the disease attack is tentatively upheld as the reason for the difference in the degree to which the plants of the two varieties in the present study were attacked by the sheath rot disease.

Nevertheless, closer observation of Table 9 would suggest that *Azolla* alone and that in combination with 40 kg fertiliser N/ha, and the control (with neither *Azolla* nor

Table 9. Effect of nitrogen sources on panicle excertion of two rice varieties* (an average of three replications)

Nitrogen source	Full panicle excertion (% of all panicles)		
	V1	V2	N source mean
1. No *Azolla*, no inorganic fertiliser N	18.2	1.4	9.8
2. *Azolla* alone	35.4	0.7	18.1
3. *Azolla* + 40 kg N/ha	32.3	4.1	18.2
4. *Azolla* + 80 kg N/ha	12.4	1.1	6.8
5. 160 kg N/ha without *Azolla*	17.6	0.3	8.9
Variety means	23.2 a	1.5 b	
$LSD_{0.05}$ to compare variety means	21.3		
CV (%) for variety means	93.63		
CV (%) for N source means	70.51		

*V1, V2 refer to *Wahiwahi* (indigenous) and IR54 (improved) rice varieties, respectively.

In a row, means followed by a common letter are not significantly (P = 0.05) different according to the *t*-test.

fertiliser) gave comparatively higher panicle excertion than all other N source treatments.

These treatments could have indirectly influenced panicle excertion by causing rice plants to mature comparatively early, therefore escaping the attack of sheath rot disease which, as has already been explained, occurred late in the season.

4.5 Effect of the experimental treatments on yield determinants and on the grain yield

4.5.1 Number of panicles per square metre

For this parameter, only the effect of the N sources was statistically significant, meaning that the N sources affected the number of panicles independently of each variety.

As seen from Table 10, *Azolla* alone or the highest rate of inorganic fertiliser alone did not increase the number of panicles per square metre over the untreated control. Only supplementing *Azolla* with inorganic fertiliser N significantly (P = 0.05) increased the number of panicles over the control. In fact, supplementing *Azolla* with 80 kg N/ha significantly (P = 0.05) increased the number of panicles over, even the highest rate of the inorganic fertiliser.

It is tempting to suggest that the failure of *Azolla* alone to significantly increase the number of panicles per square metre was due to an inadequate N supply from *Azolla* treatment to the rice plants, hence the need for additional inorganic N fertilisers.

This suggestion may be considered valid in view of the fact that the same treatment also caused low number of tillers (see Section 4.3.2.2). However, the above view is difficult to reconcile with the observation that the highest rate of fertiliser alone did not significantly increase the number of panicles over the control, while this same treatment (i.e., the highest N fertiliser rate) significantly increased the number of tillers to the same extent as *Azolla* supplemented with 80 kg N/ha (see Section 4.3.2.2). These results may imply that, although the highest fertiliser rate without *Azolla* had produced many tillers, a substantial number of the tillers could not bear panicles probably due to the poor/imbalanced nutrition resulting from the inorganic fertiliser alone.

Table 10. Effect of nitrogen sources on number of panicles of two rice varieties* (an average of three replications)

Nitrogen source	Number of panicles /m^2		
	V1	V2	N source means
1. No *Azolla*, no inorganic fertiliser N	224	303	263 c
2. *Azolla* alone	283	368	326 abc
3. *Azolla* + 40 kg N/ha	327	370	348 ab
4. *Azolla* + 80 kg N/ha	344	434	389 a
5. 160 kg N/ha without *Azolla*	254	336	295 bc
Variety means	286	362	

$LSD_{0.05}$ to compare nitrogen source means	63
CV (%) for variety means	21.75
CV (%) for N source means	15.77

*V1,V2 refer to *Wahiwahi* (indigenous) and IR54 (improved) rice varieties, respectively.

In a column, means followed by a common letter are not significantly (P = 0.05) different according to the Duncan's New Multiple Range Test.

On the other hand, *Azolla* treatment enabled the rice plants to produce less, but effective tillers which could bear panicles. It can be speculated that the balanced nutrition that was enjoyed by plants grown with *Azolla* was a result of nutrient scavenging ability of *Azolla*, coupled with its moderate release of the retained nutrients. The large number of panicles exhibited by rice plants that were grown with *Azolla* supplemented with 80 kg fertiliser N/ha may support prior inferences made by the author, that, this treatment (i.e., *Azolla* with 80 kg N/ha) could have supplied enough N and other nutrients at the rate consistent with the ability of the rice plants to make maximum use of it. Subramani and Kannaiyan (1987) also noted that the incorporation of *Azolla pinnata* in combination with 90 kg N/ha had resulted in the highest panicle number per square metre, compared to any other treatment. In that study, the effect was consistently observed in two separate seasons.

4.5.2 Number of grains per panicle

The effect of the varieties and of the N sources on the number of grains per panicle was found to be statistically significant but their interaction effect was not (Table 11). Overall, panicles of the improved variety had significantly (P = 0.05) more grains than those of the indigenous variety across the N sources. This property is, however, known to be genetic and therefore, the observed result was not surprising. On examining the effect of the N sources across the two varieties, it can be seen that panicles of plants in the control plots had a significantly (P = 0.05) lower number of grains than of those in the treated plots. This could be due to the conditions of inadequate N experienced by plants in the untreated plots as compared to those where *Azolla* and inorganic fertiliser N were applied.

