Ecology and Development Series No. 2, 2002

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The role of *Azolla* cover in improving the nitrogen use efficiency of lowland rice

Cuvillier Verlag Göttingen

Abstract

The use of the aquatic fern *Azolla* to reduce the high NH_3 volatilization losses and improve the low N use efficiency of applied urea in lowland rice fields was evaluated for 3 cropping seasons. Two on-station field experiments were established at the PhilRice experimental farms in Los Banos during the dry season of 1998-99. The influence of an *Azolla* cover in urea-amended plots applied at the rates of 0, 40, 80, 120 and 160 kg N ha^{-1} as compared to plots with urea only was assessed with respect to floodwater chemistry, NH_3 volatilization, ^{15}N recovery, N uptake, crop growth and consequently, to grain yield. The *Azolla*-cover approach was further investigated and verified in farmers´ fields during the wet and dry seasons of 2000-01 at lower N rates, *i.e.*, up to 80 kg N ha^{-1} in the wet season and up to 100 kg N ha^{-1} in the dry season. Eight identical experiments per season were carried out in selected fields in four municipalities in Laguna.

Findings revealed that a full *Azolla* cover on the floodwater surface at the time of urea application prevented the rapid and large increase in floodwater pH associated with urea hydrolysis and the photosynthetic activities of the algae. In the presence of an *Azolla* cover, the mean floodwater pH was reduced by as much as 1.9 pH units and the maximum pH value was kept below 8.3. In contrast, in the absence of a cover, floodwater pH rose above 8.5 and reached a maximum of 10.1. The floodwater temperature was lowered by as much as 5°C. As a consequence, the partial pressure of ammonia (ρNH_3), which is an indicator of potential NH_3 volatilization, was significantly depressed.

Using the ^{15}N tracer technique to determine the amount of N recovered and lost, data at harvest showed that ^{15}N recovery was higher in plots covered with *Azolla*. The total ^{15}N recovery varied between 77 and 99% and the aboveground (grain and straw) recovery by rice varied between 32 and 61%. The ^{15}N not accounted for in the *Azolla*-rice-soil system and presumed lost ranged from 0.01 to 23%. In the absence of an *Azolla* cover, ^{15}N losses ranged from 21 to 49%.

The total N uptake increased by as much as 42% and the total dry matter yield by as much as 36% on *Azolla*-covered plots. The tiller and panicle count, the most important yield components, with an *Azolla* cover were significantly increased by 50% more than the uncovered plots at all urea levels. Consequently, the grain yield was likewise improved. Grain yields from the 16 on-farm trials increased by as much as 40% at lower N rates (40 and 50 kg N ha^{-1}) and by as much as 19% at higher N rates (80 and 100 kg N ha^{-1}). In addition, response of the crop to treatments with lower N rates with an *Azolla* cover was comparable to that obtained with the higher N rates without a cover. Thus, using *Azolla* as a surface cover in combination with urea can be an alternative management practice worth considering to reduce NH_3 volatilization losses and improve N use efficiency.

Kurzfassung

In zwei Feldversuchen im Zeitraum 1998-1999 über drei Anbauperioden wurde untersucht, inwieweit im Nassreisanbau der Einsatz des Wasserfarns *Azolla* zur einer Verringerung der gasförmigen Stickstoffverluste in Form von Ammoniak und zu einer Steigerung der Effizienz der Harnstoff-N-Düngung beitragen kann. Die Untersuchungen wurden auf den Versuchsflächen der philippinischen Reisforschungsanstalt "PhilRice" in Los Baños durchgeführt. Hier wurden Versuchsparzellen angelegt, auf denen Reis zusammen mit *Azolla* und Düngergaben von 0, 40, 80, 120, und 160 kg Harnstoff-N ha^{-1} angebaut wurde. Stauwasserchemie, NH_3-Verflüchtigung, N-Rückgewinnung, N-Aufnahme durch die Pflanzen und der Reiskornertrag dieser Parzellen wurden mit Versuchsparzellen verglichen, auf denen lediglich im gleichen Maße nur mit Harnstoff gedüngt wurde.
Darüber hinaus wurde der *Azolla*-Ansatz auf acht Feldern von Kleinbauern in vier verschiedenen Kommunen der Provinz Laguna getestet.

Die Ergebnisse zeigten, dass eine geschlossene *Azolla*-Decke auf der Stauwasseroberfläche zum Zeitpunkt der Harnstoffdüngung eine Erhöhung des Stauwasser-pH verhinderte, der im Zuge der Harnstoffhydrolyse und Photosyntheseaktivität der Algen generell zu beobachten ist. Auf den Parzellen mit *Azolla*-Bedeckung wurde der mittlere pH-Wert um bis zu 1,9 Einheiten reduziert und der maximale pH-Wert unter 8,3 gehalten. Im Gegensatz hierzu stieg bei fehlender *Azolla*-Bedeckung der Stauwasser-pH auf über 8,5 und erreichte ein Maximum von 10,1. Die Stauwassertemperatur wurde durch die Azolla-Bedeckung um bis zu 5^oC gesenkt. Infolge dessen verringerte sich der Partialdruck des NH_3, ein Indikator der potentiellen N-Verflüchtigung, signifikant.

Die Höhe der N-Rückgewinnung und des N-Verlustes konnte durch ^{15}N Markierung quantifiziert werden. Die Ernteergebnisse zeigten, dass die ^{15}N Rückgewinnung auf Parzellen mit *Azolla*-Bedeckung höher war als auf Parzellen ohne Bedeckung. Die Gesamt-^{15}N-Rückgewinnung erreichte 77 bis 99 %, wobei der Körner- und Stroh-Anteil 32 bis 61 % ausmachte. Der im *Azolla*-Reis-Boden-System nicht berücksichtigte und wahrscheinlich verlorengegangene ^{15}N lag zwischen 0 und 23 %. Bei fehlender *Azolla*-Bedeckung betrugen diese ^{15}N-Verluste zwischen 21 und 49 %.

Auf Parzellen mit *Azolla*-Bedeckung stieg die N-Gesamtaufnahme um bis zu 42 % und die Gesamttrockenmasse um bis zu 36 %. Die Anzahl der Bestockungstriebe und Rispen, die wichtigsten Komponenten einer Ernte, war bei einer *Azolla*-Bedeckung im direkten Vergleich signifikant um 50 % höher als auf Parzellen ohne *Azolla*-Bedeckung, unabhängig davon, wie viel Harnstoff gedüngt wurde. Der Reisertrag in den 16 Versuchen auf Kleinbauernflächen erhöhte sich bei geringeren N-Gaben (40 bzw. 50 kg N ha^{-1}) um bis zu 40 % und um immerhin bis zu 19 % bei höheren N-Gaben (80 bzw. 100 kg N ha^{-1}). Dabei waren die Reiserträge bei niedrigeren N-Gaben zusammen mit einer *Azolla*-Bedeckung vergleichbar mit den Erträgen bei höheren N-Gaben ohne *Azolla*-Bedeckung.
Der Einsatz von *Azolla* in Kombination mit einer Stickstoffdüngung in Form von Harnstoff stellt folglich eine Anbaumethode dar, bei durch geringeren gasförmigen N-Austrag und verbesserte N-Effizienz Reiserträge erhöht werden können.

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ANR : Apparent nitrogen recovery
BY1 : Bay site 1
BY2 : Bay site 2
CEC : Cation exchange capacity
cm : Centimeter
cmol kg^{-1} : Centimole per kilogram
CES : Central Experiment Station
DAT : Days after transplanting
E longitude : East longitude
GY$_3$: Plot grain yield$_{\text{at 3\% moisture content}}$
GY$_{\text{coyod}}$: Grain yield$_{\text{component of yield oven dry}}$
g cc^{-1} : Gram per cubic centimeter
IFIA : International Fertilizer Industry Association
IFPRI : The International Food Policy and Research Institute
INSFFER : Network on Soil Fertility and Fertilizer Evaluation for Rice
IRRI : International Rice Research Institute
JSIDRE : Japanese Society of Irrigation, Drainage, and Reclamation Engineering
kg ha^{-1} : Kilogram per hectare
kg m^{-2} : Kilogram per square meter
kms : Kilometer
K_2O : Muriate of potash (0-0-60)
K : Potassium
LAI : Leaf area index
LB1 : Los Banos site 1
LB2 : Los Banos site 2
NAAP : National *Azolla* Action Program
m^2 : Square meter
m : Meter
Mt : Metric tons
mg kg^{-1} : Milligram per kilogram
ml : Milliliter
mm : Millimeter
Ndff : Nitrogen derived from fertilizer
N latitude : North latitude
NH_3 : Ammonia
NH_4^+ : Ammonium
NUE : Nitrogen use efficiency
PAN : Panicle count per hill
Pan$_{\text{12hills}}$: Panicle count$_{\text{12 hills}}$
ρNH_3 : Partial pressure of ammonia
PCARR : Philippine Council for Agriculture and Resources Research
PhilRice : Philippine Rice Research Institute
PSB : Philippine Seedboard Rice
P_2O_5 : Superphosphate (0-18-0)
P : Phosphorus
RCBD : Randomized complete block design
SC1 : Sta Cruz site 1

SC2 : Sta Cruz site 2
SF1 : San Francisco site 1
SF2 : San Francisco site 2
Si cl : Silty clay
$\text{StODW}_{\text{12hill}}$: Straw oven dry $\text{weight}_{\text{12 hills}}$
StODW_{ss} : Straw oven dry $\text{weight}_{\text{sub-sample}}$
$\text{StFW}_{\text{12hills}}$: Straw fresh $\text{weight}_{\text{12 hills}}$
StY_{OD} : Straw $\text{yield}_{\text{oven dry}}$
TDMY_{OD} : Total dry matter $\text{yield}_{\text{oven dry}}$ (above ground)
t ha^{-1} : Tons per hectare
UPLB : University of the Philippines Los Banos

Acknowledgments

I would like to express my sincere gratitude and appreciation to the following individuals and organizations for their guidance and support, without which the completion of this doctoral work would not have been achieved:

My deep gratitude to Prof. Dr. Paul L.G. Vlek, my first advisor, for providing me with the opportunity to pursue my doctoral studies, for his expert advice, for his guidance and support throughout my study, for his constructive comments and suggestions that were invaluable in refining my work.

I wish to sincerely thank Dr. Genaro O. San Valentin, my supervisor in the Philippines, for his constant encouragement, for his insightful comments on my write-up, for his unlimited support and advice, and for all his concern.

Special thanks to Dr. Eric T. Craswell for taking the time to read my drafts and for critically reviewing my write-up. His technical and editorial advice greatly improved the presentation and contents of this dissertation.

My thanks also go to Prof. Dr. Mathias Becker, my second advisor, for his evaluation.

I also wish to express my thanks to Ms. Sabine Aengenendt-Baer and Ms. Sina Bremer of ZEFc, Dr. Günther Manske and Ms. Hanna Peters for their kind assistance in everything, to Ms. Deborah Rupprecht for analyzing my samples for ^{15}N and to Ms. Margaret Jend for editing the final draft and for translating the abstract and summary into German.

It was a pleasure to work at PhilRice for my experimental research. My thanks to its entire staff for the technical support, for the numerous enjoyable discussions, and for the friendship.

I am thankful to the farmers, my laborers and field workers for their generous help in the experimental part of this research.

I gratefully acknowledge the financial support from the Zentrum für Entwicklungsforschung for the whole duration of my study.

The support and encouragement of my friends are greatly appreciated and acknowledged: Dr. Abay Asfaw, Ms. Mie Mie Aung, Ms. Lala de la Cruz, Ms. Vienna Fandialan, Dr. Dougbedji Fatondji, Ms. Olive Fillone, Mr. Albert Garcia, Mr. Tadesse Gole, Mr. Li Zhaohua, Ms. Rebecca Pick, Ms. Rahayu Widyastuti, Ms. Roukaya Zimmermann and Dr. Christian and Ms. Alice Witt.

The many other friends, the countless individuals, while not named here but certainly not forgotten, who helped me throughout my study, I thank each of them sincerely.

Most importantly, I wish to profoundly thank my parents, Efren Sr. and Letty and my brothers, Efren Jr. and Erwin, who have been my continuous source of inspiration and encouragement, for their never-ending prayers, unconditional love and untiring support.

1 Introduction

Nitrogen (N) is an essential element influencing rice productivity. The inefficient use of N by lowland rice (*Oryza sativa* L.) is a matter of concern to farmers, researchers and environmentalists. Nitrogen recovery by rice can be as low as 10% and rarely exceeds 60% (Craswell and Vlek, 1979; De Datta, 1981; Simpson *et al.*, 1984; Vlek and Byrnes, 1986; Schnier *et al.*, 1988). In Asia, the average fertilizer N recovery efficiency in farmers' fields is currently only about 30% (Dobermann *et al.*, 2002). Nitrogen losses are high particularly at low plant demand during the early growth stages when urea, the major N fertilizer used by farmers in Asia, is broadcast onto the floodwater surface (Schnier, 1995). Ammonia volatilization, the gaseous emission of NH_3 to the atmosphere, is reportedly the major cause of this low N fertilizer efficiency and an important mechanism for N losses in lowland rice fields (Vlek and Craswell, 1981; Jayaweera and Mikkelsen, 1990; Reddy *et al.*, 1990; Freney *et al.*, 1993). The conditions in lowland rice fields in the tropics, particularly in the first 2 weeks after urea application, are especially conducive for NH_3 volatilization (Purakayastha and Katyal, 1998). The high floodwater pH and high ammoniacal-N arising from the rapid hydrolysis of urea (Vlek and Stumpe, 1978) coupled with high floodwater temperature, lead to a high potential for volatilization losses of applied urea (Son and Buresh, 1993). Earlier studies have shown that the total N losses due to NH_3 volatilization are in the range of 20 to 80% (Craswell and Vlek, 1979; Rao, 1987; De Datta *et al.*, 1989; Freney *et al.*, 1990).

The low efficiency of N utilization by rice and the high losses of N applied cause substantial economic loss to farmers and create negative impacts in the environment. The challenge, therefore, is to develop a technology that can curtail the high N losses and improve the poor N use efficiency by rice. An increase in the N use efficiency would allow yield increases using the same or smaller quantities of N fertilizer. Technologies must be simple, inexpensive, and thus economically practical ensuring adoptability and greater returns to farmers, and at the same time, minimizing adverse environmental impacts.

Simpson *et al.* (1988) reported that techniques, which reduce NH_3 volatilization losses, could be expected also to reduce total gaseous N losses. In the past, several approaches have been generated and employed to control NH_3 volatilization losses and improve N use efficiency. These include, among others, the use of urease inhibitors (Freney *et al*., 1993), algicides (Simpson *et al*., 1988) and monomolecular surface films (Cai *et al*., 1987). Primarily, these treatments reduced NH_3 volatilization losses through their effects on algal growth and floodwater pH (Simpson *et al*., 1988). Most of them, however, are expensive (Damodar Reddy and Sharma, 2000) and would entail more costs to farmers in excess to their savings. Recently, the use of *Azolla* in improving the efficiency of applied urea has generated interest (Villegas and San Valentin, 1989; Vlek *et al*., 1992; Boadilla, 1993; Vlek *et al*., 1995; Cissé, 2001). This aspect of *Azolla* utilization is relatively new and has good potential but has not yet been thoroughly explored under field conditions.

Azolla is a small aquatic fern found in temperate and tropical regions of the world. Its importance in lowland rice production has been mainly related to its contribution to the N nutrition of the rice through biological N fixation (BNF). As such, it has been used for centuries as a biofertilizer. The fern provides N for rice through its symbiotic relationship with the blue-green algae, *Anabaena azollae*, living in the cavity of its leaf. *Azolla* is noted for its rapid growth. It can multiply reproductively through the production of spores and vegetatively through fragmentation. The significant contributions of *Azolla* aside from supplying N on flooded rice fields were extensively reviewed by Mandal *et al*. (1999).

The hypothesis is that NH_3 volatilization can be reduced if a barrier is present at the surface of the floodwater. By covering the floodwater surface, *Azolla* could act as a physical barrier and influence some physical, chemical and microbiological processes in the floodwater. It could conserve N by trapping the NH_3 liberated from the urea, absorb the incoming solar radiation and suppress the algae-induced rise in floodwater pH. Results from experiments conducted under greenhouse conditions in Germany and in the Philippines seem promising and support the above hypothesis. They have demonstrated that an *Azolla* mat growing on the floodwater surface can minimize NH_3 volatilization losses and improve N use efficiency by preventing a large increase in floodwater pH and by maintaining lower floodwater temperatures (Villegas and San Valentin, 1989; Vlek *et al*., 1992; Vlek *et al*., 1995; Cissé, 2001). Field research verifying these results is, however, very limited and the results obtained were inconclusive. It is necessary, therefore, to verify and provide concrete evidence of the positive impacts of an *Azolla* cover with regards to minimizing NH_3 volatilization losses and enhancing urea efficiency under field conditions, in order to promote the adoption of this management approach to farmers. Thus, the aim of this study was to elucidate the potential benefits of having an *Azolla* cover at the time of urea application under flooded rice conditions in on-station and on-farm fields in a province in the Philippines.

Specifically, the study intended to:

1. Assess the influence of an *Azolla* cover on the floodwater chemistry and its relation to NH_3 volatilization losses;
2. Compare the N recoveries from urea-amended, *Azolla*-covered treatments with those of urea applied alone using the ^{15}N tracer technique;
3. Evaluate the response of rice to the presence of an *Azolla* cover in terms of N uptake, crop growth and yield.

2 Review of literature

2.1 Fertilizer trend and usage

By the year 2050, the world population will double its current level of more than 6 billion. The Asian population, in particular, is expected to rise by 53% over the next 30 years (Hossain and Singh, 2000). Consequently, the demand for food grains will also escalate. The International Food Policy and Research Institute (1996) forecasts a 39% increase in cereal demand between 1995 and 2020, while Hossain and Singh (2000) report a 65% increase by the year 2020.

To meet this high demand, 80% of the additional grain will have to come from yield increases rather than from farmland expansion (Maene, 2000). The developing world, where the potential for crop area expansion is limited, will be responsible for most of these increases. Therefore, the growing demand for staple grains will rely on the intensive use of chemical fertilizers.

FAO data show that chemical fertilizer use has steadily increased over the last decades and this trend is likely to continue in the coming years. The annual fertilizer use is expected to rise from an average of 134 million tons between 1995 and 1997 to about 180 million tons by 2030, with a range of plus or minus 10% depending on the improvement in the efficiency of fertilizer use. This represents an annual growth rate of about 1% per annum (FAO, 2000).

Nitrogen fertilizer consumption, in particular, increased from 12 to 81 million tons between 1960 and 1998 (Maene, 2000). Other statistics report a rise from 3.6 million tons in 1950 to 85 million tons in 1990 (Ayoub, 1999). Gilland (1998) asserts that the 74 million tons per year of nitrogen fertilizer currently used for agricultural production will increase to 200 million tons by 2050 – an annual growth rate of ~1.9%. This intensive use of nitrogen and other fertilizers was one of the main factors in the increase in the average world cereal grain yields from 1.13 t ha^{-1} in 1950 to 2.76 t ha^{-1} in 1990 (Brown, 1996).

Rice, wheat and maize are the major users of fertilizer, and account for over 50% of the global fertilizer use (FAO, 2000). Rice, in particular, accounts for more than

40% of the total fertilizer consumption, and 20% of all N fertilizer production (Hossain and Singh, 2000).

In the Philippines, there was a significant positive correlation (r=0.73*) between the growth of rice production and urea consumption from 1981 to 1999. Urea consumption has been increasing for the last two decades. In 1999 alone, Filipino farmers used ~294,172 Mt of urea which is equivalent to ~135,319 Mt of nitrogen. For the same year, the Philippines imported 313,719 Mt of urea, an increase of 23.9% from the previous year (FAO statistics). With price of US$ 267 per Mt, cost to the country amount to about US$ 8.4 M, 15% higher than the 1998 cost of urea imports.

Nitrogen fertilizers are produced using natural gas, petroleum and coal that supply the energy and hydrogen required to convert atmospheric N to ammonia. The process, therefore, draws fossil fuel reserves, which are non-renewable resources. In this sense, modern agricultural production systems are non-sustainable (Hossain and Singh, 2000).

2.2 Nitrogen use efficiency

Nitrogen use efficiency (NUE) is defined as the production of grain per unit of N absorbed from the soil. The efficiency with which N is used by rice depends on factors such as the physiological efficiency with which plant N is used to increase grain yield; the uptake per unit of N applied; and the agronomic efficiency which is the increase in grain yield per unit of N input (Moll *et al*., 1982).

Nitrogen use efficiency is commonly studied in terms of the amount of fertilizer N applied and referred to as fertilizer N use efficiency (Liu, 2000). Parr (1973) defined fertilizer N use efficiency as the percentage recovery of fertilizer N by a crop. It is calculated by taking the difference in the N uptake by the aboveground parts of fertilized plants with that of the unfertilized plants.

Fertilizer N use efficiency is determined primarily by the crop's growth rate and its nutrient demand, and also by the ability of the plant to compete effectively with other processes that draw off nutrients (Zaman, 1987; Buresh *et al*., 1988a; De Datta *et al*., 1990). The amount of N taken up by plants depends on several factors such as the N-supplying capacity of the soil, the previous N uptake, the developmental stage of the plant when N was applied, and the crop's yield potential (Wuest and Cassman, 1992b).

There seems to be a general consensus that recovery of applied N by rice is very inefficient (Craswell and Vlek, 1979; Vlek and Fillery, 1984; Vlek and Byrnes, 1986) and thus, is a major drawback in achieving high agronomic efficiency (Rasmussen and Rohde, 1991; Finck, 1992; Awasthi, 1999). The uptake efficiency of applied N ranges from 20 to 60% with an average efficiency of only 30 to 40% in most areas (http://riceweb.org). Recent on-farm studies found no strong evidence to indicate that this low fertilizer N efficiency by rice has increased over the past years (Dobermann *et al.*, 2002). This low N efficiency reflects poor agronomic management and the highly dynamic nature of N in the soil-floodwater system, which leads to gaseous losses. Aside from high losses as gas, N is transported to the ground and surface waters. These factors cause direct economic loss to farmers and exert negative impacts on the atmosphere and water quality (Xing, 2000).

An article from the Rice Web (http://riceweb.org) reported that in most Asian countries, irrigated rice farmers apply N at the rate of 60 to 90 kg N ha^{-1} during the wet season and 100 to 150 kg N ha^{-1} during the dry season. If a grain yield of 6 t ha^{-1} is to be achieved, the crop should take up approximately 100 kg N ha^{-1}. Assuming that the N uptake efficiency is 50%, and that the soil can support a yield of 3 t ha^{-1} without any N fertilizer being applied (N uptake of 45 kg ha^{-1}), a farmer must apply N at the rate of 110 kg N ha^{-1} to obtain a 6 t ha^{-1} grain yield. If the average N uptake efficiency of 30 to 40% is used, the N rate would have to be increased to ~180 and 135 kg N ha^{-1}, respectively.

In view of the large quantities of N involved, poor N use efficiency by rice not only causes significant economic loss to farmers but to the national economy as well. For example, at a wholesale price of US$ 0.66 per kg of N in urea, US$ 10.6 billion could be lost if only 80% of the approximately 80 Mt of N used in world agriculture in 1998 was utilized. According to the International Fertilizer Industry Association (IFIA), a 1% increase in cereal NUE would today be worth US$ 400,000,000 (http://fertilizer.org/ifa).

On the basis of the above-mentioned economic and environmental reasons, it is imperative to improve the management of N fertilizers applied to rice. Management of N is not only important to farmers engaged in agricultural production, but also to researchers and environmentalists concerned with the effects of lost N on climate change and the ozone layer (Peoples *et al.*, 1995). The interests of each are not mutually exclusive. Responsible management aimed at increasing the efficiency of N fertilizer use by crops will ensure greater returns to farmers, and provide incentives for reducing its negative impacts on the environment (Freney *et al.*, 1995). Efficient N fertilization, which can be the key to sustainable productivity, is synonymous with minimizing N losses to the environment, without sacrificing crop yields (Maene, 2000).

2.3 Ammonia volatilization

Ammonia volatilization is the transfer of NH_3 from floodwater to the atmosphere across a water-air interface (Jayaweera and Mikkelsen, 1990). Ammonia is produced from applied fertilizers containing ammonium N or from ammonium formed in the process of urea hydrolysis. The chemical dynamics of NH_3 from floodwater is as follows (Jayaweera and Mikkelsen, 1990):

$$NH_4 \xleftrightarrow[\kappa_a]{\kappa_d} H^+ + NH_{3(aq)} \xrightarrow{\kappa_{vN}} NH_{3(air)}$$

where,

κ_d = dissociation rate constant for NH_4/NH_3 equilibrium, first order,

κ_a = association rate constant for NH_4/NH_3 equilibrium, second order, and

κ_{vN} = volatilization rate constant for NH_3, first order.

Research results indicate that NH_3 volatilization is a major process contributing to N losses in flooded rice (Bouldin and Alimagno, 1976; Mikkelsen *et al.*, 1978; Vlek and Stumpe, 1978; Craswell and Vlek, 1979; Fillery *et al.*, 1984; Weeraratna and Craswell, 1984; Fillery and de Datta, 1986). The application of urea provides conditions in the soil-floodwater system conducive for NH_3 volatilization to proceed. Fillery *et al.*

(1984) reported NH_3 losses amounting to 47% when urea is applied at transplanting. When it is broadcast onto the floodwater 10 days after transplanting, the measured losses ranged from 10 to 56% (Freney *et al.*, 1990). In the Philippines, Fillery *et al.* (1986) found extensive N losses ranging from 45 to 60%, 14 to 21 days after transplanting. Depending on the N source, method of application, and management, NH_3 volatilization from flooded rice fields typically ceases about 7 to 14 days after fertilizer N application (Fillery and de Datta 1986; Fillery *et al*., 1984).

Ammonia losses from the floodwater have been evaluated using micrometeorological techniques, but they are expensive to install and maintain. Vlek and Craswell (1981) proposed an alternative method that assesses the potential loss by measuring floodwater chemistry parameters such as total ammoniacal-N, floodwater pH and temperature that determine the NH_3 partial pressure. This approach is simple, inexpensive and easier to adopt (Rao, 1987).

Floodwater pH influences the equilibrium between ammonium and ammonia. It fluctuates markedly and closely in parallel with NH_3 losses (Fillery *et al*., 1984), and must be considered a major factor contributing to NH_3 losses in the field. The relative concentration of NH_3 increases from 0.1 to 1, 10 and 50% as the pH changes from 6 to 7, 8 and 9, respectively (Peoples *et al*., 1995). Other findings show an increase in the ratio of NH_3 to NH_4^+ from 0.056 to 5.6 (at 25^oC) as the pH increases from 8 to 10 (Freney *et al*., 1985; Leuning *et al*., 1984).

Several authors have noted a correlation between the increase in daytime pH and the volatilization of ammonia. Mikkelsen *et al*. (1978) noted a daytime pH of up to 10, and measured NH_3 losses representing up to 20% of the urea-N broadcast onto the floodwater. Biological activity influences the pH of the floodwater, thus contributing significantly to NH_3 losses in the rice fields. The depletion of CO_2 in floodwater during photosynthesis increases floodwater pH in the day (Bouldin and Alimagno, 1976; Mikkelsen *et al*., 1978; Craswell *et al*., 1981).

Temperature exerts its influence on volatilization losses in such a way that, as the temperature rises, the relative proportion of ammonia to ammonium present at a given pH increases, while the solubility of NH_3 in water decreases. High temperatures also increase the diffusion of NH_3 through the soil, and affect the rate of microbial transformations (Freney *et al.*, 1983).

There are other factors, however, aside from these floodwater factors that influence NH_3 volatilization. Wind speed through its influence on the rate of transport of NH_3 away from the air-water or air-soil interface is another major factor influencing volatilization (Freney *et al*., 1981; Vlek and Craswell, 1981; Denmead *et al*., 1982; Fillery *et al*., 1984). Other variables influencing NH_3 volatilization include pH-buffer capacity and cation exchange capacity of the soil, levels of urease activity, availability of moisture, soil texture, nitrification rate, and the presence of plants or plant residues (Freney and Black, 1988). In rice systems, factors such as fertilizer composition, rate, time and method of application, floodwater depth, and algal growth, exert their influence through the primary variables – ammoniacal-N concentration, pH and temperature of floodwater, and wind speed (Peoples *et al*., 1995).

Ammonia volatilized from fertilizer N not only leads to N losses but also becomes a source of NO_x when it reacts with OH in the stratosphere (Xing, 2000). Furthermore, the volatilized NH_3 serves as a second source of N_2O and NO when it returns to the soil through wet and dry deposition, and thus, reduces the soil capability as an atmospheric methane sink (Mosier *et al*., 1991).

2.4 Management techniques to minimize ammonia losses

Several techniques have been devised in the past to reduce NH_3 losses from floodwater in rice fields. These include gypsum coating of urea (Tripathy *et al*., 1999), the use of algicides and biocides (Simpson *et al*., 1988), and the use of urease inhibitors to delay urea hydrolysis (Freney *et al*., 1993; Chaiwahnakupt *et al*., 1996). Slow release fertilizers such as the sulfur coated urea which works by reducing the release rate of N was also tested (Craswell and Vlek, 1979; Vlek and Craswell, 1981). These techniques, however, have met limited success in the field.

A potential method in minimizing NH_3 losses under lowland conditions is to provide a physical barrier on the surface of the floodwater to prevent aqueous NH_3 from leaving the water. Monomolecular films and long chain alcohols were tested in Australia and found to reduce NH_3 volatilization. The surface film, however, increased floodwater temperature, was dispersed easily by wind, and was therefore, unstable. Its effect was also short-lived due to the microbiological decomposition of the alcohol (Cai *et al*., 1987). The search for other ways to lower the high NH_3 losses and improve the poor N use efficiency has led to the use of *Azolla* as a possible alternative management technique.

2.5 *Azolla*

Azolla is a small, floating, water fern found all over the world. There are six species recognized, namely, *A. pinnata, A caroliniana, A. filiculoides, A. mexicana, A. microphylla* and *A. nilotica* (Moore, 1969). The agronomic importance of *Azolla* for lowland rice is well recognized and mainly lies in its capability to provide N for rice due to its symbiotic relationship with *Anabaena azollae* living in the cavities of its leaf. Farmers in Vietnam and China have been using the fern as green manure for centuries (Lumpkin and Plucknett, 1982).

2.5.1 *Nitrogen fixed by Azolla*

The growth and amount of N fixed by *Azolla* in the field depends among others on climatic factors, rice growth, and the nutrient status of the medium. Singh and Singh (1987) also reported a variation in N fixed depending on the species and their growth.

The growth and N_2-fixation of *Azolla* were generally higher in the dry season than in the wet season because of higher solar radiation (Singh and Singh, 1995). The density of the rice crop also significantly influences the growth and N_2-fixation of the fern. Higher leaf area index (LAI) of rice due to increasing rates of fertilizer N application can cause more shading to *Azolla* plants and reduce their growth and N_2-fixation later in the season (Singh and Singh, 1988).

Singh and Singh (1989) reported that fresh *Azolla* grown in a fallow rice field with a biomass of approximately 20 t ha^{-1} contains 28.7 to 36.8 kg N ha^{-1}. On the basis of the growth and N uptake by rice, Singh *et al*. (1985) also showed that one layer of *Azolla* was equivalent to 30 kg N ha^{-1}. Oliveros *et al*. (1983) found that an average N accumulation of *Azolla* grown continuously with a rice crop in dual culture reached a maximum of 1 to 2 kg N ha^{-1} day^{-1}. As much as 60 to 120 kg N ha^{-1} was produced with 3 to 4% N content of *Azolla* with a doubling time of 7 to 10 days.

2.5.2 *Release and availability of Azolla-N to rice*

Azolla growing with rice releases some mineral N in the floodwater. The fern's nitrogen contribution to rice, however, is highest when it is incorporated into the soil as a green manure (Rosenani *et al.*, 1992). Singh (1979) reported that 80% of the NH_4^+ is released from fresh *Azolla* 3 weeks after incubation. Data obtained by Saha *et al*. (1982) showed that 56 to 75% *Azolla*-N was released after 3 to 6 weeks of incubation. Watanabe *et al*. (1977) showed that about 75% of total nitrogen is mineralized in 6 to 8 weeks.

It is reported that the availability of *Azolla*-N is closely related to its C:N ratio and that the C:N ratio is the main indicator of *Azolla* quality. Liu (1979) observed that *Azolla* decomposes and supplies N quickly if the C:N ratio is about 10; and thus *Azolla* can be considered an efficient biofertilizer for increasing soil fertility as well as rice productivity (Satapathy, 1998). However, if the C:N ratio is 17, it cannot supply N quickly and so N is not available to the current crop.

Ito and Watanabe (1985) showed that approximately 50% of the *Azolla*-N incorporated into the soil was recovered by the rice plants at 42 days after transplanting (DAT), indicating that *Azolla*-N is rapidly mineralized and becomes available to the plants. Galal (1997) reported that under Egyptian conditions, approximately 43% of the total N taken up by the rice plant comes from the N fixed and released by *Azolla*. The distribution pattern of N depends upon the time of incorporation. When *Azolla* was incorporated at 30 DAT, 65% of the *Azolla*-N was recovered in the straw, and only 15% in the grain. Incorporating *Azolla* at 78 DAT increased the amount of N recovered in the grain to about 50% (Ito and Watanabe, 1985).

2.5.3 *Effect of Azolla on grain yield and yield components*

Azolla incorporated into the soil, or applied in combination with urea, or used as a dual crop floating on the floodwater surface of rice plants produced higher grain yields than treatments without *Azolla* (Singh and Singh, 1986; Guthbrod, 1987; Lales *et al.*, 1987; Setty *et al*., 1987). The increase in rice yield due to *Azolla* incorporation is well established (Kannaiyan, 1995). Earlier studies by Moore (1969) reported a 14 to 40% increase in rice yield with *Azolla* incorporation. Oliveros *et al*. (1983) found grain yield with *Azolla* higher by as much as 1.5 t ha^{-1}, which was mainly attributed to the increase in plant height, tiller number and dry weight of the crop. From the International Network for Soil Fertility and Fertilizer Efficiency for Rice (INSFFER) experiments, an increase of 44 kg grain per ton of applied *Azolla* was estimated (Ito and Watanabe, 1985). Kolhe and Mittra (1989) found a 51% increase in grain yield when 30 kg N ha^{-1} urea was combined with *Azolla* incorporated at the rate of 10 t ha^{-1} before transplanting as compared with only urea. Incorporating *Azolla* at the rate of 5, 10 and 15 t ha^{-1} increased grain yield of rice in India by 32, 47 and 56% compared to the control during the wet season (Singh and Mandal, 1997). Application of *Azolla* at 10 t ha^{-1} was comparable to the application of inorganic fertilizer at 30 kg N ha^{-1}. Recent experiments by Jayanthi *et al*. (1998) showed that N use efficiency increased by 15% with the combined use of 40 kg N ha^{-1} urea and *Azolla*. Their results further suggest that *Azolla* incorporated at the rate of 10 t ha^{-1} is equivalent to 40 kg N ha^{-1}.

Even when *Azolla* is not incorporated, an increase in the grain yield of rice was observed. *Azolla* dual cropped with rice led to a rice yield increase of 6% (Singh, 1977), 40% (Singh, 1985) and 23 to 67% (Talley *et al*., 1977).

2.5.4 *Other beneficial effects of Azolla*

Dual cropping of rice with *Azolla* results in the enhancement of soil organic matter (Vendan, 1998). *Azolla* showed superiority over the chemical N fertilizer application alone by increasing the organic carbon, total N and available phosphorus (P) of the soil (Singh *et al*., 1988). Saha *et al*. (1982) observed an increase in soil total N due to the incorporation of *Azolla* compared to the control. Part of this increase could be due to the NH_3 excreted by *Azolla* during its growth. Their investigation showed that incorporation of *Azolla* in rice field soils can bring about appreciable changes in some of the electro-chemical and chemical properties of the soils particularly in the redox potential, and the availability of N and P.

