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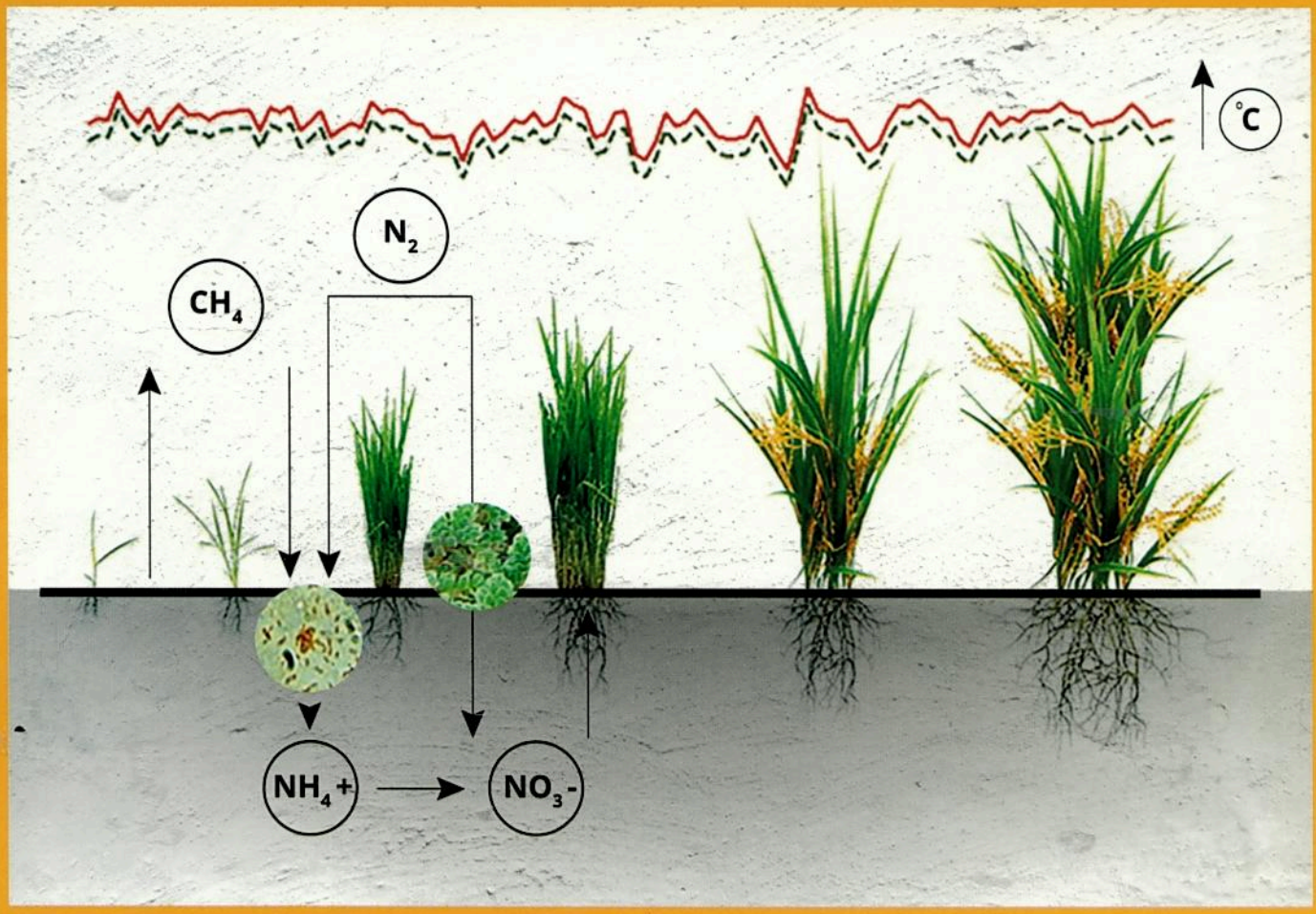
VOLUME 1 | ISSUE 1



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ABOUT THE COVER

Rice lives with its environment and this most important interaction between the two brings challenges and opportunities to the crop. This issue presents the role of azolla and vermi (worm)-composting of organic wastes in rice and rice-based production systems and the relationship between microorganisms and methane emissions. Breeding technique and growth model were also studied for increased rice productivity.

ABOUT THE LOGO

By taking the first step, we experience growth. With growth of rice as the base, questions from the very core of rice to the complexities of rice-based cropping and biological systems are up for answers leading to increased understanding and productivity.