The highest fertiliser rate gave grain number which did not differ significantly from that obtained with *Azolla* alone or that supplemented with fertiliser N. On the other hand, supplementing *Azolla* with 80 kg N/ha significantly (P = 0.05) increased the number of grains over those obtained with *Azolla* alone or *Azolla* supplemented with 40 kg N/ha. It would be plausible to speculate that the rice plants could have taken up N from *Azolla* supplemented or not supplemented with fertiliser N in amounts equal to that from the highest rate of inorganic fertiliser. In that case, the potential of *Azolla* to accumulate and supply N is indicated.

Table 11. Effect of nitrogen sources on number of grains of two rice varieties* (an average of three replications)

Nitrogen source	Number of grains/panicle		
	V1	V2	N source means
1. No *Azolla*, no inorganic fertiliser N	42	55	48 c
2. *Azolla* alone	52	69	61 b
3. *Azolla* + 40 kg N/ha	53	70	62 b
4. *Azolla* + 80 kg N/ha	71	79	75 a
5. 160 kg N/ha without *Azolla*	66	70	68 ab
Variety means	57 b	69 a	

$LSD_{0.05}$ to compare variety means	13
$LSD_{0.05}$ to compare nitrogen source means	10
CV (%) for variety means	4.72
CV (%) for N source means	13.16

*V1,V2 refer to *Wahiwahi* (indigenous) and IR54 (improved) rice varieties, respectively.

In a row or column, means followed by a common letter are not significantly (P = 0.05) different according to the *t*-test and the Duncan's New Multiple Range Test, respectively.

A positively-close relationship between the number of spikelets (grains) per panicle and the amount of N absorbed by the rice plant had previously been reported by Yamada *et al.*(1957), Matsushima (1962), Shimizu (1967) and Wada (1969). The fact that supplementing *Azolla* with 80 kg N/ha gave a significantly larger number of grains than *Azolla* alone and when the *Azolla* was supplemented with 40 kg N/ha, would indicate that more benefit could only be realised by supplementing *Azolla* with fertiliser N at rates not less than 40 kg N/ha at the site of the study. A similar observation, that, supplemented *Azolla* increases the number of grains per panicle has also been reported in a study by Singh (1979b), which indicated that supplementing *Azolla pinnata* with 30 kg N/ha (urea) increased the grain yield mainly by increasing the number of grains per panicle.

4.5.3 Panicle fertility

Panicle fertility is important as it is related to the final yield in that, for the same amount of grains, high panicle fertility is positively correlated with high grain yield (Matsushima, 1967; Tsuboi, 1992).

The statistical analysis did not show any significant effect of the N sources and that of the interaction between the N source and variety on panicle fertility. Only the effect of variety was found to have determined the panicle fertility (Table 12).

Panicle fertility of the indigenous variety was significantly (P = 0.05) higher than that of the improved variety (Table 12). This phenomenon was probably genetically-controlled because when the plant possesses a large number of grains per panicle (as was the case with the improved variety in the present study), it generally exhibits a lower percentage of ripened grains (panicle fertility), and vice versa (Matsushima, 1967) because, in the case of a large number of grains per panicle, and assuming that the amount of carbohydrates assimilated or translocated is fairly constant, each grain will receive a relatively smaller amount of the carbohydrates than that received by each grain on a panicle having fewer number of grains (Matsushima, 1967). Under normal cases, the yield of the high-yielding varieties is not reduced by the low percentage of ripe grains because the large number of grains totally nullifies the effect of the low panicle fertility.

Table 12. Effect of nitrogen sources on panicle fertility (ripe grains) of two rice varieties* (an average of three replications)

Nitrogen source	Ripe grains (% of all of grains)		
	V1	V2	N source means
1. No *azolla*, no inorganic fertiliser N	81.3	69.1	75.2
2. *Azolla* alone	76.1	66.3	71.2
3. *Azolla* + 40 kg N/ha	79.6	66.4	73.0
4. *Azolla* + 80 kg N/ha	76.7	63.8	70.2
5. 160 kg N/ha without *Azolla*	80.5	66.4	73.4
Variety means	78.81 a	66.4 b	
$LSD_{0.05}$ to compare variety means	11.1		
CV (%) for variety means	5.00		
CV (%) for N source means	8.83		

*V1,V2 refer to *Wahiwahi* (indigenous) and IR54 (improved) rice varieties, respectively.

In a row, means followed by a common letter are not significantly (P = 0.05) different according to the *t*-test.

The other speculation as to the low panicle fertility exhibited by the improved variety as compared to the indigenous was due to the sheath rot disease which caused poor excertion of panicles of the improved variety, hence preventing many spikelets from adequate exposure to pollination. The N source and rate appear to have no effect on panicle fertility as evident from Table 12.