2.5.5 *Critical factors affecting Azolla growth*

Being an aquatic fern, water is *Azolla*'s most fundamental requirement. *Azolla* grows well both on deepwater surfaces and on shallow ponds (Quebral, 1989).

Among the essential elements, phosphorus is the nutrient that most commonly limits *Azolla* growth. Results of greenhouse studies showed that a floodwater P concentration greater than 0.1 mg kg^{-1} is needed to obtain a maximum biomass production of *Azolla* (Ali and Watanabe, 1986). Soils with low levels of available P likewise have severely reduced *Azolla* growth (Tilo *et al*., 1989). For good *Azolla* growth, the soil must contain more than 20 mg kg^{-1} available P (Olsen P) (San Valentin *et al*., 1986). In soils deficient in P, split application of 15 kg P_2O_5 or basal application of 30 kg P_20_5 is recommended (Watanabe *et al*., 1980). An economic analysis done by Kikuchi *et al.* (1984) using the 1980 and 1981 prices showed that the return to N fertilizer savings after deducting the cost of P fertilizer for *Azolla* was comparable to the minimum estimate in South Cotabato, Philippines where P fertilizer is not required.

Light intensity is the most important microclimatic factor affecting the growth of *Azolla* (Kröck and Watanabe, 1985). Singh and Singh (1989) observed that at low solar intensity, the growth of *Azolla* inside a full rice canopy is restricted.

The reported influence of N fertilizer varies. Lumpkin and Plucknett (1982) suggested that N fertilizer does not directly influence *Azolla* growth. The poor growth of *Azolla* when N fertilizer was applied was attributed to the increased competition with other organisms whose growth is stimulated by the nitrogen. Singh and Singh (1988), however, found that the growth and N_2-fixation of *Azolla* are significantly reduced at higher rates of fertilizer N. Singh (1998) made similar observations. *Azolla* growth is not affected by urea applied at the rate of 30 kg N ha^{-1}, but increasing the rate to 60 kg N ha^{-1} caused a significant reduction in the fresh weight and N yield of *Azolla*. Uheda and Kitoh (1992) noted similar findings. The high concentration of ammonium ions in the medium effectively inhibits the nitrogen-fixing ability of the fern, but they have no adverse effects on the growth of *Azolla* when their concentration is low (Peters *et al.*, 1981).

2.5.6 *Amount of Azolla inoculum and time of inoculation*

Singh (1979a) recommended an initial inoculum of 0.1 to 0.3 kg m^{-2} (1.0 to 3.0 t ha^{-1}) for multiplication plots in India. Singh (1989) recommended a lower rate of *Azolla* inoculum (0.5 to 1.0 t ha^{-1}) when applied one week after transplanting. Singh and Singh (1995) suggested an *Azolla* inoculum of 0.5 t ha^{-1} applied a week after transplanting for intercropping *Azolla*. In Vietnam, Tran and Dao (1973) suggested a 0.5 kg m^{-2} (5 t ha^{-1}) inoculum, while in the Philippines, recommendations of 0.2 to 0.5 kg m^{-2} (2.0 to 5.0 t ha^{-1}) are common (Quebral, 1989).

A higher inoculum rate produces full cover in a relatively short time. *Azolla* inoculated at the rate of 3.0 t ha^{-1} can cover the floodwater surface in 10 days (Singh and Singh, 1986) while *Azolla* inoculated at 2.0 t ha^{-1} filled up a fallow rice field in 15 days (Singh and Singh, 1989). At a lower inoculum rate (0.5 t ha^{-1}), *Azolla* covered the fields in 20 days (Singh *et al*., 1985). The inoculum requirement is lower when *Azolla* is inoculated at the early stages of rice growth (Liu, 1979). However, at low density, *Azolla* maybe overgrown by other plants like algae and weeds.

Azolla inoculated at high rates before transplanting and attaining full cover at transplanting tends to damage the rice seedlings. Therefore, it is recommended that *Azolla* be applied one week after rice has been transplanted (Singh, 1982b). It is likewise recommended that *Azolla* be used with 20 to 22 day-old rice seedlings.

2.5.7 *The use of Azolla to improve N use efficiency*

In the Philippines, *Azolla* is being promoted as a supplement for chemical N. Farmers claim that the fern helps reduce farm expenses and suppresses weeds in the paddy (Suva *et al*., 1989). *Azolla* is likewise being utilized as feed for fish, poultry and livestock, and as compost for upland crops. Its culture, management and utilization in the Philippines have been described fully in a book published by the National *Azolla* Action Program (NAAP) in 1989.

The use of *Azolla* to reduce NH_3 losses and increase the low fertilizer use efficiency of N is a relatively new aspect in *Azolla* utilization. Results of a laboratory study conducted in the Philippines (Villegas and San Valentin, 1989) showed that the amount of NH_3 that volatilized was low in the presence of an *Azolla* cover. *Azolla* reduced the floodwater pH by 2.0 pH units and the floodwater temperature by 1 to 2^oC. Boadilla (1993) found a reduction of 0.5 to 1.4 units in floodwater pH and 2 to 5^oC in floodwater temperature in the presence of an *Azolla* cover that ultimately led to an 80 to 90% reduction in NH_3 losses. Experiments conducted by Vlek *et al*. (1992), Vlek *et al*. (1995) and Cissé (2001) under greenhouse conditions in Germany confirmed these results. *Azolla* can reduce NH_3 volatilization losses from rice fields by forming a physical barrier to the NH_3 liberated; by intercepting the incoming solar radiation necessary for algal growth; and by absorbing part of the N applied (Mandal *et al*., 1999). At the same time, it can fix N without altering or increasing the floodwater pH (Tel-Or *et al*., 1991; Vlek *et al*., 2002).

Liu (1979) reported that, unlike chemical N fertilizers and other N_2-fixers, *Azolla* neither contaminates the environment nor consumes the photosynthates of rice plants. Therefore, the use of *Azolla* in rice cultivation can be considered a promising technique to improve the poor N use efficiency, thus increasing the farmers' profits and providing protection from the environmental pollution caused by the intensive use of chemical fertilizers (Galal, 1997).

3 Materials and methods

On-station field experiments were carried out in 1998-99 to evaluate the use of *Azolla* in combination with different rates of urea as a management technique to improve the N use efficiency of rice. The technique was later verified and assessed in farmers' fields in 2000-01.

3.1 Location

The study was conducted in the province of Laguna, Philippines (14°23'N and 21°25'N latitude and 116°E and 127°E longitude) (Figure 1).

3.1.1 *On-station field experiments*

Two field experiments were conducted at the Central Experiment Station (CES) inside the University of the Philippines Los Banos (UPLB) compound (~14°11'N latitude, 121°15'E longitude, 21 m above sea level) during the 1998-99 dry season. Experiment 1 was established in November, and Experiment 2 in February. The second experiment was located about 300 m from the site of the first experiment. Selected physico-chemical properties of the soil at the experimental sites are shown in Table 1.

Table 1: Physico-chemical characteristics of the soil at the Central Experiment Station fields. Los Banos, Philippines. Dry season, 1998-99.

Characteristics	Experiment 1 Soil depth (cm) 0-15	 15-30	Experiment 2 Soil depth (cm) 0-15	 15-30
pH	6.05	6.44	5.51	6.61
Total nitrogen (%)	0.16	0.06	0.23	0.09
Available P (mg kg^{-1})	11.79	3.86	16.37	12.09
Exchangeable K (cmol kg^{-1})	1.14	0.35	1.28	0.90
Organic matter (%)	2.58	0.69	3.89	1.30
CEC (cmol kg^{-1})	41.1	36.8	44.7	41.3
Bulk density (g cc^{-1})	0.69	0.96	0.52	0.94
Texture	Clay	Clay	Clay	Clay

pH	Potentiometric (1:1 Water) (PCARR, 1980)	CEC	Ammonium acetate extraction (PCARR, 1980)
Total nitrogen	Kjeldahl method (PCARR, 1980)		
Available P	Olsen P (PCARR, 1980)	Bulk density	JSIDRE (1983)
Exchangeable K	Ammonium acetate extraction (PCARR, 1980)	Texture	Hydrometer method (PCARR, 1980)
Organic matter	Walkley and Black (PCARR, 1980)		

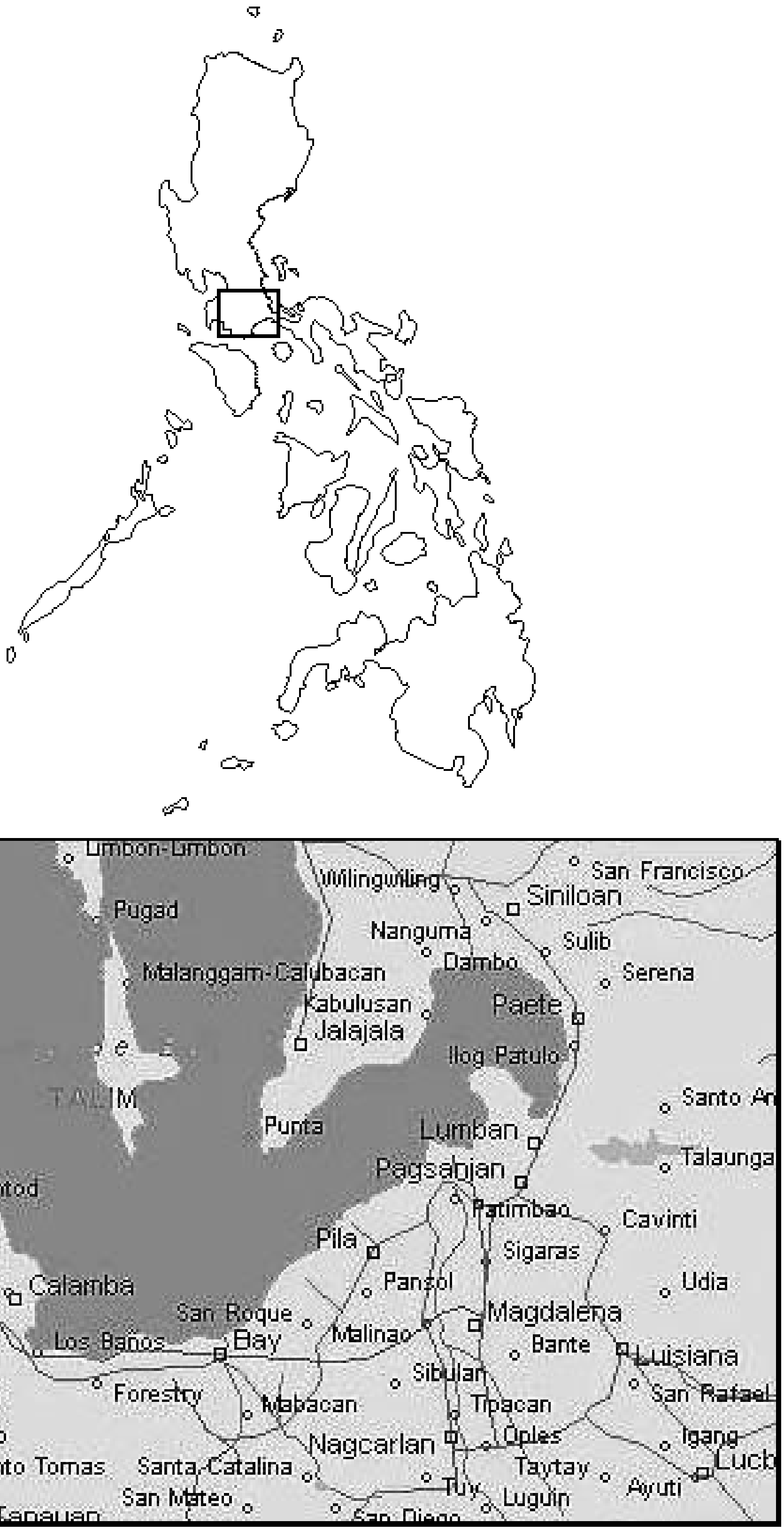

Figure 1: Map showing the Laguna province, Philippines.

Daily solar radiation, rainfall, and maximum and minimum temperature data during the season (Figure 2) were obtained from the International Rice Research Institute (IRRI) weather station ($14^o11'$ latitude and $121^o15'$ longitude). The monthly rainfall throughout the cropping period (November to April) was unusually high when compared with the 18-year rainfall record (1979-1997) of the same months (Appendix I). The total monthly rainfall in December and March were more than fourfold and sevenfold higher than the 18-year average rainfall of the same months. This heavy rainfall was attributed to the La Nina weather phenomenon, which affected the country during this period (www.uswaternews.com).

3.1.2 *On-farm field experiments*

To assess the feasibility of applying the technology in farmers' fields, 10 identical experiments were conducted during the wet season (May to October) and 8 identical experiments during the dry season (November to April) of 2000-01 in fields with low N status. Sites were chosen on the basis of the results of pot experiments conducted prior to the actual field experiments using PhilRice's "minus one element kit" for the diagnosis of multiple nutrient deficiencies (PhilRice Technoguide, 2001). Two experiments each were established in three municipalities (Maahas, Los Banos; Puypuy, Bay; and San Francisco, Victoria) and four experiments in Sta. Cruz (Labuin and Bubukal) during the wet season. The two experiments, however, in Bubukal, Sta. Cruz, were terminated at the late vegetative stage because of heavy rat infestation.

During the 2000-01 dry season, the field trials were carried out in the same municipalities but several changes were made. First, the farmer-cooperators in Los Banos and Bay were replaced because they declined to have their farms used for the experiments for another season. Second, field experiments were not established in Bubukal, Sta. Cruz because of the above mentioned rat infestation. Table 2 presents selected physico-chemical properties of the soil at the sites.

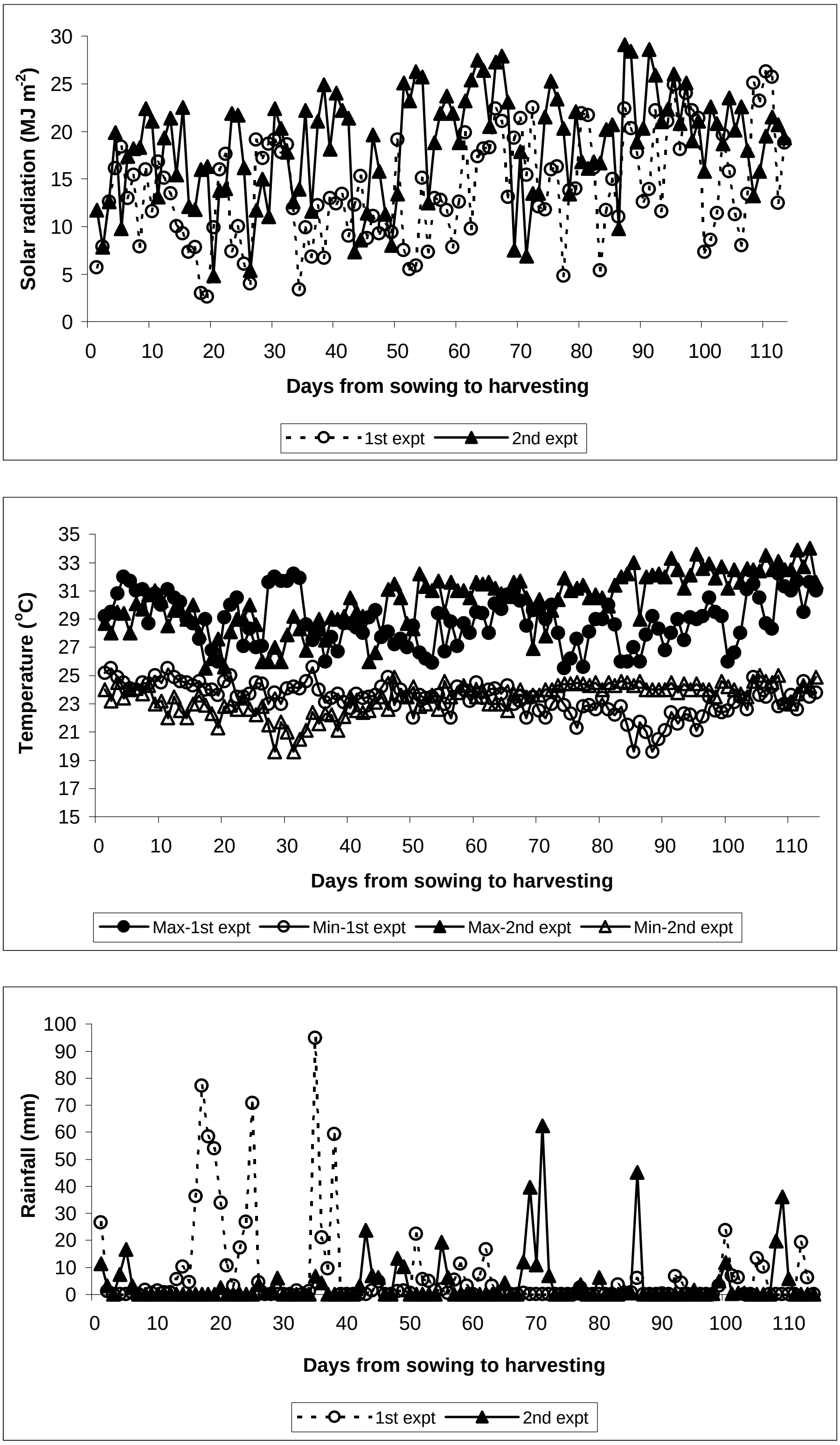

Figure 2: Weather conditions during the growth stages of rice. Los Banos, Philippines. Dry season, 1998-99. (First experiment sown on November 23; second experiment sown on January 17)

Table 2: Physico-chemical characteristics of soil in farmers' fields. Laguna, Philippines. Wet and dry seasons, 2000-01.

Characteristics	Maahas Los Banos	Puypuy Bay	San Francisco Victoria	Labuin Sta Cruz	Bubukal Sta Cruz
pH	5.80	5.90	6.50	5.50	5.60
Total nitrogen (%)	0.26	0.22	0.19	0.15	0.17
Available P (mg kg^{-1})	20.96	39.86	9.44	85.96	60.69
Exchangeable K (cmol kg^{-1})	0.79	0.76	0.64	0.13	0.28
Organic matter (%)	5.11	4.43	3.76	3.24	3.49
CEC (cmol kg^{-1})	37.5	39.7	31.3	18.4	27.4
Texture	Clay	Silty clay	Clay loam	Loam	Si cl loam

pH	Potentiometric (1:1 Water) (PCARR, 1980)
Total nitrogen	Kjeldahl method (PCARR, 1980)
Available P	Olsen P (PCARR, 1980)
Exchangeable K	Ammonium acetate extraction (PCARR, 1980)
CEC	Ammonium acetate extraction (PCARR, 1980)
Organic matter	Walkley and Black (PCARR, 1980)
Texture	Hydrometer method (PCARR, 1980)

The experimental fields were plowed once, harrowed twice and leveled before the onset of the cropping seasons. Details of the cultural management practices are presented in Appendices II, III and IV.

3.2 Experimental layout and treatments

Ten treatment combinations consisting of five nitrogen levels applied alone or combined with *Azolla* were laid out in a Randomized Complete Block Design (RCBD) with four replicates during the 1998-99 dry season experiments.

Plot size was 20 m^2 (4 m x 5 m). Each individual plot was surrounded by a levee (30 cm wide) to prevent cross contamination of treatments between plots. All plots were flooded to a depth of ~5 cm two days after transplanting up to 2 weeks before harvest.

The treatment combinations employed were as follows:

T_1	Control (without N and without *Azolla*)	
T_2	0 kg N ha^{-1}	with *Azolla* cover
T_3	40 kg N ha^{-1}	
T_4	40 kg N ha^{-1}	with *Azolla* cover
T_5	80 kg N ha^{-1}	

T_6	80 kg N ha^{-1}	with *Azolla* cover
T_7	120 kg N ha^{-1}	
T_8	120 kg N ha^{-1}	with *Azolla* cover
T_9	160 kg N ha^{-1}	
T_{10}	160 kg N ha^{-1}	with *Azolla* cover

The N rate was tested up to 160 kg N ha^{-1} to determine if *Azolla* would still improve the efficiency of applied N at this high level.

The treatments in the 2000-01 wet and dry season experiments in farmers' fields were identical to the on-station field experiments except that N rates were reduced from five to three. For the wet season, the treatments were arranged as follows:

T_1	Control	(without N and *Azolla*)
T_2	0 kg N ha^{-1}	with *Azolla* cover
T_3	40 kg N ha^{-1}	
T_4	40 kg N ha^{-1}	with *Azolla* cover
T_5	80 kg N ha^{-1}	
T_6	80 kg N ha^{-1}	with *Azolla* cover

For the 2000-01 dry season plantings, N rates were increased from 40 to 50 kg N ha^{-1}, and from 80 to 100 kg N ha^{-1}.

3.3 Planting materials

3.3.1 *Rice plant*

Rice (*Oryza sativa* L.) varieties PSBRc 54 (114-day maturity) and PSBRc 80 (112-day maturity) obtained from the Philippine Rice Research Institute (PhilRice) Los Banos were planted during the 1998-99 dry season on-station trials and during the 2000-01 wet and dry season on-farm experiments. Seeds at the rate of 40 kg ha^{-1} were soaked in water for 24 hours and incubated for 48 hours. Pre-germinated seeds were sown uniformly in a well-prepared seedbed. A wet seedbed was used for on-station field experiments, and modified dapog[1] for on-farm experiments. Twenty-day old seedlings were transplanted 20 cm x 20 cm apart at 2 to 3 seedlings per hill for on-station and on-farm field experiments. Replanting of dead and weak seedlings was done four days after transplanting (DAT) to ensure uniformity of growth and plant density per hill.

Appropriate control measures were applied to minimize insects, diseases, weeds, snails, birds, and rat infestation. In 2 of the 10 on-farm experiments during the wet season of 2000, however, rat damage was so severe that these measures were not sufficient to control the rats.

3.3.2 *Azolla*

Azolla was obtained and multiplied in the propagation ponds of the National *Azolla* Action Program (NAAP), PhilRice in Los Banos, and Luzon Polytechnic College in Siniloan, Laguna. As a result of heavy rainfall (610 mm within 10 days) during the start of the 2000-01 dry season, the propagation ponds overflowed and the *Azolla* was washed out. Inoculum was then obtained from the Don Mariano Marcos State University in Rosario, La Union (280 km north of Los Banos).

Azolla was harvested, drained and weighed a day before the scheduled inoculation time, *i.e.*, four days before the first urea application. Fifty percent of the floodwater surface was inoculated with *Azolla*, containing between 2.76 and 3.10% N, at the rate of 5 t ha^{-1} (0.5 kg m^{-2}) in plots with *Azolla* treatments. The aim was that at the time of urea application, the floodwater surface would be completely covered with *Azolla*.

3.4 Inorganic fertilizer

Based on the farmers' N application schedule, two thirds of the urea was topdressed onto the 5 cm standing water 7 days after transplanting, and the remaining one third at 7 days before panicle initiation.

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[1] By 'modified dapog' is meant seedbed on which the rice seeds are sown on soil spread on concrete floors, or plastic sheets.

Phosphorus and potassium were broadcast at a uniform dose of 30 kg P_2O_5 ha^{-1} and 30 kg K_2O ha^{-1} at the time of transplanting in the on-station experiments. For experiments carried out in farmers' fields, 60 kg P_2O_5 ha^{-1} and 90 kg K_2O ha^{-1} were applied.

3.5 ^{15}N balance determination

The effect of the *Azolla* cover on the recovery of applied N was assessed using the ^{15}N tracer technique in the on-station experiments during the 1998-99 dry season. Microplots enclosed in polyethylene plastic sheets (1.0 m x 1.0 m x 0.3 m) were inserted in the center of each main plot. The sheets were embedded ~15 cm below the soil surface, leaving ~15 cm projected above the soil surface, to prevent possible run-off. Plots were flooded a few hours before urea application and remained flooded until 2 weeks before harvest.

Except for the 160 kg N ha^{-1} treatment and the control plots, each microplot received ^{15}N-labeled urea at 4.8737%^{15}N atom excess for the first experiment and 4.7333% ^{15}N atom excess for the second experiment. ^{15}N-labeled urea was applied at the same rate and in the same way as the non-labeled urea for the first urea application in the main plots.

3.5.1 *Microplot sampling*

Plant and soil samples were taken at harvest for ^{15}N analysis. Four hills from the center of the microplot were cut at ground level and washed to remove any adhering soil. Grains were threshed and the chaff added back to the straw. Straw and grain samples were placed in separate labeled bags and dried to constant weight at 80°C in a forced-draft oven. Dry weights were recorded and samples were ground with a grinder.

Composite soil samples were taken from depths of 0 to 15 and 15 to 30 cm using an auger and placed in labeled plastic bags. They were spread on a paper in a room until they were air-dried. The clods were pulverized using a mallet and passed through a 2-mm sieve.

All samples were sent to the Institute of Agricultural Chemistry, University of Bonn for analysis.

3.5.2 *^{15}N analysis*

Plant and *Azolla* samples were further ground and milled to a homogenous fine powder at the Institute of Agricultural Chemistry. A small portion each of the straw, grain and soil samples were weighed in tin cups, balled and placed into the auto sampler of the mass spectrometer (ANCA SL coupled to 20-20 stable isotope analyzer IRMS).

The %^{15}N recovery by the plant, *Azolla* and soil was computed using the following formula (Zapata, 1990):

$$\text{Ndff}(\%) = \frac{\%^{15}\text{N atom excess of sample}}{\%^{15}\text{N atom excess of labeled urea}} \text{x} 100 \qquad \textbf{Equation 1}$$

The %^{15}N atom excess is obtained by subtracting the %^{15}N natural abundance (0.3663 atom %^{15}N) from the %^{15}N abundance of the enriched materials (urea, plant, soil, and *Azolla*).

$$\text{N yield} = \text{Dry matter yield}\left(\%\,\text{Total N of the sample}/100\right) \qquad \textbf{Equation 2}$$

$$\text{Fertilizer N yield}\left(\text{kg ha}^{-1}\right) = \text{N yield}\left(\%\,\text{Ndff}/100\right) \qquad \textbf{Equation 3}$$

$$\text{Fertilizer N recovery}\left(\%\right) = \frac{\text{Fertilizer N yield}}{\text{Rate of labeled urea applied}} \text{x} 100 \qquad \textbf{Equation 4}$$

3.6 Sampling methods and analyses

3.6.1 *Floodwater measurements (1998-99 dry season)*

Floodwater samples of about 200 ml were collected daily between 1200 and 1400 hours from the day of the initial urea application up to day 10. Samples were placed in 50 ml plastic vials, treated with 2 drops of sulfuric acid, brought to the laboratory, shaken and filtered through Whatman #42 filter paper. The concentration of ammoniacal-N in the floodwater was determined colorimetrically using the salicylate method (Keepers and Zweers, 1986).

During floodwater sampling, floodwater pH and temperature were measured *in situ* with a portable pH meter (Milwaukee/Cole Parmer pH meter-pen type) and a mercury-in-glass thermometer (maximum of 100^{o}C). Additionally, floodwater and soil temperatures were measured with a data logger on plots with and without *Azolla* cover adjacent to each other in the second experiment (Figure 3).

The partial pressure of ammonia (ρNH_3) in the floodwater was calculated from total ammoniacal-N concentration, floodwater pH, and temperature using the corrected equation of Denmead *et al*. (1983).

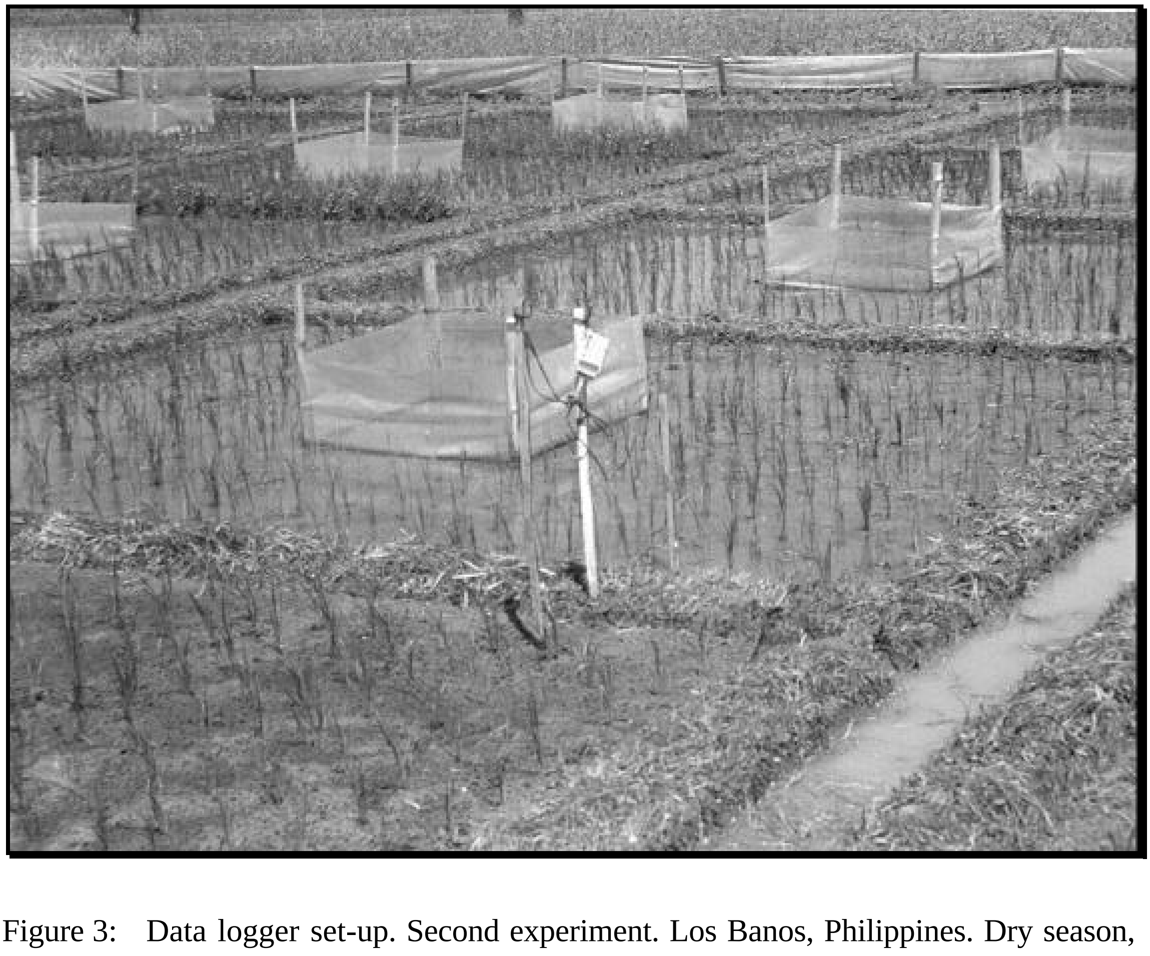


Figure 3: Data logger set-up. Second experiment. Los Banos, Philippines. Dry season, 1998-99.

3.6.2 *Soil samples*

Soils were sampled at the 0 to 15 and 15 to 30 cm depth for the on-station field experiments. Soil samples for bulk density determination were likewise taken. For on-farm trials, only surface (0 to 15 cm) soil samples were collected at each location. Soils from 20 to 30 randomly selected spots were taken after the final land preparation using a soil auger. Soils were thoroughly mixed to make a composite sample, air-dried, pulverized, and chemically and mechanically analyzed for pH, organic matter, total nitrogen, available P, exchangeable K, cation exchange capacity, and particle size analysis (clay, silt and sand).

3.6.3 *Azolla samples*

Prior to inoculation, *Azolla* was sub-sampled for total nitrogen analysis. It was dried in a forced draft oven at 80^{o}C to constant weight, ground and analyzed.

3.6.4 *Plant samples*

Tiller number Tiller number was measured from eight random hills at maximum tillering stage and from 12 random hills at harvest.

Grain yield Plant sampling at harvest followed the procedures of soil and plant sampling, and measurements of IRRI (1994).

At maturity, 125 hills (5 m^2) from each plot were cut at the base and threshed to separate the grains. Grain samples were cleaned and sun-dried to reduce the moisture content. When the moisture content was about 10 to 16%, unfilled spikelets and chaff were removed with a blower. The plot grain yield (PlotGY) was then weighed and the grain moisture content (MC_{PlotGY}) measured with a moisture tester. The plot grain yield from the harvest area was corrected to 14% moisture content ($Plot_{GY14}$) using the formula:

$$PlotGY_{14} = PlotGY \times [(100 - MC_{PlotGY})/86] \qquad \textbf{Equation 5}$$

Because drying grain to constant weight at 70°C typically results in grain with about 3% moisture content, this correction was made by adjusting the grain yield (GY_{14}) from the grain yield harvest area to 3% moisture content (GY_3) as described in this formula:

$$GY_3 = GY_{14} \times (86/97) \qquad \textbf{Equation 6}$$

Yield components Twelve additional hills were randomly selected from each plot at harvest. The 12-hill samples were used to measure the yield components, estimate total aboveground biomass, and analyze N concentration for N uptake determination.

To avoid deterioration of the samples, they were immediately processed after sampling. Any adhering soil on the stems or leaves was rinsed-off with tap water.

Panicles per hill were counted from the 12-hill samples. After counting the panicles, all spikelets (both filled and unfilled) from the panicles were stripped and placed in paper bags and properly labeled. Spikelet samples were then oven-dried at 70°C for 3 days to reduce the moisture content. After this initial drying, the weight of each sample was measured and recorded (SpW_{12hill}). A 30 to 40 gram sub-sample was immediately removed and the sub-sample weight recorded (SpW_{ss}).

To ensure a representative sub-sample, each sample was poured onto a tray and mixed thoroughly to avoid segregation of filled and unfilled spikelets. After weighing the spikelet sub-samples, the total number of filled ($FSpNo_{ss}$) and unfilled spikelets ($UFSpNo_{ss}$) in the sub-sample were separated and counted. Filled and unfilled spikelets were placed in separate bags properly labeled, and again, oven-dried as before to attain complete oven dryness. The oven-dry weights of filled ($FSpODW_{ss}$) and unfilled spikelets ($UFSpODW_{ss}$) in each sub-sample were weighed and recorded. These sub-samples were saved for grinding and N analysis.

Total spikelets per panicle (SpPan), filled spikelets per panicle (FspPan), filled spikelet percentage (FSpPct), and 1000-filled grain weights were computed using the following formulas:

$$FSpPct = \{(FspNo_{ss})/[(FspNo_{ss}) + (UFSpNo_{ss})]\} \times 100$$ **Equation 7**

$$FSpPAN = FspNo_{12hill}/PAN_{12hill}$$ **Equation 8**

$$SpPan = FSpPAN/(FSpPct/100)$$ **Equation 9**

The oven-dry thousand-grain weight ($TGODW_{COYOD}$) in grams was determined by the oven-dry filled spikelets per sub-sample ($FSpODW_{ss}$) and number of filled spikelets per sub-sample ($FspNo_{ss}$):

$$TGODW_{COYOD} = (FSpODW_{ss}/FspNo_{ss}) \times 1000$$ **Equation 10**

Straw yield To measure the straw oven-dry weight (StODW) from the 12-hill samples ($StODW_{12hill}$), the total fresh straw weight of the 12-hill samples ($StFW_{12hill}$) was first weighed and recorded after removing all spikelets. Because of limited drying space, sub-sampling was done. To avoid moisture loss, sub-sampling was done immediately after weighing the total fresh weight.