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FOREWORD

Research is a progression, and this process leads to innovations that change lives and helps man rise from the toils of constant evolution. This peer-reviewed journal, managed by the Philippine Rice Research Institute, was launched in September 2014 to be a venue for intellectual pursuits and conversations on the biodiversity that interacts with rice.

Specifically, the peer-reviewed journal encourages publication of original research articles, review articles, commentaries, case reports, and short communications that have impact on and are interconnected with rice and rice-based crops.

As research cultivates the mind, this specialized journal publishes rice and rice-based researches on soil and crop improvement, resource use efficiency, biofertilizers, biopesticides, biomaterials, machinery, food processing, high-value pharmaceuticals, nutraceuticals, and systems analysis and simulation. It also covers the economics, social, and communication systems that may influence the landscape of rice and rice-based production.

While agricultural research tries to provide solutions, it is continually faced with growing demands to sustain food security amid increasing population, changing ecosystems, and decreasing land area for agriculture production. We published two reviews in this maiden issue: the growth of azolla on the floodwater and its role in reducing ammonia volatilization loses from the irrigated lowland rice floodwater system and vermi (worm)-composting of organic wastes and its potential role in sustaining rice and rice-based production systems.

In keeping with environmental concerns, results of the studies on the biological activities of populations of methanogens and methanotrophs in relation to emissions of methane from irrigated lowland rice field to the atmosphere were also presented in this issue. The emission of gases to the atmosphere can increase global temperatures and impact on crop productivity. Hence, a breeding technique was evaluated to test the tolerance of rice genotypes to high air temperatures. A rice crop growth model was calibrated to simulate potential rice yields and assess the potential impact of increased air temperatures on rice crop productivity. You will also find them in this issue.

Research is not only a way of life; it also helps facilitate our own way of life and appreciate complexities. With this noble goal, we invite you to contribute to the upcoming editions of our journal.

Rolando T. Cruz

ROLANDO T. CRUZ, Ph.D.
Editor-in-Chief

EFFECT OF AZOLLA COVER ON FLOODWATER CONDITION AND AMMONIA VOLATILIZATION: A REVIEW

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ABSTRACT
Ammonia volatilization remains a major mechanism for the loss of nitrogen (N) from rice fields, which contributes to low recovery of fertilizer N applied to flooded rice (*Oryza sativa* L.). The transformation of N from organic or inorganic sources to NH_4 and NH_3 through biological and chemical processes and how these biological and chemical processes depend on submerged soil and floodwater condition and above-ground environment is now understood. Irradiance, concentration of NH_4, pH, temperature, and wind speed or turbulence above the floodwater influence NH_3 volatilization losses in flooded soils. From planting until the closing of leaves at the end of vegetative phase, the floodwater is exposed to the sun. Anything that could partially or fully shield the floodwater and soil from solar radiation would influence temperature and pH of the floodwater and submerged soil. Azolla, among other aquatic macrophytes, can play the role of a shield when fully grown and entirely cover the floodwater surface. The focus of this review is on various studies conducted in the Philippines that illustrate the important roles of Azolla cover on the floodwater in rice paddies.

KEYWORDS
Azolla cover, ammonia volatilization, floodwater pH, floodwater temperature

INTRODUCTION

Considered as one of the fastest growing plants, Azolla gained the distinction of superorganism when the carbon-rich sediment collected from the Arctic Sea during the Arctic Coring Expedition was confirmed to be from Azolla (Bujak and Bujak, 2014). During the Arctic Azolla Event that took place almost 1 million years during the Eocene period, Azolla is believed to have sequestered 0.9-3.5 x 10^{18} g atmospheric CO_2 and speculated to have caused the change in Earth's climate from the warmer pre-Azolla greenhouse climate toward our modern icehouse climate (Speelman et al., 2009).

Azolla grows in the flood waters of rice paddies for green manure and livestock feed. The unique characteristic of Azolla is its high N_2-fixing capability through its symbiotic association with the filamentous nitrogen-fixing bacteria, *Anabaena azollae.* The *Azolla-Anabaena* symbiotic association has the potential to provide the solution to some of the global problems such as the rising atmospheric CO_2, depleting supply of non-renewable energy, and food security (Salehzadeh et al., 2014, Muradov et al., 2014). The high N_2-fixing capability of the Azolla-Anabaena symbiotic association is attributed to combined activities of ammonia-assimilating enzymes in the association rather than independently by *Anabaena* and *Azolla* (Ray et al., 1978).