4.5.4 Grain weight

The effects of variety and that of the N sources were statistically significant in determining the grain weight (Table 13). The ripe grains obtained from variety *Wahiwahi* were significantly (P = 0.05) heavier than those of IR54, and this is because the grains of variety *Wahiwahi* are inherently bigger and heavier than those of IR54. When the overall effect of N sources (averaged over varieties) is examined, the grains of the plants from the treated plots were significantly (P = 0.05) heavier than those from the control (untreated) plots. The amount of N that was naturally supplied by the soil was low, causing a generally poor growth of the rice plants. It is important to note that *Azolla* alone gave rice grains which, in terms of weight, did not differ significantly (P = 0.05) from the grains produced by plants which

Table 13. Effect of nitrogen sources on weight of grains of two rice varieties* (an average of three replications)

Nitrogen source	Weight of 1000 grains (g)		
	V1	V2	N source means
1. No *Azolla*, no inorganic fertiliser N	30.3	21.4	25.9 b
2. *Azolla* alone	31.1	21.7	26.4 a
3. *Azolla* + 40 kg N/ha	31.5	21.9	26.7 a
4. *Azolla* + 80 kg N/ha	31.3	21.9	26.6 a
5. 160 kg N/ha without *Azolla*	31.1	22.6	26.9 a
Variety means	31.1 a	21.9 b	

$LSD_{0.05}$ to compare variety means	0.6
$LSD_{0.05}$ to compare nitrogen source means	0.5
CV (%) for variety means	0.4
CV (%) for N source means	1.52

*V1,V2 refer to *Wahiwahi* (indigenous) and IR54 (improved) rice varieties, respectively.

In a row or column, means followed by a common letter are not significantly (P = 0.05) different according to the *t*-test and the Duncan's New Multiple Range Test, respectively.

received *Azolla* supplemented with 40 or 80 kg N/ha or even the plants which received the highest rate of inorganic fertiliser N. The results could not be attributed to the amount of N that the *Azolla* had accumulated and availed to the crop because, the *Azolla* accumulated low amounts of N (see Section 4.1). The amount of N accumulated by *Azolla* and hence made available to the associated rice crop could, in no way, match that from the highest rate of inorganic fertiliser (albeit its speculated high losses). The probable explanation could be based on the previous proposition that *Azolla* (alone or when supplemented with fertiliser N) accumulated many other plant nutrients and modified the growing conditions in favour of the rice plants, which responded by increasing also the grain weight as it did to the other growth and yield parameters.

4.5.5 Grain yield

The grain yield, especially that of the variety IR54 was less than what was expected from a high-yielding variety and this was attributed to sheath rot disease.

The different N source treatments which included the *Azolla* (supplemented or not supplemented with urea) and the highest rate of inorganic fertiliser N increased the grain yield over the untreated control. This observation was expected since all the yield determinants (see Sections 4.5.1-4) were poorer in the control than in the treated plots. It is also seen from Table 14 that supplementing *Azolla* with 80 kg inorganic N/ha gave significantly (P = 0.05) higher grain yield than the highest rate of inorganic fertiliser N. That the combination of *Azolla* with 80 kg inorganic N/ha gave higher grain yield than the addition of 160 kg N/ha is not entirely surprising because, the same treatment had surpassed the highest rate of inorganic fertiliser in increasing the number of panicles per unit area (see Section 4.5.1) although the same treatment had a comparable effect in increasing the number and weight of the grains (see Sections 4.5.2 and 4.5.4)

This would underscore earlier arguments that much benefits could be realised when *Azolla* is supplemented with some levels of inorganic fertiliser N than when *Azolla* or inorganic fertiliser N are singularly used. The results of this study (on the effect of supplemented *Azolla* on grain yield) are also consistent with those of previous workers.

Table 14. Effect of nitrogen sources on grain yield of two rice varieties* (an average of three replications)

Nitrogen source	Grain yield (t/ha)		
	V1	V2	N source means
1. No *Azolla*, no inorganic fertiliser N	2.05	2.36	2.20 c
2. *Azolla* alone	3.49	3.47	3.48 ab
3. *Azolla* + 40 kg N/ha	3.75	3.67	3.71 ab
4. *Azolla* + 80 kg N/ha	3.93	4.03	3.98 a
5. 160 kg N/ha without *Azolla*	3.65	3.14	3.39 b
Variety means	3.37	3.34	

$LSD_{0.05}$ to compare nitrogen source means	0.56
CV (%) for variety means	11.04
CV (%) for N source means	13.77

*V1,V2 refer to *Wahiwahi* (indigenous) and IR54 (improved) rice varieties, respectively.

In a column, means followed by a common letter are not significantly (P = 0.05) different according to the Duncan's New Multiple Range Test.

For example, in a study to examine the effect of partial substitution of inorganic N by *Azolla*, Arvadia *et al.* (1989) found that *Azolla* supplemented with 60 kg N/ha (as urea) increased rice yield by 64% over the control, as compared to an increase of 56% due to the application of 100 kg N/ha (as urea) alone. Jeyaraman and Purushothaman (1989) found that supplementing *Azolla pinnata* with 75 kg N/ha increased the yield of the variety IR20 by 61% as compared to a 50% increase due to the application of 100 kg N/ha as inorganic fertiliser alone.