A representative sub-sample of 200 to 250 grams was taken for drying. The sub-sample weight ($StFW_{ss}$) was recorded. Straw sub-samples were then dried at 70^{o}C to constant weight. The final oven-dry weight ($StODW_{ss}$) was recorded. The oven-dry straw yield from 12 hills ($StODW_{12hill}$) was then calculated as:

$$StODW_{12hill} = (StODW_{ss}/StFW_{ss}) \times StFW_{12hill}$$ **Equation 11**

Grain:straw ratio (GSR) was then calculated as the ratio of oven-dry filled spikelets to straw yield from the 12-hill sample:

$$GSR = FSpODW_{12hill}/StODW_{12hill}$$ **Equation 12**

The plot straw yield on a kilogram per hectare basis (StY_{OD}) was calculated by dividing the oven-dry grain yield (GY_3) by the grain-straw ratio (GSR).

$$StY_{OD} = GY_3/GSR$$ **Equation 13**

Total aboveground biomass Total aboveground biomass included the oven-dry grain yield, straw yield, and the oven-dry yield of unfilled spikelets ($UFSpY_{OD}$).

The unfilled spikelet yield ($UFSpY_{OD}$) was calculated from 12-hill and grain yield harvest area data, namely:

-Unfilled spikelets, oven-dry weight
-Filled spikelets, oven-dry weight
-Grain yield, oven-dry weight

$$UFSpY_{OD} = (UFSpODW_{ss}/FSpODW_{ss}) \text{ x } GY_3 \qquad \textbf{Equation 14}$$

Total aboveground biomass yield ($TDMY_{OD}$) was the sum total of oven-dry grain yield, straw yield, and unfilled spikelets yield.

$$TDMY_{OD} = GY_3 + StY_{OD} + UFSpY_{OD} \qquad \textbf{Equation 15}$$

The harvest index (HI) is the ratio of oven-dry grain yield to the total aboveground biomass:

$$HI = GY_3/(GY_3 + StY_{OD} + UFSpY_{OD}) \qquad \textbf{Equation 16}$$

Tissue N content and N uptake Plant samples for N concentration analysis were taken from sub-samples collected at maximum tillering stage and at harvest. Oven-dried sub-samples were ground and sent to the Institute of Agricultural Chemistry in Bonn for analysis. The N concentration was measured using an automated C-N analyzer (EURO EA Elemental Analyzer).

Nitrogen uptake was calculated as the product of the dry matter yield on an oven-dry basis and the N concentration of the plant part analyzed:

$$\text{N uptake (kg ha}^{-1}\text{) = Dry matter yield x N concentration} \qquad \textbf{Equation 17}$$

The "apparent" N uptake efficiency was estimated as:

$$\%\,\mathrm{N\,recovery} = \frac{\mathrm{N\,uptake_{fert}} - \mathrm{N\,uptake_{control}}}{\mathrm{N\,fertilizer\ applied}}\,\mathrm{x}100 \qquad \textbf{Equation 18}$$

3.7 Statistical analysis

A two-factorial analysis of variance and the LSD comparison of treatment means were computed with the IRRISTAT software Version 3.1. The significance of the presence of an *Azolla* cover, N rate and *Azolla* x N interaction on the floodwater chemistry, N uptake, apparent N recovery, total dry matter yield, yield and yield parameters were determined. Results were presented as means of four replicates.

4 Results and discussion

4.1 Floodwater chemistry

4.1.1 *Floodwater pH*

Figure 4 shows the effect of a full *Azolla* cover on the floodwater pH determined *in situ* between 1200 and 1400 hours for 10 days from the initial urea application.

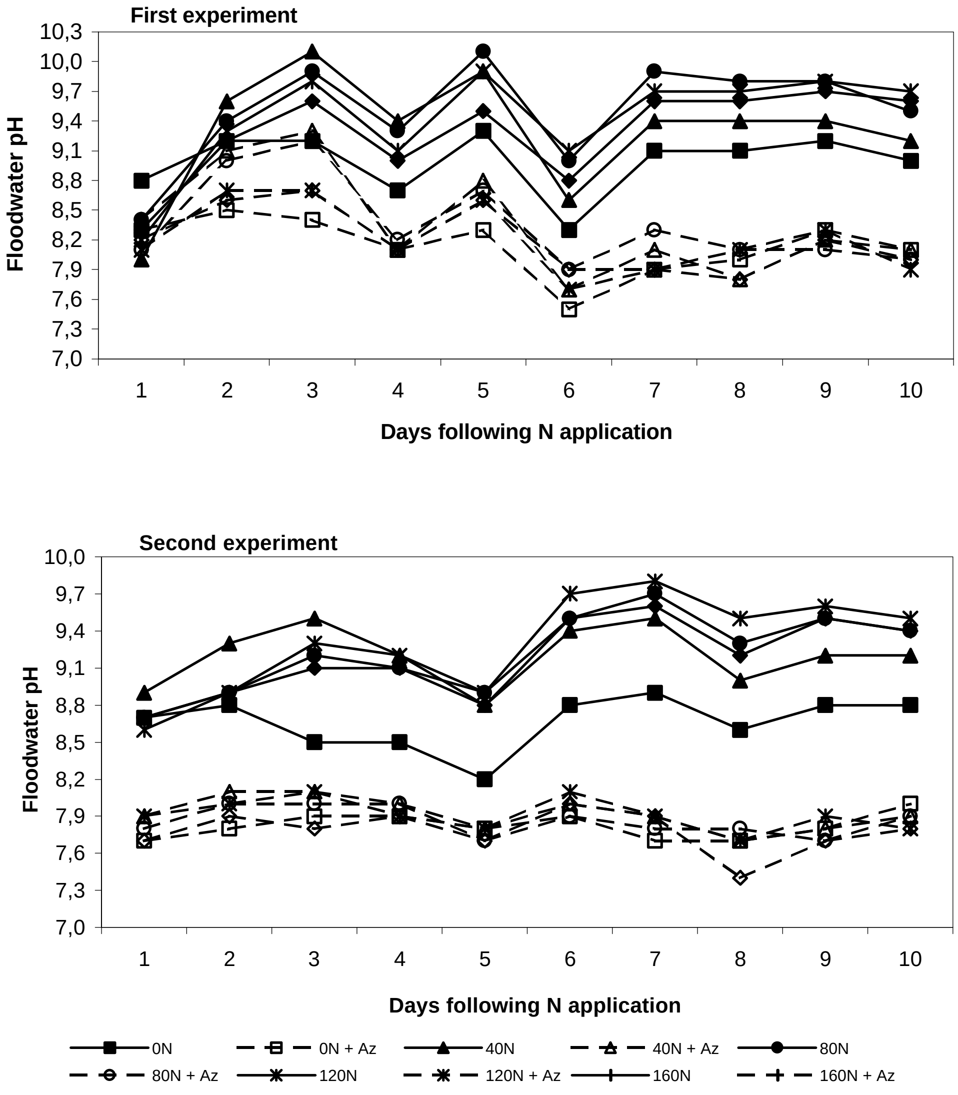


Figure 4: Effect of an *Azolla* cover on the floodwater pH for 10 days from the initial urea application. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

In the first experiment, floodwater pH without *Azolla* cover on day 1 were 8.0, 8.4, 8.2, and 8.3 in the 40, 80, 120 and 160 kg N ha^{-1} treatments. These floodwater pH values were reduced by 0.1 to 0.3 units in the presence of an *Azolla* cover, except in the 40 kg N ha^{-1} plus *Azolla* treatment, where the floodwater pH was higher by 0.4 units than in the uncovered plot. The highest floodwater pH (8.8) was measured in the non-fertilized plot without *Azolla* cover. In the second experiment, floodwater pH ranged from 8.6 to 8.9 in the uncovered plots, whereas floodwater pH was maintained below 8.0 in the *Azolla*-covered plots. *Azolla* significantly reduced ($P<0.01$) floodwater pH by as much as 1.2 units.

A day after urea application (day 2), floodwater pH increased in all treatments. In the first experiment, floodwater pH without an *Azolla* cover rapidly increased by 1.6, 1.0, 1.1 and 0.9 pH units in the 40, 80, 120 and 160 kg N ha^{-1} treatments. In contrast, for the same N levels but with an *Azolla* cover, the rise of the floodwater pH was less than 1.0 pH unit. The magnitude of pH increase was less in all treatments in the second experiment. Floodwater pH without an *Azolla* cover increased by 0.2 to 0.4 units while that with an *Azolla* cover increased by 0.1 to 0.2 units. *Azolla* significantly reduced ($P<0.01$) the floodwater pH by 0.5 to 0.7 units in the first experiment and by 0.9 to 1.2 units in the second experiment.

Floodwater pH rose higher on day 3 in both experiments. A maximum pH value of 10.1 was recorded in the 40 kg N ha^{-1} treatment in the first experiment. The same pH value was measured on day 5 in the 80 kg N ha^{-1} treatment in the same experiment. Floodwater pH higher than 10.0 has been noted in earlier studies. Simpson *et al*. (1984) observed floodwater pH values in excess of 10.0 during the day, while Mikkelsen *et al*. (1978) reported pH maxima of up to 10.0. In the second experiment, floodwater pH reached a peak of 9.5 in the 40 kg N ha^{-1} treatment on the same day (day 3). It was reduced by 1.4 units in the presence of an *Azolla* cover.

Statistical analysis showed that the presence of an *Azolla* cover significantly lowered ($P<0.01$, $P<0.05$) the daily floodwater pH a day after urea application until the last day of sampling for all N levels. The presence of an *Azolla* cover reduced floodwater pH by as much as 1.8 units in the first experiment, and by as much as 1.9 units in the second experiment. In greenhouse experiments, Cissé (2001) reported a pH difference of up to 4.5 units, while Vlek *et al*. (1995) found a pH reduction of up to 2.5 units between plots with and without an *Azolla* cover.

The *Azolla* cover kept the mean floodwater pH below 8.3 for 10 days in the first experiment and below 8.0 in the second experiment. The magnitude of reduction in floodwater pH tends to become higher with increasing N rate. In the first experiment, the mean floodwater pH was reduced by 0.9, 1.0, 1.2 and 1.1 units when *Azolla* was combined with 0, 40, 80, 120 and 160 kg N ha^{-1}. The magnitude of difference in floodwater pH between plots with and without an *Azolla* cover was greater in the second experiment. Mean floodwater pH was reduced by 0.9 units for 0 kg N ha^{-1}, 1.3 units for 40 and 80 kg N ha^{-1}, and 1.4 units for 120 and 160 kg N ha^{-1}.

The rise in floodwater pH following urea application is attributed to the hydrolysis of urea and the subsequent formation of $(NH_4)_2CO_3$ (Chauhan and Mishra, 1989; Phongpan *et al*., 1988; Sherlock and Goh, 1985). This reaction produces OH^- and bicarbonates, which consequently lead to an increase in floodwater pH (Singh and Singh, 1986). This is further affected by the presence of algae, which have been postulated to increase daytime pH (Thind and Rowell, 1999; Simpson *et. al.*, 1988). The photosynthetic uptake of CO_2 by algae reduces its concentration in the floodwater and influences the carbonate equilibria (Cissé, 2001; Fillery *et al*., 1984; Stumm and Morgan, 1981) such that, even without urea, the pH values of floodwater remain high.

In both experiments, algae were visible in the floodwater 2 days after urea application in treatments without an *Azolla* cover. They multiplied rapidly, presumably because the urea was used as an N source for growth. This upsurge in the growth of algae in the floodwater is a common observation when N and also P fertilizers are applied (Roger, 1996). Though algae were not sampled and identified in the present studies, it is assumed that these algae are green algae. Fillery *et al*. (1986) reported that broadcast application of urea favors the growth of green algae, which cause an increase in floodwater pH. Cao *et al*. (1984) reported an increase in pH values in urea-treated plots as compared with the control treatments after the appearance of algae in the floodwater surface 3 days after urea application.

The lower floodwater pH under an *Azolla* cover is explained by the fern's influence on the absorption of light. By intercepting the incoming solar radiation, *Azolla* inhibits the growth of algae, thus suppressing the rise in floodwater pH. In addition, it was surmised that the respiration of *Azolla*-derived CO_2 by *Anabaena* resulted in an increase in the ρ_{CO2} of the floodwater and a reduction in floodwater pH (Vlek *et al.*, 1995). In combination with urea, *Azolla* acts as a counterbalance against the rise in pH due to urea hydrolysis (Cissé, 2001).

4.1.2 *Floodwater temperature*

The floodwater temperature for 10 days following the initial urea application is plotted in Figure 5.

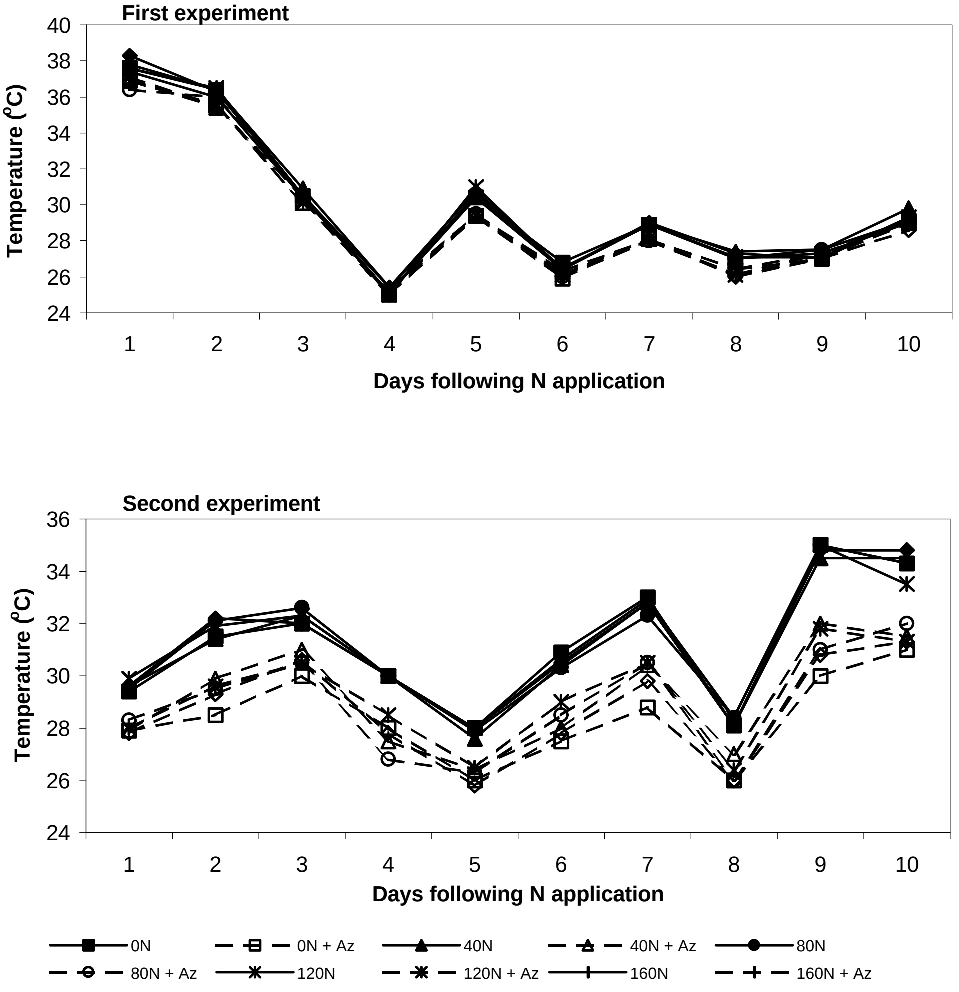


Figure 5: Effect of an *Azolla* cover on the floodwater temperature for 10 days from the initial urea application. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

Plots receiving urea combined with *Azolla* showed consistently lower temperatures than plots with only urea. On the first day of urea application, floodwater temperatures in the first experiment varied between 37.4 and 38.3°C on plots without *Azolla* cover, whereas temperatures on plots with *Azolla* cover were slightly lower, ranging from 36.4 to 37.1°C. In the second experiment, temperatures on the no *Azolla* cover varied between 29.4 and 29.9°C, whereas in the presence of an *Azolla* cover, temperatures ranged from 27.8 to 28.3°C.

The magnitude of reduction in the daily floodwater temperatures between the *Azolla*-covered plots *vs.* the uncovered plots was greater in the second experiment, where a difference of as much as 5°C as compared to the first experiment was recorded. The smallest difference recorded was 1.2°C.

Azolla in combination with urea reduced the mean temperature in the first experiment by 0.6°C in the 0 kg N ha^{-1}, 0.7°C in the 40 and 160 kg N ha^{-1}, 0.5°C in the 80 and 120 kg N ha^{-1} treatments. In the second experiment, the magnitude of reduction was higher, *i.e.*, 2.8°C in the 0 kg N ha^{-1}, 1.9°C in the 40 kg N ha^{-1}, 2.3°C in the 80 kg N ha^{-1}, 2.1°C in the 120 kg N ha^{-1}, and 2.6°C in the 160 kg N ha^{-1} treatments.

A two-factorial statistical analysis computed daily for the whole sampling period shows that the floodwater temperature in the presence of an *Azolla* cover is significantly different ($P<0.05$) to the treatments with no *Azolla* cover in both experiments. This is similar to the results obtained by Cissé (2001) who found a significant reduction in floodwater temperatures between the *Azolla*-inoculated and *Azolla*-free plots.

In floodwater, temperature depends among others on the meteorological conditions such as air temperature, wind, rainfall and sunlight intensity, and on the density of the rice canopy and aquatic plants (Roger, 1996). In general, floodwater temperature was slightly higher in the first experiment, which was established in December, than in the second experiment established in February. The outside average maximum and minimum temperature for the 10-day sampling period in December (28.2 and 23.8°C) was slightly higher than that in February (27.9 and 21.2°C).

Floodwater temperature is an important factor affecting NH_3 volatilization. It affects the relative proportion of NH_3 to NH_4 present at a given pH (Peoples *et al.*, 1995). As the temperature increased from 20 to 30^{o}C, the degree of dissociation increased approximately twofold, from 0.11 to 0.20, and nearly doubled the initial $NH_{3(aq)}$ in the system from 0.44 to 0.82. In an earlier study, Jayaweera and Mikkelsen (1990) found an increase in aqueous NH_3 as the temperature increased from 10 to 40^{o}C at various pH levels resulting in an increase in NH_3 loss per day.

Floodwater temperature as measured by a data logger To clearly illustrate the effect of an *Azolla* cover on floodwater and soil temperatures, a data logger was set-up on plots with and without an *Azolla* cover, adjacent to each other, concurrent with the measurement of floodwater parameters in Experiment 2. The logger was installed in the morning and removed late in the afternoon except on day 3, where the logger was maintained throughout the night to measure nighttime floodwater and soil temperatures. Figure 6 illustrates the effect of an *Azolla* cover on the floodwater and soil temperatures as measured by the data logger.

Day 1. The plot with an *Azolla* cover had a higher floodwater temperature until approximately 10.00 in the morning. It then decreased such that its temperature was lower by 1.7^{o}C than that of the uncovered plot a few minutes before 11.00 am. Floodwater temperature was maximum at 12.58 pm.

The soil temperatures in the *Azolla*-covered and uncovered plots were the same at 8.28 am. Both increased through time, but the magnitude of increase was lower in the *Azolla*-covered plot. The temperature difference between the uncovered and the covered plot was highest (1.4^{o}C) at the time when the maximum floodwater temperature was recorded.

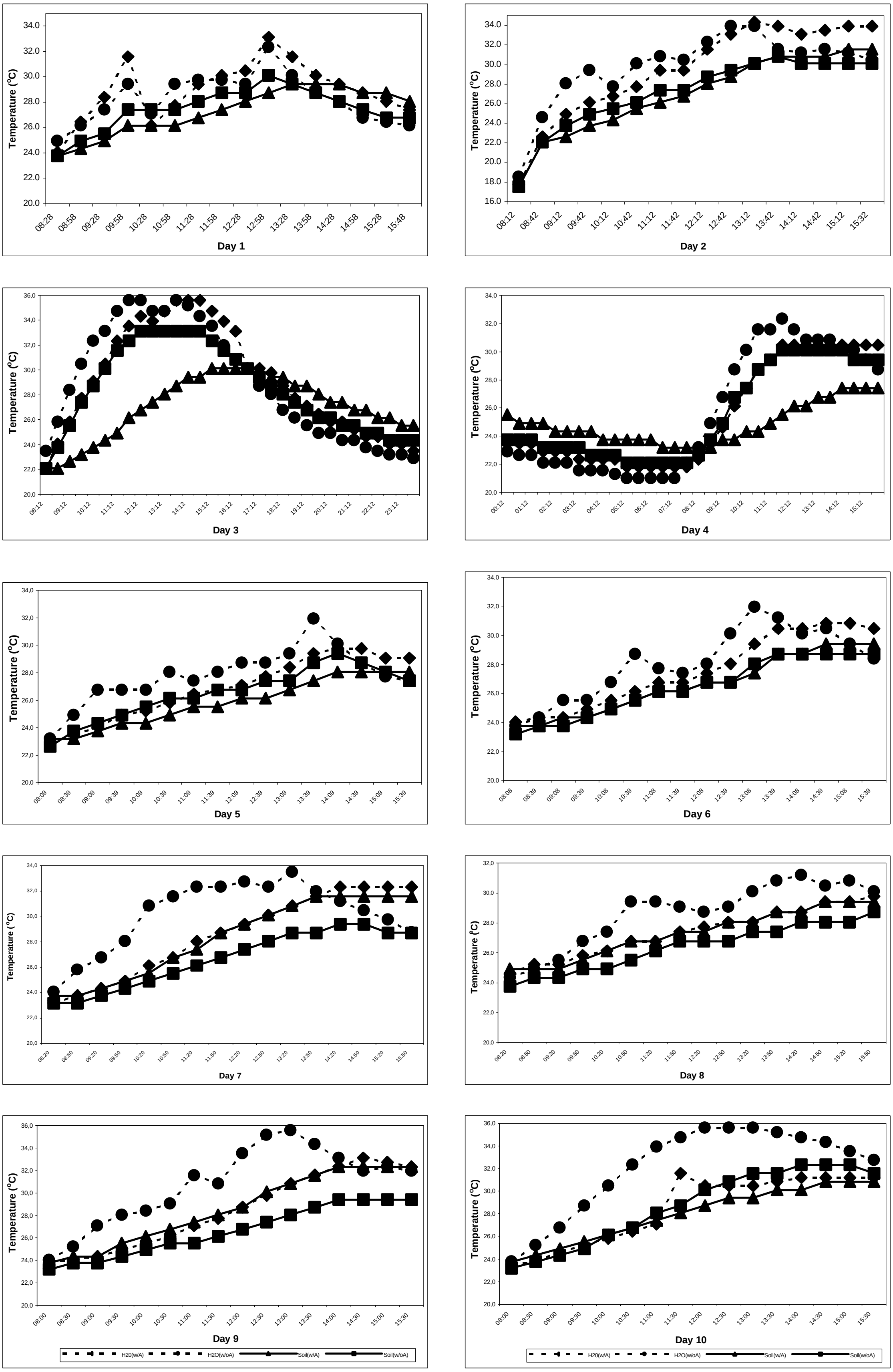


Figure 6: Effect of an *Azolla* cover on the floodwater and soil temperatures as measured by a data logger. Second on-station field experiment. Los Banos, Philippines. Dry season, 1998-99.

Day 2. The floodwater and soil temperatures of the plot without *Azolla* cover were higher than the plot with *Azolla* cover from 8.12 am until 12.42 pm. At 9.42 and 11.12 am, the maximum floodwater and soil temperature difference between treatments were recorded. The floodwater and soil temperatures of the uncovered plot were higher than those of the *Azolla*-covered plot by as much as 3.3 and 1.3^{o}C. Floodwater temperature was highest at 13.12 pm. During this time, the *Azolla*-covered plot had higher temperature than the uncovered plot. This trend continued until the late afternoon.

Day 3. A similar trend was observed. Floodwater and soil temperatures of the *Azolla*-covered plot were lower than those in the uncovered plot in the morning until early in the afternoon. The difference in floodwater temperature was highest at 10.12 am where the temperature in the *Azolla*-free plot was 3.3^{o}C higher than that in the *Azolla*-covered plot. The difference in soil temperature was highest at 11.12 am where the *Azolla*-free plot had a temperature higher by as much as 6.7^{o}C. At 2.12 pm, the floodwater temperature was higher in the *Azolla*-covered plot until 7.12 in the morning of day 4. Soil temperature was higher in the *Azolla*-covered plot from 5.12 until 8.12 in the morning of day 4.

Day 4. The floodwater temperature of the *Azolla*-covered plot remained lower than that of the *Azolla*-free plot until 13.42 pm. The highest temperature difference between the plots with and without *Azolla* cover was recorded at 10.42 am (2.8^{o}C). The soil temperature in the *Azolla*-covered plot was lower than in the *Azolla*-free plot until late in the afternoon. The highest difference recorded between the *Azolla*-free plot and the *Azolla*-covered plot was 4.6^{o}C at 11.42 am.

Days 5 to 10. The floodwater temperature in *Azolla*-covered plots remained lower than that in the *Azolla*-free plots in the succeeding days. The difference in the floodwater temperature was highest between 9.00 until 12.30 in the afternoon, where a maximum difference of 2.7 to 6.8^{o}C was observed between the *Azolla*-covered and the uncovered plots.

In general, the floodwater temperature of the *Azolla*-covered plot was lower than that of the *Azolla*-free plot in the morning until approximately 14.00 pm. After this time, the trend was reversed such that the *Azolla*-free plot had a lower floodwater temperature than the *Azolla*-covered plot. The floodwater in the uncovered plot heats up faster than in the *Azolla*-covered plot, but it also tends to cool down faster. In contrast, the floodwater temperature in the *Azolla*-covered plot takes time to cool down, which explains its higher temperature as compared to the *Azolla*-free plot in the late afternoon.

Roger (1996) reported that the highest temperatures in the rice field ecosystem usually occur in the floodwater and the soil surface. This is because floodwater transmits short-wave radiations to the soil while reducing the upward escape of emitted long-wave radiation, in effect producing a greenhouse effect that heats the floodwater and the soil surface.

4.1.3 *Floodwater total ammoniacal-N*

In general, treatments with an *Azolla* cover contained more total ammoniacal-N (NH_3 + NH_4-N) than treatments without cover during the entire sampling period (Figure 7). Total ammoniacal-N in plots with an *Azolla* cover was higher by as much as 5.3 and 5.5 g N m^{-3} as compared to the *Azolla*-free plots in the first and second experiments. The difference in the mean floodwater ammoniacal-N between *Azolla*-covered and *Azolla*-free plots was highest in the 120 kg N ha^{-1} plus *Azolla* treatment in both experiments, where differences of 1.0 and 1.7 g N m^{-3} were recorded. This higher concentration of total ammoniacal-N in the floodwater in the presence of an *Azolla* cover could be due to a reduction of NH_3 losses resulting from the low partial pressure of ammonia in *Azolla*-covered plots, and could also be due to a lower uptake by the green algae. In a previous study (unpublished) conducted by the author, a similar trend was observed. Likewise, a similar pattern was reported by Villegas and San Valentin (1989), where total ammoniacal-N concentrations were higher in the 60 and 120 kg N ha^{-1} plots covered with *Azolla* by as much as 2.84 and 0.77 g N m^{-3} as compared with the uncovered plots. Several studies in the past have shown that in the presence of a barrier in the floodwater interface such as surface films (Cai *et al*., 1987), a higher ammoniacal-N was measured. The same effect was obtained when algicides such as terbutryn were added to the floodwater (Muirhead *et al*., 1989; Simpson *et al*., 1988) or when urea was broadcast with PPD, a urease inhibitor (Phongpan *et al*., 1988).

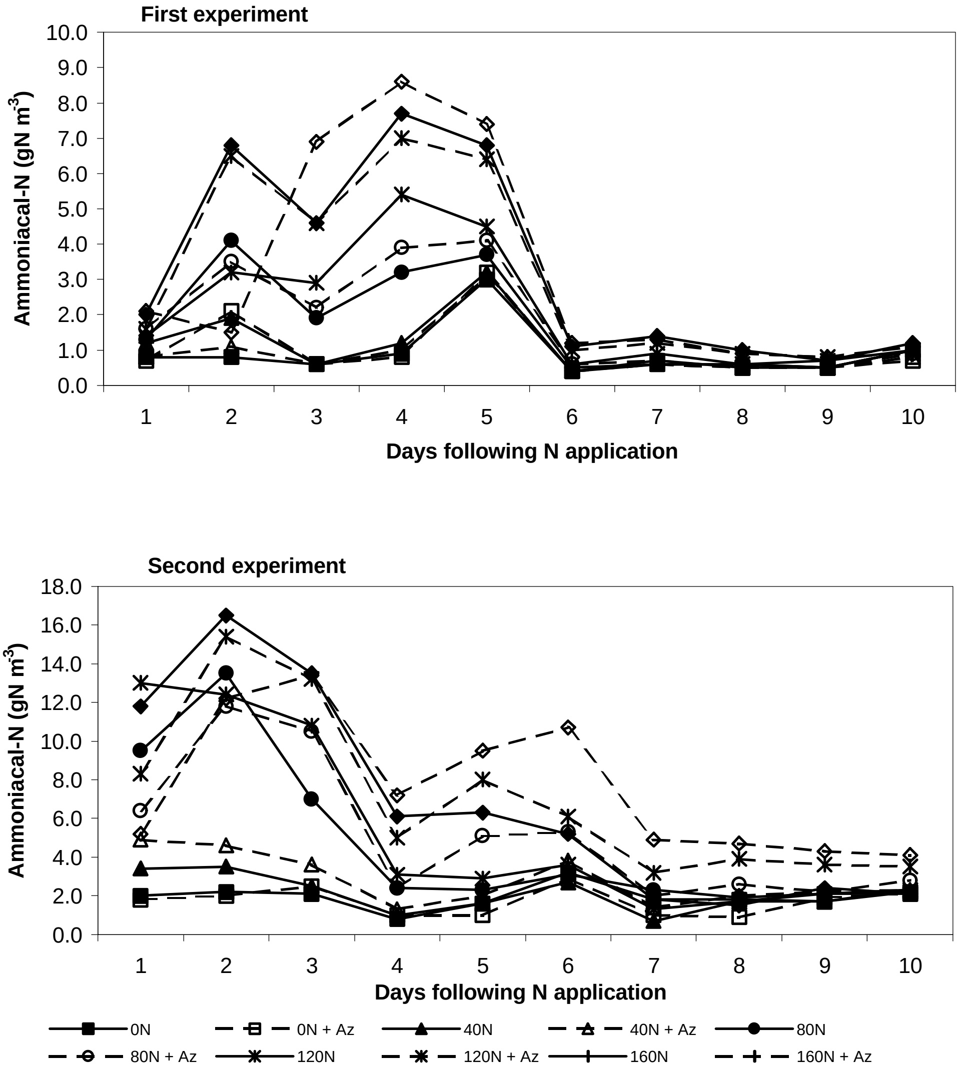


Figure 7: Effect of an *Azolla* cover on the total ammoniacal-N concentration for 10 days from the initial urea application. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

Data show that the concentration of total ammoniacal-N in floodwater increased markedly following urea application. It rose steeply to a maximum value within two to four days in both experiments. Simpson *et al.* (1984) explained that urea that is dissolved in the floodwater shortly after application has to be transported to the soil surface by diffusion or convection before it can be hydrolyzed. The present results are in close conformity with the results obtained by Chauhan and Mishra (1989), Fillery *et al.* (1984, 1986) and Mikkelsen *et al.* (1978), where the concentration of ammoniacal-N in the floodwater was found to increase gradually and attained a peak on the third or fourth day after urea application. This pattern of increase can be attributed to the delay in hydrolysis of urea.

The application of 160 kg N ha^{-1} combined with an *Azolla* cover produced the highest concentration of total ammoniacal-N (8.6 g N m^{-3}) in the first experiment. Another peak, however, was observed on the fourth and fifth days. According to Son and Buresh (1993), at any given time, more ammoniacal-N coming from urea is found in the soil than in the floodwater. Thus, it is highly possible that, because of the disturbance of the soil surface caused by the heavy rains, ammoniacal-N was released from the soil to the floodwater. Alternatively, the decline in the total ammoniacal-N on day 3 can be attributed to NH_3 losses, and its increase on days 4 and 5 to further urea hydrolysis and diffusion. The decrease after rains (on day 6) can be due to dilution (Roger, 1996) and mixing with soil. After the sixth or seventh day, none of the treatments in the first experiment had more than 2 g N m^{-3} total ammoniacal-N in the floodwater. On the ninth up to the tenth day, less than 1 g N m^{-3} of total ammoniacal-N was detected. Cao *et al*. (1984) found that the significant proportion of urea that moved into the floodwater immediately after application disappeared within 3 to 5 days and the losses were attributed to NH_3 volatilization. Other authors attributed this reduction in ammoniacal-N concentrations in the floodwater not only to gaseous N losses, but also to the immobilization of ammoniacal-N by aquatic flora, and adsorption by NH_4^+-N on the soil exchange sites (Phongpan *et al*., 1988; Fillery *et al*., 1984; Craswell and Vlek, 1979).

Total ammoniacal-N concentrations in the floodwater were generally higher and persisted for a longer time (up to the tenth day) in the second experiment. The maximum concentration of total ammoniacal-N (16.5 g N m^{-3}) was recorded on the second day in the 160 kg N ha^{-1} treatment. The immediate occurrence of the ammoniacal-N peak soon after N application might imply the presence of a high urease activity in the floodwater of the soil. An appreciable amount of total ammoniacal-N (>2.0 g N m^{-3}) still remained in the floodwater when sampling was terminated on day 10.

4.1.4 *Floodwater aqueous ammonia*

Figure 8 illustrates the aqueous NH_3 in the floodwater after urea application on *Azolla*-covered and *Azolla*-free plots. Aqueous NH_3 is an important parameter governing NH_3 volatilization from solutions (Vlek *et al.*, 1980). It is calculated as:

$$A = C/[1 + 10^{(0.09018 + 2729.92/T - pH)}]$$, (Denmead *et al.*, 1982)

where, A = aqueous NH_3 concentration in floodwater in g N m^{-3},

C = ammoniacal-N concentration in the water (aqueous ammonia plus ammonium) in g N m^{-3}, and

T = floodwater temperature in degrees Kelvin.