Several species and varieties of Azolla were found and reported (Dunham and Fowler, 1987). The Biofertilizer Germplasm Collection of the International Rice Research Institute (IRRI) is maintaining more than 500 live accessions belonging to eight Azolla species and two varieties, namely: *A. pinnata var. imbricata, A. pinnata var. pinnata, A. mexicana, A. filiculoides, A. carolinana, A. microphylla, A. rubra,* and *A. nilotica* (Watanabe et. al., 1992). The Azolla accessions were collected from 55 countries including Asia and Oceana (56%), North America and South America (24.8%), Africa (7.8%), and Europe (4.8%). *A. pinnata* var. *imbricata* and *A. microphylla* are the more common species. *Azolla pinnata* var *imbricata* is among the first few accessions of IRRI in 1975.

Azolla has been well studied as biofertilizer for rice. However, other functions that Azolla perform in flooded rice ecosystem should be exploited to be benefited by farmers. Among these functions is the alteration of floodwater condition contributing to NH_3 volatilization and increased availability of micronutrient elements associated with the alkalinity in submerged soils. Studies revealed that Azolla cover on floodwater of rice reduced NH_3 volatilization while it suppresses the rise in daytime floodwater pH and temperature (Villegas and San Valentin, 1989, Vlek et al.,1995, Vlek et al., 2002, De Macale et al., 2002). Lowering pH will favor solubility of zinc (Zn) among other metallic nutrients in submerged soils. Cagauan (1999) also observed suppression of weed growth in rice fields where Azolla, ducks, and fish were grown together.

This review focuses on the evidence indicating how floodwater condition influences ammonia volatilization and how Azolla in flooded rice ecosystem can modify floodwater condition utilizing data from limited studies conducted in the Philippines.

GROWTH AND NITROGEN ACCUMULATION OF AZOLLA

In 1979, IRRI inoculated rice paddies in the municipalities of Tantangan, Banga, and Koronadal in the province of South Cotabato, Philippines with *Azolla pinnata* var. *imbricata* (Bangkok strain). By 1981, this fast-growing Azolla species covered more than 5,000 ha of rice lands and by 1985, the area expanded to about 84,000 ha (Mabbayad, 1985). This experience in South Cotabato led the Philippine government to establish the National Azolla Action Program (NAAP) with the primary objective of reducing the dependence of Filipino rice farmers on imported inorganic N fertilizer in irrigated rice production (Mabbayad, 1985).

Growth rate, heat tolerance, and resistance to pests and diseases were used in the selection of best variety of Azolla for use as biofertilizer in flooded rice culture. The studies conducted by NAAP showed that the growth of Azolla varies depending on species and location in the Philippine. Soil must have at least 18 ppm P extractable by 0.5 M $NaHCO_3$ (Olsen P extraction method) to assure biomass production with growth rate of almost 7 days doubling time (Leyese and Tilo, 1970; San Valentin et al., 1986). A coordinated evaluation was conducted by NAAP at different locations in the Philippines using similar set of Azolla species and strains pre-selected for their relative tolerance to temperature and resistance to pests and diseases (Mabbayad, 1985). Table 1 shows the growth rates of the different Azolla species evaluated and recommended by NAAP for the different regions. The doubling time (DT) shown in Table 1 represents the average number of days for the Azolla species to double its biomass. Growth rate (r), another growth parameter, can be calculated from DT using the equation r = 69/DT.

The species that performed well in the various regions in the Philippines exhibited DT from 3.0 to 7.8 days as shown in Table 1. *A. microphylla* 403 and *A. microphylla* 417 were the fastest growing species in Albay with DT=3, while A. *pinnata* 704, A. *microphylla* 407, and *A. caroliniana* 405 performed almost similarly in Camarines Sur with DT 7.3 to DT 7.8. Location specific environmental factors played significant role in the performance of the species particularly the effect of temperature, solar radiation, and

Table 1
Growth rate of recommended Azolla species in the Philippines (Data from UPLB-NAAP Training Manual on Biology and Culture of Azolla)