What is more interesting at this point is the fact that *Azolla* alone (not supplemented with any fertiliser N) supplied a grain yield comparable to that obtained with *Azolla* supplemented with 40 or 80 kg N/ha, and that obtained with the highest rate of inorganic fertiliser alone. This trend appears to be consistent with many other parameters as explained in Section 4.3.2.1. It would therefore appear that *Azolla* benefited the rice plants in some other aspects apart from it being a source of N *per se*. This is because, as it has already been explained, the amount of N that *Azolla* accumulated and hence made available to the rice plants was quite low. It is recognised that yield is a function of many factors which include a balanced supply of plant nutrients (macro- and micro-nutrients). The study was conducted at a site where soil-related factors which could limit yield were determined and all of them found to be non-limiting except phosphorus (which had to be added to all plots) and nitrogen which was one of the treatments in this study. As their levels were not different among the plots, their effect would be expected to be the same in all plots in the absence of *Azolla*. It has already been explained that *Azolla* is a nutrient scavenger (see Section 4.3.1.2) so, it could have accumulated many other plant nutrients such as potassium, zinc and iron, from the soil and flood water. The accumulated nutrients might have been released back into the soil (in the vicinity of the plant roots) by *Azolla*, especially after decomposition, where the rice plants could have taken them up more efficiently. The mat of *Azolla* is also known to have a moderating effect on the pH and temperature of both soil and flood/irrigation water (Talley and Rains, 1980a; Kroeck *et al.*, 1988; Villegas and San Valentin, 1989). The moderation of soil and water pH and temperature, and the availing of various nutrients to the rice plants by *Azolla*, might have created a more favourable growth environment for the rice plants, which may have culminated in the observed higher grain yields.

The potential of *Azolla* in promoting rice growth and yield may therefore be due to many other factors apart from it being a source of nitrogen.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

The main objective of this study was to quantify the green-manuring potential of *Azolla* when grown as a dual crop with rice, alone or in combination with inorganic nitrogenous (urea) fertiliser. To meet this objective, the experiment was carried out to assess the biomass and nitrogen accumulation by one crop of *Azolla*, then, its effect on the growth performance of the rice crop was determined as described in Chapter 3. A parallel study was also conducted to determine the decomposition rate of *Azolla* under field conditions, also as described in Chapter 3. The results of these experiments led to the following conclusions and recommendations:

1. *Azolla* biomass and N_2 accumulation decreased with the increase in the density of the rice canopy as was exemplified by the lower amount (25 kg/ha) of N accumulated by *Azolla* growing under the more dense canopy of the variety IR54, as compared to 38 kg N/ha accumulated by the *Azolla* that was growing under the less dense canopy of the variety *Wahiwahi*. The full potential

of *Azolla* in accumulating biomass and nitrogen in the present study could not be fully established.

2. The decomposition of *Azolla* after its incorporation into the soil was fast, taking only three weeks for the *Azolla* to lose 90% of its dry matter (DM). This meant that a large portion of its N content, and probably of the other plant nutrients it had accumulated, were mineralised within this period.

3. The rice plants grown with *Azolla* showed positive response in about two weeks, after *Azolla* incorporation into soil, indicating the fertilising potential of *Azolla*. In this study, *Azolla* alone managed to improve not only many growth and yield parameters, but also grain yield to the same extent as the highest level of inorganic fertiliser N. Considering the low amount of N that *Azolla* accumulated in this study, the beneficial effects of *Azolla* on rice plants could not be explained merely by its N supply only.

In view of the above observations/conclusions, it is recommended that:

(a) the full potential of *Azolla* be established by growing it as a monocrop, and then comparing the economics of this practice with those of intercropping so as to establish the most economical way to make use of *Azolla* in rice production. This should be done in different locations and seasons, with various promising rice varieties, to come out with reasonable conclusions that are location- and variety-specific.

(b) *Azolla* should be introduced into the rice fields at most one week after transplanting of rice seedlings so as to synchronise the release of its nutrients with the peak period of N (and other nutrients) requirement by the rice crop.

(c) further research be done to establish other factors (biotic and abiotic), apart from its supply of N, that make *Azolla* promote the rice plant growth and yield.

REFERENCES

Alam, S.M. (1989) Effect of *Azolla* and nitrogen on rice grain and straw yield. In: *International Rice Research Newsletter*, Volume 14 No.6, pp 20.

Alexander, D. (1988) Effect of *Azolla* and other fertilisers on rice yields. In: *International Rice Research Newsletter*, Volume 13 No.5, pp 35.

Alli, F.A. (1996) *Azolla* utilisation on rice cropping systems in Zanzibar. In: *Special seminar on potentials and constraints for improvement of rice cultivation in Tanzania*. 11 - 15 March 1996, KATC Moshi, Tanzania.

Anderson, J.M. and Ingram, J.S. (Eds) (1993) *Tropical Soil Biology and Fertility. A Handbook of Methods*. Commonwealth Agricultural Bureau International. pp 221.

App, A.; Bouldin, D.R.; Dart, P.J. and Watanabe, I.(1980) Constraints to biological nitrogen fixation in soil of the tropics. In: *Priorities for alleviating soil-related constraints to food in the tropics.* International Rice Research Institute, Los Banos, Philippines. pp 317-319.

Arvadia, M.K.; Shah T.M.; Saiyed, F.N.; Pavagadhi. C.B.; Seth, R.D.; Patel, D.K.; Rathore, S.S. and Raman, S. (1989) Effect on rice of partial substitution of nitrogen by *Azolla*. In: *International Rice Research Newsletter*, Volume 14 No.6, pp 20.

Ashton, P.J. (1974) The effect of some environmental factors on the growth of *Azolla filiculoides* Lam. In: *The Orange River Progress Report* (Edited by Zinderen-Bakker, E.M.V.). Institute of Environmental Sciences, University of Orange Free State, Bloemfontein, South Africa. pp 123-138.