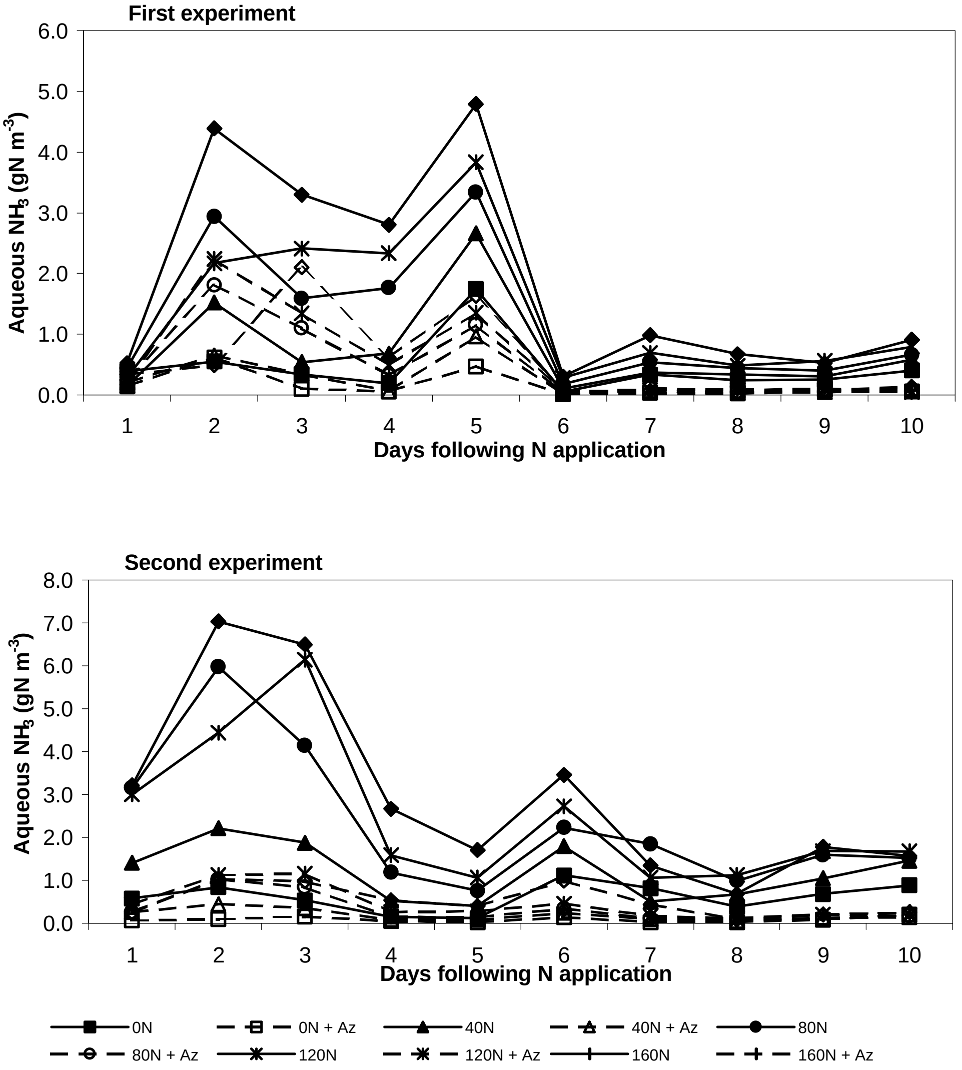


Figure 8: Effect of an *Azolla* cover on the aqueous NH_3 for 10 days from the initial urea application. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

The graph indicates high aqueous NH_3 for all treatments where an *Azolla* cover was absent. Inoculation of *Azolla* onto the floodwater surface prior to urea application effectively reduced aqueous NH_3 in all *Azolla*-covered treatments in both experiments due entirely to low floodwater pH and temperature. With each successive increase in the N levels, the aqueous NH_3 also increased such that at the highest N rate, 160 kg N ha^{-1}, the highest aqueous NH_3 (4.79 g N m^{-3}) was recorded in the first experiment. This was reduced to 1.63 g N m^{-3} in the presence of *Azolla*. The same treatment likewise produced the highest aqueous NH_3 (7.04 g N m^{-3}) in the second experiment, which was lowered to 1.03 g N m^{-3} in the presence of an *Azolla* cover.

Statistical analysis of daily data showed that an *Azolla* cover significantly decreased ($P<0.01$) the aqueous NH_3 from day 2 to day 10 in the first experiment, and for the whole 10-day sampling period in the second experiment. A significant *Azolla* x N interaction was noted on days 2, 4, 6, 7, 9 and 10 ($P<0.01$), and day 8 ($P<0.05$) in the first experiment. A significant interaction ($P<0.01$) on days 2, 3, 5, and 9, and on day 4 ($P<0.05$) was observed in the second experiment.

The $NH_{3(aq)}$ in the floodwater is governed by NH_4-N concentration in the floodwater, the pH, and the temperature (Jayaweera and Mikkelsen, 1990; Sherlock and Goh, 1985; Denmead *et al.*, 1982; Vlek and Craswell, 1981). The high aqueous NH_3 in treatments without an *Azolla* cover in both experiments was mainly attributed to the high floodwater pH and temperature. According to Vlek and Craswell (1981) and the equation used to calculate it, the content of aqueous NH_3 in floodwater increases about tenfold per unit increase in pH in the pH range 7.5 to 9.0. It increases approximately linearly with increasing temperature at a given total concentration of ammoniacal-N.

4.1.5 *Partial pressure of ammonia*

The partial pressure of ammonia is used as a measure of the potential for NH_3 volatilization losses. It is mainly determined by the concentration of aqueous NH_3 and the temperature, and computed as:

$$\rho_o = 0.00594\ AT/10^{(1477.8T-1.6937)}$$ (Denmead *et al.*, 1983)

where, ρ_o = partial pressure of ammonia in Pascals,
A = aqueous NH_3 concentration in the floodwater in g N m^{-3},
T = floodwater temperature in degrees Kelvin.

The resulting effects of the variations in aqueous NH_3 and temperature on the partial pressure of ammonia for the various treatments are shown in Figure 9.

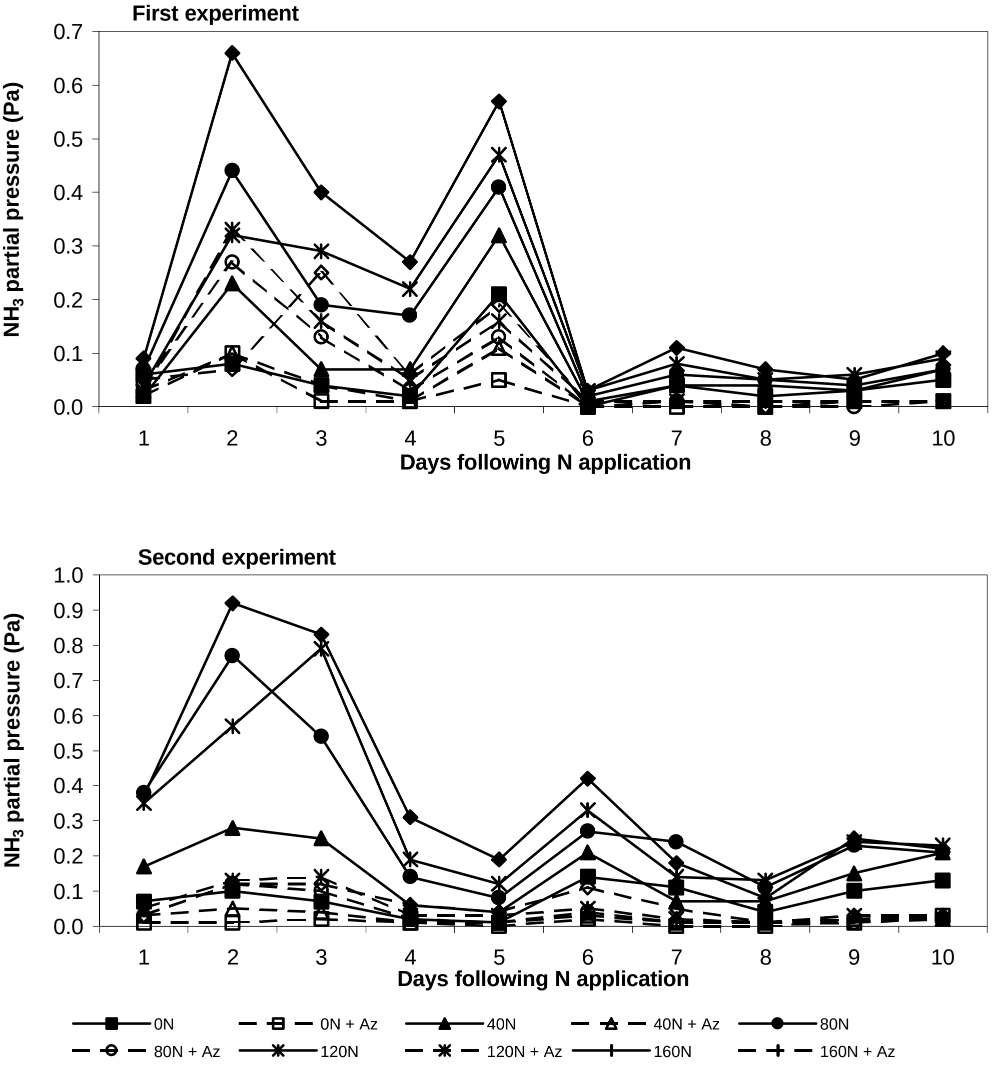


Figure 9: Effect of an *Azolla* cover on the NH_3 partial pressure for 10 days from the initial urea application. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

The NH_3 partial pressure (ρNH_3) calculated for both experiments more or less followed the same pattern as the aqueous NH_3. It was substantially reduced when urea was combined with *Azolla*. The partial pressure of ammonia was likewise greater in the second experiment than in the first and became higher, the greater the quantity of N applied. The highest ρNH_3 was measured on the second day in the 160 kg N ha^{-1} treatment in which 0.66 and 0.92 Pa were obtained in the first and second experiments. These high ρNH_3 values indicate a high potential for NH_3 volatilization. Covering the floodwater surface with *Azolla* significantly reduced ($P<0.01$) the ρNH_3 to 0.07 and 0.12 Pa, so that the potential for NH_3 losses was much lower.

The magnitude of reduction in the NH_3 partial pressure between *Azolla*-covered and *Azolla*-free plots tends to increase with higher N rates. The mean ρNH_3 in the first and second experiments was lowered by 0.04 and 0.29 g N m^{-3} in the 0 kg N ha^{-1}, 0.06 and 0.13 g N m^{-3} in the 40 kg N ha^{-1}, 0.09 and 0.26 g N m^{-3} in the 80 kg N ha^{-1}, 0.09 and 0.26 g N m^{-3} in the 120 kg N ha^{-1}, and 0.13 and 0.32 g N m^{-3} in the 160 kg N ha^{-1} in *Azolla*-covered plots.

Statistical analysis shows that an *Azolla* cover significantly reduced ($P<0.01$) the partial pressure of ammonia from day 2 to 10 and for the entire 10-day sampling period for the first and second experiments. A significant *Azolla* x N interaction was likewise noted on days 2, 4, 7, and 8 ($P<0.01$) and days 6, 9 and 10 ($P<0.05$) for the first experiment. In the second experiment, a significant interaction was observed on days 2 and 3 ($P<0.01$) and days 4, 5, and 9 ($P<0.05$).

This marked reduction in ρNH_3 was mainly due to the low floodwater pH and temperature obtained in treatments with an *Azolla* cover. Based on findings of Vlek *et al.* (1995), most of the reduction in the NH_3 volatilization potential is brought about by the reduction in floodwater pH, which appears to be the primary contributing factor controlling NH_3 losses from flooded soil. Similarly, Jayaweera and Mikkelsen (1990) showed in a sensitivity analysis that floodwater pH is the most sensitive determinant influencing NH_3 volatilization. These findings were supported by Anila Kumar and Rajaram (1991) and Chauhan and Mishra (1989) who noted a significant positive correlation between floodwater pH and cumulative NH_3 losses.

Urea-N losses occur mostly during the first 10 days after urea application (Watanabe *et al*., 1989). In the present experiments, the partial pressure of ammonia was low on day 1 and increased to a maximum immediately on day 2. Following the peak, the NH_3 partial pressure in both experiments gradually decreased until it became almost negligible by the end of the 10-day sampling period, negating further NH_3 losses. This is consistent with reports that the NH_3 volatilization increases rapidly in the first 3 to 4 days following N fertilization, and then decreases with time (Ouyang *et al*., 1998; Reddy *et al*., 1990; Schnier *et al*., 1990; Buresh and Austin, 1988; Fillery and De Datta, 1986).

As the *Azolla* cover results in lower floodwater pH and temperature, lower NH_3 losses are expected. The present results confirm this hypothesis. Despite the high amount of total ammoniacal-N in the floodwater, which can lead to substantial NH_3 losses, an *Azolla* cover was able to effectively depress the high potential for NH_3 volatilization after urea was broadcast onto the floodwater. *Azolla* mainly exerted its influence in inhibiting the rise in floodwater pH and temperature. This agrees with an earlier study conducted by Vlek *et al*. (1995), where the reduction in the NH_3 volatilization potential was attributed to the reduction in floodwater pH.

4.2 ^{15}N recovery

Tables 3 and 4 present the ^{15}N recovery in the rice-soil system with and without an *Azolla* cover at different N rates in the two experiments.

4.2.1 ^{15}N *recovery by the rice*

The presence of an *Azolla* cover on the floodwater surface prior to the initial urea application resulted in an improved ^{15}N recovery by the aboveground biomass (grain plus straw) at harvest in both experiments. ^{15}N recovery by rice on *Azolla*-covered plots ranged from 31.7 to 42.1% in the first experiment, an increase of 3.6 to 20.3% over the ^{15}N recovery in the *Azolla*-free plots. The increase was proportionally higher in the second experiment, where ^{15}N recovery by rice on *Azolla*-covered plots increased by approximately 25 to as much as 95% as compared with the ^{15}N recovery by rice on *Azolla*-free plots. The percent ^{15}N recovery difference between the *Azolla*-covered and the *Azolla*-free plots decreased as the N rate increased in the second experiment, whereas no specific trend was observed in the first experiment. ^{15}N plant recovery was highest in the 120 kg N ha^{-1} combined with *Azolla* (42.1%) in the first experiment and in the 40 kg N ha^{-1} plus *Azolla* treatment (61.2%) in the second experiment.

Table 3: Recovery (%) of ^{15}N-labeled urea in *Azolla*, plant and soil at harvest. First on- station field experiment. Los Banos, Philippines. Dry season, 1998-99.

Treatment	**Straw**	**Grain**	**Total Plant**	***Azolla***	**Soil (0-15 cm)**	**Soil (15-30 cm)**	**TOTAL**
40N	10.5 ± 4.5	20.1 ± 3.8	30.6 ± 8.3		42.6 ± 25.4	5.8 ± 1.5	79.0 ± 33.2
40N + Az	9.7 ± 3.8	22.0 ± 3.2	31.7± 6.5	10.7 ± 5.6	29.0 ± 6.4	5.9 ± 1.5	77.3 ± 4.3
80N	7.8 ± 2.1	22.2 ± 4.0	30.0 ± 5.7		20.4 ± 8.8	3.8 ± 1.3	54.2 ± 6.3
80N + Az	10.1 ± 3.9	26.0 ± 5.4	36.1 ± 7.4	11.6 ± 2.8	24.3 ± 8.1	6.2 ± 1.5	78.2 ± 8.5
120N	14.7 ± 2.8	24.8 ± 4.7	39.5 ± 7.5		23.1 ± 10.2	3.5 ± 0.8	66.1 ± 15.7
120N + Az	14.6 ± 4.1	27.5 ± 3.5	42.1 ± 6.7	13.9 ± 3.1	34.3 ± 11.2	4.3 ± 1.1	94.6 ± 15.8

Az = *Azolla*

Table 4: Recovery (%) of ^{15}N-labeled urea in *Azolla*, plant and soil at harvest. Second on-station field experiment. Los Banos, Philippines. Dry season, 1998-99.

Treatment	**Straw**	**Grain**	**Total Plant**	***Azolla***	**Soil (0-15 cm)**	**Soil (15-30 cm)**	**TOTAL**
40N	20.1 ± 1.8	11.3 ± 3.5	31.4 ± 5.0		32.0 ± 14.2	3.2 ± 1.8	66.6 ± 17.3
40N + Az	31.3 ± 1.7	29.9 ± 7.5	61.2 ± 9.0	5.6 ± 2.6	30.7 ± 14.4	2.3 ± 0.4	99.9 ± 13.2
80N	19.3 ± 4.4	10.3 ± 6.0	29.6 ± 10.2		19.7 ± 8.9	1.3 ± 0.1	50.6 ± 18.0
80N + Az	24.7 ± 4.1	24.3 ± 3.6	49.0 ± 6.6	6.5 ± 6.7	38.0 ± 11.5	2.2 ± 0.7	95.6 ± 14.2
120N	18.2 ± 1.5	18.1 ± 3.0	36.3 ± 2.5		31.2 ± 7.5	1.3 ± 0.2	68.8 ± 9.9
120N + Az	22.7 ± 4.4	22.8 ± 2.0	45.5 ± 5.3	3.0 ± 2.1	30.2 ± 11.2	2.3 ± 0.7	80.9 ± 12.5

Az = *Azolla*

The improved ^{15}N recovery by the rice in the *Azolla*-covered treatments is partly attributed to the lower NH_3 volatilization losses in the earlier stage of rice as supported by the low ρNH_3 in the floodwater. Furthermore, *Azolla*, upon its decomposition could have released part of the ^{15}N it absorbed and its availability led to a better ^{15}N utilization by the crop. Singh and Singh (1989) reported that about 45% of the N from *Azolla* is released in 60 days and the rice plants take up about 34% of it during this time. An earlier study by Vlek *et al*. (1995) attributed the increased recovery by rice partly to the uptake of urea-N by the actively growing *Azolla*.

In the absence of an *Azolla* cover on the floodwater surface, plant recovery of ^{15}N ranged from 30 to 39.5% in the first experiment. These values, though lower than the plant recovery in the presence of an *Azolla* cover, were slightly higher than the values obtained by Katyal *et al*. (1985), where ^{15}N recovery by the plant varied from 21 to 31%. Singh and Yadav (1981) attributed these high recoveries to the priming effect; N application encourages root proliferation and better plant growth causing the plant to extract more N from the soil. It could also be due to mineralization and immobilization of ^{15}N.

In general, the ^{15}N recovery of labeled urea by the aboveground biomass increased with increasing N rate both in the absence and in the presence of an *Azolla* cover in the first experiment. This trend is similar to the results obtained by Raun *et al*. (1999), where fertilizer N recovery by the plants increased with increasing N rate. In the second experiment, the opposite trend was observed. In the presence of an *Azolla* cover, total plant ^{15}N recovery decreased with an increase in fertilizer N rate. In another experiment, the same authors (Raun *et al*., 1999) noted a decrease in fertilizer N recovery by the plants with an increase in N applied. Similarly, Katyal and Gadalla (1990) found a tendency for a marginal decrease in ^{15}N recovery by plants with increasing N rate.

Plant recovery of ^{15}N at the maximum N rate (120 kg N ha^{-1}) was less than 10% higher than that in the lowest N rate (40 kg N ha^{-1}). This result suggests that, in the absence of an *Azolla* cover, using ^{15}N-labeled urea for two thirds of the N applied at transplanting did not greatly influence the amount of plant ^{15}N recovery.

The grain recovered more ^{15}N than the straw in all treatments regardless of *Azolla* cover in the first experiment. Grain ^{15}N recovery was proportionally higher by as much as 185% than straw ^{15}N recovery. In the second experiment, more ^{15}N was recovered by the straw up to the 80 kg N ha^{-1} treatment. In treatments 80 and 120 kg N ha^{-1} with *Azolla*, ^{15}N recovery by the straw was almost equal to the ^{15}N recovery by the grain.

The *Azolla* cover increased ^{15}N grain recovery from approximately 9 to 17% in the first experiment. In the second experiment, the difference in the ^{15}N grain recovery of treatments with and without an *Azolla* cover was greater. ^{15}N recovery by grain in the 40, 80 and 120 kg N ha^{-1} treatments combined with *Azolla* was significantly higher ($P<0.01$) by 165, 139 and 26% in proportion to the 40, 80 and 120 kg N ha^{-1} applied alone, respectively. ^{15}N recovery by the straw likewise significantly increased ($P<0.01$) in the presence of an *Azolla* cover. A significant interaction ($P<0.01$) between *Azolla* cover and N in the percent ^{15}N recovery by rice straw occurred. *Azolla* in combination with 40, 80 and 120 kg N ha^{-1} significantly increased ($P<0.01$) recovery of applied ^{15}N by straw by 56, 28 and 25% in proportion to the no *Azolla* cover.

4.2.2 ^{15}N *recovery by Azolla*

Earlier findings that show *Azolla* taking up nutrients from the floodwater were confirmed in the present experiments. Data show that the aquatic fern assimilated a fraction of the applied ^{15}N. At harvest, the *Azolla* plant retained 10.7 to 13.9% of the labeled urea in the first experiment. The ^{15}N in *Azolla* increased with increasing N rate and recovery was highest in the 120 kg N ha^{-1} treatment (13.9%). The retention was lower in the second experiment, where ^{15}N recovery by the *Azolla* plant at harvest ranged only from 3.0 to 6.5%. On the basis of its biomass and appearance, it was observed that the *Azolla* grew better in the first experiment than in the second experiment, which was initially infested with webworm (*Ephestiopsis vishnu*). Thus, the *Azolla* in the first experiment could have assimilated more N.

Cissé (2001) found that *Azolla* immobilized up to 67.8% of applied ^{15}N six weeks after application. Initially, nitrogen might be temporarily locked up in the *Azolla*, which limits availability of N to rice plants. In the process, however, this protects N from immediate gaseous losses. Nitrogen is, in fact, being conserved within the system and is mineralized later, becoming available for plant use (Keeney and Sahrawat, 1986; Craswell and Vlek, 1979). During the entire rice growing season, *Azolla* can remineralize up to 44.8% of the N it previously immobilized under greenhouse conditions (Cissé, 2001). Mineralization of *Azolla*-N is quite rapid in flooded rice soils. Its decomposition is almost complete within 30 days, and N then becomes equally available like urea-N (Rosenani and Chulan, 1992).

4.2.3 *^{15}N recovery in the soil*

Much of the labeled urea not taken up by the rice plant remained in the soil at crop maturity. Across rates of N application, no regular pattern was observed in the soil ^{15}N recovery.

Data show that at harvest, more than 30% of the labeled urea applied was found in the 0 to 15 cm layer in both experiments. According to Alfaia *et al*. (2000), N immobilization in the soil occurs mainly in the upper 30 cm layer and others (Simpson *et al.*, 1984) report that most residual ^{15}N is recovered in this layer.

Leaching losses in both experiments were apparently negligible, as very little labeled urea was detected below the 15 cm depth. As leaching losses were insignificant and run-off was prevented, it was assumed that all losses were gaseous. It was observed however, that more ^{15}N was found in the 15 to 30 cm depth in the first experiment than in the second experiment. An average of 4.4 and 5.5% were recovered in plots with and without an *Azolla* cover in the first experiment. The mean ^{15}N recovery in the second experiment, on the other hand, was 2.3% in the plots with *Azolla* cover and 1.9% in the plots without *Azolla* cover. The slightly higher ^{15}N in the 15 to 30 cm soil depth in the first experiment could be due to the intense rainfall that occurred a few days after urea application (Figure 10), which moved the labeled urea down the soil profile. In contrast, in the second experiment, rainfall was minimal during the first 2 weeks after urea application.

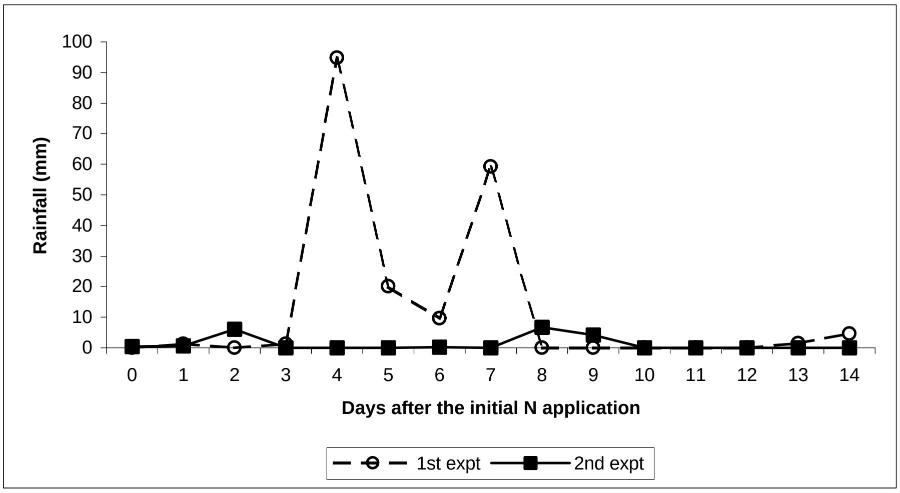


Figure 10: Rainfall pattern in the on-station experimental sites two weeks after the initial urea application. Los Banos, Philippines. Dry season, 1998-99.

4.2.4 *Total ^{15}N recovery in the Azolla-plant-soil system*

In general, the presence of an *Azolla* cover markedly improved the total recovery of ^{15}N in the rice-soil system. In the first experiment, the recovery of applied ^{15}N significantly increased ($P<0.05$) from an average of 66.4% in treatments without *Azolla* cover to an average of 83.3% with *Azolla* cover. The highest ^{15}N recovery (94.6%) was obtained in the 120 kg N ha^{-1} plus *Azolla* treatment. The highest proportional difference between the *Azolla*-covered and *Azolla*-free plots was obtained, however, in the 80 kg N ha^{-1} rate, where ^{15}N recovery with *Azolla* (78.2%) was 44% higher than the ^{15}N recovery without (54.2%). In the second experiment, the average ^{15}N recovery of 62.0% on *Azolla*-free plots was significantly increased ($P<0.01$) to 92.1% when *Azolla* was present. ^{15}N recovery was highest in the 40 kg N ha^{-1} plus *Azolla* treatment, where almost all of the ^{15}N applied was recovered (99.9%). The increase was highest in the 80 kg N ha^{-1} level, where the presence of an *Azolla* cover increased ^{15}N recovery by 89%.

The improvement in the ^{15}N recovery in the presence of an *Azolla* cover indicates the important contribution of *Azolla* in the N economy in rice fields. Its beneficial effects on the floodwater chemistry, and its assimilation and conservation of N led to an enhancement in the ^{15}N recovery.

4.2.5 ^{15}N losses in the system

The ^{15}N balance technique provides a measure of the magnitude of applied ^{15}N losses (Katyal *et al.*, 1985). Results show that, irrespective of the rate of ^{15}N applied, the fraction of urea-^{15}N unaccounted for in the plant and in the soil, and presumably lost through NH_3 volatilization was lower in treatments with urea combined with *Azolla* than that with urea applied alone in both experiments (Figure 11). Losses ranged from 5.4 to 22.7% in the first experiment and from a low of 0.01 to 19.1% in the second experiment. Vlek *et al.* (1995) reported a significant reduction in NH_3 losses in the presence of an *Azolla* mat.

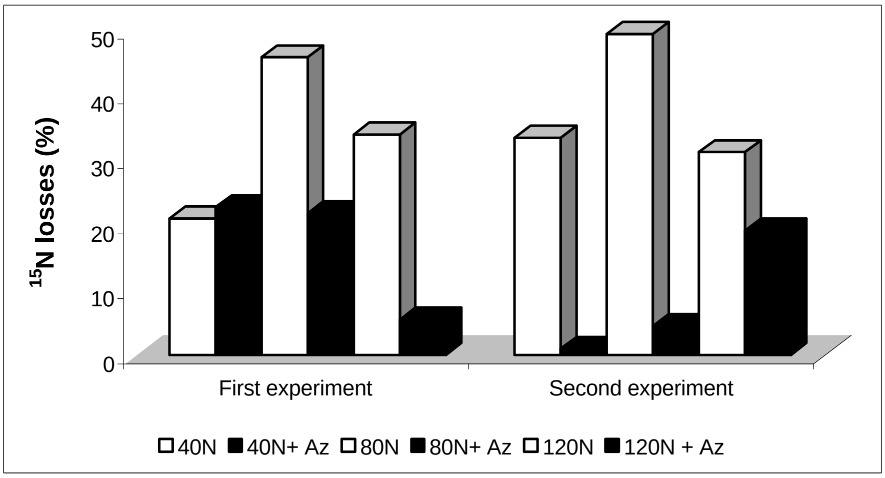


Figure 11: Effect of an *Azolla* cover on the ^{15}N losses (%). On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

The trend for ^{15}N losses in the *Azolla*-covered plots in the two experiments differs. In the first experiment, ^{15}N losses from the soil-plant system were highest in the 40 kg N ha^{-1} plus *Azolla* (22.7%) and lowest in the 120 kg N ha^{-1} plus *Azolla* (5.4%). The reverse was observed in the second experiment. ^{15}Nitrogen losses were highest in the 120 kg N ha^{-1} plus *Azolla* (19.1%) and lowest in the 40 kg N ha^{-1} plus *Azolla* (0.01%). This latter trend is similar to findings of others (Rozas *et al.*, 1999; De Datta *et al.*, 1990; Katyal and Gadalla 1990; Azis *et al.*, 1987), which show that the unrecovered ^{15}N tends to increase as the amount of basally applied N increased.

In contrast, extensive losses of applied ^{15}N were noted in treatments without *Azolla* cover. ^{15}Nitrogen losses ranged from 21.0 to as high as 49.4%. This range is comparable to the 46 to 50% losses from urea reported by Katyal *et al*. (1985). The high partial pressure of ammonia resulting from a high floodwater pH and temperature in *Azolla*-free plots indicates that NH_3 volatilization most probably played a significant role in increasing the N losses. These high N losses led to the poor recoveries of applied N by the rice crop and resulted in lower N use efficiency.

4.3 Apparent N recovery

The apparent N recovery (ANR) of rice in the on-station experiments is shown in Figure 12. Results show that the ANR was improved in the presence of an *Azolla* cover. In the first experiment, ANR increased from 45.8 to 70.2%, or a relative increase of 53% when plots applied with 40 kg N ha^{-1} were covered with *Azolla*. The ANR in plots treated with 80 and 120 kg N ha^{-1} increased relatively by 20 and 10% respectively, when they were covered with *Azolla*. The presence of an *Azolla* cover at the highest N rate (160 kg N ha^{-1}) though, led to a 7% lower ANR than that in plots applied with 160 kg N ha^{-1} alone. The effect of an *Azolla* cover on the ANR was more pronounced in the second experiment. The ANR increased from 54.3 to 93.3% or a relative increase of 72% when *Azolla* covered the floodwater surface of plots applied with 40 kg N ha^{-1}. In the *Azolla*-covered plots amended with 80 and 120 kg N ha^{-1}, the ANR was proportionally higher by 40 and 26%, respectively. In contrast to the results from the first experiment, the highest relative increase was achieved at the highest N rate with *Azolla* cover (103%). In general, the ANR of rice decreased as the rate of N increased in both experiments.

In comparison with the ^{15}N recovery, the values obtained for the ANR were higher. Such deviations according to Buresh *et al*. (1990) are common and most likely relate to a better exploitation of the soil-N in the covered plots. Though the values are different, the apparent N recovery was highly correlated (r=0.91**) with the ^{15}N recovery by the rice in the second experiment but not in the first (r=0.21).

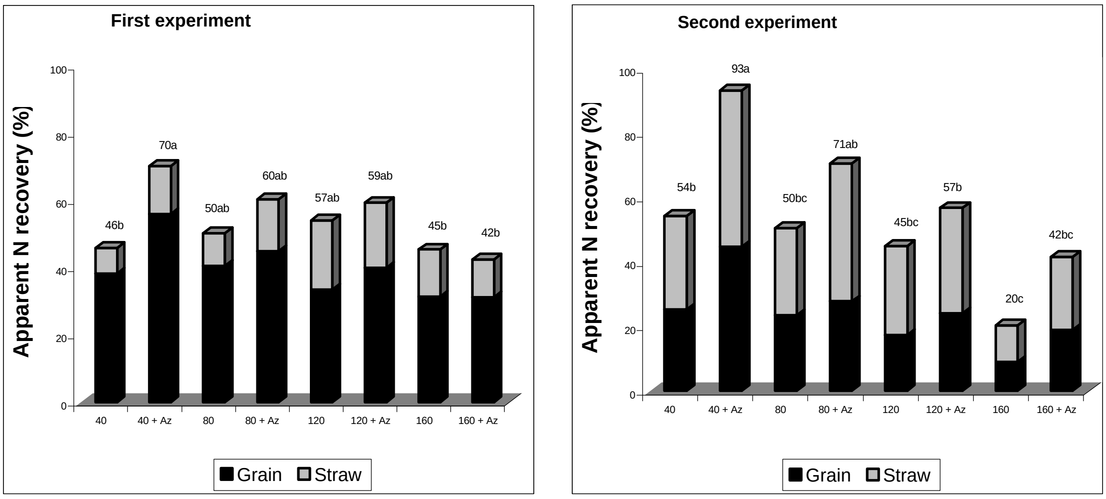


Figure 12: Apparent N recovery (%) of rice as affected by an *Azolla* cover. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

4.4 Nitrogen uptake

4.4.1 *On-station field experiments*

Straw yield at harvest Straw yield of rice at harvest significantly increased ($P<0.01$) in the presence of an *Azolla* cover in the second experiment but not in the first. Straw yield was higher by 6.8 to 34.6% in treatments with *Azolla* cover. The highest straw yield (5924 kg ha^{-1}) was obtained in the treatment applied with 80 kg N ha^{-1} combined with an *Azolla* cover (Figure 13).

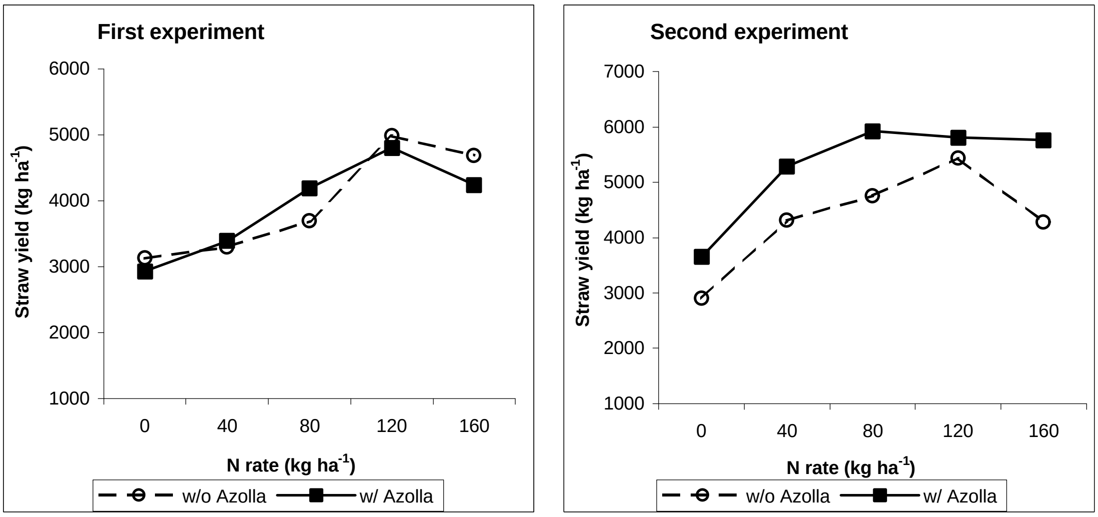


Figure 13: Effect of an *Azolla* cover on the straw yield of rice at harvest. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

Grain and straw N concentration The presence of an *Azolla* cover increased the N concentration of the grain in both experiments (Figure 14). In the first experiment, N concentration was higher by as much as 5.0% in urea-treated plots with an *Azolla* cover, but the increase was not statistically significant. In the second experiment, a significant increase ($P<0.01$) in the N concentration was observed in *Azolla*-covered, urea-amended plots. The highest N concentration (1.6%) recorded in plots with 160 kg N ha^{-1} and an *Azolla* cover was 8% significantly higher ($P<0.01$) than that of the uncovered plots. The grain N concentration in the treatment with 120 kg N ha^{-1} and *Azolla* cover (1.5%) was 9.0% higher than that in the 160 kg N ha^{-1} without the cover.

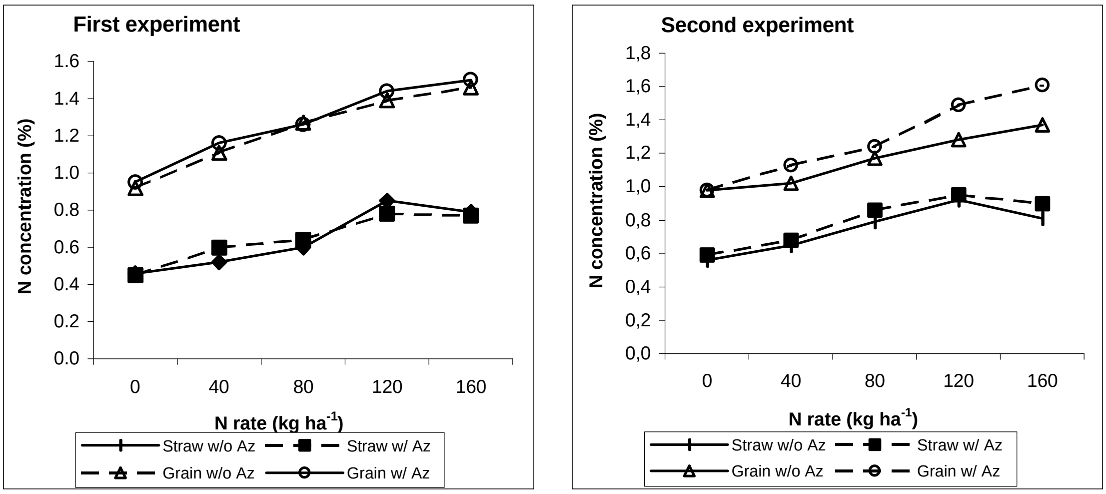


Figure 14: Effect of an *Azolla* cover on the grain and straw N concentration at harvest. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

The N concentration of the straw (Figure 14) improved with both urea application and *Azolla* cover. In the first experiment, the presence of an *Azolla* cover in plots with 40 and 80 kg N ha^{-1} increased straw N by 15.0 and 6.0%, respectively. At higher N levels (120 and 160 kg N ha^{-1}), however, *Azolla* cover did not further increase the N concentration in the straw. In the second experiment, plots with an *Azolla* cover at the different N rates had straw N concentrations significantly higher ($P<0.05$) by 4.0 to 12.0% in proportion to the treatments without *Azolla*. Straw N concentration in the non-fertilized plots with *Azolla* was comparable to that of the 40 kg N ha^{-1} without cover.