Region	Sub-Center/Location	Recommended Species[a]	Doubling Time[b], days
1	DMMMSU, Bacnotan, La Union	A. microphylla 403	7.1
		A. microphylla 407	6.4
		A. pinnata 5	7.5
		A. microphylla 418	7.0
2	CSU, Gonzaga, Cagayan	A. mexicana	3.5
		A. microphylla 418	4.0
3	PSU, Magalang Pampanga	A. microphylla 418	6.0
		A. pinnata 704	7.0
4	UPLB, Los Banos, Laguna	A. microphylla 418	5.0
		A. caroliniana 302	6.0
		A. pinnata 5	7.0
		A. pinnata 704	7.0
5	CBSUA, Pili, Camarines Sur	A. pinnata 704	7.5
		A. microphylla 407	7.3
		A. caroliniana 405	7.8
	BSU-CA, Guinobatan, Albay	A. microphylla 418	3.0
		A. microphylla 403	3.0
		A. pinnata 704	6.0
6	CPU, Iloilo City	A. caroliniana 305	6.5
		A. microphylla 418	7.0
		A. microphylla 403	7.0
		A. microphylla 407	7.0
7	SU, Dumaguete City	A. microphylla 403	5.0
		A. microphylla 407	7.5
		A. microphylla 418	6.0
		A. pinnata 5	7.0
8	VSU, Baybay, Leyte	A. pinnata 704	6.5
		A. pinnata Java	7.0
10	XU-CA, Cagayan de Oro City	A. pinnata 704	7.0
		A. pinnata Java	7.0

[a] *Original accession number of IRRI's Biofertilizer Germplasm Collection (Watanabe et al. 1986).*
[b] Doubling time in number of days is based on the exponential growth equation $N(t) = N(0)\, e^{rt}$ where N(t) is biomass at time t, N(0) is biomass at time t=0, and r is growth rate. *Growth rate r is equal to the natural log N(t)/N(0) divided by t. When N(t)/(N0)=2, log 2=0.69 and doubling time DT = 0.69/r. For example if growth rate r = 10%, DT = 69/10 = 6.9 days*

exposure to pests and diseases. The biology and culture team of NAAP also found that biomass production of Azolla fluctuated during the year in Los Baños, Laguna, Philippines following changes in temperature. Average net production of *A. microphylla* 417, *A. caroliniana, A. pinnata* (Bangkok), and *A. pinnata* (Australia) was low during the hottest months of the year from April to May. *A. microphylla* produced the highest net biomass, averaging 1.2 kg/m^2/week from September to March and 0.85 kg/m^2/week from April to August.

On the basis of biomass production, Azolla is expected to supply the N requirement of a high-yielding rice crop within 20 days (Lumpkin, 1987). Accumulation of N after 10 days of growth can reach 88 kg N/ha, assuming 5 t/ha initial fresh biomass and N content of 4% N based on dry weight, with growth rate of 20% per day or doubling time of 3.8 days. Watanabe (1985) summarized data from field trials conducted in 10 countries and reported Azolla fresh biomass production ranges from 5-25 t/ha with 10-50 kg N/ha when grown before or after transplanting. Roger

and Ladha (1992) reported N_2-fixation rate ranging from 0.4 to 3.6 kg N/ha/day and averaged 2 kg N/ha/day.

AZOLLA COVER ON FLOODWATER OF SUBMERGED SOIL

Conditions in the rice paddies can favor the fast-growing Azolla to fully cover the floodwater of the entire paddy field before planting time. Azolla can produce fresh biomass of 1.5-2.0 kg/m^2 which is enough to cover the entire floodwater surface like a floating mat. The floodwater cover can significantly reduce the amount and intensity of solar radiation reaching the soil, possibly restricting biological and chemical processes depending on available photosynthetically active radiation (PAR), heat, and energy.

Chemical and biological changes following submergence of an oxidized soil as the reduction process takes place and effects on rice had been well studied (Ponnamperuma et al., 1966; Ponnamperuma, 1972; Patrick and Reddy, 1978; Yoshida, T., 1978; Kyuma, 2004). At the early stage of submergence when there is sufficient O_2 organic matter is decomposed by heterotrophic microorganisms to produce CO_2 while respiratory processes occurring in plant roots and other organisms in the soil also continuously produce CO_2(g), which reacts with water to produce H_2CO_3. In an open system such as the bare soil, there is opportunity for CO_2(g) to escape to the atmosphere and re-enter.

Soil microbiological functions in submerged soils are influenced by the presence of Azolla cover on floodwater in rice paddy which include fixation of atmospheric N, organic matter decomposition leading to ammonification and transformations to NH_3(aq) and NH_3(air), and utilization of CO_2 by photosynthetic filamentous algae. These microbiological functions are directly or indirectly influenced by temperature, solar radiation, and pH of the system. The following presentation will focus on how the presence of shield on floodwater surface can influence these floodwater condition and chemical processes leading to the formation of NH_3. Table 2 summarizes the change in the general condition of the floodwater in the presence and absence of surface cover.