Bar, E.; Kulasooriya, S.A. and Tel-or, E. (1991) Regulation of nitrogenase activity by light in the *Azolla-Anabaena* symbiosis. *Bioresource Technology* 38, 171 - 178.

Becking, J.H. (1976) Contribution of plant-algal associations. In: *Proceedings of the 1st international symposium on nitrogen fixation* (Edited by Newton, W.E. and Nyman, C.J.). June 1974, Washington State University Press, Pullman. Volume 2, pp 556 - 580.

Becking, J.H. (1979) Environmental requirements of *Azolla* use in tropical rice production. In: *Nitrogen and Rice.* International Rice Research Institute, Los Banos, Philippines. pp 345 - 373.

Cary, P.R. and Weerts, P.G.J. (1992) Growth and nutrient composition of *Azolla pinnata* R. Brown and *Azolla filiculoides* Lamarck as affected by water temperature, nitrogen and phosphorus supply, light intensity and pH. *Aquatic Botany* 43, 163 - 180.

Chung-chu, L. (1984) Recent advances on *Azolla* research. In: *Practical Application of Azolla for Rice production.* (*Edited by Silver, W.S. and Schroder, E.C.*) Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht. pp 45 - 54.

Chung-Chu, L. (1987) Re-evaluation of *Azolla* utilization in agricultural production. In: *Azolla Utilization: Proceedings of the 1st international workshop on Azolla use*. (Edited by Smith, W.H. and Emerita, P.C.) International Rice Research Institute, Manila, Philippines. pp 67 - 76.

Gee, G.W. and Bauder, J.W. (1986) Particle size analysis. In: *Methods of soil analysis part 1. Agronomy series No. 9, 2nd edition.(Edited by Klute, A.)* American Society of Agronomy-Madison, Wisconsin, USA. pp 383 - 409.

Gomez, K.A. and Gomez, A.A. (1984) *Statistical Procedures for Agricultural Research*, 2nd edition. John Wiley and Sons Inc. Toronto, Canada. Pp 680.

Hamdi, Y.A. (1982) Application of nitrogen fixing systems in soil improvement and management. In: *FAO Soils Bulletin* No.49. pp 188.

Horibata, T. (1992) *Rice Production Manual*. Kilimanjaro Agricultural Development Project, Moshi, Tanzania. pp 35.

Ito, O. and Watanabe, I. (1985) Availability to rice plants of nitrogen fixed by *Azolla*. *Soil Science Plant Nutrition* 31, 91 - 104.

Jeyaraman, S. and Purushotham, S. (1989) Biofertilizer efficiency in lowland rice. In: *International Rice Research Newsletter*, Volume 13 No.3, pp 25.

Kannaiyan, S.; Thangaraju, M. and Oblisami, G. (1983) Effect of *Azolla* green manuring on the rice crop. *Science Culture* 49, 217 - 219.

Kanyeka, Z.L.; Msomba, S.W.; Kihupi, A.N. and Penza, M.S.F. (1994) Rice ecosystems in Tanzania: Characterisation and classification. *Rice Research and Training Newsletter* 9, 13 - 15.

Kihupi, A.L. (1997) *Rice research at Sokoine University of Agriculture*. A paper presented at the Workshop on Rice Cultivation Survey Methods, Kilimanjaro Agricultural Training Centre, Moshi, Tanzania, 2-11 December, 1997.

Kondo, M.; Kobayashi, M. and Takayashi, E. (1989) Effect of phosphorus on *Azolla* and its utilization in rice culture in Niger. *Plant Soil* 120, 165 - 170.

Kroeck, T.; Alkdemper, J. and Watanabe, I. (1988) Effect of an *Azolla* cover on the conditions in flood water. *Journal of Agronomy and Crop Science* 161, 185 - 189.

Kulasooriya, S.A.; Hirimburegana, W.K. and Abeysekera, S.W. (1987) Use of *Azolla* in Sri Lanka. In: *Azolla Utilization: Proceedings of the 1st international workshop on Azolla use.* (Edited by Smith, W.H. and Emerita, P.C.) International Rice Research Institute, Manila, Philippines. pp 131 - 139.

Lay, W.L.; Huang, S.N. and Wang, C.T. (1989) Effect of *Azolla* application on growth and yield of rice. In: *Bulletin of Taichung District Agricultural Improvement Station* No.24, pp 3-12.

Lindsay, W.L. and Norvel, W.A. (1978) Development of DTPA Soil test for zinc, iron, manganese and copper. *Soil Science Society of American Journal* 42, 421 - 428.

Little, T. M. and Hills, F.G. (1978) Agricultural Experimentation: Design and Analysis. John Wiley and Sons, New York, USA. pp 350.

Lowendorf, H.B. (1982) *Biological nitrogen fixation in flooded rice fields*. Cornell International Agriculture Mimeograph No.96. pp 76.

Lumpkin, T.A. (1987a) Collection, maintenance and cultivation of *Azolla*. In: *Symbiotic Nitrogen Fixation Technology*. *(Edited by Elkan, G.H.)* Marcel Dekker, Inc., New York. pp 55 - 94.