Total N uptake by rice at harvest Total N uptake (straw and grain) by rice in plots where urea was combined with *Azolla* was greater than that with urea only in both experiments (Table 5). In the first experiment, total N uptake by rice in *Azolla*-covered plots was higher by 1.9 to 16.5%, though, the increase was not significant. *Azolla* in conjunction with 120 kg N ha^{-1} gave an N uptake (112.4 kg N ha^{-1}) comparable to that obtained in plots with only 160 kg N ha^{-1} (114.0 kg N ha^{-1}). In combination, however, with the highest N rate (160 kg N ha^{-1}), the presence of an *Azolla* cover insignificantly reduced the total N uptake by rice (109 *vs.* 114 kg N ha^{-1}). Manna and Singh (1989) reported an increase in the total N uptake by rice when *Azolla* was combined with urea at the rate of 60 and 90 kg N ha^{-1}. Beyond the 90 kg N ha^{-1} rate, however, the authors noted the *Azolla* no longer significantly affected the total N uptake. As their experiment focused on the contribution of *Azolla* in terms of supplying N, the authors attributed this decrease to the reduced N contribution of *Azolla*.

Table 5: Effect of an *Azolla* cover on the total N uptake by rice at harvest. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

N rate (kg ha^{-1})	Total nitrogen uptake (kg ha^{-1})			
	First experiment		Second experiment	
	No *Azolla*	With *Azolla*	No *Azolla*	With *Azolla*
0	41.2 d	42.0 d	48.6 d	56.8 c
40	59.5 c	69.3 c	70.2 c	85.8 b
80	81.4 b	89.4 d	89.0 ab	105.1 a
120	109.1 a	112.4 a	102.6 a	116.7 a
160	114.0 a	109.0 a	81.2 bc	115.1 a

Source of variation	**df**	First experiment	Second experiment
Azolla (A)	1	ns	**
Nitrogen (N)	4	**	**
A x N	4	<1	ns
cv (%)		10.1	12.7

**, * = significant at 0.01 and 0.05 probability levels, respectively; ns = not significant

The total N uptake response was greater in the second experiment, where an *Azolla* cover significantly increased ($P<0.01$) the total rice N uptake. On the basis of the floodwater chemistry data, this difference in the total N uptake between the two experiments could be partly explained by the greater persistence and peak levels of the partial pressure of ammonia in the absence of *Azolla* in the first experiment as compared to the second experiment. This led to higher N losses and hence, poor efficiency.

In the second experiment, the effect of an *Azolla* cover was highest in the 160 kg N ha^{-1} treatment where a significant increase ($P<0.01$) in the total N uptake of 41.7% was recorded. Furthermore, in the presence of an *Azolla* cover, the total N uptake in the 120 kg N ha^{-1} treatment was 35.4 kg ha^{-1} more than that obtained in the 160 kg N ha^{-1}, whereas *Azolla* in combination with 80 kg N ha^{-1} gave an N uptake (105.1 kg N ha^{-1}) comparable to that obtained in plots treated with 120 kg N ha^{-1} (102.6 kg N ha^{-1}).

Even in the non-fertilized treatment, the mere presence of an *Azolla* cover on the floodwater surface increased the total N uptake by the rice plant. It was higher by 1.9 and 16.9% over the control (no N, no *Azolla* cover) in the first and second experiments.

Grain and straw N uptake In general, grain N uptake increased with the increasing rate of N applied (Figure 15). In both experiments, the presence of an *Azolla* cover significantly increased ($P<0.01$, $P<0.05$) the grain N uptake by 5.9 to 16.9% in the first experiment, and by 6.7 to 34.3% in the second experiment. In the first experiment, grain N uptake in the plots applied with 80 kg N ha^{-1} (62.6 kg N ha^{-1}) and 120 kg N ha^{-1} (74.7 kg N ha^{-1}) with an *Azolla* cover were comparable to the grain N uptake in the plots applied with 120 kg N ha^{-1} (66.9 kg N ha^{-1}) and 160 kg N ha^{-1} (77.0 kg N ha^{-1}) without cover. In the second experiment, grain N uptake in the *Azolla*-covered plots applied with 40 kg N ha^{-1} (50.1 kg N ha^{-1}) and 80 kg N ha^{-1} (54.4 kg N ha^{-1}) were similar to those obtained in the uncovered treatments with 80 kg N ha^{-1} (51.0 kg N ha^{-1}) and 120 kg N ha^{-1} (53.0 kg N ha^{-1}). Furthermore, grain N uptake in the 120 kg N ha^{-1} with *Azolla* cover was higher by 14.6 kg ha^{-1} than that obtained in the 160 kg N ha^{-1}applied alone. The *Azolla* cover in the non-fertilized plots improved grain N uptake by 7.9 and 9.9% in the first and second experiments.

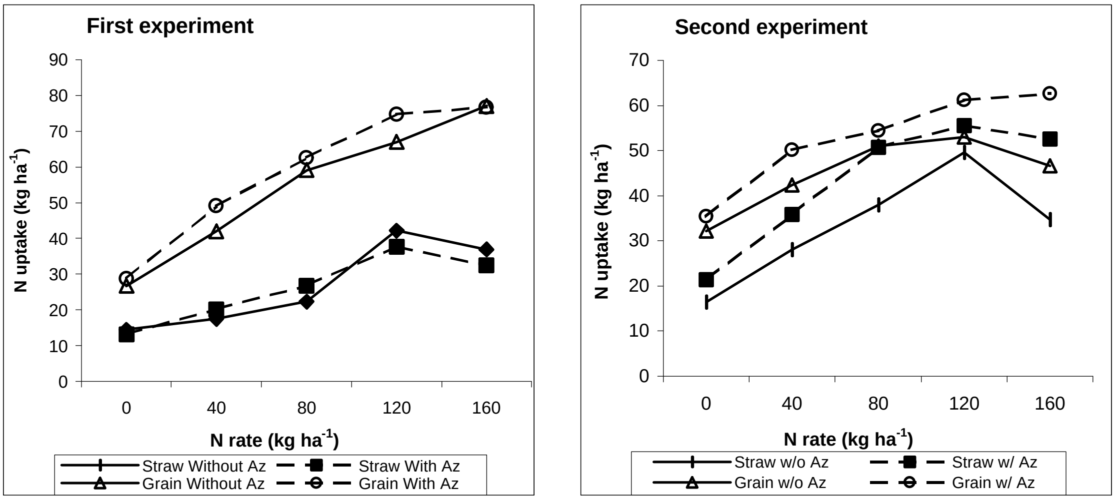
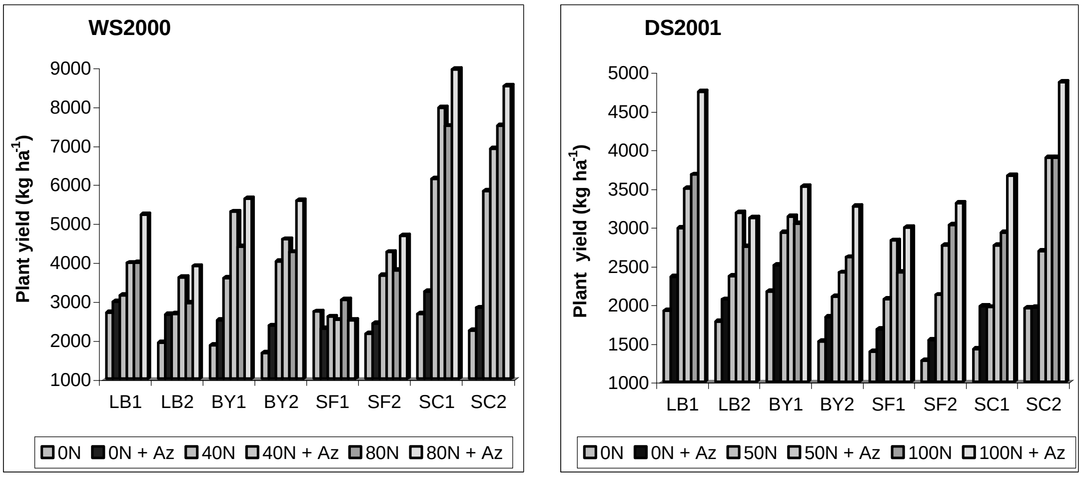


Figure 15: Effect of an *Azolla* cover on the grain and straw N uptake at harvest. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

Similar to the trend in the grain N uptake, straw N uptake increased as the N fertilizer rate increased (Figure 15). It was only slightly improved in the presence of an *Azolla* cover in the first experiment. In the second experiment, the presence of an *Azolla* cover significantly increased ($P<0.01$) the straw N uptake at the different N rates. The magnitude of increase was likewise greater, reaching 51.6% at 160 kg N ha^{-1}. Straw N uptake in the plots applied with 40 and 80 kg N ha^{-1} with an *Azolla* cover gave similar N uptake to that obtained in the 80 and 120 kg N ha^{-1} applied alone. The maximum straw N uptake (116.7 kg N ha^{-1}) was recorded in the 120 kg N ha^{-1} with *Azolla*. It was 20.8 kg N ha^{-1} higher than the straw N uptake in the 160 kg N ha^{-1} applied alone.

4.4.2 *On-farm field experiments*

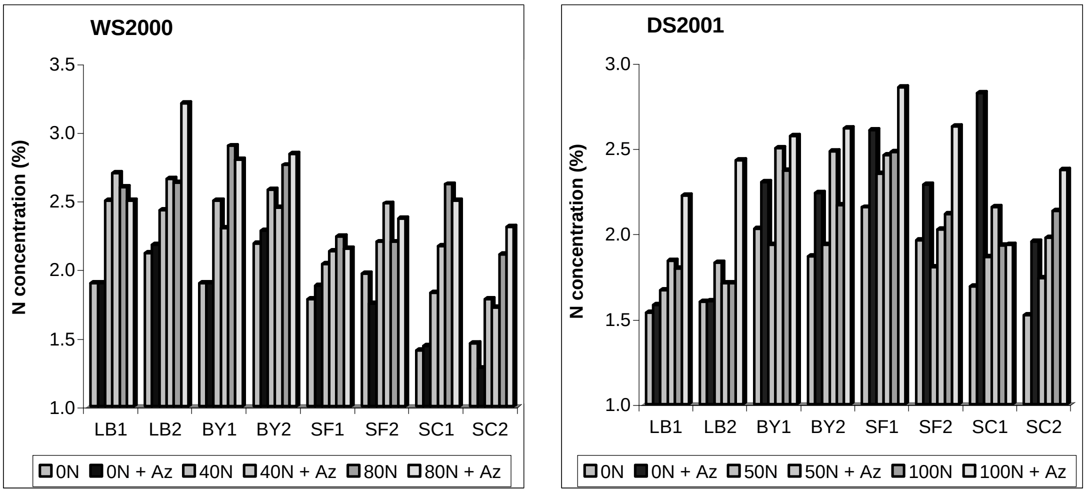
Plant yield at maximum tillering stage Plant yields of rice were higher for urea with *Azolla* than for urea alone in 7 out of 8 sites in the wet season and at all sites in the dry season (Figure 16). The gain was significant ($P<0.01$) at the 40 kg N ha^{-1} rate in the wet season (47.5%), and at the 50 kg N ha^{-1} in the dry season (45.0%).



LB1: Los Banos site 1
LB2: Los Banos site 2
BY1: Bay site 1
BY2: Bay site 2
SF1: San Francisco site 1
SF2: San Francisco site 2
SC1: Sta Cruz site 1
SC2: Sta Cruz site 2

Figure 16: Effect of an *Azolla* cover on the plant yield of rice at maximum tillering stage. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

Plant N concentration at maximum tillering stage In the on-farm experiments, the *Azolla* cover not only increased plant yield but also the N concentration. In the wet season, the increase in plant N concentration in the 40 and 80 kg N ha^{-1} plots with *Azolla* cover was relatively small with no specific trend. The highest N concentration was 3.21%, which was obtained in the 80 kg N ha^{-1} with *Azolla* cover (Figure 17). In the dry season, N concentration was significantly higher ($P<0.05$) in 7 out of 8 sites, with one site (Sta. Cruz site 1) showing a significant *Azolla* x N interaction.



LB1: Los Banos site 1
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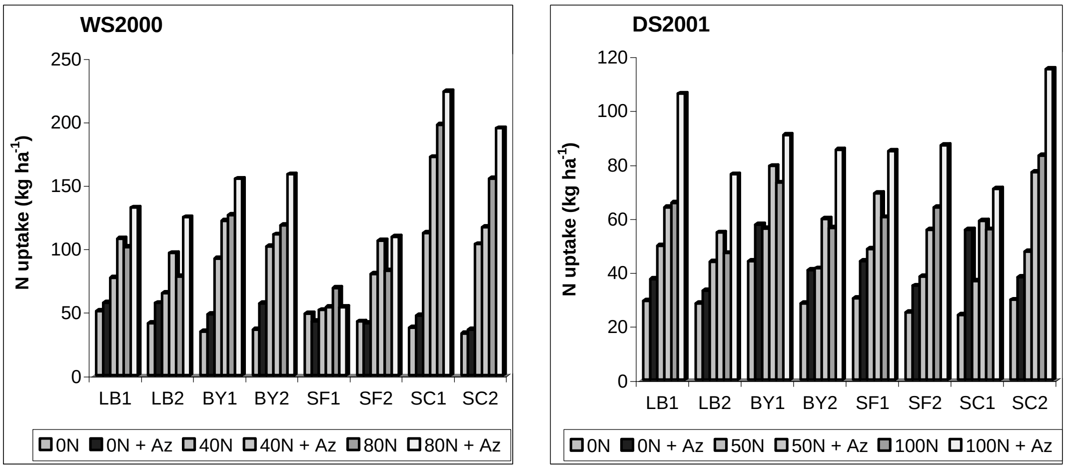
BY1: Bay site 1
BY2: Bay site 2
SF1: San Francisco site 1
SF2: San Francisco site 2
SC1: Sta Cruz site 1
SC2: Sta Cruz site 2

Figure 17: Effect of an *Azolla* cover on the plant N concentration at maximum tillering stage. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

Plant N uptake at maximum tillering stage Irrespective of season, *Azolla* in conjunction with urea led to higher plant N uptake (Figure 18). The response was greater in the dry season than in the wet season, presumably due to the higher solar radiation and higher mean daily temperature.



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SF1: San Francisco site 1
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SC1: Sta Cruz site 1
SC2: Sta Cruz site 2

Figure 18: Effect of an *Azolla* cover on the plant N uptake at maximum tillering stage. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

In the wet season, plant N uptake significantly increased in response to an *Azolla* cover in 7 out of 8 sites whereas in the dry season, all sites (8) showed a significant *Azolla* effect with one site (Los Banos site 1) having a significant *Azolla* x N interaction.

Plant N uptake in the wet season increased by 4.6 to 53.4% when plots receiving 40 kg N ha^{-1} were covered with *Azolla*. In the dry season (50 kg N ha^{-1}), this increase was 24.4 to 62.0%. Plant N uptake at the maximum N rates (80 and 100 kg N ha^{-1}) increased by 22.5 to 60.2% in the wet season and by 24 to 62.3% in the dry season due to *Azolla* cover. Even the plots without urea but inoculated with *Azolla* produced a plant N uptake higher than the control. In one of the sites (Sta. Cruz site 1), plant N uptake in the unfertilized plot with *Azolla* cover was even more than twice the plant N uptake in the equivalent plot without *Azolla*. It should be noted though, that there were sites where the effect of *Azolla* on the plant N uptake was negative. The San Francisco site 1, for instance, had 21.5% lower plant N uptake in the plots applied with 80 kg N ha^{-1}. At the same location (San Francisco), plant N uptake in the non-amended but *Azolla*-covered plot was 12.7% (San Francisco site 1) and 3.1% (San Francisco site 2) lower than the control.

At Los Banos site 1, plant N uptake in the 100 kg N ha^{-1} (65.6 kg N ha^{-1}) was significantly increased by 40.5 kg N ha^{-1} in the presence of *Azolla* or an increase of 61.8%. A significant *Azolla* x N interaction was observed at that location, indicating a positive synergistic effect of an *Azolla* cover (Vlek *et al*., 1995).

The significant rise in the plant N uptake by the rice at most sites with increasing doses of urea with *Azolla* corresponded with increases in dry matter production. It is generally agreed that crops, which produce large amounts of dry matter, consume more N than those producing less dry matter (Liu, 2000). The increase was also due to a marked increase in the concentration of N in the rice straw. Thus, the nitrogen conserved by the reduction of NH_3 volatilization in the beginning contributed to greater vegetative growth, as reflected in the higher plant dry matter yield and N concentration, leading to a higher plant N uptake. In addition, the N fixed and supplied by the *Azolla* presumably could have contributed to the higher plant N uptake, particularly later in the season.

Straw yield of rice at harvest In general, there was an increase in the straw yield of rice at harvest due to the combined effect of the *Azolla* cover and urea application (Figure 19). A significant increase in the straw yield was observed in 5 out of 8 sites in the wet season and in 6 out of 8 sites in the dry season. Straw yield was at its maximum in the 80 kg N ha^{-1} with *Azolla* cover (6.5 t ha^{-1}) in the wet season and in the 100 kg N ha^{-1} with *Azolla* cover (3.9 t ha^{-1}) in the dry season. One site (San Francisco site 2), however, showed a negative response to an *Azolla* cover in combination with 100 kg N ha^{-1} where straw yield decreased by 7.6%. In the dry season, the treatment *Azolla* and 50 kg N ha^{-1} produced a straw yield 1.9 to 24.4% higher than that in the 50 kg N ha^{-1}. Three sites (Bay site 1 and Sta Cruz sites 1 and 2), however, gave negative response to the presence of *Azolla* in this treatment. Straw N uptake in these sites declined by 12.9, 11.3 and 7.1%. An *Azolla* cover together with 100 kg N ha^{-1} increased straw yield by 8.1 to 50.8%.

In the non-fertilized plots, the presence of an *Azolla* cover increased straw yield by 1.1 to 49.6% in the wet season and by 7.0 to 40.1% in the dry season.

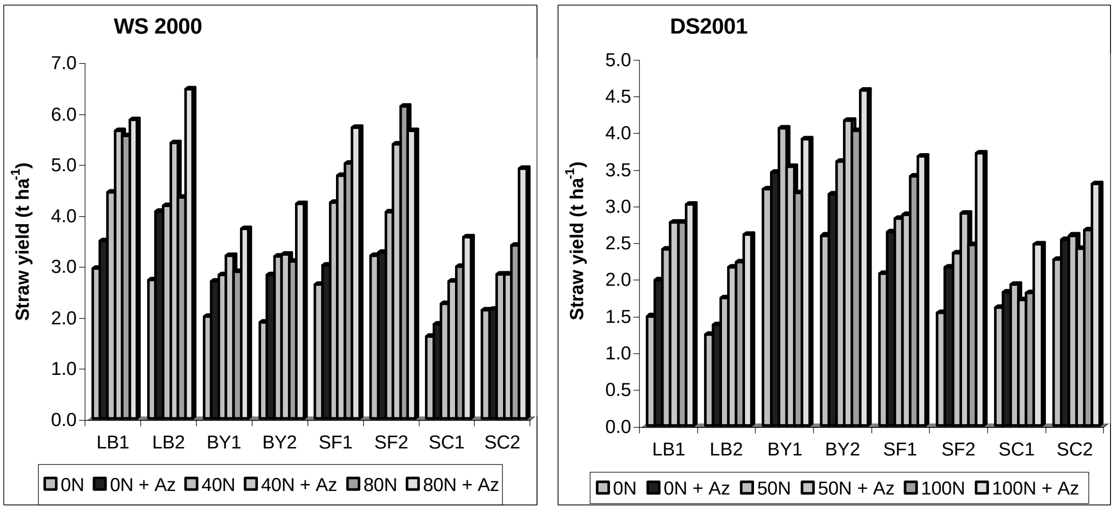

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Figure 19: Effect of an *Azolla* cover on the straw yield of rice at harvest. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

Nitrogen concentration in grain and straw at harvest The grain and straw N concentration at harvest in the wet and dry seasons did not show any consistent trends with regard to the effect of an *Azolla* cover. The grain N concentration in plots with urea and an *Azolla* cover was higher by 4.3 to 11% only at one site (Los Banos site 2) in the wet season. There were sites where plots covered with *Azolla* gave a lower grain N concentration than the uncovered plots (Figure 20). The straw N concentration followed a similar trend (Figure 21).

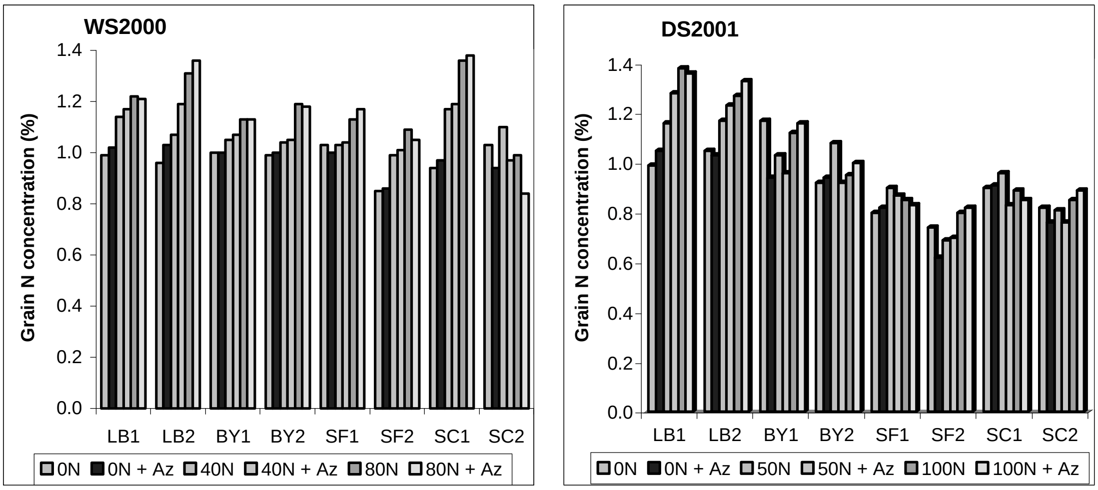

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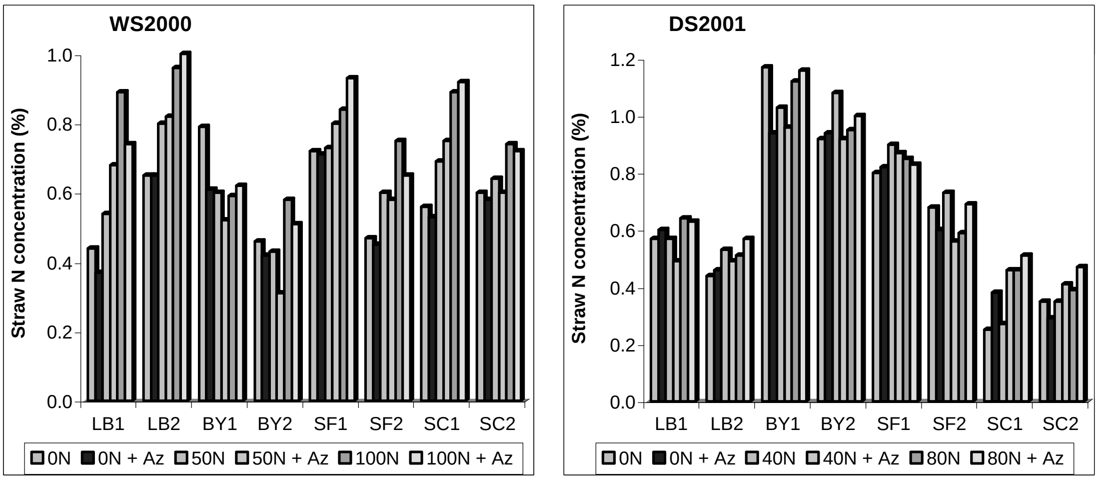
Figure 20: Effect of an *Azolla* cover on the grain N concentration at harvest. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.



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BY1: Bay site 1
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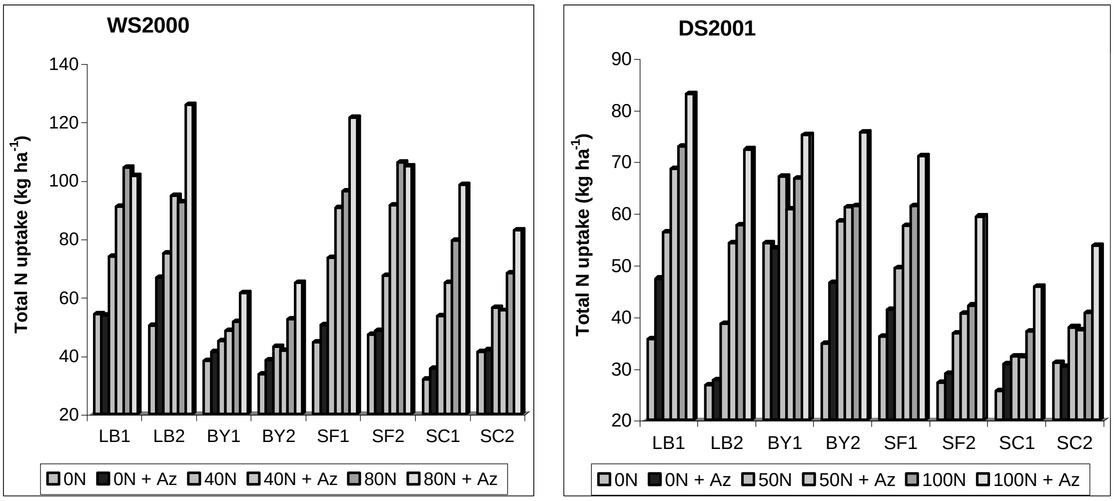
SF1: San Francisco site 1
SF2: San Francisco site 2
SC1: Sta Cruz site 1
SC2: Sta Cruz site 2

Figure 21: Effect of an *Azolla* cover on the straw N concentration at harvest. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

Total N uptake by rice at harvest At harvest, an *Azolla* cover significantly increased the total N uptake by rice in 2 out of 8 sites in the wet season and in 7 out of 8 sites in the dry season. The rate of total N uptake was higher in the dry season than in the wet season. Higher mean daily temperature and greater solar radiation during the entire growing season of rice in the dry season contributed to the higher total N uptake of the crop at harvest.

Total N uptake increased with both N rates and *Azolla* cover, resulting in the maximum N uptake at the highest N rates together with *Azolla* (Figure 22). In combination with *Azolla*, total N uptake in the whole plant increased by as much as 36.0% with the 80 kg N ha^{-1} in the wet season and by as much as 40.8% with the 100 kg N ha^{-1} in the dry season. In the dry season, one site (Los Banos site 2) showed a significant *Azolla* x N interaction ($P<0.05\%$), reflecting a reduction in N losses, and N-fixation by the fern. The total N uptake of 38.5 kg N ha^{-1} in the 50 kg N ha^{-1} treatment increased by 40.7% to 54.2 kg N ha^{-1} in response to an *Azolla* cover. At the highest N rate (100 kg N ha^{-1}), the total N uptake of 57.6 kg N ha^{-1} increased to 72.3 kg N ha^{-1}, or an increase of 25.6%. This increase in the total N uptake we attributed to the N fixed and to the N conserved by the *Azolla*, which were later released and absorbed by the plant.



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SC2: Sta Cruz site 2

Figure 22: Effect of an *Azolla* cover on the total N uptake at harvest. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

In the on-farm experiments, the rice crop took up ~32 to 52 kg N ha^{-1} in the wet season and from 26 to 36 kg N ha^{-1} in the dry season, without any N applied. Long-term fertility trials in temperate and tropical regions have shown that about 50 kg N ha^{-1} is absorbed by each crop of rice grown without addition of N fertilizer (Koyama and App, 1979). The effect of an *Azolla* cover on the total N uptake in non-fertilized plots was equivalent to the application of 40 kg N ha^{-1} in the wet season and 50 kg N ha^{-1} in the dry season. Similar findings were reported by Vlek *et al*. (1992). An increase in the N uptake by the rice crop at harvest with the use of *Azolla* has been reported before (Singh and Singh, 1986). During *Azolla* decomposition, N is released gradually; rice can thus utilize *Azolla*-N more effectively.

Grain and straw N uptake at harvest Grain N uptake significantly increased at half of the sites in the wet season and in 5 out of 8 sites in the dry season due to the combined effect of *Azolla* and urea (Figure 23). One site (San Francisco site 2) in the wet season and two sites (Los Banos site 1 and Bay site 2) in the dry season showed a significant *Azolla* x N interaction. Grain N uptake at San Francisco site 2 significantly increased by 17.5 kg N ha^{-1} in the 40 kg N ha^{-1} and by 8.3 kg N ha^{-1} in the 80 kg N ha^{-1} when these treatments were covered with *Azolla*. These data represent a 41.2 and 14.0% increase over the no *Azolla* cover treatments. The percentage increase in the N uptake by grain in the presence of *Azolla* ranged from 0.6 to 41.2% in the 40 kg N ha^{-1} and from 3.6 to 27.4% in the 80 kg N ha^{-1}. In the dry season, *Azolla* significantly increased the grain N uptake in Los Banos site 2 by 14.2 kg N ha^{-1} in the 50 kg N ha^{-1} treatment and 7.7 kg N ha^{-1} in the 100 kg N ha^{-1} treatment. These data represent a 48.1 and 7.5% increase. At Bay site 2, a 5.2% reduction in the grain N uptake in the 50 kg N ha^{-1} treatment with *Azolla* cover was noted. The percentage increase in uptake of N by grain in the presence of *Azolla* ranged from 26.2 to 41.2% in the 50 kg N ha^{-1} and from 7.5 to 25.2% in the 100 kg N ha^{-1}. The maximum grain N uptake of 67.9 kg N ha^{-1} in the wet season and 64.3 kg N ha^{-1} in the dry season were recorded at the highest N rates (80 and 100 kg N ha^{-1}) with *Azolla* cover.

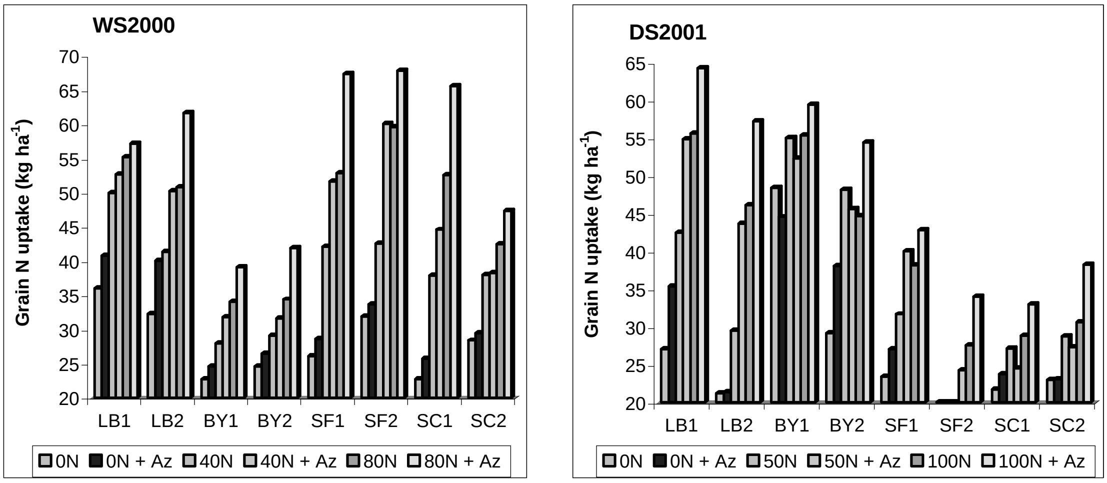

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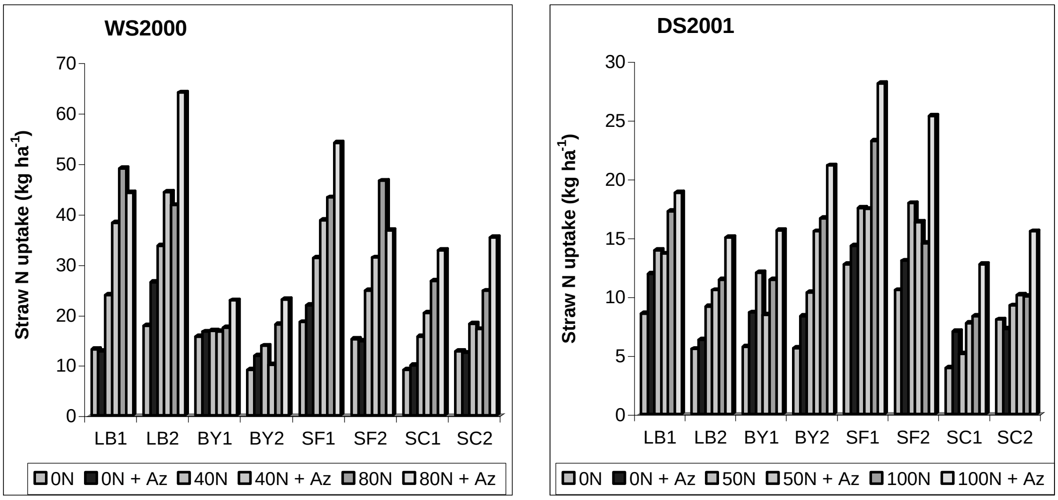
Figure 23: Effect of an *Azolla* cover on the grain N uptake at harvest. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

In non-fertilized plots, increases in grain N uptake in treatments with *Azolla* cover ranged from 3.7 to 24% in the wet season and from 1.1 to 30.9% in the dry season.

The presence of an *Azolla* cover increased straw N uptake by 24.3 to 60.1% in the 40 kg N ha^{-1} plots in the wet season, and by 16.4 to 51.6% in the 50 kg N ha^{-1} plots in the dry season (Figure 24). At the highest N rate, an *Azolla* cover led to an increase in the straw N uptake of 22.8 to 53.6% in the wet season and 9.2 to 74.1% in the dry season. *Azolla* in combination with 40 kg N ha^{-1} gave a straw N uptake similar to that of the 80 kg N ha^{-1} application.

Overall, the significant rise in the N uptake by the rice crop achieved with urea together with an *Azolla* cover was consistent with increases in dry matter production. This significant increase in plant growth suggests that there was a better supply of N to the rice crop during the vegetative stage and this supply sustained a high dry matter accumulation until harvest.