SOIL CONDITION CONTRIBUTING TO AMMONIA VOLATILIZATION

One of the effects of submergence is the change in pH of acid soils or alkaline soils toward neutrality (Ponnamperuma, 1972; Patrick and Reddy, 1978; Kyuma, 2004). The increase in pH of acid soils is favorable to microbial processes that release nutrients and increase availability of P chemically bound to oxides and hydroxides of Fe and Al. The decrease in pH of alkaline soils favors availability of P in the form of Ca and Mg compounds with P and solubility of Zn, Cu, Fe, and Mn, which form compounds with carbonates with very low dissociation (Adams, 1971).

Change in pH in submerged soil and floodwater is associated with the CO_2-H_2O system. Carbon dioxide dissolves in water and various forms of carbonate compounds (Lindsay, 1979): $CO_2(g) + H_2O \rightleftharpoons H_2CO_3^\circ$ with equilibrium constant K° based on chemical activity, $K^\circ = 10^{-1.46}$; $H_2CO_3^\circ \rightleftharpoons H^+ + HCO_3^-$ ($K^\circ=10^{-6.36}$); and $HCO_3^- \rightleftharpoons H^+ + CO_3$ ($K^\circ=10^{-10.33}$). The equilibrium will shift as the activities of the reactants (e.g. partial pressure of CO_2 and H^+) change. The distribution of various carbonate species in solution at a given partial pressure of CO_2(g) will follow their equilibrium constants (Lindsay, 1979). For example, CO_2 in air is about 0.000355 atm but in flooded soils the CO_2(g) levels often range from 0.01 to 0.3 atm (Lindsay, 1979). The equilibrium reactions of each carbonate species dictates the change in pH when CO_2(g) changes (Lindsay, 1979). For example, decreasing the CO_2(g) by 10-fold (e.g. from 0.0355 to 0.00355 atm) will shift the equilibrium by one pH unit lower. Utilization of CO_2(g) by photosynthetic microorganisms can bring about such change in partial pressure of CO_2 in the soil. For instance, biological N_2 fixation by blue-green algae utilizes CO_2 and water for the production of their own photosynthate (Roger, 1984).

Azolla cover reduces solar radiation penetrating the submerged soil. There is reduction in light penetration by about 50% due to presence of Azolla cover in the floodwater. On clear cay, the amount and quality of light that reach the surface of the submerged soil depend on time of the day, stage of rice plant, and

Table 2
Relative floodwater condition of submerged soil and presence of Azolla cover.

Floodwater Surface	Light Penetration	Temp	CO_2	pH	NH_3(aq)
Without Azolla	Good to Excellent	High	High	>pH 8.5	Low
With Azolla Cover	Poor to Very Poor	Low	Low	<pH 8.0	High

floodwater condition including water depth, amount of dissolved materials in the water and presence of partially decomposed organic matter on the surface of the submerged soil. The reduction in solar radiation by the Azolla cover influences biological activity particularly to the photosynthetic microorganisms.

FLOODWATER CONDITION AND NITROGEN TRANSFORMATION FROM NH_4 TO NH_3

Nitrogen from NH_4 containing fertilizers and from hydrolysis of Urea is converted to NH_4^+ in the soil solution which then reacts with hydronium ion (H_3O^+) to form NH_3(aq) and finds its way to the atmosphere as NH_3 (air). The escape of NH_3 from the liquid phase to the atmosphere in response to difference in partial pressure in the liquid phase, pNH_3(aq) and the atmosphere, pNH_3(air), at the water-air interphase. All these processes are strongly dependent on temperature based on the kinetic theory and thermodynamic laws. Kinetic theory postulates that temperature and pNH_3 are related by way of thermodynamic dissociation constant of the equilibrium between NH_4 and NH_3 (Bates and Pinching, 1949) and the increase in kinetic energy of gas molecules. As temperatures increases, there is relative proportion of NH_3 to NH_4^+ in the system at a given pH increases, while the solubility of NH_3 in water decreases. Temperature increases the diffusion of NH_3 through the soil and affects the rate of microbial transformations. Wind speed and turbulence also influence NH_3 volatilization (Denmead, 1983) by way of altering the rate of diffusion from floodwater and air. Movement of air mass (wind speed and wind sweep) over the floodwater surface will influence diffusion of NH_3(g) at the interface of floodwater surface and air mass over the floodwater.