Lumpkin, T.A. (1987b) Environmental requirements for successful *Azolla* growth. In: *Azolla Utilization: Proceedings of the 1st international workshop on Azolla use*. (Edited by Smith, W.H. and Emerita, P.C.) International Rice Research Institute, Manila, Philippines. pp 89-97.

Lumpkin, T.A. and Plucknett, D.L. (1980) *Azolla* : Botany, physiology and use as a green manure. *Economic Botany* 34, 111 - 153.

Lumpkin, T.A. and and Bartholomew, D.P. (1986) Predictive models for the growth response of eight *Azolla* accessions to climatic variables. *Crop Science* 26, 107 - 111.

Malavolta, E.; Acorsi, W.R.; Ruschel, A.P.; Krug, F.J.; Nakayama, L.I. and Eimori, I. (1981) Mineral nutrition and nitrogen fixation in *Azolla*. In: *Associative nitrogen fixation, Vol.2. (Edited by Vose, P.B. and Ruschel, A.P.)* CRC Press, Inc, Boca Raton, Florida. pp 205 - 211.

Manna, A.B.; Singh, P.K. (1989) Rice yields as influenced by *Azolla* nitrogen fixation and urea nitrogen fertilization. *Plant and Soil* 114: 63 - 68.

Matsushima, S. (1962) Some experiments on soil-water-plant relationship in rice. *Division of Agriculture Bulletin* No. 112, Malaysia. pp 32.

Matsushima, S. (1967) *Crop Science in Rice: A Theory of Yield Determination and Its Application*. Fuji Publishing Co.Ltd 1-26, Nishigahara, Kita-ku, Tokyo, Japan. pp 365.

Mclean, E.O. (1982) Soil pH and lime requirements. In: *Methods of soil analysis part 2. Agronomy series No. 9, 2nd edition. (Edited by Page, A.T.; Miller, R.H. and Keeney, D.R.)* American Society of Agronomy-Madison, Wisconsin, USA. pp 199 - 223.

Meelu, O.P.; Singh, Y.; Singh, B. (1994). *Green manuring for soil productivity improvement. World Soil Resources Reports*. FAO, Rome. pp 80.

National Academy of Sciences (1979) *Microbial Processes: Promising Technologies for Developing Countries Report of an Ad hoc Panel of the Advisory Committee on Technology Innovation, Board on Science and Technology for International Development, Commission on International Relations*. Washington DC. pp 200.

Nelson, D.W. and Sommers, L.E. (1982) Total carbon, organic carbon, and organic matter. In: *Methods of soil analysis part 2. Agronomy series No. 9, 2nd edition* (*Edited by Page, A.T.; Miller, R.H. and Keeney, D.R.*) American Society of Agronomy-Madison, Wisconsin, USA. pp 539 - 577.

Nikokwe, C.A. (1992) Decomposition of *Clotalaria* and *Leucaena* green manures and their nutrient release patterns. *Msc. Thesis*, Sokoine University of Agriculture, Morogoro, Tanzania.

Okalebo, J.R.; Gathua, K.W. and Woomer, P.L. (1993) *Laboratory Methods of Soil and Plant Analysis: A working manual*. Soil Science Society of East Africa Technical Publication No.1. and Tropical Soil Biology and Fertility Programme, Nairobi, Kenya.

Pande, H.K. (1978) *Azolla*: A precious organic nitrogen fertilizer for rice. In: *Proceedings of India/FAO/Norway Seminar on complementary use of mineral fertilizers and organic materials in India*. pp 232 - 236. Ministry of Agriculture and Irrigation, Krishi Bhavan, New Delhi, India.

Peters, G.A. (1976) Studies on the *Azolla-Anabaena azollae* symbiosis. In: *Proceedings of the 1st international symposium on nitrogen fixation, Volume 2* (Edited by Newton W.E. and C.J. Nyman C.J.). Washington State University Press, Pullman. pp 592 - 610.

Peters, G.A. (1978) The *Azolla-Anabaena* symbiosis: morphology and physiology. In: *Proceedings of the INPUTS 2nd review meeting*. The East-West-Center Resources Institute, Honolulu, Hawaii, pp 153-165.

Peters, G.A.; Ray, T.B.; Mayne B.C. and Toia, R.E. Jr. (1979). The *Azolla-Anabaena association*: morphological and physiological studies. In: *Proceedings of the Steenbock-Kettering international symposium on nitrogen fixation* (Edited by Newton, W.E. and Orme-Johnson, W.H.) University Park Press, Baltimore.

Rains, D.W. and Talley, S.N. (1978a) Use of *Azolla* as a source of nitrogen for temperate zone rice culture. In: *Proceedings of the INPUTS 2nd review meeting*. The East-West-Center Resources Institute, Honolulu, Hawaii. pp 167 - 174.

Rains, D.W. and Talley, S.N. (1978b) Use of *Azolla* in North America. In: *Nitrogen and Rice*. International Rice Research Institute, Manila, Philippines. pp 417-431.

Ramasamy, S.; Dawood, A.S. and Chinnaswami, K.N. (1988) Organic and inorganic nitrogen effect on rice. In: *International Rice Research Newsletter*, Volume 13 No.5, pp 35.

Roger, P.A. and Ladha, J.K. (1992) Biological nitrogen fixation in wetland rice fields: Estimation and contribution to nitrogeen balance. *Plant and Soil* 141, 41-55.