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SC2: Sta Cruz site 2

Figure 24: Effect of an *Azolla* cover on the straw N uptake at harvest. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

4.5 Tiller and panicle count

4.5.1 *On-station field experiments*

Tiller count at harvest A small negative effect of an *Azolla* cover on the tiller count was observed initially in the non-fertilized treatment. Tiller count was reduced by 4.8% in the first experiment and by 1.7% in the second experiment. In the first experiment, no specific trend in the tiller count was observed in the urea-amended, *Azolla*-covered treatments (Figure 25). At low fertilizer N rates, the presence of an *Azolla* cover led to higher tiller number. Plots with *Azolla* cover had both fewer (6.9%) and more (between 3.2 and 15.8%) tillers than the uncovered treatments, but the change was not significant. The plots with *Azolla* cover and treated with 160 kg N ha^{-1} produced the highest tiller number (17). The similar response to an *Azolla* cover was observed in the second experiment, with higher tiller count in treatments with an *Azolla* cover up to the 120 kg N ha^{-1} (8.9 to 12.2%). Above the 120 kg N ha^{-1}, no increase in the tiller count arising from the use of *Azolla* as a cover was found.

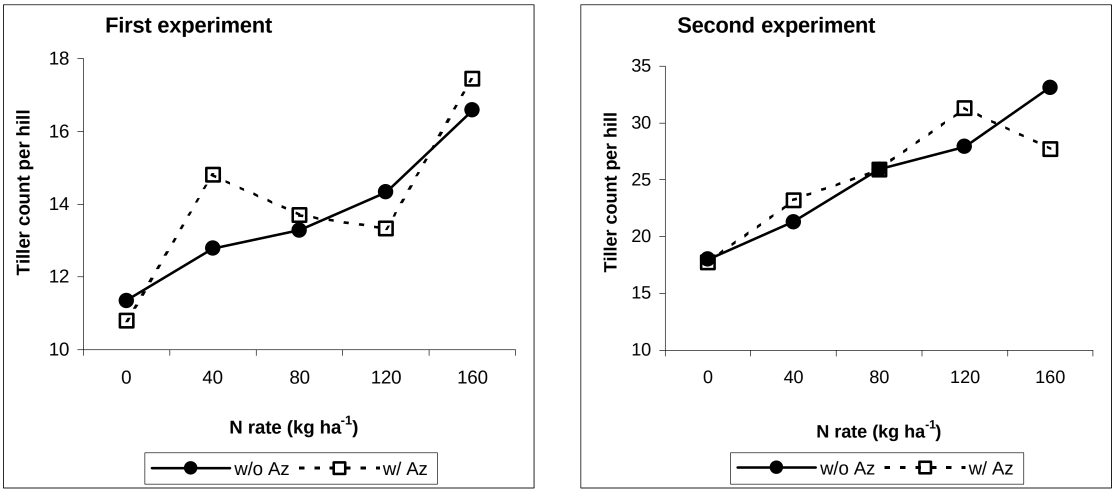


Figure 25: Effect of an *Azolla* cover on the tiller count at harvest. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

Panicle count at harvest The panicle count per hill was significantly affected by the *Azolla* cover in the first experiment but not in the second. In the first experiment, panicle count increased by 2.2 to 10.3% in treatments with *Azolla* cover. In the second experiment, panicle count increased by 4.7 and 9.4% in the plots applied with 80 and 120 kg N ha^{-1} with *Azolla* cover (Figure 26).

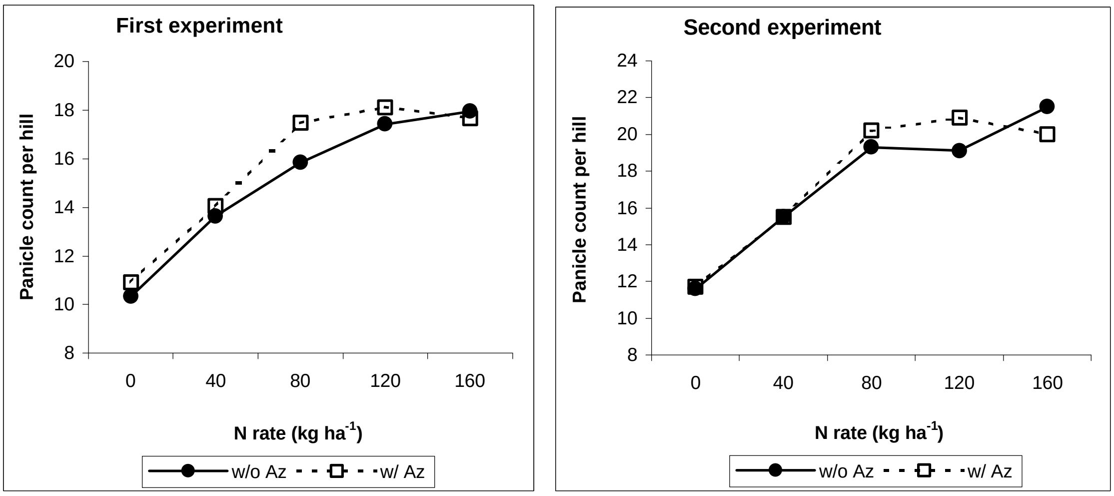
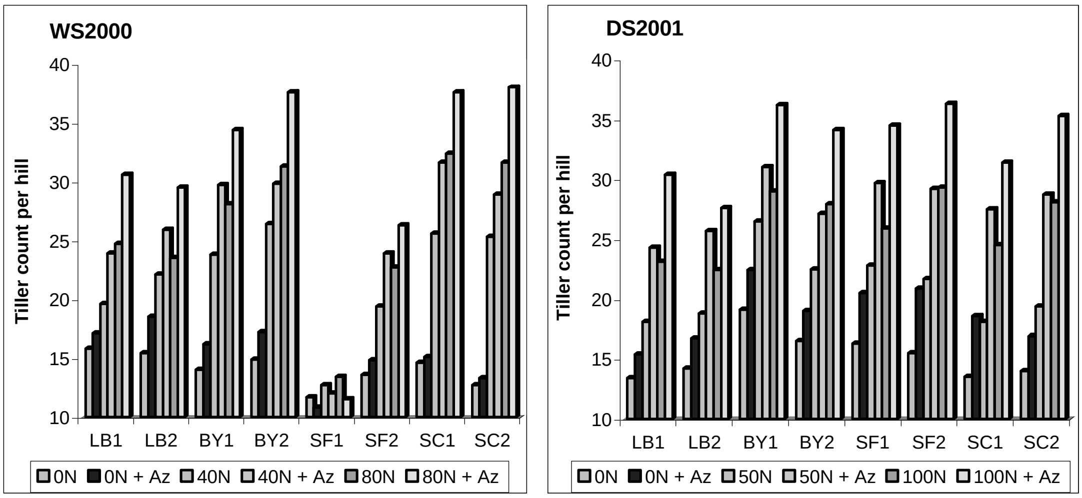


Figure 26: Effect of an *Azolla* cover on the panicle count at harvest. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

4.5.2 *On-farm field experiments*

Tiller count at maximum tillering stage The mid-season tiller count from the on-farm field experiments clearly reflected the benefits of having an *Azolla* cover on the floodwater surface at the time of urea application (Figure 27). The presence of an *Azolla* cover significantly increased ($P<0.01$) the tiller count at all sites in both seasons. The tiller count for the lower N rates with an *Azolla* cover were comparable if not higher than that obtained with the higher N rates applied alone. In the wet season, the *Azolla* cover increased the tiller count by 19% (12.7 to 24.8%) for the 40 kg N ha^{-1}, and 21% (15.6 to 25.8%) for the 80 kg N ha^{-1}. One site (Sta Cruz site 2) showed a significant *Azolla* x N interaction ($P<0.01$) indicating greater benefits from the combined *Azolla* and urea treatment than for the sum of the treatments alone.

In the dry season, a similar trend was observed, but the response was greater than in the wet season. Results from three sites (Los Banos site 1, San Francisco site 1, and Sta Cruz site 2) produced a significant *Azolla* x N interaction. The highest percent increase (51.7%) was recorded in the 50 kg N ha^{-1} plus *Azolla* treatment, whereas the highest tiller number (36) was recorded in the 100 kg N ha^{-1} with *Azolla* cover.



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SC2: Sta Cruz site 2

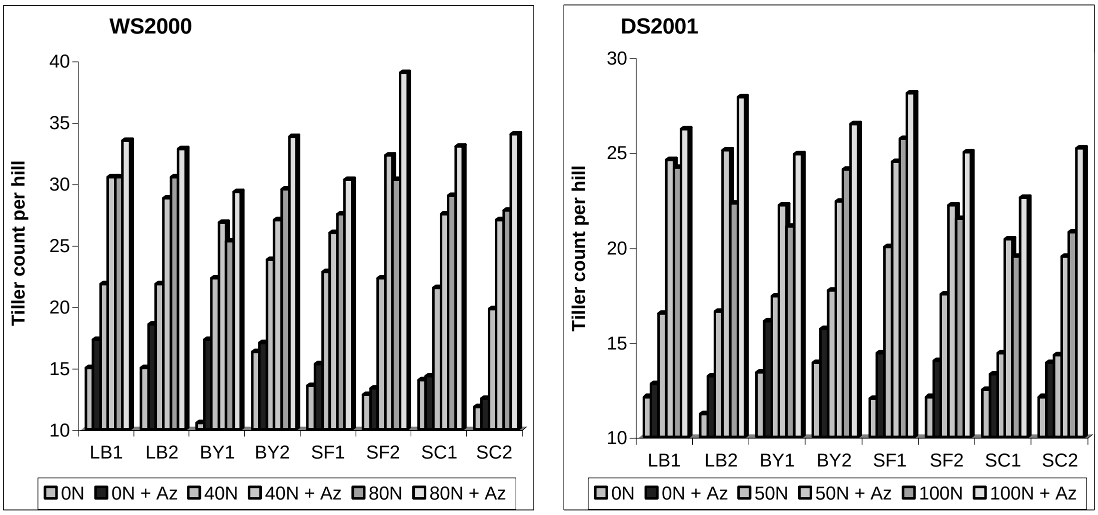
Figure 27: Effect of an *Azolla* cover on the tiller count at maximum tillering stage. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

Plots with *Azolla* cover in the non-fertilized treatments likewise had tiller numbers 3.4 to 20.2% higher in the wet season and 14.9 to 37.6% higher in the dry season as compared to the control plots (no N, no *Azolla* cover).

According to Mae and Shoji (1984), a close correlation exists between the number of tillers and the amount of N absorbed during the vegetative period. The N conserved at the time of urea application and the N supplied by the *Azolla* were taken up by the rice plants in their vegetative stage (as shown in the plant N uptake data) promoting growth and increasing tiller number at the maximum tillering stage.

Tiller count at harvest At harvest, all treatments with *Azolla* cover had a significantly higher ($P<0.01$) number of tillers than the uncovered treatments in both seasons. The higher N treatments with *Azolla* cover produced the maximum number of tillers, *i.e.*, 39 and 28 (Figure 28). In the wet season, five sites (Los Banos sites 1 and 2, San Francisco site 2 and Sta Cruz sites 1 and 2) showed a significant *Azolla* x N interaction, with 27.9 to 44.8% more tillers with *Azolla* at 40 kg N ha^{-1} and 7.5 to 28.7% more at 80 kg N ha^{-1}. In the dry season, a significant *Azolla* x N interaction ($P<0.01$) was observed in 4 out of 8 sites (Los Banos sites 1 and 2 and Sta Cruz sites 1 and 2). The magnitude of increase in the tiller count due to an *Azolla* cover in the dry season was greater than that in the wet season. At 50 kg N ha^{-1} plus *Azolla*, the tiller count increase ranged from 36.1 to 51.7%, whereas with 100 kg N ha^{-1} plus *Azolla* cover, the increase was 8.3 to 25.1%. The *Azolla*-covered plots with 40 kg N ha^{-1} in the wet season or 50 kg N ha^{-1} in the dry season produced tiller numbers comparable to those of 80 and 100 kg N ha^{-1}.

Consistent with the tiller count at the maximum tillering stage, the *Azolla* cover in the non-fertilized plots increased the tiller number at harvest by as much as 64.5 and 20.8% over the control plots in the wet and dry seasons, respectively.



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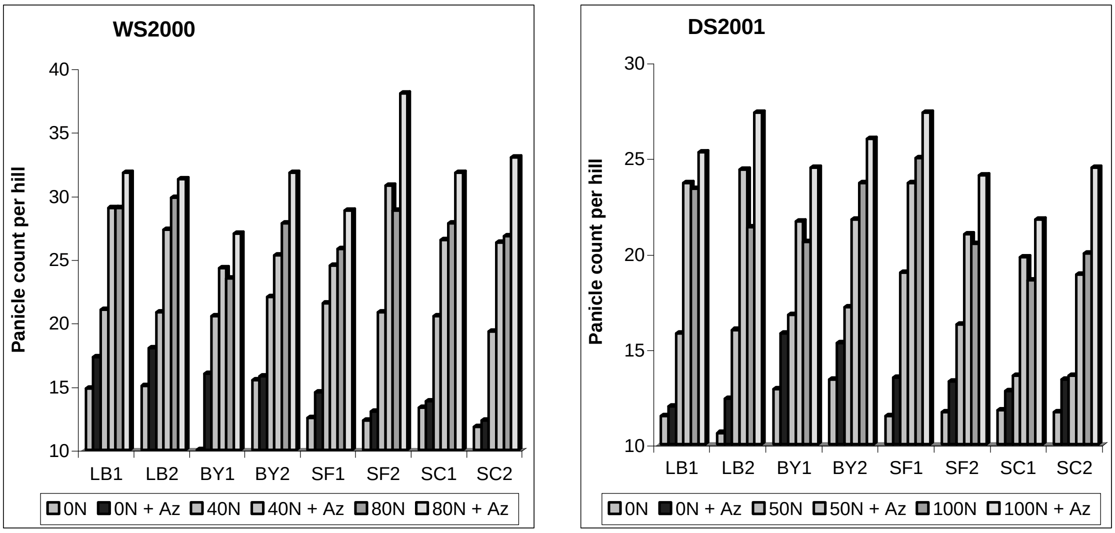
SC1: Sta Cruz site 1
SC2: Sta Cruz site 2

Figure 28: Effect of an *Azolla* cover on the tiller count at harvest. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

Tillering ability is one of the most important traits of rice. It significantly influences the production of panicles, which in turn is highly correlated with grain yield (Gravois and Helms, 1992). The higher number of tillers in *Azolla*-covered plots observed at maximum tillering stage was still observed at harvest. Presumably, the presence of an *Azolla* cover in the vegetative period of the rice allowed more N to be utilized by the rice crop because of reduced volatilization. At the same time, Singh (1986) reported that N fixed by *Azolla* is supplied to the rice at the late tillering, heading and milk stage. The present study confirmed earlier results by Parot (1991) showing that the N supplied by *Azolla*, together with the urea conserved, increased the tiller numbers of rice. Tiller number, in turn, determines the potential number of panicles.

Panicle count at harvest Several authors (Samonte *et al*., 1998; Miller e*t al*., 1991) reported the positive direct effect of tiller count on panicle density. The period that most influences the panicle number is the most active tillering stage (Matsushima, 1967). In the present investigation, the beneficial effect of an *Azolla* cover earlier reflected on the tiller count was also observed on the panicle count, which was significantly higher in the presence of an *Azolla* cover. In the wet season, urea application together with *Azolla* cover produced a significant *Azolla* x N interaction in 5 out of 8 sites (Los Banos sites 1 and 2, San Francisco site 2, and Sta Cruz sites 1 and 2). An *Azolla* cover together with

40 kg N ha^{-1} increased the panicle count per hill by 31.3 to 48.1% over the 40 kg N ha^{-1} alone. In combination with 80 kg N ha^{-1}, the *Azolla* cover increased the panicle count by 5.0 to 31.9%. In the dry season, a significant *Azolla* x N interaction (P<0.01) was observed in 4 out of 8 sites (Los Banos sites 1 and 2, and Sta Cruz sites 1 and 2). The plots with 50 kg N ha^{-1} and with an *Azolla* cover produced 39.0 to 53.2% more panicles than the uncovered plots. The 100 kg N ha^{-1} plus *Azolla* cover had a panicle number 8.4 to 27.9% higher than that in plots with only 100 kg N ha^{-1} (Figure 29).



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SF1: San Francisco site 1
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SC1: Sta Cruz site 1
SC2: Sta Cruz site 2

Figure 29: Effect of an *Azolla* cover on the panicle count at harvest. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

The highest panicle count was obtained in the plots with higher N rates combined with *Azolla* (38 and 27). The lower N rates with *Azolla* cover produced a panicle number comparable to the higher N rates without cover, irrespective of season. Panicle count per hill was likewise higher in the control plots with *Azolla* cover than without. In the wet season, panicle count per hill ranged from 10 to 16 in the control plot without *Azolla* cover, which increased by 1.9 to 60.0% due to *Azolla*. In the dry season, the panicle count was increased by 3.9 to 21.8%. This increase in panicle count even without application of urea could be due to the reduction in losses of the N mineralized from the soil in the presence of an *Azolla* cover. The contribution, however, of *Azolla*-N maybe more important.

In general, other yield parameters such as spikelets per panicle, filled spikelets per panicle, thousand grain weight, grain:straw ratio and harvest index did not show any significant effect of *Azolla* nor any definite trend in both seasons in the farmers' fields. Hence, the data were not presented.

4.6 Total dry matter yield at harvest

4.6.1 *On-station field experiments*

The main effect of *Azolla* cover showed that in conjunction with urea, total dry matter yield was significantly higher than that obtained with urea alone (Figure 30). The total dry matter yield at harvest increased by 4.6 to 25.5%. The highest dry matter yield (10.4 t ha^{-1}) was found in the treatment where 120 kg N ha^{-1} was applied with an *Azolla* cover. The highest difference (25.5%) between the *Azolla*-covered and the uncovered treatment was recorded at the highest N rate (160 kg N ha^{-1}).

In the first experiment, an increase in the total dry matter yield of up to 3.4% was noted in *Azolla*-covered plots. In general, however, the total dry matter yields produced in treatments with *Azolla* cover were not significantly different to the *Azolla*-free plots.

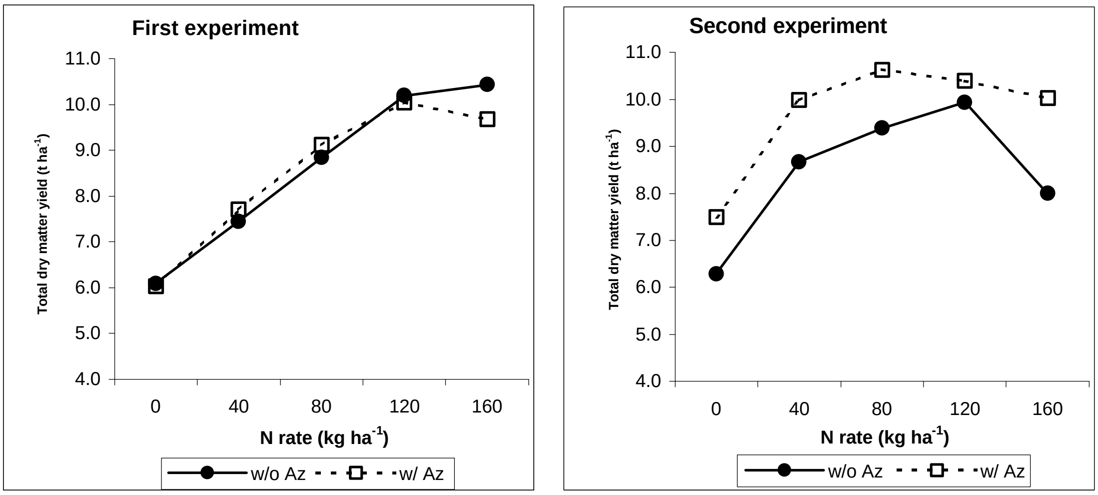
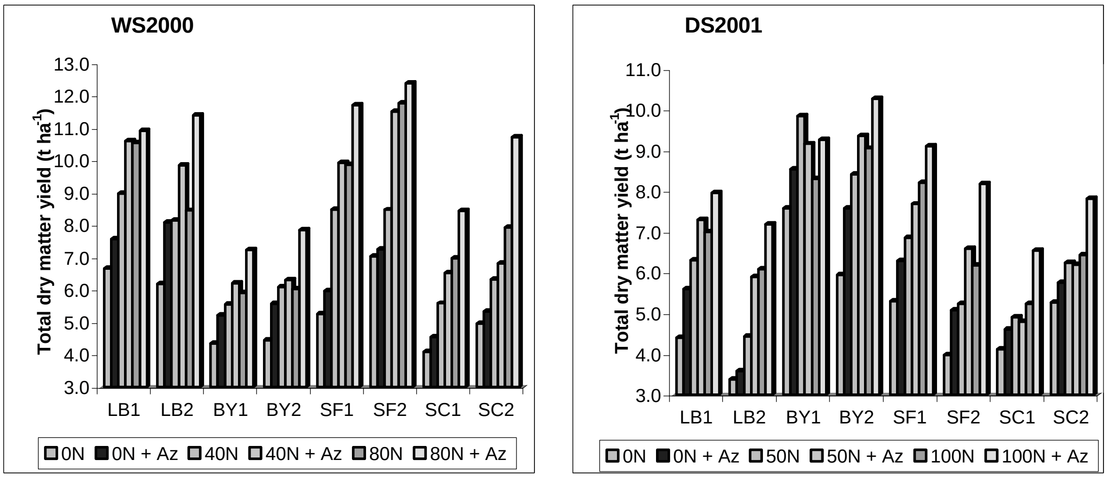


Figure 30: Effect of an *Azolla* cover on the total dry matter yield of rice at harvest. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

4.6.2 *On-farm field experiments*

In the on-farm experiments, in general, a significantly higher dry matter yield was produced in *Azolla*-covered plots as compared to *Azolla*-free plots (Figure 31).



LB1: Los Banos site 1
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BY2: Bay site 2
SF1: San Francisco site 1
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SC1: Sta Cruz site 1
SC2: Sta Cruz site 2

Figure 31: Effect of an *Azolla* cover on the total dry matter yield of rice at harvest. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

In the wet season, the total dry matter yield at five sites was significantly higher in treatments with *Azolla* cover. A significant *Azolla* x N interaction occurred at San Francisco site 2, where total dry matter yield significantly increased by 35.9% in the plots with 40 kg N ha^{-1}. In the plots treated with 80 kg N ha^{-1}, total dry matter yield increased by 5.2% in the presence of an *Azolla* cover. The highest total dry matter yield (12.4 t ha^{-1}) was also recorded in this treatment. For the rest of the sites, the total dry matter yield rose by 3.8 to 35.9% in the 40 kg N ha^{-1} in response to an *Azolla* cover. The 80 kg N ha^{-1} combined with *Azolla* increased total dry matter yield by 3.6 to 35.3%. The non-fertilized plots covered with *Azolla* yielded 3.3 to 30.7% more total dry matter yield than the control plots.

In the dry season, *Azolla* cover significantly increased the total dry matter yield of rice in 6 out of 8 sites. The total dry matter yield increased by 11.2 to 33.2% in plots with 50 kg N ha^{-1}. Three sites (Bay site 2, Sta. Cruz sites 1 and 2), however, produced a lower total dry matter yield in this treatment. At the highest N rate (100 kg N ha^{-1}), the total dry matter yield increased by 11.0 to 32.3% in the presence of an *Azolla* cover. The total dry matter yield in the control plots with *Azolla* cover was 6.1 to 27.7% higher than those without *Azolla*.

Total dry matter yield in treatments with 40 kg N ha^{-1} and *Azolla* in the wet season, and 50 kg N ha^{-1} with *Azolla* in the dry season were comparable if not greater than the corresponding total dry matter yield in the 80 and 100 kg N ha^{-1} treatments, respectively.

4.7 Grain yield

4.7.1 *On-station field experiments*

In general, an *Azolla* cover in combination with the different levels of urea increased the grain yield of rice (Figure 32). The extent of the increase in both experiments, however, was too small to be of statistical significance.

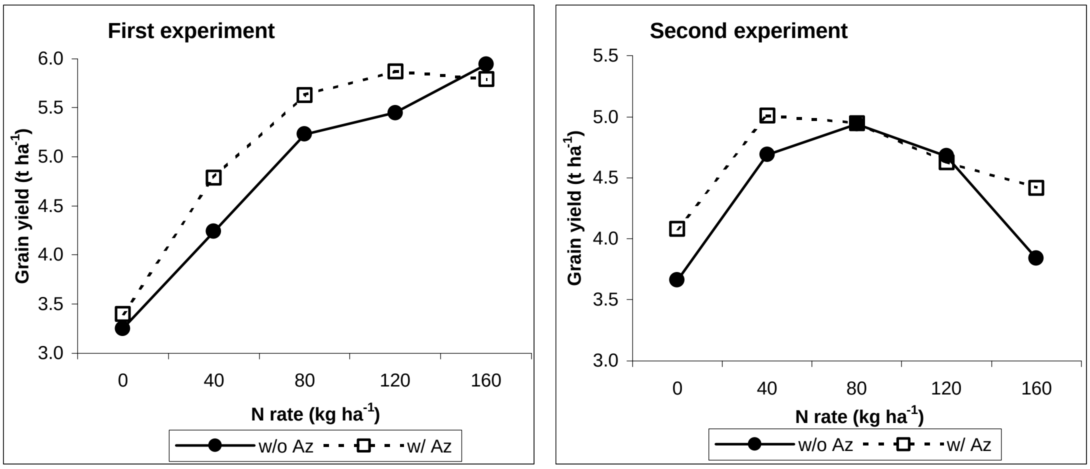


Figure 32: Effect of an *Azolla* cover on the grain yield at harvest. On-station field experiments. Los Banos, Philippines. Dry season, 1998-99.

In the first experiment, rice responded positively up to the 160 kg N ha^{-1} rate without *Azolla* cover. It produced the maximum grain yield of 5.9 t ha^{-1}. In the presence of an *Azolla* cover, rice responded positively up to the 120 kg N ha^{-1} rate where grain yield equaled that obtained in the 160 kg N ha^{-1} treatment. With 40, 80, 120 and 160 kg

N ha^{-1}, grain yield increased by 30.6, 61.0, 67.6 and 82.7% over the control (3.2 t ha^{-1}). An *Azolla* cover increased the grain yield further, appreciably though not significantly, by 12.8, 7.5 and 7.7% up to the 120 kg N ha^{-1} level. At the 40 kg N ha^{-1} rate, the yield increase amounts to half a ton of rice. Yield started to plateau at the highest N rate, where no further increase in grain yield due to an *Azolla* cover was observed. It should be noted, though, that the application of 40 and 120 kg N ha^{-1} in combination with an *Azolla* cover resulted in a grain yield response comparable to those of 80 and 160 kg N ha^{-1} without cover. Grain yield for the 80 kg N ha^{-1} with *Azolla* cover (5.6 t ha^{-1}) was even higher than that obtained for the 120 kg N ha^{-1} (5.4 t ha^{-1}).

In the second experiment, grain yield was depressed due to a heavy rainfall that occurred only weeks before harvest. As a result, plants in treatments with higher N lodged. It is thus difficult to say that the graph above depicts the actual yield response and, therefore, only a hypothetical trend can be postulated. In the control and at the urea rate of 40 kg N ha^{-1}, the presence of an *Azolla* cover gave a yield advantage over the no *Azolla* cover. Without *Azolla*, yield increased up to the 80 kg N ha^{-1} rate and then started to decline in the 120 kg N ha^{-1} rate. At the 80 and 120 kg N ha^{-1} rates, the advantage of having an *Azolla* cover disappeared. The effect of an *Azolla* cover became smaller and insignificant in its influence on grain yield.

It should be noted, however, that even without N applied and without an *Azolla* cover, the grain yield of rice was 3.3 t ha^{-1} in the first experiment and 3.7 t ha^{-1} in the second experiment. Singh and Singh (1987) reported that lowland rice could be grown continuously with reasonable yields without N fertilizers. The soil apparently can mineralize enough native N to adequately supply the crop with N (Buresh *et al.*, 1990). In our experimental fields, which are frequently used for experiments and rice production, the level of N in the soil can support a 3 to 4 t ha^{-1} crop annually. Soil analysis showed that the first experimental site has 0.16% N and the second experimental site has 0.23% N.

In addition to N losses, there are other factors, which probably limited the yield of rice in our experiments. Adverse weather such as heavy rains and soil factors such as high native soil N limited the response of rice to the combined treatment of urea and *Azolla*. Consequently, the *Azolla* effects on NH_3 volatilization losses and N conservation in the early rice stages of rice growth did not result in a significant increase in grain yield. Thus, the statistically significant treatment difference between *Azolla*-covered and *Azolla*-free plots observed in the floodwater chemistry has not reflected significant differences in grain yields at maturity.

Potential crop yield index The spikelets per hill were computed to determine the potential yield index (PYI) of rice. The index is based on the panicle density and the spikelet number of rice, which are fixed at the time of heading, and reflects the N status of the plant between transplanting and heading (Vlek *et al.*, 1979).

In contrast with the actual grain yield, where no significant effect of *Azolla* was noted, the PYI of rice was significantly higher ($P<0.05$) under an *Azolla* cover in the first experiment. It should be noted, though, that the growth period of rice between transplanting and heading was exposed to low solar radiation and low mean temperature (13.7 MJ m^{-2} and 25.9^{o}C on the average, respectively). Under these climatic conditions, the yield potential increased linearly up to the 80 kg N ha^{-1} rate where the highest PYI was recorded (2036) (Figure 33). Increasing the N level in combination with *Azolla* beyond the 80 kg N ha^{-1} did not further enhance the potential yield of rice. With the actual grain yield, the rice responded positively up to the 120 kg N ha^{-1} with an *Azolla* cover. This improvement could have come from the gain from other yield components, which were established after heading.

The PYI would have indicated the possible yield response of the plants in the second experiment if lodging had not occurred. It shows that the yield potential of rice increased with increasing levels of urea combined with *Azolla* up to 120 kg N ha^{-1}. At this rate, the spikelets per hill with an *Azolla* cover were significantly higher ($P<0.05$) by 26% than that without.

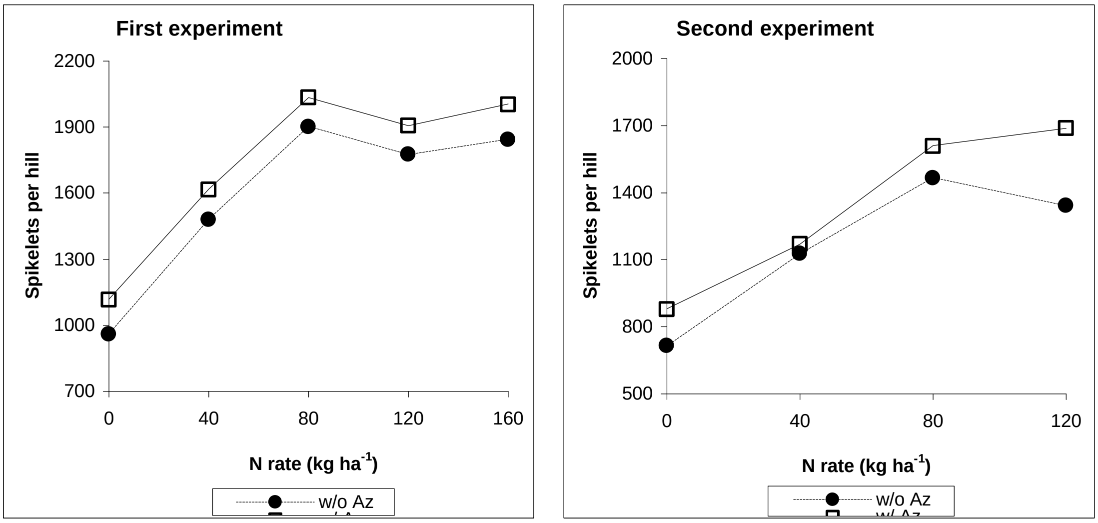


Figure 33: Effect of an *Azolla* cover on the potential crop yield index (PYI). On-station field experiments. Los Banos, Philippines. Dry season 1998-99.

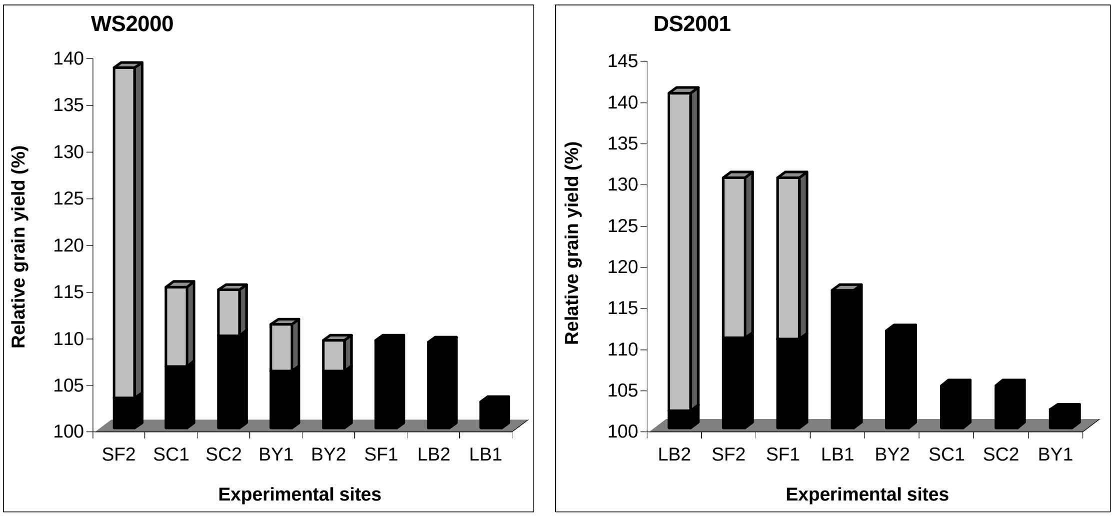
4.7.2 *On-farm field experiments*

The influence of an *Azolla* cover on grain yield was clearly manifested in the on-farm field experiments, where most of the sites had insufficient levels of N. Since N was limiting, yield responded better to the combined treatment of urea and *Azolla* cover. Plots with urea and *Azolla* cover gave consistently higher grain yields than plots with urea alone in the wet and dry seasons. Maximum grain yields were obtained in *Azolla*-covered plots applied with 80 kg urea-N ha^{-1} (7.3 t ha^{-1}) in the wet season and with 50 kg N ha^{-1} (6.2 t ha^{-1}) in the dry season. For the same N rates but without *Azolla* cover, grain yield was 0.2 and 1.17 t ha^{-1} lower.

In the wet season, the presence of an *Azolla* cover significantly increased ($P<0.01$, $P<0.05$) the grain yield in 6 out of 8 sites. One site (San Francisco site 2) produced a significant *Azolla* x N interaction ($P<0.05$). The grain yield in plots with 40 kg urea-N ha^{-1} plus an *Azolla* cover (6.7 t ha^{-1}) was significantly higher ($P<0.01$) by 38.6% than that in the 40 kg N ha^{-1} applied alone (4.8 t ha^{-1}). The grain yield in plots with 80 kg N ha^{-1} and *Azolla* cover (7.3 t ha^{-1}) was significantly higher ($P<0.01$) by 19.0% than the yield in the 80 kg N ha^{-1} (6.1 t ha^{-1}) treatment. The yield in the 40 kg N ha^{-1} with *Azolla* cover treatment was 8.7% higher than the yield in the 80 kg N ha^{-1} treatment.

In the dry season, the response of rice to the combined treatment of urea and *Azolla* cover was better than that in the wet season. Seven out of 8 sites showed a significant ($P<0.01$, $P<0.05$) *Azolla*-cover effect on the grain yield. A positive significant *Azolla* x N interaction ($P<0.05$) occurred at one site (Los Banos site 2), where *Azolla* cover significantly increased ($P<0.01$) the grain yield in the 50 kg N ha^{-1} treatment by 40.6% as compared with the 50 kg N ha^{-1} without cover. At the highest N rate (100 kg N ha^{-1}), the presence of *Azolla* raised grain yield by 19.4% over the no-*Azolla* plot.