Fertilizer N recovery by rice seldom exceeds 50% of the N applied as fertilizer (Vlek and Byrnes, 1986). In the Philippines, ammonia volatilization can account from 20% to more than 80% of the losses from N fertilizers in flooded soils (Mikkelsen et al., 1978; De Datta et al., 1989; Freney et al., 1990). Losses from urea range from 11% to 54% when it is broadcast to the floodwater immediately after transplanting (Schnier,1995). Urea, $CO(NH_2)_2$, can dissolve in the floodwater and hydrolyze to form NH_4CO before the granules fall on the surface of the submerged soil.

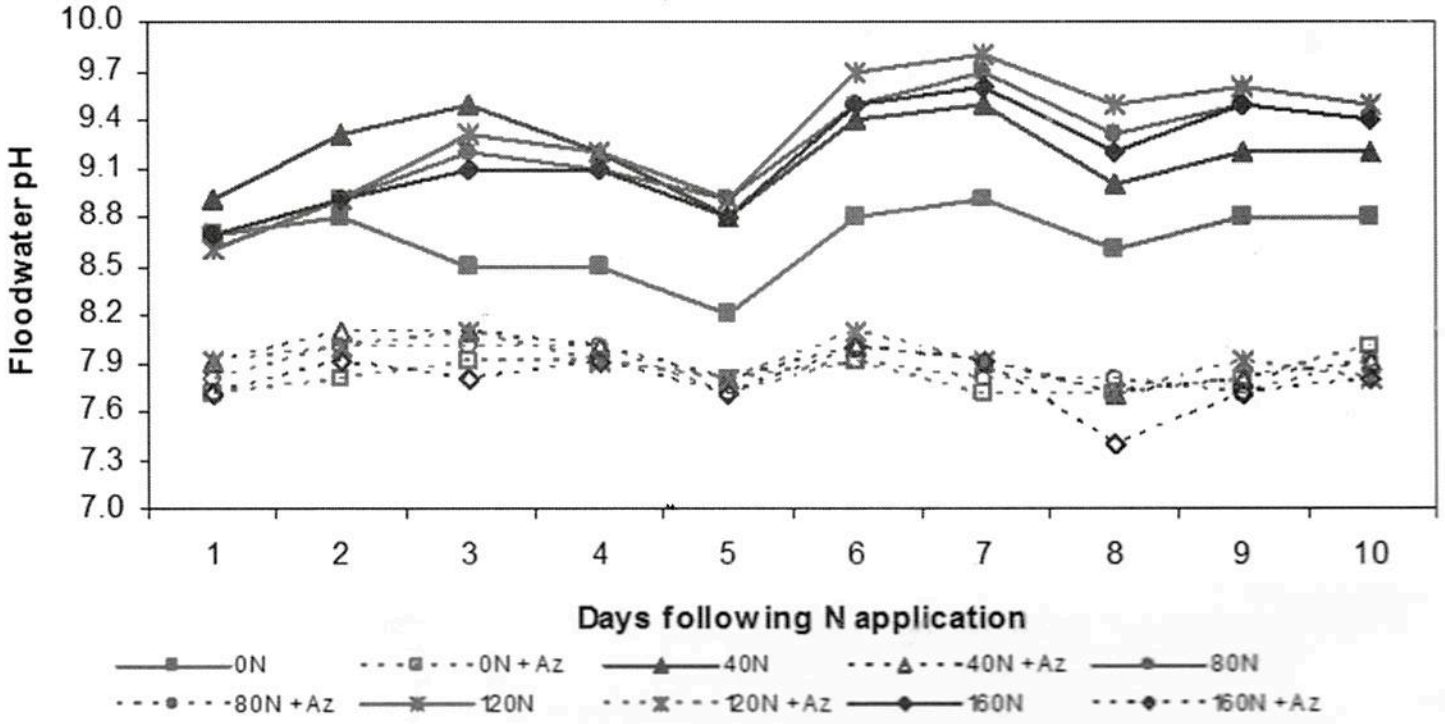
Ammonia volatilization from flooded soil system has been thoroughly assessed (Jayaweera and Mikkelsen, 1990). Concentration of NH_3, temperature, pH of floodwater and submerged soil, and wind speed and turbulence over the floodwater surface constitute the major factors that markedly influence partial pressure of ammonia (pNH_3) in the floodwater and volatilization of NH_3 to the atmosphere (Freney et al.,1995; Fillery and Vlek, 1986; and Jayaweera and Mikkelsen, 1990). The effect of floodwater pH can be easily understood on the basis of the dissociation of NH_4 ion in the presence of hydroxide (OH^-) ion. Acting as a week base, ammonium dissociates in water to form NH_3(aq) and the dissociated H^+ forms water molecules with the OH^{-1}. Ammonia concentration increases by a factor of 10 for every unit increase in pH up to pH 9.0. The pH, in particular, affects the equilibrium between NH_4^+ and NH_3, so that 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 (Freney et al., 1983). A computer model proposed by Jayaweera and Mikkelsen (1990) relating NH_3 volatilization in flooded soil system consists of chemical aspect of the NH_4/NH_3(aq) equilibrium in the floodwater and the volatilization aspect that determine transfer of NH_3(aq) in the floodwater to the air. Equilibrium between NH_4 and NH_3(aq) and NH_3(air) involves dissociation of H^+ from NH_4 to form NH_3(aq) and simultaneously the re-association of H^+ with NH_3(aq) and finally the formation of NH_3(air), referred to as the dissociation rate constant, association rate constant, and volatilization rate constant, respectively. It is in the dissociation and association reactions that pH and temperature of the floodwater play an important role while the volatilization rate is influenced by temperature and wind speed and wind sweep over the floodwater.