Rosenani, A.B. and Chulan, H.A. (1992) Availability of nitrogen from N-15 labelled *Azolla pinnata* and Urea to flooded rice. *Plant Soil* 143, 153 - 161.

Satapathy, K.B. (1993) Effect of different plant spacing pattern on the growth of *Azolla* and rice. *Indian Journal of Plant Physiology* 36, 98 - 102.

Shimizu, T. (1967) Process of yield-formation in rice plants from the point of dry matter production. *Dry matter production in crops* 4, 12 - 26.

Sikander, A. and Watanabe, I. (1987) Response of *Azolla* to phosphorus, potassium and zinc in different paddy soils. In: *Azolla Utilization: Proceedings of the* 1^{st} *international workshop on Azolla use.* (Edited by Smith, W.H. and Emerita, P.C.) International Rice Research Institute, Manila, Philippines. p 279.

Silvester, W.B. (1972) Dinitrogen fixation by plant associations excluding legumes. In: *A Treatise on Dinitrogen Fixation (Section IV): Agronomy and Ecology. (Edited by Hardy, R.W.F. and Gibson, A.H.)* Wiley-Interscience, New York. pp 141.

Singh, P.K. (1979a) Multiplication and utilization of fern *Azolla* containing nitrogen-fixing algae symbiont: a green manure in rice cultivation. *Il Riso* 26, 125 - 137.

Singh, P.K. (1979b) Effect of *Azolla* on the yield of paddy with and without application of N fertilizer. *Current Science* 46, 624 - 644.

Singh, D.P. and Singh, P.K. (1988) The effects of phosphorus and carbofuran on the growth and nitrogen fixation of *Azolla pinnata* and the yields of rice. *Experimental Agriculture* 24, 183 - 189.

Singh, P.K.; Singh, D.P. and Singh, R.P. (1992a) Growth, acetylene reduction activity, nitrate uptake and nitrate reductase activity of *Azolla caroliniana* and *Azolla pinnata* at varying nitrate levels. *Biochemistry and Physiology* 188, 121 - 127.

Singh R.P. and Singh, P.K. (1989) Effect of nitrogen fertilizers on nitrogen fixation and heterocyst frequency of cyanobacterium *Anabaena azollae* in seven species of *Azolla*. *Biochemistry and Physiology* 185, 429 - 433.

Sisworo, E.L.; Eskew, D.L.; Sisworo, W.H.; Rasjid, H.; Kadarusman, H.; Solahuddin, S. and Soepardi, G. (1990) Studies on the availability of *Azolla* nitrogen and Urea nitrogen for rice growth using nitrogen-15. *Plant Soil* 128, 209 - 220.

Subramani, S. and Kannaiyan, S. (1987) The effect of incorporation and unincorporation of *Azolla* biofertilizer on the grain yield of rice. *Journal of Agronomy and Crop Science* 159, 308 - 311.

Subudhi, B.P.R. and Watanabe, I. (1981) Differential phosphorus requirements of *Azolla* species and strains in phosphorus-limited continuous culture. *Soil Science and Plant Nutrition* 27, 237 - 247.

Talley, S.N. and Rains, D.W. (1980a) *Azolla* as a nitrogen source for temperate rice. In: *Nitrogen fixation: Proceedings of the Steenbock-Kettering Symposium, Volume 2 (Edited by Newton, W.E. and Orme-Johnson, W.H.)*. 1978, Madison. University Park Press, Baltimore. pp 311 - 320

Talley, S.N. and Rains, D.W. (1980b) *Azolla filiculoides* as a fallow season green manure for rice in a temperate climate. *Agronomy Journal* 72, 11 - 18.

Tsuboi, T. (1992) *Handbook of Tropical Rice Cultivation*. Association for International Cooperation of Agriculture and Forestry, Tokyo, Japan. pp 30.

Thomas, G.W. (1982) Exchangeable bases. In: *Methods of soil analysis part 2. Agronomy series No. 9, 2nd edition (Edited by Page, A.T.; Miller, R.H. and Keeney, D.R.)* American Society of Agronomy-Madison, Wisconsin, USA. pp 539 - 577.

Tung, H.F. and Shen, T.C. (1985) Studies of the *Azolla pinnata-Anabaena azollae* symbiosis: Concurrent growth of *Azolla* with rice. *Aquatic Botany* 22, 145 - 152.

van Hove, C. (1989) *Azolla and its multiple uses, with emphasis on Africa.* Food and Agriculture Organization of the United Nations, Rome. pp 53.

Ventura, W. and Watanabe, I. (1991) *Azolla* and *Sesbania*: organic fertilizers. In: *The Philippine Environment: Opportunities in Conservation and Rehabilitation.* The Philippine Futuristic Society, Manila. pp 171 - 178.

Ventura, W.; Watanabe, I. and Mascarina, G.B. (1992) Mineralization of Azolla-N and its availability to wetland rice: II. Fertilizer effect and N-45 uptake by rice from different species of *Azolla* with varying N contents. *Soil Science Plant Nutrition* 38, 505 - 516.

Villegas, G.G. and San Valentin, G.O. (1989) Effect of *Azolla* cover on nitrogen and rice in flooded Mahaas clay. In: *Azolla*: *Its culture, management and utilisation in the Philippines*. National *Azolla* Action Program, The University of the Philippines, Los Banos, Philippines. pp 65-90.