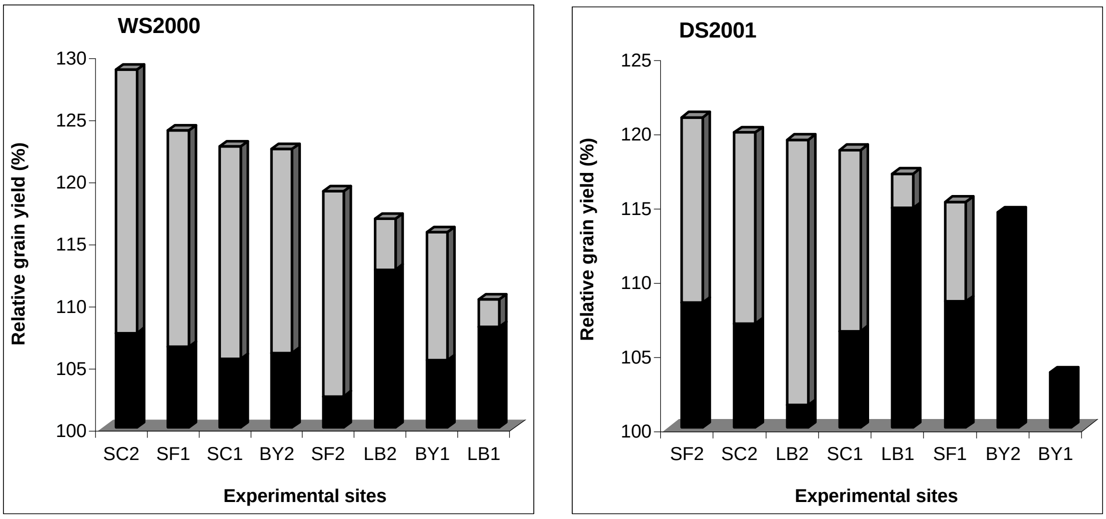
The relative grain yield of rice in plots applied with 40 kg N ha^{-1} and covered with *Azolla* was 2.8 to 38.6% higher than the grain yield obtained in the uncovered 40 kg N ha^{-1} treatment in the wet season (Figure 34). Of this relative grain yield increase, the increase due to an *Azolla* x N interaction is indicated by the red portion in each bar. Five out of 8 sites showed an increase due to an interaction of *Azolla* and urea. At the highest relative grain yield increase (38.6%), an *Azolla* cover increased grain yield by nearly 1.9 t ha^{-1}, 92% (1.7 t ha^{-1}) of which was due to the *Azolla* x N interaction. In the dry season, the plots with 50 kg N ha^{-1} and with *Azolla* cover had a relative grain yield higher by 2.2 to 40.6% than the grain yield in the 50 kg N ha^{-1} treatment. In half of the total sites, part of the increase in relative yield was due to an *Azolla* x N interaction. At the highest relative grain yield increase (40.6%), the presence of an *Azolla* cover increased grain yield by nearly 1.2 t ha^{-1}, of which 1.1 t ha^{-1} was due to the *Azolla* x N interaction. Five sites in the wet season had a relative yield 10% higher in the 40 kg N ha^{-1} with *Azolla* cover than the plots with only 40 kg N ha^{-1}. The same number of sites in the dry season had a relative yield 10% higher in the 50 kg N ha^{-1} plus *Azolla* cover than the 50 kg N ha^{-1} alone. An *Azolla* cover together with 40 kg N ha^{-1} in the wet season and 50 kg N ha^{-1} in the dry season produced a comparable grain yield response with that obtained in the 80 and 100 kg N ha^{-1} without *Azolla*. Singh (1986) found that *Azolla* inoculated once in addition to 30 kg N ha^{-1} as urea produced the same amount of grain as with the application of 60 kg N ha^{-1} as urea. Earlier results show that dual cropping of *Azolla* and rice with 30, 60 and 90 kg N ha^{-1} as urea showed grain yields similar to those obtained by the application of 60, 90 and 120 kg N ha^{-1} as urea (Singh *et al*., 1989).



LB1: Los Banos site 1	BY1: Bay site 1	SF1: San Francisco site 1	SC1: Sta Cruz site 1
LB2: Los Banos site 2	BY2: Bay site 2	SF2: San Francisco site 2	SC2: Sta Cruz site 2

Figure 34: The relative grain yield of rice at 40 and 50 kg N ha^{-1} with *Azolla* cover over the 40 and 50 kg N ha^{-1} applied alone. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

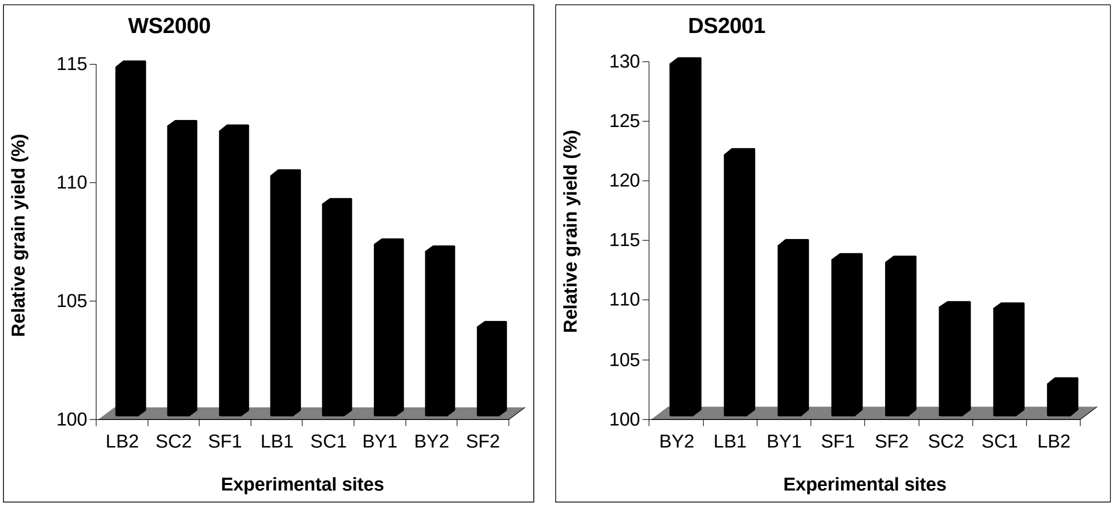
Grain yield differences were significant even at the highest N rate (80 kg N ha^{-1} in the wet season and 100 kg N ha^{-1} in the dry season) (Figure 35). *Azolla* cover in combination with 80 kg N ha^{-1} increased the relative grain yield by 4.8 to 28.8% over the 80 kg N ha^{-1} rate applied alone in the wet season. All sites showed an interaction effect of *Azolla* and urea. The highest *Azolla* x N interaction effect on grain yield was observed at San Francisco site 2. Of the 1.2 t ha^{-1} increase due to an *Azolla* cover, 1.0 t ha^{-1} was attributed to the interaction effect. Seven out of 8 sites had a relative grain yield higher by 10% and more in the *Azolla*-covered plots. In the dry season, the relative grain yield at the 100 kg N ha^{-1} plus *Azolla* cover treatment was higher by 3.7 to 20.9% than that at the 100 kg N ha^{-1}. An *Azolla* x N interaction effect on the grain yield was recorded in 6 out of 8 sites. The interaction effect was highest from the grain yield at Los Banos site 2, where 92% (0.7 t ha^{-1}) of the 0.8 t ha^{-1} increase was due to the *Azolla* x N interaction. The same number of sites (7 out of 8) had a relative grain yield higher by 10% and more.



LB1: Los Banos site 1
LB2: Los Banos site 2
BY1: Bay site 1
BY2: Bay site 2
SF1: San Francisco site 1
SF2: San Francisco site 2
SC1: Sta Cruz site 1
SC2: Sta Cruz site 2

Figure 35: The relative grain yield of rice at 80 and 100 kg N ha^{-1} with *Azolla* cover over the 80 and 100 kg N ha^{-1} applied alone at harvest. On-farm field experiments. Laguna. Philippines. Wet and dry seasons, 2000-01.

A consistent trend was observed in the yield comparison of *Azolla*-covered and *Azolla*-free plots in the non-fertilized treatments (Figure 36). In both seasons, grain yield in the 0 kg N ha^{-1} plus *Azolla* treatment was higher than that of the control. In the wet season, grain yield in the control plots ranged from 2.6 to 4.3 t ha^{-1}. This increased to 2.8 and 4.5 t ha^{-1} in the presence of an *Azolla* cover. The relative grain yield of the *Azolla*-covered plots was 3.7 to 14.7% higher than that of the control (no *Azolla* cover). Three out of 8 sites produced a relative grain yield 10% higher in the presence of an *Azolla* cover than the control. In the dry season, grain yield in the control plots ranged between 2.3 and 4.6 t ha^{-1}, whereas in the presence of an *Azolla* cover, grain yield varied from 2.2 to 5.3 t ha^{-1}. The relative grain yield increase in the *Azolla*-covered plots during this season ranged from 2.6 to 29.4%. Five out of 8 sites showed a grain yield higher by 10% in the *Azolla*-covered plots as compared with the control.



LB1: Los Banos site 1
LB2: Los Banos site 2
BY1: Bay site 1
BY2: Bay site 2
SF1: San Francisco site 1
SF2: San Francisco site 2
SC1: Sta Cruz site 1
SC2: Sta Cruz site 2

Figure 36: The relative grain yield of rice at 0 kg N ha^{-1} with *Azolla* over the 0 kg N ha^{-1} at harvest. On-farm field experiments. Laguna, Philippines. Wet and dry seasons, 2000-01.

The higher grain yield obtained in the *Azolla*-covered plots can mainly be attributed to the production of a significantly higher tiller number, presumably because of the adequate N supply during the vegetative and reproductive stages of the rice. In a recent experiment, productive tillers were found to be positively correlated (r=0.88) with grain yield (Bronson *et al.*, 2000). As a result of the higher tiller number, the number of panicles is likewise increased. The panicle number, which is determined at the early vegetative stage, is also a major factor influencing the rice yield. Gravois and Helms (1992) found panicle density exerting the largest positive direct effect on rice among the different yield components of rice. Samonte *et al.* (1998) found panicle density significantly correlated with grain yield (r=0.65*).

Increases in the grain yield of rice dual cropped with *Azolla* (unincorporated) have been reported (Singh, 1985). However, these results were mainly explained by the N contributed from *Azolla*. The fern's low C:N ratio allowed a fast mineralization of its N, which is eventually taken up by the rice plants. In the present experiments, it was shown that the presence of an *Azolla* cover reduced the potential for volatilization losses. In addition, besides fixing N from the atmosphere, *Azolla* took up N from urea that was applied. The urea-N was thus conserved. Unfortunately, no effort was made to assess how much of the N taken up by the *Azolla* from the urea was released and subsequently taken up by the rice plant. Nor was it assessed how much of the N fixed by the *Azolla* from the atmosphere was given off by the fern upon its decomposition. Singh and Singh (1989) reported that about 34% of the ^{15}N applied as *Azolla* was taken up by the rice plant in 60 days. About 45% of the *Azolla*-N was released in 60 days, 55% remained in the soil undecomposed and 11% was lost as gas. In another study, (Singh, 1981a) reported that *Azolla*-N mineralization was 41 to 67% in 7 to 35 days. If *Azolla* is inoculated at transplanting, the time of N release coincides with the vegetative period where N is needed for the production of tillers. Shiga and Ventura (1976) observed that the period of most rapid N absorption by rice was 20 to 45 days after transplanting. Thus, more nutrients should be made available during this period. It is not only through decomposition, however, that N from the *Azolla* becomes available for crop uptake. *Azolla* plants floating on the paddy water detach old tissues and slough-off root tissues from their bodies. When *Azolla* is grown with rice, such substances may be an important source of N to the rice plants.

Satapathy (1999) reported that the use of *Azolla* is as effective as the application of urea or organic manures in increasing the grain yield of rice. Being endowed with a high nutrient status and low C:N ratio, *Azolla* can be considered an efficient biofertilizer in increasing soil fertility as well as the productivity of rice.

Having an *Azolla* cover at the time of urea application established a larger number of tillers and improved the overall growth of the crop, which translated to a higher grain production. Not all sites, however, showed the positive effect of the high tiller count and panicle count on yield. This is an indication that, besides N nutrition, grain yield depends on other factors during the reproductive stage. Environmental conditions prevailing during the reproductive and ripening stages such as solar radiation and temperature have a profound influence in determining grain yields at harvest (Ntamatungiro, 1999).

There are many interlinking processes in the use of an *Azolla* cover on the floodwater surface. Aside from providing N and suppressing weeds, which compete for N nutrition, an *Azolla* cover can bring about, directly or indirectly, certain changes in the physical, chemical and biological properties of the soil-water interface in rice fields, which are of agronomic importance (Yanni, 1992). Results show that *Azolla* can prevent the sudden increase in floodwater pH induced both by algae and the application of N fertilizer, prevent the heating up of floodwater and in turn, reduce NH_3 volatilization losses from urea which tend to be high in flooded rice environments.

5 General discussion

The poor N use efficiency by lowland rice is a major constraint to lowland rice production in the tropics. Research efforts of the past, quantifying fertilizer N losses from the soil-floodwater system, showed that this low efficiency to a large extent is due to high N losses arising from NH_3 volatilization, a major pathway whereby a considerable amount of the surface-applied urea is lost. Urea, most commonly used by farmers because of its high N content, is subject to N losses of up to 50% when surface-applied.

The rate of NH_3 volatilization under flooded rice conditions is influenced mainly by the floodwater pH, temperature, and the level of ammoniacal-N (Fillery and Vlek, 1986). Ammonia volatilization proceeds rapidly because of the rapid increase in floodwater pH brought about by the hydrolysis of urea (Reddy *et al*., 1990) and the photosynthetic activities of the algae. In order to reduce NH_3 volatilization losses and enhance the efficiency of urea, it is essential to avoid pH increase in the floodwater. Theoretically, this can be achieved by placing a barrier on the floodwater surface (Vlek *et al*., 1995)

The possible use of the water fern *Azolla* has recently been identified as such a potential barrier (Vlek *et al*., 1992). Earlier studies on *Azolla* dealt mainly with its utilization as biofertilizer for rice and other upland crops, and its use as an animal feed for ducks, poultry and swine. Its utilization, however, as a means of reducing NH_3 volatilization losses was recognized only in the late 80’s. Laboratory studies conducted in the Philippines showed that an *Azolla* cover prevented the large increase in the floodwater pH, maintained lower temperatures but higher total ammoniacal-N, suggesting a reduction in NH_3 losses (Villegas and San Valentin, 1989). Vlek *et al*. (1992), Vlek *et al*. (1995) and Cissé (2001) found a similar trend in the floodwater chemistry under greenhouse conditions in Germany. With the use of the tracer technique, they showed a reduction in the N losses from the system and an increase in the ^{15}N recovery by the rice plant. This was attributed in part to the reduced volatilization potential (lower pH of the floodwater) and partly to the urea-N taken up by the *Azolla*. This higher ^{15}N recovery data is consistent with the results obtained in small-plot experiments conducted in China, Sri Lanka and Thailand, where the inoculation of *Azolla* increased the ^{15}N recovery by rice from urea applied at transplanting by 10, 60 and 53% (Kumarasinghe and Eskew, 1993). Studies verifying these laboratory and greenhouse results under field conditions are rare (Boadilla, 1993; de Macale, 1997) and inconclusive. The present experiments which were conducted under both on-station and on-farm field conditions tried to evaluate and confirm the potential benefits of using *Azolla* as a means to reduce NH_3 volatilization losses and improve N use efficiency. The two on-station experiments provided detailed information about the floodwater dynamics and the fate of ^{15}N-labeled urea. The 16 on-farm experiments were designed to assess the effects of *Azolla* on the growth and yield response in multi-location trials. Experiments in both series were conducted in the wet season and the dry season.

5.1 Floodwater chemistry

The increase in the floodwater pH immediately after urea application due to OH^- production during urea hydrolysis is well established, and was observed in both on-station field experiments. At the same time, a further increase in the floodwater pH is brought about by the algal activity. Fertilization of urea stimulates the growth of algae and increases their photosynthetic activity (Simpson *et al.*, 1994). In turn, the dissolved CO_2 in the floodwater is reduced during the daytime leading to a rise in the floodwater pH (Mikkelsen *et al.*, 1978; Stumm and Morgan, 1981; Thind and Rowell, 1997). The higher the floodwater pH, the higher the potential for NH_3 volatilization losses.

Application of urea onto the floodwater in the presence of an *Azolla* cover resulted in a lower floodwater pH increase during the day. Without an *Azolla* cover, the floodwater pH increased by 0.9 to 1.6 pH units one day after urea application whereas, in its presence, the increase was less than 1.0 pH units. The *Azolla*-covered treatments consistently had a significantly lower floodwater pH for the entire 10-day sampling period than those in the no-*Azolla* cover treatments. Floodwater pH was maintained below 8.3 in the first experiment and 8.0 in the second experiment. In contrast, floodwater pH in treatments without cover rose above 8.5 with a peak of 10.1.

These data suggest that *Azolla* provided conditions unfavorable for algal growth as indicated by the lower floodwater pH. The effect of an *Azolla* cover on the floodwater pH is partly explained in terms of its absorption of available light (Vlek *et al*., 2002). According to Saito and Watanabe (1978), shading is one of the most important factors limiting the photosynthesis of algae in lowland rice fields. With *Azolla* covering the floodwater surface, less light penetrated the floodwater. *Azolla* absorbs incoming solar radiation, reducing light intensity (Kröck *et al*., 1988a). In effect, the photosynthetic activity of the algae was reduced in the presence of an *Azolla* cover. Vlek *et al*. (1995) speculated that this reduction in the floodwater pH might be partly due to the respiration of *Azolla*, which increases the CO_2 partial pressure in the water, but found this to be insignificant (Vlek *et al*., 2002). Furthermore, the blue-green alga, *Anabaena azollae*, living in the fern's cavities, derives its carbon from the *Azolla* (Tel-Or *et al*., 1991). Therefore, it does not contribute to increasing the floodwater pH.

Another result of the shading effect was that the floodwater temperatures of the *Azolla*-covered plots were significantly lower during the midday sampling than those in the *Azolla*-free plots. An *Azolla* cover resulted in a mean floodwater temperature reduction of 0.6 to 2.6°C. The maximum floodwater temperature difference between *Azolla*-covered and *Azolla*-free plots was 5°C. In the presence of an *Azolla* cover, the rapid heating of the floodwater from morning until midday was prevented. It should be noted, however, that measurements using a data logger showed higher floodwater temperatures in *Azolla*-covered plots in the late afternoon as compared to the *Azolla*-free plots. Thus, an *Azolla* cover also slowed the cooling of the floodwater. Cissé (2001) made similar observations in greenhouse experiments.

A higher total ammoniacal-N in the floodwater was measured in plots with an *Azolla* cover. *Azolla*-covered plots contained, at the maximum, 5.5 g N m^{-3} more total ammoniacal-N than the *Azolla*-free plots. This could be due to the reduction in the NH_3 volatilization losses and also to a lower uptake by the fern. Without a cover, such high total ammoniacal-N can result in substantial NH_3 losses. However, this appeared to be prevented. Despite the higher concentration of total ammoniacal-N, the partial pressure of ammonia (ρNH_3) in *Azolla*-covered plots was significantly reduced in both experiments. Without an *Azolla* cover, the ρNH_3 reached a peak of 0.66 and 0.92 Pa in the first and second experiments, respectively. These high ρNH_3 values are conducive to high NH_3 volatilization losses (Vlek and Craswell, 1981; Simpson *et al*., 1984).

Covering the floodwater surface with *Azolla* markedly reduced these ρNH_3 values by more than 85%. Overall, the calculated ρNH_3 in plots covered with *Azolla* was very low, virtually eliminating the danger of NH_3 losses. This low ñNH_3 was mainly attributed to the significantly lower floodwater pH, which appears to be the most important factor controlling NH_3 volatilization, and the lower floodwater temperature under an *Azolla* cover. Vlek *et al*. (1995) found that under greenhouse conditions, the NH_3 volatilization potential is reduced when floodwater pH is controlled. The minimal NH_3 partial pressure in *Azolla*-covered treatments provided evidence that, under the conditions of our field experiments, *Azolla* is capable of curtailing NH_3 volatilization losses. In contrast, the high floodwater pH and temperatures in *Azolla*-free plots that led to a high ρNH_3, could result in high N losses via NH_3 volatilization.

5.2 ^{15}N recovery

The significant effects of the *Azolla* cover on the potential for NH_3 volatilization as indicated from the floodwater chemistry was reflected in the ^{15}N recovery data. The average total ^{15}N recovery improved by 25 to 49% in the presence of an *Azolla* cover. The highest relative increase recorded between *Azolla*-covered and *Azolla*-free plots was 89%. ^{15}N recoveries by the rice plant from the *Azolla*-covered plots were 3.6% to as much as 95% higher than those from the *Azolla*-free plots. Of the ^{15}N-labeled urea applied, 5 to 14% were found in the *Azolla* plant at harvest (Tables 3 and 4). Earlier studies with ^{15}N showed that applied urea is taken up by the *Azolla* (Vlek *et al*, 1995; de Macale, 1997; Cissé, 2001). Initially, this would mean a limited availability of N to the rice plants. Through this process, however, the *Azolla* also contributes to the reduction of NH_3 volatilization losses. The fern, by assimilating part of the urea applied, protects N from immediate gaseous N losses. The N temporarily locked up within the *Azolla* is actually being conserved. It is remineralized later and becomes available to the rice crop (Keeney and Sahrawat, 1986; Vlek and Craswell, 1981). This observation was confirmed under greenhouse conditions by Cissé (2001), who found that, of the 67.8% ^{15}N immobilized by the *Azolla* 6 weeks after urea application, 44.8% is remineralized during the growing season and taken up by the rice plant. Vlek *et al*. (1995) largely attributed the increased recovery of rice to the uptake of urea-N by the actively growing *Azolla*. In addition, the presence of an *Azolla* cover increased the ^{15}N recovery through the maintenance of a higher total ammoniacal-N content, but lower partial pressure of ammonia in floodwater (Figure 7) during the first 10 days after the application of urea, which is crucial for the N uptake by the plant. The low potential for NH_3 volatilization in the early vegetative stage could have likewise allowed more N to be available for plant assimilation.

The fraction of the ^{15}N-labeled urea unaccounted for in the plants and soil and presumed lost was lower for urea-amended, *Azolla*-covered plots, with values ranging from 0.01 to 22.7%, than for plots with urea applied alone. Plots without *Azolla* cover had losses of applied ^{15}N ranging from 21 to 49%. These high losses likely account for the poor ^{15}N recoveries by the rice crop. Lower quantities of N were available for plant uptake resulting in lower N use efficiency. As leaching and surface run-off were prevented, it can be concluded based on the floodwater chemistry, that these N losses were largely due to NH_3 volatilization. An *Azolla* cover thus reduced the high potential for NH_3 volatilization losses by preventing the rapid rise in floodwater pH and temperature, and by immobilizing urea-N in the floodwater.

5.3 Total N uptake

The presence of an *Azolla* cover enhanced the plant N uptake by the rice plants at the maximum tillering stage in on-farm trials. In the wet season, the plant N uptake increased by as much as 53.4 and 60.2% at the 40 and 80 kg N ha^{-1} rates; and in the dry season, by as much as 62.0% at the 50 and 100 kg N ha^{-1}. This increase in the plant N uptake with *Azolla* cover may have arisen partly from the reduced NH_3 volatilization losses immediately after urea application. Likewise, the N immobilized early on by the *Azolla* and later released would have also contributed to the improved N nutrition of the rice crop. The higher availability of N contributed to the development of more vigorous rice plants during the vegetative stage, which were more efficient in taking up native N.

At harvest, the combined use of *Azolla* cover and urea in the on-station experiments resulted in a 1.9 to 41.7% higher total N uptake. In farmers' fields, the total N uptake increased by as much as 36% in the 80 kg N ha^{-1} and by as much as 41% in the 100 kg N ha^{-1} treatments with *Azolla* cover. The total N uptake at lower N rates with *Azolla* cover was comparable to that obtained at higher N rates without cover. These findings suggest that N was still available to the rice crop until the later stages of its growth.

The apparent N recovery (ANR) data is in agreement with the ^{15}N recovery results. *Azolla*-covered treatments had higher ANR than those without cover.

Even in the non-fertilized plots, total N uptake increased in the presence of an *Azolla* cover. Rosenani and Azizah Chulan (1992) reported that growing *Azolla* with rice resulted in a significant release of N into the floodwater. This is presumably coming from the N fixed from the decomposed *Azolla* (Jokela and Randall, 1997). Watanabe *et al*. (1981) found that rice took up 15% of the N fixed by the *Azolla* floating on the floodwater surface. Though most of the N coming from *Azolla* becomes available to the rice crop after its decomposition, the *Azolla* plants detach old tissues and sloughed-off root tissues from their bodies when floating on the floodwater surface. This tissue becomes a source of N, which is gradually available to rice and can contribute to the N supply of the rice plants (Singh and Singh, 1989).

5.4 Crop growth and yield

The presence of an *Azolla* cover in combination with urea was effective in increasing the grain yield of rice compared with that recorded with urea alone. This was manifested most clearly in the results of the on-farm trials. Factors such as unfavorable weather, which affected grain yield, limited the response of rice to the combined application of urea and *Azolla* cover in the on-station trials. In farmers' fields, however, the grain yield of rice was approximately 40% higher at the 40 and 50 kg N ha^{-1} rates and 19% higher at the 80 and 100 kg N ha^{-1} rates in the presence of an *Azolla* cover. Interestingly, an increase in the grain yield due to an *Azolla* x N interaction in most on-farm sites in both seasons was also observed. At lower N rates, the effect of an interaction account for 33 to 92% of the main *Azolla* effect during the wet season and 2 to 95% in the dry season. At higher N rates, 22 to 87% of the *Azolla* effect in the wet season and 13 to 92% in the dry season were due to the interaction. The highest increase in the grain yield at lower N rates due to an *Azolla* cover was 1.86 and 1.15 t ha^{-1} in the wet and dry seasons, respectively. Of this increase, 1.71 and 1.09 t ha^{-1} were due to an *Azolla* x N interaction, which may be directly related to the conservation of N. At higher N rates, of the maximum yield increase of 1.17 and 0.79 t ha^{-1} in the wet and dry seasons, 1.01 and 0.74 t ha^{-1} were attributed to an *Azolla* x N interaction. In the non-fertilized plots, an *Azolla* cover resulted in a grain yield increase of as much as 15% in the wet season and as much as 29% in the dry season. The response of rice to the combined treatment of urea and *Azolla* cover was greater in the dry season than in the wet season. More sites showed a significant effect of an *Azolla* cover and an *Azolla* x N interaction on the grain yield.

The reduction of NH_3 volatilization losses from the urea application with *Azolla* on the floodwater surface a week after transplanting resulted in the establishment of a higher tiller number and improvement of the overall growth of the rice. This translated into higher grain yield. The tiller and panicle count data in Section 4.5 and the total dry matter yield in Section 4.6 support this interpretation. Besides minimizing NH_3 losses, *Azolla* conserved N by taking part of the urea applied, and together with the N it fixed, released it later. The process not only increased the availability of N for the rice crop, it also ensured a continuous supply of N throughout the rice growing period. Thus, higher grain yield resulting from an *Azolla* cover was also due to a higher recovery of applied urea by the plant. The lower yields from plots treated with urea alone indicate that N losses could have led to lower N use efficiency and consequently, lower rice response. The occurrence of an *Azolla* x N interaction effect on the grain yield indicates that the influence of an *Azolla* cover in the enhancement of the response of rice to the applied urea-N was not only additive in nature, but synergistic as well. This means that, besides the benefits achieved with the application of urea-N alone or from the N fixation by *Azolla*, an additional benefit was noted that could not be obtained from the separate treatments. This would have been the conservation of applied N by the *Azolla* cover. In fact, it appears that the latter effect might in some cases exceed the main effects of urea, and *Azolla* through N fixation. (Vlek *et al.*, 1995).

6 Conclusions

The present study evaluated the use of *Azolla* as an alternative approach to increase the poor N use efficiency of rice and reduce the high potential for NH_3 volatilization losses from urea under lowland conditions.

The results from field experiments provided convincing evidence that *Azolla* used as a cover on the floodwater of rice could help curb NH_3 volatilization losses. The study provided strong evidence that losses of N are being suppressed. The *Azolla* cover brought about changes in the floodwater chemistry, and in the physical and microbiological environment. Under the conditions of our experiments, we have demonstrated that a full *Azolla* cover effectively prevented the sudden rise in the floodwater pH following urea application, the primary contributing factor influencing the potential for high NH_3 volatilization losses. As a result, the large NH_3 losses that commonly occur when urea is broadcast onto the floodwater of rice shortly after transplanting were reduced. In minimizing NH_3 volatilization losses, the N use efficiency was improved. *Azolla* likewise brought about appreciable changes with regard to the availability of N, which influences the growth and mineral nutrition of the rice plants.

With an *Azolla* cover on the floodwater surface, a higher grain yield can be achieved with a reduced rate of urea applied. In the present investigations, combining *Azolla* with urea produced yields, which were generally higher by 10% or more than those without cover. Moreover, N at lower rates (40 to 50 kg N ha^{-1}) with an *Azolla* cover produced yield comparable to that obtained at higher N rates (80 to 100 kg N ha^{-1}) without cover. As such, a considerable amount of urea can be saved when it is combined with *Azolla*. Availability of *Azolla* will not be a constraint as *Azolla* can be easily propagated and can thus be readily available to farmers. This prospect is especially attractive in lieu of the high cost of N fertilizer and the growing need to improve grain yield with minimum adverse environmental effects associated with the intensive use of N fertilizer.

There are many interlinking processes associated with the use of an *Azolla* cover on the floodwater surface. *Azolla* brings about changes in the physical, chemical and microbiological properties of the floodwater that lead to N conservation and benefits to rice. The combined application of urea with *Azolla* can thus be an efficient fertilizer management method to reduce NH_3 losses from urea applied to lowland rice, can introduce an N-fixing species into the system, and can lead to increased grain yields. These benefits can surpass those from either urea or *Azolla* alone.

The fine-tuned system should time the *Azolla* inoculation such that an *Azolla* cover is present at the time of fertilizer N application. In our experiments, this took place one week after transplanting. An initial cover of 50%, three days before fertilizer N application was sufficient to assure full coverage. Less *Azolla* may be applied if the time span between inoculation and urea application can be extended. No further manipulation of *Azolla* is needed in this system.

7 Recommendations

The use of an *Azolla* cover on the floodwater surface was found to be effective in improving the N use efficiency by rice under the environmental conditions of the experiments. It therefore, deserves to be included in the suit of management packages offered in the area studied. Its effect, however, varies with factors such as weather, soil and the rate of algae development in the floodwater. Thus, it is suggested that further research on its use under different agro-climatic conditions be carried out. It would likewise be worthwhile to consider and assess the economics (*e.g.*, labor costs) of *Azolla* use.

It was presumed that, aside from the N conserved by the *Azolla*, the fern also supplied N through biological N fixation during the growing period of the rice. The extent, however, of its supply was not measured. In addition, the study did not determine the amount of the applied ^{15}N-labeled urea, recovered by the plant coming from the ^{15}N conserved and then released by the *Azolla*, and the ^{15}N coming from the initial urea applied. No direct measurement of ^{15}N coming from the *Azolla* and taken up by the plant was attempted. For future research, these factors would be worth taking into consideration. Further field investigation is needed on the availability of N immobilized by the *Azolla* and later remineralized and taken up by the rice plant. The results of such work will complement the present study and help fine-tune *Azolla* and urea management practices for the ultimate benefit of rice farmers.

8 Summary

In the tropics, the development of management techniques to improve the poor N use efficiency by lowland rice (*Oryza sativa* L.) and reduce the high N losses is an important focus of agronomic research. An alternative approach based on the use of the water fern *Azolla* in combination with urea was considered in the present experiments.

The potential of an *Azolla* cover in reducing NH_3 volatilization losses in lowland rice fields and increasing the low N use efficiency and grain yield of rice were assessed under field conditions in Laguna, Philippines. Two field trials were carried out at the experimental station in the 1998-1999 dry season. Ten treatment combinations consisting of five N levels (0, 40, 80, 120 and 160 kg N ha^{-1}) applied alone or combined with *Azolla* were evaluated. The nitrogen rate was tested up to 160 kg N ha^{-1} to determine if an *Azolla* cover would still improve the efficiency of applied N at this high level. The feasibility of using the *Azolla* cover to improve the N use efficiency was further studied in farmers' fields during the wet and dry seasons of 2000-2001. Eight identical field experiments per season were carried out in selected fields in four municipalities in Laguna. The sampling design from the on-station experiments was followed. The N rates, however, were reduced to the level where the benefits of the *Azolla* cover are visible (*i.e.*, up to 80 kg N ha^{-1} during the wet season and up to 100 kg N ha^{-1} during the dry season).

An isotope microplot study using ^{15}N-labeled urea was included in the 1998-99 on-station trials to trace the fate of the urea applied and to provide evidence supporting the results obtained in macroplots.

Findings on floodwater chemistry in the on-station experiments revealed that an *Azolla* cover present on the floodwater surface prior to urea application significantly suppressed the rise in daytime pH. The mean floodwater pH was reduced by 0.9 to 1.4 pH units in the presence of an *Azolla* cover. Furthermore, the floodwater pH in urea-amended, *Azolla*-covered plots was maintained below 8.3 in the first experiment and below 8.0 in the second experiment. In contrast, without the *Azolla* cover, floodwater pH in plots with different rates of urea rose above 8.5 reaching a maximum of 10.1. These reductions in the floodwater pH suggest that *Azolla* could have influenced the algal growth, which is responsible for the rapid rise in floodwater pH. By providing a cover on the floodwater surface, *Azolla* directly absorbed the incoming solar radiation. Light intensity, which is essential for the growth of algae, is then reduced and the consequent pH elevation is abated. The *Azolla* cover likewise prevented the floodwater surface from heating up quickly during the day. The floodwater temperature, which is another important factor influencing NH_3 volatilization, was significantly lower by as much as 5°C in the presence of an *Azolla* cover. The concentration of total ammoniacal-N was as much as 5.5 g N m^{-3} higher in plots with *Azolla* cover. Despite the higher total ammoniacal-N measured in *Azolla*-covered plots, the partial pressure of ammonia (ρNH_3), which is indicative of the potential for NH_3 losses, was substantially reduced. In the absence of an *Azolla* cover, the ρNH_3 reached a peak of 0.66 and 0.92 Pa on the second day in the first and second experiments. These values were significantly lowered to 0.07 and 0.12 Pa, respectively, a proportional reduction of more than 85%. This marked decrease in the partial pressure of ammonia is attributed mainly to the low floodwater pH in plots with an *Azolla* cover.

At harvest, the presence of an *Azolla* cover led to a relative increase in the total ^{15}N recovery of applied urea of up to 89%. The highest total ^{15}N recovery obtained in the *Azolla*-rice-soil system was 99.9%. Of the total ^{15}N recovered in the *Azolla*-rice-soil system, 32 to 61% of the N applied was recovered by the aboveground biomass (grain and straw). Without the cover, only 30 to 40% was recovered. Overall, the aboveground recovery increased by as much as 95%. Interestingly, 6.5 to 13.9% of the labeled N was found in the *Azolla* plants at final harvest. Consequently, the fraction of the ^{15}N unaccounted for and presumably lost through NH_3 volatilization was lower in *Azolla*-covered plots, with losses not exceeding 23%. In contrast, in the absence of an *Azolla* cover extensive ^{15}N losses ranging from 21 to 49% were recorded. In general, the apparent N recovery data, though higher than the ^{15}N recovery data showed a similar trend. It was improved in the presence of an *Azolla* cover.