EFFECT OF AZOLLA COVER ON FLOODWATER CONDITION

In the Philippines, Villegas and San Valentin (1990) and de Macale (2002) studied the changes pH and temperature of both floodwater and the submerged soil brought about by the presence of Azolla mat covering the floodwater. Average temperature of the floodwater was lower with Azolla cover than without Azolla cover by 1°C to 2°C. Lemna, a common aquatic macrophyte in Philippine rice paddies, was found also to reduce pH and temperature but to a lesser extent than Azolla. The presence of Azolla cover reduced floodwater pH by almost 2 units. The pH values of uncovered treatments ranged from pH 8.65 to pH 8.95 while the covered treatments ranged from pH 7.7 to pH 7.9.

Diurnal and daily temperature of air, floodwater, and submerged soil; and pH of floodwater were measured in plots applied with different rates of Urea and with or without Azolla cover (de Macale, 2002). From 8 am to 2 pm, floodwater and soil temperature was lower in plots with Azolla cover than without cover with difference of 1.7°C to 6.8°C (Figure 1). After 2 pm the Azolla covered plots appeared to cool faster than plots without Azolla, thus, temperature was higher in the Azolla covered plots than the plots without Azolla. The magnitude of difference in temperature between the Azolla covered plots and without Azolla depends on time of the day and cloud cover.

Figure 1.
Effect of Azolla cover on changes in floodwater pH from the time of application of different rates of Urea. 1st Dry season trial 1998-1999 (Data from de Macale, 2002).



In Laguna, de Macale (2002) observed floodwater alkalinity (pH) between 12 pm and 2 pm, the period when temperature was usually high during the day (Figure 2). Data were examined for the effect of Azolla cover and levels of Urea applied. Urea treatments were 0, 40, 80, 120, and 160 kg N ha^{-1}.

While pH fluctuates daily, plots with Azolla cover had consistently lower pH than plots without Azolla cover but pH values increased with rate of Urea applied. The highest pH value recorded was pH 10 in the first experiment and pH 9.7 in the second experiment and the lowest was observed in plots with Azolla cover and without Urea.

REDUCTION IN NH_3 VOLATILIZATION LOSSES BY AZOLLA COVER ON FLOODWATER

The effect of Azolla cover on floodwater was studied by Villegas (1985) in relation to floodwater condition and rate of N fertilizer applied under laboratory and screen house conditions. In the laboratory studies, it was found that there was an increase in pH from pH 7.0 to 8.5 and 10.0 and increase in temperature from 24°C to 44°C and when there was an imposed air movement over the floodwater, an increased amount of NH_3 lost by volatilization from solution containing about 400 ppm NH_3 was observed. The effects of pH, temperature, and air movement on the loss of NH_3 by volatilization were reduced in the presence of Azolla cover on the floodwater (Figure 3).