Wada, G. (1969) The effect of nitrogenous nutrition on the yield-determining process of rice plants. *Bulletin of National Institute of Agricultural Science (Japan)*, Serial No. A16, 27-167.

Wagner, G.M. (1996) *Potential biofertilizers for rice in Tanzania*. A paper presented at a special seminar on potentials and constraints for improvement of rice cultivation in Tanzania, Kilimanjaro Agricultural Training Centre, Moshi, Tanzania, 11 - 15th March, 1996.

Wang, D.; Zhao, M. and Chen, D. (1987). Studies on *Azolla* mineralisation rate and nutrient releasing dynamics. In: *Azolla utilisation: Proceedings of the* 1^{st} *international workshop on Azolla use*. (Edited by Smith, W.H. and Emerita, P.C.) International Rice Research Institute, Manila, Philippines. pp 275.

Wangari, N. (1995) Decomposition and nutrient release patterns of *Sesbania sesban* and *Lantana camara* and the use of *Lantana* as a green manure source in vegetable production. *Msc. Thesis*, Sokoine University of Agriculture, Morogoro, Tanzania.

Watanabe, I. (1982) *Azolla-Anabaena* symbiosis : Its physiology and use in tropical agriculture. In: *Microbiology of Tropical Soils and Plant Productivity. (Edited by Dommergues, Y. R. and Diem H.G.)* Martinus Nijhoff/Dr. W. Junk Publishers, The Hague . pp 169 - 185.

Watanabe, I. (1984) Use of symbiotic and free-living blue-green algae in rice culture. *Outlook Agriculture* 13, 166 - 172.

Watanabe, I. (1996) *Azolla use and rice production in Tanzania*. A paper presented at a seminar on *Azolla* and its use, Kilimanjaro Agricultural Training Centre, Moshi, Tanzania, 7 June, 1996.

Watanabe, I. and Liu, C.C. (1992) Improving nitrogen-fixing systems and integrating them into sustainable rice farming. *Plant and Soil* 141, 57 - 67.

Watanabe, I; Espinas, C.R.; Berja, N.J. and Alimagno, D. (1977) Utilisation of *Azolla-Anabaena* complex as a nitrogen fertiliser for rice. International Rice Research Institute Research Paper Series 11, p 15.

Watanabe, I.; N.S. Berja and D.C. Del Rosario (1980) Growth of *Azolla* in paddy field as affected by phosphorus fertilizer. *Soil Science Plant Nutrition* 26, 301 - 307.

Wen, Q.; Cheng, L. and Shi, S. (1987) Decomposition of *Azolla* in the field and availability of *Azolla* nitrogen to plants. In: *Azolla Utilization: Proceedings of the 1st international workshop on Azolla use*. (Edited by Smith, W.H. and Emerita, P.C.) International Rice Research Institute, Manila, Philippines. pp 241 - 253.

Yamada, N.; Ota, Y. and Kushibuchi, K. (1957) Studies on ripening of rice. 1. Role of nitrogen process of ripening of rice. *Proceedings of Crop Science Society of Japan* 26, 111 - 115.

Yatazawa, M.; Tomatsu, N.; Hosoda, N. and Nunome, K. (1980) Nitrogen fixation in *Azolla-Anabaena* symbiosis as affected by mineral nutrient status. *Soil Science Plant Nutrition* 26, 415 - 426.

You, C.; Zhang, R. and Song, W. (1987) Some aspects of rice-*Azolla* association in northern China. In: *Azolla Utilization: Proceedings of the 1st international workshop on Azolla use*. (Edited by Smith, W.H. and Emerita, P.C.) International Rice Research Institute, Manila, Philippines. pp 189 - 195.

APPENDIX

Appendix Table. A summary of the effect of nitrogen sources* (averaged across varieties) for the various parameters studied [Derived from Tables 4 - 14]

Nitrogen source	Leaf chlorophyll content (SPAD value)	Plant height at maturity (cm)	Tiller count at maximum tillering stage (no./4 hills)	Flowering/heading time (DAT)	Maturity time (DAS)	Full panicle excertion (% of all panicles)	Panicles/ m^2	Grains/ panicle	Ripe grains (% of all grains)	Weight of 1000 grains (g)	Grain yield (t/ha)
1. No *Azolla*, no inorganic fertiliser N	25.4 d	83.1	60 c	68	108 b	9.8	263 c	48 c	75.2	25.9 b	2.20 c
2. *Azolla* alone	29.5 bc	96.9	72 b	69	107 b	18.1	326 abc	61 b	71.2	26.4 a	3.48 ab
3. *Azolla* + 40 kg N/ha	30.2 ab	99.3	74 a	71	109 b	18.2	348 ab	62 b	73.0	26.7 a	3.71 ab
4. *Azolla* + 80 kg N/ha	31.5 a	104.5	78 a	72	112 a	6.8	384 a	75 a	70.2	26.6 a	3.98 a
5. 160 kg N/ha without *Azolla*	28.0 c	103.8	84 a	73	112 a	8.9	295 bc	68 ab	73.4	26.9 a	3.39 b

*Letters are assigned where the effect of N sources was statistically (P = 0.05) significant

In a column, means followed by a common letter are not significantly (P = 0.05) different according to the Duncan's New Multiple Range Test.