The total N uptake by rice increased in fertilized and non-fertilized plots in response to *Azolla* cover in both on-station and on-farm field experiments. At the maximum tillering stage, the plant N uptake in farmers' fields increased by 4.6 to 53.4% in the wet season, and by 24.4 to 62.0% in the dry season when plots receiving 40 and 50 kg N ha^{-1} were covered with *Azolla*. At the higher N rates (80 and 100 kg N ha^{-1}), plant N uptake increased by 22.5 to 60.2% in the wet season and by 24.0 to 62.3% in the dry season in the presence of an *Azolla* cover. At harvest, the total N uptake data from on-station trials showed an increase of up to 41.7% in the presence of an *Azolla* cover. In farmers' fields, the maximum total N uptake was obtained at the highest N rates with *Azolla* cover. It increased by 36% in the wet season and by 41% in the dry season. The total N uptake in plots with 40 kg N ha^{-1} and *Azolla* cover was comparable to that obtained with 80 kg N ha^{-1} without the cover. The effect of an *Azolla* cover in non-fertilized treatments was equivalent to the application of 40 to 50 kg N ha^{-1}.

The positive effects of the *Azolla* cover on rice were reflected on the tiller count in the farmers' fields in both seasons. The maximum number of tillers was obtained in treatments with 80 and 100 kg N ha^{-1} and *Azolla* cover. At the maximum tillering stage, the presence of an *Azolla* cover significantly increased the tiller count by as much as 25.5% in the wet season and by as much as 51.7% in the dry season. At harvest, this had diminished to 8 and 25%. At the lower N rate, the tiller number was still around 50% higher with *Azolla*. Plants in the non-fertilized plots with *Azolla* cover likewise produced a higher tiller number than those with no cover. A significant positive *Azolla* x N interaction was observed at harvest in half of the total sites.

Similarly, the panicle count significantly improved with the use of an *Azolla* cover. The panicle count in plots with the lower N rate and *Azolla* cover significantly increased by as much as 48 and 53% as compared to those without cover. At higher N rates (80 and 100 kg N ha^{-1}), *Azolla* cover increased the panicle count by 32 and 28%. Plots with 40 and 50 kg N ha^{-1} and *Azolla* cover had tiller and panicle counts comparable to those in the 80 and 100 kg N ha^{-1} plots, respectively, without cover. A significant *Azolla* x N interaction was observed in the same sites, where a significant *Azolla* x N interaction effect on tiller count was earlier noted.

Generally, the main effect of an *Azolla* cover in combination with urea is an increase in the total dry matter yield of rice at harvest. The *Azolla*-covered plots in the on-station field experiments produced a 26% higher total dry matter yield compared with the *Azolla*-free plots. In the farmers' fields, the total dry matter yield at low and high N rates increased by more than 30% when they were combined with an *Azolla* cover. The total dry matter yields in plots with 40 and 50 kg N ha^{-1} and covered with

Azolla were comparable or higher than those obtained in the 80 and 100 kg N ha^{-1} without cover.

The benefits of having an *Azolla* cover on the floodwater surface prior to urea application were subsequently reflected on the grain yields of rice which were higher than those obtained without cover. The response of rice to the combined application of urea and *Azolla* cover in the on-station trials was, however, limited by other factors such as adverse weather, which caused lodging and affected grain yield. The influence of an *Azolla* cover on grain yield was manifested more clearly in the results of the on-farm trials. The presence of an *Azolla* cover increased the grain yield of rice by approximately 40% at lower N rates (40 and 50 kg N ha^{-1}), and by 19% at higher N rates (80 and 100 kg N ha^{-1}). Furthermore, an increase in the grain yield in both seasons due to an *Azolla* x N interaction was observed. In the farmers' trials, when plots with 40 and 50 kg N ha^{-1} were covered with *Azolla*, the increase in grain yield in the best responding fields amounted to 1.7 and 1.2 t ha^{-1}. Of this amount, 1.6 and 1.1 t ha^{-1} were attributed to the interaction effect. At higher N rates, an *Azolla* x N interaction accounted for the 1.0 t ha^{-1} increase in the grain yield in 2 of the sites in the wet season and for more than 0.5 t ha^{-1} increase in grain yield in the dry season. Thus, in addition to the additive effects of urea fertilization and biological N fixation by *Azolla*, an extra yield increase was observed, which was most likely due to the conservation of applied N.

In the non-fertilized plots, the grain yields of rice increased by as much as 15% in the wet season and by as much as 29% in the dry season in the presence of an *Azolla* cover, which is mainly attributed to the production of higher tiller and panicle numbers.

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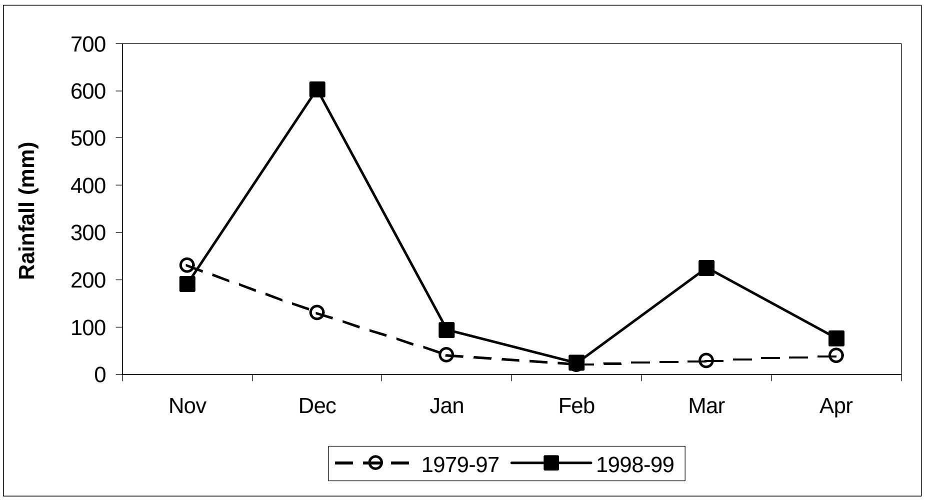
Appendices

Appendix I: Dry season total monthly rainfall from 1979 to 1997 and 1998 to 99. Los Banos, Philippines. (Source: IRRI weather station)



Appendix II: Cultural management practices. First and second experiments. Los Banos, Philippines. Dry season 1998-99.

DAT	**Activities**	**First experiment**	**Second experiment**
-23	Soaking of seeds for 24 hours	November 20	January 16
-22	Seed incubation for 48 hours	November 21 - 23	January 17 - 19
-20	Sowing of seeds	November 23	January 19
-14	Field layout		January 26
-2	Soil sampling	December 14	February 6
-1	Pulling of seedlings	December 15	February 7
	Microplot installation	December 15	February 7
0	Application of P and K	December 16	February 8
	Transplanting	December 16	Ferbruary 8
3	Irrigation of field	December 19	February 11
4	*Azolla* inoculation	December 20	February 12
5	Replanting of missing hills	December 21	February 13
7	Application of 2/3 N	December 23	February 15
25-30	Application of 1/3 N	January 26	March 22
90 up	Harvest	March 19 – April 5	May 11 - 21

DAT: Days after transplanting

Appendix III: Cultural management practices. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

DAT	Activities	LB1	LB2	BY1	BY2	SF1	SF2	SC1	SC2	SC3	SC4
-23	Soaking of seeds for 24 hours	May 29	May 29	May 29	May 29	May 17	May 29	May 29	May 29	June 15	June 15
-22	Seed incubation for 48 hours	May 30	May 30	May 30	May 30	May 18	May 30	May 30	May 30	June 16	June 16
-20	Sowing of seeds	June 2	June 2	June 2	June 2	May 20	June 2	June 2	June 2	June 18	June 18
-14	Land preparation	June 3-4	June 3-4	June 14-15	June 14-15	May 25-26	June 1-2	June 16-17	June 16-17	July 1-2	July 1-2
-14	Field lay-out	June 3-4	June 3-4	June 14-15	June 14-15	May 25-26	June 1-2	June 16-17	June 16-17	July 1-2	July 1-2
-1	Pulling of seedlings	June 16	June 16	June 24	June 24	June 9	June 20	June 21	June 21	July 9	July 9
0	Application of P and K	June 17	June 17	June 25	June 25	June 10	June 21	June 22	June 22	July 10	July 10
0	Transplanting	June 17/23	June 17/23	June 25	June 25	June 10	June 21	June 22	June 22	July 10	July 10
0	Application of Bayluscide for snails	June 17/23	June 17/23	June 25	June 25	June 10	June 21	June 22	June 22	July 10	July 10
3	Irrigate	June 19	June 19	June 28	June 28	June 13	June 24	June 25	June 25	July 13	July 13
4	Inoculation of Azolla	June 20/26	June 20/26	June 29	June 29	June 14	June 25	June 26	June 26	July 14	July 14
5	Replanting of missing hills	June 21	June 21	June 30	June 30	June 15	June 26	June 27	June 27	July 15	July 15
7	Application of 2/3 N	June 29	June 29	July 2	July 2	June 17	June 28	June 29	June 29	July 17	July 17
25-30	Application of 1/3 N	August 11	August 11	August 15	August 15	July 26	August 3	August 8	August 8	August 29	August 29
90 up	Harvest	September 22	September 22	September 22	September 22	September 7	September 22	September 22	September 22	October 8	October 8

LB1: Los Banos site 1
LB2: Los Banos site 2
BY1: Bay site 1
BY2: Bay site 2
SF1: San Francisco site 1
SF2: San Francisco site 2
SC1: Sta Cruz site 1
SC2: Sta Cruz site 2
SC3: Sta Cruz site 3
SC4: Sta Cruz site 4

DAT: days after transplanting

Appendix IV: Cultural management practices. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

DAT	Activities	LB1	LB2	BY1	BY2	SF1	SF2	SC1	SC2
-23	Soaking of seeds for 24 hours	October 27	October 27	December 1	December 1	November 6	November 6	November 29	November 29
-22	Seed incubation for 48 hours	October 28	October 28	December 2	December 2	November 7	November 7	November 30	November 30
-20	Sowing of seeds	October 30	October 30	December 4	December 4	November 9	November 9	December 2	December 2
-14	Land preparation			-1st week of December-		Nov 10, 12, 18	Nov 10, 12, 18	-1st week of December-	
-14	Field lay-out	November 14	November 14	December 10	December 10	November 22	November 22	December 7	December 7
-1	Pulling of seedlings	November 17	November 17	December 19	December 19	November 27	November 27	December 17	December 17
0	Application of P and K	November 18	November 18	December 20	December 20	November 28	November 28	December 18	December 18
0	Transplanting	November 18	November 18	December 20	December 20	November 28	November 28	December 18	December 18
0	Application of Bayluscide for snails	November 18	November 18	December 20	December 20	November 28	November 28	December 18	December 18
3	Irrigate	November 21	November 21	December 23	December 23	December 2	December 2	December 21	December 21
4	Inoculation of Azolla	November 22	November 22	December 24	December 24	December 3	December 3	December 22	December 22
5	Replanting of missing hills	November 23	November 23	December 26	December 26	December 4	December 4	December 23	December 23
7	Application of 2/3 N	November 25	November 25	December 27	December 27	December 6	December 6	December 26	December 26
25-30	Application of 1/3 N	January 17	January 17	January 22	January 22	January 16	January 16	January 20	January 20
90 up	Harvest	February 22/26	February 22/26	March 25/29	March 25/29	March 5/9	March 5/9	March 26/30	March 26/30

LB1: Los Banos site 1
LB2: Los Banos site 2
BY1: Bay site 1
BY2: Bay site 2
SF1: San Francisco site 1
SF2: San Francisco site 2
SC1: Sta Cruz site 1
SC2: Sta Cruz site 2

DAT: days after transplanting

Appendix V: Effect of an *Azolla* cover on the daily floodwater pH. First experiment. Los Banos, Philippines. Dry season, 1998-99.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	2.20	ns	90.08	**	137.80	**	362.45	**	235.02	**
Nitrogen	1.52	ns	9.66	**	18.02	**	6.64	**	9.44	**
Azolla x Nitrogen	1.93	ns	1.16	ns	<1		5.78	**	1.19	ns

	Day 6		Day 7		Day 8		Day 9		Day 10	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	94.17	**	228.13	**	411.58	**	345.20	**	228.37	**
Nitrogen	3.78	*	3.33	*	3.86	*	1.79	ns	1.14	ns
Azolla x Nitrogen	<1		1.10	ns	2.29	ns	4.21	**	2.82	*

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix VI: Effect of an *Azolla* cover on the daily floodwater pH. Second experiment. Los Banos, Philippines. Dry season, 1998-99.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	191.56	**	160.79	**	146.10	**	324.00	**	190.58	**
Nitrogen	1.21	ns	2.79	*	4.99	**	4.63	**	3.74	*
Azolla x Nitrogen	<1		<1		1.94	ns	3.79	*	5.07	**

	Day 6		Day 7		Day 8		Day 9		Day 10	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	470.11	**	522.44	**	341.18	**	1118.41	**	397.83	**
Nitrogen	7.48	**	6.18	**	3.61	*	10.92	**	1.47	ns
Azolla x Nitrogen	3.86	*	3.08	*	5.33	**	12.37	**	4.90	**

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix VII: Effect of an *Azolla* cover on the daily floodwater temperature. First experiment. Los Banos, Philippines. Dry season, 1998-99.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	11.09	**	51.97	**	6.99	*	3.20	ns	44.02	**
Nitrogen	<1		<1		<1		2.37	ns	<1	
Azolla x Nitrogen	<1		<1		1.65	ns	<1		<1	

	Day 6		Day 7		Day 8		Day 9		Day 10	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	13.71	**	132.92	**	91.95	**	1.06	ns	3.19	ns
Nitrogen	<1		<1		1.18	ns	<1		<1	
Azolla x Nitrogen	1.53	ns	<1		1.05	ns	<1		<1	

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix VIII: Effect of an *Azolla* cover on the daily floodwater temperature. Second experiment. Los Banos, Philippines. Dry season, 1998-99.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	54.05	**	51.97	**	69.75	**	111.59	**	165.87	**
Nitrogen	<1		<1		1.19	ns	1.77	ns	<1	
Azolla x Nitrogen	<1		<1		<1		1.77	ns	1.78	ns

	Day 6		Day 7		Day 8		Day 9		Day 10	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	112.86	**	93.77	**	198.39	**	86.74	**	42.44	**
Nitrogen	1.27	ns	<1		1.96	ns	<1		<1	
Azolla x Nitrogen	2.09	ns	2.23	ns	2.41	ns	1.08	ns	<1	

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix IX: Effect of an *Azolla* cover on the daily total ammoniacal-N. First experiment. Los Banos, Philippines. Dry season, 1998-99.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	<1		<1		10.97	**	1.91	ns	3.38	ns
Nitrogen	12.06	**	9.88	**	58.33	**	47.70	**	21.12	**
Azolla x Nitrogen	1.10	ns	10.31		3.37	*	<1		1.08	ns

	Day 6		Day 7		Day 8		Day 9		Day 10	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	2.59	ns	<1		<1		<1		7.07	*
Nitrogen	13.47	**	13.50	**	7.55	**	4.96	**	3.60	*
Azolla x Nitrogen	<1		<1		1.06	ns	<1		<1	

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix X: Effect of an *Azolla* cover on the daily total ammoniacal-N. Second experiment. Los Banos, Philippines. Dry season, 1998-99.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	7.42	*	<1		1.16	ns	<1		5.37	*
Nitrogen	11.05	**	11.81	**	11.17	**	6.91	**	6.37	**
Azolla x Nitrogen	2.46	ns	<1		<1		<1		1.18	ns

	Day 6		Day 7		Day 8		Day 9		Day 10	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	4.47	*	3.50	ns	6.82	*	4.71	*	6.47	*
Nitrogen	2.90	*	2.50	ns	2.19	ns	2.18	ns	1.44	ns
Azolla x Nitrogen	<1		2.03	ns	2.81	*	<1		1.62	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XI: Effect of an *Azolla* cover on the daily aqueous NH_3. First experiment. Los Banos, Philippines. Dry season, 1998-99.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	3.37	ns	19.69	**	17.07	**	61.95	**	114.90	**
Nitrogen	1.60	ns	8.52	**	34.77	**	14.38	**	12.52	**
Azolla x Nitrogen	<1		7.87	**	1.84	ns	5.81	**	2.58	ns

	Day 6		Day 7		Day 8		Day 9		Day 10	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	60.22	**	206.72	**	307.54	**	141.04	**	225.26	**
Nitrogen	10.79	**	11.91	**	11.24	**	5.14	**	6.02	**
Azolla x Nitrogen	4.34	**	10.25	**	10.05	**	3.85	*	4.40	*

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XII: Effect of an *Azolla* cover on the daily aqueous NH_3. Second experiment. Los Banos, Philippines. Dry season, 1998-99.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	35.97	**	57.66	**	85.53	**	29.65	**	25.51	**
Nitrogen	3.00	*	9.07	**	15.82	**	8.01	**	7.58	**
Azolla x Nitrogen	2.19	ns	4.83	**	8.37	**	3.56	*	2.84	*

	Day 6		Day 7		Day 8		Day 9		Day 10	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	54.91	**	33.46	**	77.83	**	312.46	**	174.60	**
Nitrogen	4.77	**	2.45	ns	3.41	*	11.43	**	2.21	ns
Azolla x Nitrogen	1.15	ns	1.75	ns	2.14	ns	7.89	**	2.22	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XIII: Effect of an *Azolla* cover on the partial pressure of ammonia. First experiment. Los Banos, Philippines. Dry season, 1998-99.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	3.74	ns	19.85	**	17.21	**	65.31	**	113.98	**
Nitrogen	1.72	ns	8.08	**	33.34	**	14.75	**	11.43	**
Azolla x Nitrogen	<1		7.74	**	1.90	ns	6.24	**	2.46	ns

	Day 6		Day 7		Day 8		Day 9		Day 10	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	44.20	**	197.87	**	238.19	**	147.88	**	189.60	**
Nitrogen	9.38	**	11.45	**	8.04	**	4.43	**	5.27	**
Azolla x Nitrogen	3.50	*	10.71	**	6.92	**	3.61	*	3.66	*

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XIV: Effect of an *Azolla* cover on the partial pressure of ammonia. Second experiment. Los Banos, Philippines. Dry season, 1998-99.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	33.06	**	56.47	**	82.45	**	31.01	**	26.95	**
Nitrogen	2.72	ns	8.54	**	14.52	**	8.15	**	7.72	**
Azolla x Nitrogen	2.00	ns	4.98	**	7.98	**	3.86	*	3.00	*

	Day 6		Day 7		Day 8		Day 9		Day 10	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	59.17	**	33.87	**	80.48	**	326.32	**	164.10	**
Nitrogen	4.77	**	2.28	ns	3.38	*	11.05	**	1.99	ns
Azolla x Nitrogen	1.26	ns	1.54	ns	2.29	ns	8.45	**	2.08	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XV: Effect of *Azolla* cover on the ^{15}N recovery by the grain. First and second experiments. Los Banos, Philippines. Dry season, 1998-99.

	First experiment		Second experiment	
Treatment	F-value	Significance	F-value	Significance
Azolla	2.68	ns	12.85	**
Nitrogen	2.98	ns	2.57	ns
Azolla x Nitrogen	<1		1.16	ns

* = significant at 5% level; ns = not significant

Appendix XVI: Effect of *Azolla* cover on the ^{15}N recovery by the straw. First and second experiments. Los Banos, Philippines. Dry season, 1998-99.

	First experiment		Second experiment	
Treatment	F-value	Significance	F-value	Significance
Azolla	<1		85.00	**
Nitrogen	8.14	**	2.53	ns
Azolla x Nitrogen	<1		9.18	**

** = significant at 1% level; ns = not significant

Appendix XVII: Effect of *Azolla* cover on the total ^{15}N recovery by rice. First and second experiments. Los Banos, Philippines. Dry season, 1998-99.

	First experiment		Second experiment	
Treatment	F-value	Significance	F-value	Significance
Azolla	6.53	*	25.34	**
Nitrogen	1.74	ns	1.11	ns
Azolla x Nitrogen	1.98	ns	2.58	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XVIII: Effect of an *Azolla* cover on the grain N uptake. First and second experiments. Los Banos, Philippines. Dry season, 1998-99.

	First experiment		Second experiment	
Treatment	F-value	Significance	F-value	Significance
Azolla	5.19	*	21.52	**
Nitrogen	99.57	**	25.42	**
Azolla x Nitrogen	<1		1.97	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XIX: Effect of an *Azolla* cover on the straw N uptake. First and second experiments. Los Banos, Philippines. Dry season, 1998-99.

	First experiment		Second experiment	
Treatment	F-value	Significance	F-value	Significance
Azolla	<1		20.47	**
Nitrogen	44.01	**	28.89	**
Azolla x Nitrogen	<1		1.23	ns

** = significant at 1% level; ns = not significant

Appendix XX: Effect of an *Azolla* cover on the grain N concentration. First and second experiments. Los Banos, Philippines. Dry season, 1998-99.

	First experiment		Second experiment	
Treatment	F-value	Significance	F-value	Significance
Azolla	3.60	ns	21.24	**
Nitrogen	138.09	**	50.35	**
Azolla x Nitrogen	<1		2.73	ns

** = significant at 1% level; ns = not significant

Appendix XXI: Effect of an *Azolla* cover on the straw N concentration. First and second experiments. Los Banos, Philippines. Dry season, 1998-99.

	First experiment		Second experiment	
Treatment	F-value	Significance	F-value	Significance
Azolla	<1		4.74	**
Nitrogen	37.09	**	30.77	**
Azolla x Nitrogen	1.15	ns	<1	

** = significant at 1% level; ns = not significant

Appendix XXII: Effect of an *Azolla* cover on the plant N uptake at maximum tillering stage. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	11.83	**	29.42	**	6.59	*	16.94	**
Nitrogen	30.22	**	27.30	**	38.53	**	101.92	**
Azolla x Nitrogen	1.50	ns	2.37	ns	<1		2.86	ns

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	1.46	ns	7.39	*	10.72	**	9.89	**
Nitrogen	3.06	ns	30.76	**	101.35	**	187.92	**
Azolla x Nitrogen	<1	ns	2.14	ns	2.33	ns	3.35	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXIII: Effect of an *Azolla* cover on the plant N uptake at maximum tillering stage. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	24.72	**	8.64	*	11.35	**	28.24	**
Nitrogen	51.91	**	12.57	**	11.34	**	31.14	**
Azolla x Nitrogen	5.59	*	2.14	ns	<1		1.67	ns

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	34.42	**	26.19	**	28.96	**	19.60	**
Nitrogen	37.96	**	65.80	**	10.32	**	51.59	**
Azolla x Nitrogen	<1		1.37	ns	1.26	ns	2.00	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXIV: Effect of an *Azolla* cover on the plant yield at maximum tillering stage. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	11.46	**	21.73	**	35.86	**	13.20	**
Nitrogen	19.46	**	13.19	**	74.59	**	55.58	**
Azolla x Nitrogen	1.41	ns	<1		2.37	ns	<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	2.65	ns	5.24	*	14.87	**	6.94	**
Nitrogen	<1		22.87	**	92.53	**	91.79	**
Azolla x Nitrogen	<1		<1		1.23	ns	<1	

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXV: Effect of an *Azolla* cover on the plant yield at maximum tillering stage. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	27.37	**	20.57	**	10.63	**	17.49	**
Nitrogen	85.93	**	33.27	**	29.63	**	51.11	**
Azolla x Nitrogen	2.35	ns	2.30	ns	<1		1.29	ns

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	18.74	**	12.45	**	18.16	**	13.02	**
Nitrogen	323.13	**	82.46	**	32.21	**	47.98	**
Azolla x Nitrogen	1.18	ns	1.23	ns	<1		3.29	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXVI: Effect of an *Azolla* cover on the plant N concentration at maximum tillering stage. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	<1		10.81	**	<1		<1	
Nitrogen	21.26	**	25.48	**	11.99	**	6.98	**
Azolla x Nitrogen	<1		2.97	ns	<1		<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	<1		<1		1.13	ns	<1	
Nitrogen	2.78	ns	5.99	*	67.40	**	51.60	**
Azolla x Nitrogen	<1		1.46	ns	2.87	ns	2.76	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXVII: Effect of an *Azolla* cover on the plant N concentration at maximum tillering stage. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	5.05	*	2.15	ns	5.50	*	19.85	**
Nitrogen	7.44	**	3.94	*	1.63	ns	3.67	ns
Azolla x Nitrogen	1.40	ns	3.63	ns	<1		<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	8.80	**	14.76	**	15.20	**	14.94	**
Nitrogen	3.07	ns	8.14	**	2.56	ns	15.81	**
Azolla x Nitrogen	<1		<1		7.60	**	<1	

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXVIII: Effect of an *Azolla* cover on the total N uptake at harvest. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	<1		25.45	**	1.78	ns	1.17	ns
Nitrogen	33.91	**	41.17	**	5.59	*	7.40	**
Azolla x Nitrogen	1.65	ns	1.29	ns	<1		<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	3.13	ns	2.87	ns	10.42	**	1.36	ns
Nitrogen	15.17	**	49.64	**	81.00	**	22.78	**
Azolla x Nitrogen	<1		2.91	ns	1.54	ns	1.40	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXIX: Effect of an *Azolla* cover on the total N uptake at harvest. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	17.40	**	26.00	**	<1		13.22	**
Nitrogen	60.79	**	113.10	**	3.53	ns	39.09	**
Azolla x Nitrogen	<1		5.33	*	<1		1.77	ns

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	5.31	*	6.39	*	5.55	*	4.37	ns
Nitrogen	22.32	**	19.09	**	16.18	**	25.64	**
Azolla x Nitrogen	<1		2.60	ns	1.66	ns	5.79	*

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXX: Effect of an *Azolla* cover on the straw N uptake at harvest. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	1.04	ns	23.93	**	1.17	ns	<1	
Nitrogen	42.17	**	39.32	**	1.64	ns	4.77	*
Azolla x Nitrogen	3.74	*	2.27	ns	<1		<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	1.60	ns	<1		5.66	*	2.46	ns
Nitrogen	8.27	**	31.36	**	51.48	**	28.20	**
Azolla x Nitrogen	<1		2.96	ns	<1		3.72	*

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXI: Effect of an *Azolla* cover on the straw N uptake at harvest. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	1.35	ns	6.05	*	<1		12.73	**
Nitrogen	11.07	**	27.45	**	8.46	**	35.15	**
Azolla x Nitrogen	<1		1.12	ns	3.71	*	<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	1.21	ns	3.76	ns	29.44	**	3.67	ns
Nitrogen	13.44	**	5.66	**	24.46	**	9.85	**
Azolla x Nitrogen	<1		3.28	ns	<1		3.76	*

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXII: Effect of an *Azolla* cover on the straw yield at harvest. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	7.18	*	71.32	**	5.87	*	3.59	ns
Nitrogen	34.09	**	41.24	**	4.51	*	4.28	*
Azolla x Nitrogen	1.08	ns	2.22	ns	<1		<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	1.11	ns	1.67	ns	10.77	**	3.28	ns
Nitrogen	8.73	**	42.07	**	49.30	**	17.69	**
Azolla x Nitrogen	<1		5.04	*	<1		3.13	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXIII: Effect of an *Azolla* cover on the straw yield at harvest. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	9.75	**	13.33	**	1.75	ns	15.79	**
Nitrogen	32.34	**	57.92	**	5.77	*	36.47	**
Azolla x Nitrogen	<1		1.06	ns	11.05	**	<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	1.30	ns	12.19	**	4.82	*	1.82	ns
Nitrogen	6.74	**	9.84	**	6.88	**	4.09	*
Azolla x Nitrogen	<1		<1		6.67	**	1.77	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXIV: Effect of an *Azolla* cover on the straw N concentration at harvest. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	<1		<1		7.59	*	6.72	*
Nitrogen	29.59	**	15.21	**	8.20	**	11.86	**
Azolla x Nitrogen	3.81	*	<1		4.75	*	<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	1.30	ns	1.94	ns	<1		<1	
Nitrogen	5.75	*	17.31	**	32.61	**	9.10	**
Azolla x Nitrogen	<1		<1		<1		<1	

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXV: Effect of an *Azolla* cover on the straw N concentration at harvest. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	<1		<1		<1		3.30	ns
Nitrogen	1.48	ns	4.08	*	9.50	**	12.02	**
Azolla x Nitrogen	<1		1.26	ns	1.67	ns	<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	<1		3.61	ns	31.33	**	<1	
Nitrogen	3.96	*	<1		19.48	**	3.70	*
Azolla x Nitrogen	1.06	ns	8.59	**	3.05	ns	1.85	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXVI: Effect of an *Azolla* cover on the grain N concentration at harvest. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	<1		10.17	**	<1		<1	
Nitrogen	19.63	**	58.51	**	6.04	*	46.20	**
Azolla x Nitrogen	<1		<1		<1		<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	<1		<1		1.02	ns	3.77	ns
Nitrogen	10.77	**	39.25	**	78.93	**	1.18	Ns
Azolla x Nitrogen	<1		1.29	ns	<1		<1	

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXVII: Effect of an *Azolla* cover on the grain N concentration at harvest. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	5.61	*	1.68	ns	<1		1.26	ns
Nitrogen	80.34	**	40.43	**	<1		2.64	ns
Azolla x Nitrogen	3.49	ns	1.15	ns	<1		6.38	**

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	<1		<1		1.77	ns	1.20	ns
Nitrogen	2.96	ns	4.94	*	<1		4.44	*
Azolla x Nitrogen	<1		1.59	ns	1.20	ns	1.45	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXVIII: Effect of an *Azolla* cover on the grain N uptake at harvest. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	3.64	ns	11.33	**	2.07	ns	2.49	ns
Nitrogen	40.16	**	17.90	**	9.33	**	8.39	**
Azolla x Nitrogen	<1		<1		<1		<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	5.56	*	16.15	**	14.01	**	<1	
Nitrogen	25.45	**	61.63	**	99.33	**	6.09	*
Azolla x Nitrogen	<1		3.98	*	2.11	ns	<1	

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXIX: Effect of an *Azolla* cover on the grain N uptake at harvest. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	23.95	**	39.90	**	<1		6.90	*
Nitrogen	70.23	**	171.40	**	1.86	ns	22.89	**
Azolla x Nitrogen	<1		9.89	**	<1		3.70	*

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	8.74	**	4.34	ns	<1		2.68	ns
Nitrogen	23.00	**	23.05	**	6.15	*	26.74	**
Azolla x Nitrogen	<1		1.65	ns	1.12	ns	4.87	*

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXX: Effect of an *Azolla* cover on the tiller count at maximum tillering stage. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	22.00	**	41.15	**	44.49	**	20.56	**
Nitrogen	61.99	**	74.01	**	177.55	**	151.80	**
Azolla x Nitrogen	2.73	ns	1.70	ns	3.17	ns	1.76	ns

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	2.55	ns	15.93	**	11.82	**	68.31	**
Nitrogen	1.06	ns	62.26	**	108.10	**	896.79	**
Azolla x Nitrogen	<1		1.51	ns	2.29	ns	15.55	**

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXXI: Effect of an *Azolla* cover on the tiller count at maximum tillering stage. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	49.94	**	22.86	**	56.83	**	33.81	**
Nitrogen	95.18	**	31.45	**	111.05	**	98.02	**
Azolla x Nitrogen	4.91	*	1.60	ns	3.17	ns	1.96	ns

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	104.28	**	111.35	**	81.94	**	61.37	**
Nitrogen	116.10	**	179.95	**	77.40	**	128.79	**
Azolla x Nitrogen	3.82	*	<1		2.53	ns	5.36	*

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXXII: Effect of an *Azolla* cover on the tiller count at harvest. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	65.32	**	33.09	**	40.83	**	10.54	**
Nitrogen	257.69	**	136.04	**	105.11	**	104.61	**
Azolla x Nitrogen	12.65	**	3.70	*	1.13	ns	1.50	ns

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	11.10	**	26.23	**	41.00	**	39.46	**
Nitrogen	122.12	**	102.64	**	339.24	**	207.69	**
Azolla x Nitrogen	<1		5.66	*	9.98	**	7.14	**

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXXIII: Effect of an *Azolla* cover on the tiller count at harvest. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	52.25	**	60.77	**	48.84	**	14.62	**
Nitrogen	227.24	**	120.63	**	79.92	**	62.07	**
Azolla x Nitrogen	21.06	**	7.52	**	1.17	ns	1.27	ns

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	12.68	**	20.00	**	41.88	**	114.02	**
Nitrogen	84.47	**	62.05	**	85.20	**	268.63	**
Azolla x Nitrogen	<1		1.15	ns	8.54	**	8.44	**

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXXIV: Effect of an *Azolla* cover on the panicle count at harvest. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	77.03	**	25.21	**	35.38	**	8.40	*
Nitrogen	277.76	**	122.71	**	99.23	**	89.86	**
Azolla x Nitrogen	12.70	**	4.11	*	1.15	ns	1.76	ns

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	12.08	*	26.52	**	41.09	**	45.42	**
Nitrogen	112.22	**	87.64	**	300.47	**	233.44	**
Azolla x Nitrogen	<1		5.25	*	8.66	**	9.11	**

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXXV: Effect of an *Azolla* cover on the panicle count at harvest. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	48.18	**	58.42	**	54.37	**	15.11	**
Nitrogen	218.51	**	115.38	**	81.10	**	64.82	**
Azolla x Nitrogen	21.25	**	7.63	**	1.32	ns	1.32	ns

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	10.29	**	18.26	**	44.53	**	128.11	**
Nitrogen	72.07	**	53.72	**	77.15	**	275.83	**
Azolla x Nitrogen	<1		1.36	ns	8.19	**	9.80	**

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXXVI: Effect of an *Azolla* cover on the total dry matter yield at harvest. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	11.81	**	42.05	**	4.48	ns	3.45	ns
Nitrogen	58.71	**	23.65	**	5.34	*	3.88	*
Azolla x Nitrogen	1.62	ns	1.28	ns	<1		<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	2.73	ns	10.90	**	13.61	**	7.82	*
Nitrogen	14.50	**	53.21	**	56.37	**	31.73	**
Azolla x Nitrogen	<1		5.07	*	1.24	ns	3.29	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXXVII: Effect of an *Azolla* cover on the total dry matter yield at harvest. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	17.84	**	18.21	**	2.68	ns	15.23	**
Nitrogen	35.55	**	71.22	**	10.73	**	28.45	**
Azolla x Nitrogen	<1		2.98	ns	4.63	*	<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	4.75	*	16.69	**	8.01	*	3.75	ns
Nitrogen	15.96	**	17.79	**	20.41	**	8.84	**
Azolla x Nitrogen	<1		<1		4.28	*	1.75	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXXVIII: Effect of an *Azolla* cover on the grain yield at harvest. On-farm field experiments. Laguna, Philippines. Wet season, 2000.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	8.29	*	7.94	*	2.05	ns	2.32	ns
Nitrogen	25.99	**	3.75	*	5.34	*	2.44	ns
Azolla x Nitrogen	<1		<1		<1		<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	6.29	*	27.37	**	13.61	**	13.26	**
Nitrogen	23.70	**	47.43	**	51.59	**	40.32	**
Azolla x Nitrogen	<1		5.95	*	1.80	ns	2.20	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant

Appendix XXXXIX: Effect of an *Azolla* cover on the grain yield at harvest. On-farm field experiments. Laguna, Philippines. Dry season, 2000-01.

	Los Banos site 1		Los Banos site 2		Bay site1		Bay site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	18.82	**	20.51	**	2.99	ns	18.95	**
Nitrogen	29.17	**	73.03	**	11.20	**	28.81	**
Azolla x Nitrogen	<1		4.76	*	<1		<1	

	San Francisco site 1		San Francisco site 2		Sta Cruz site1		Sta Cruz site 2	
Treatment	F-value	Significance	F-value	Significance	F-value	Significance	F-value	Significance
Azolla	14.32	**	14.80	**	5.98	*	6.20	*
Nitrogen	27.69	**	26.60	**	22.22	**	14.91	**
Azolla x Nitrogen	1.14	ns	1.03	ns	1.23	ns	1.21	ns

** = significant at 1% level; * = significant at 5% level; ns = not significant