Following solar radiation changes during the day, floodwater pH and temperature increased reaching their maximum at about 2 pm. Azolla cover reduced the magnitude of increase in pH and temperature. Field trials conducted in Laguna by de Macale (2002) showed that the presence of Azolla cover slightly varied the alkalinity of floodwater around pH 8.0 with application of different levels of Urea-N but without the Azolla cover. Floodwater alkalinity rose above pH 8.5 reached a peak of pH 9.7 at 7 days after the application of fertilizer (Figure 1). This reduction in pH alone is expected to have tremendous influence on the transformation of NH_4^+ to NH_3(aq) according to the model of Jayaweera and Mikkelsen (1990). De Macale (2002) observed higher total NH_4-N in the floodwater with Azolla cover than without Azolla cover by as much as 5.3-5.5 g N m^{-2}.

In La Union, Boadilla (1993) studied the effect of Azolla cover on the dynamics of urea broadcast in flooded soils and on floodwater pH, and temperature. Using N balance procedure, the presence of Azolla cover retained greater amount of NH_3-N in the flood water and reduced N losses by about 80-90%. Boadilla (1993) observed that

Figure 2.
Effect of Azolla cover on changes in floodwater pNH3 from the time of application of different rates of Urea. 2nd Dry season trial 1998-1999 (Data from de Macale, 2002).

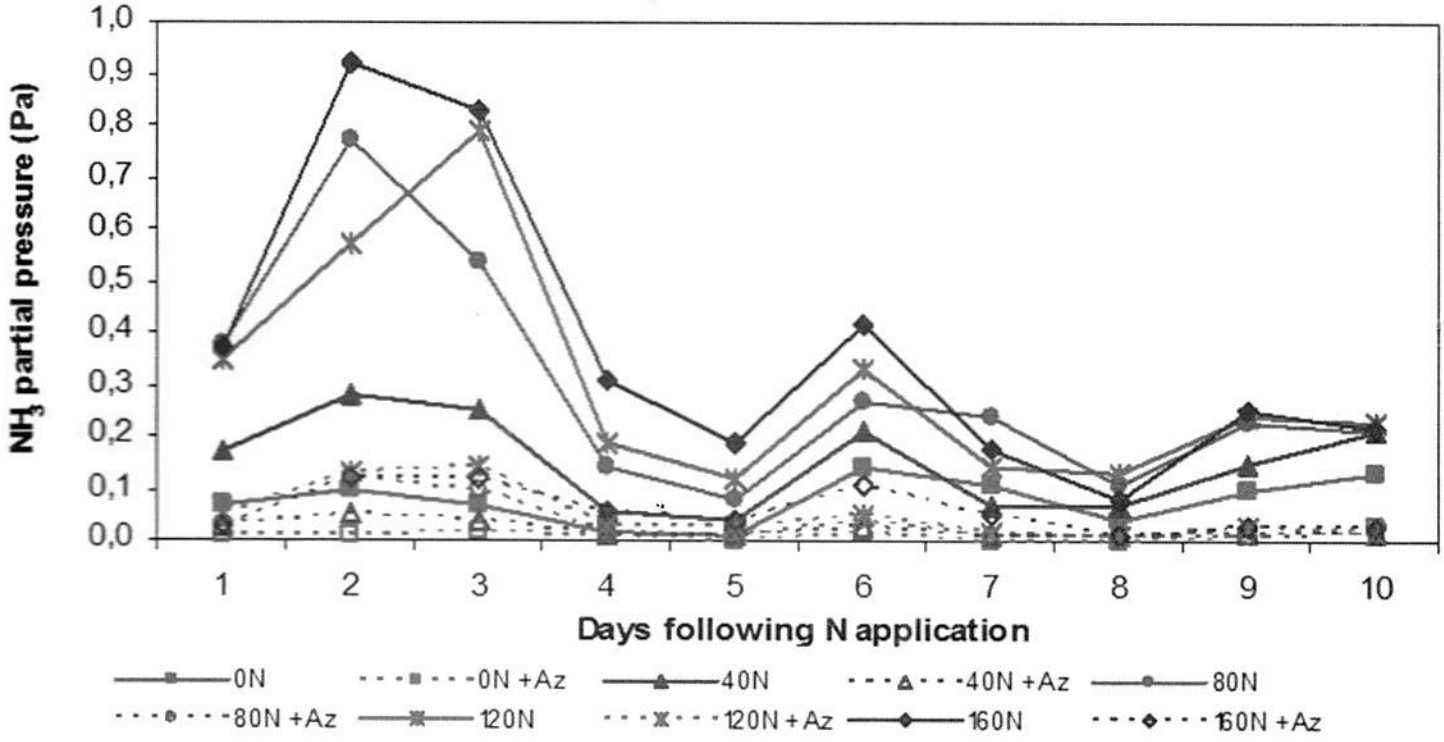
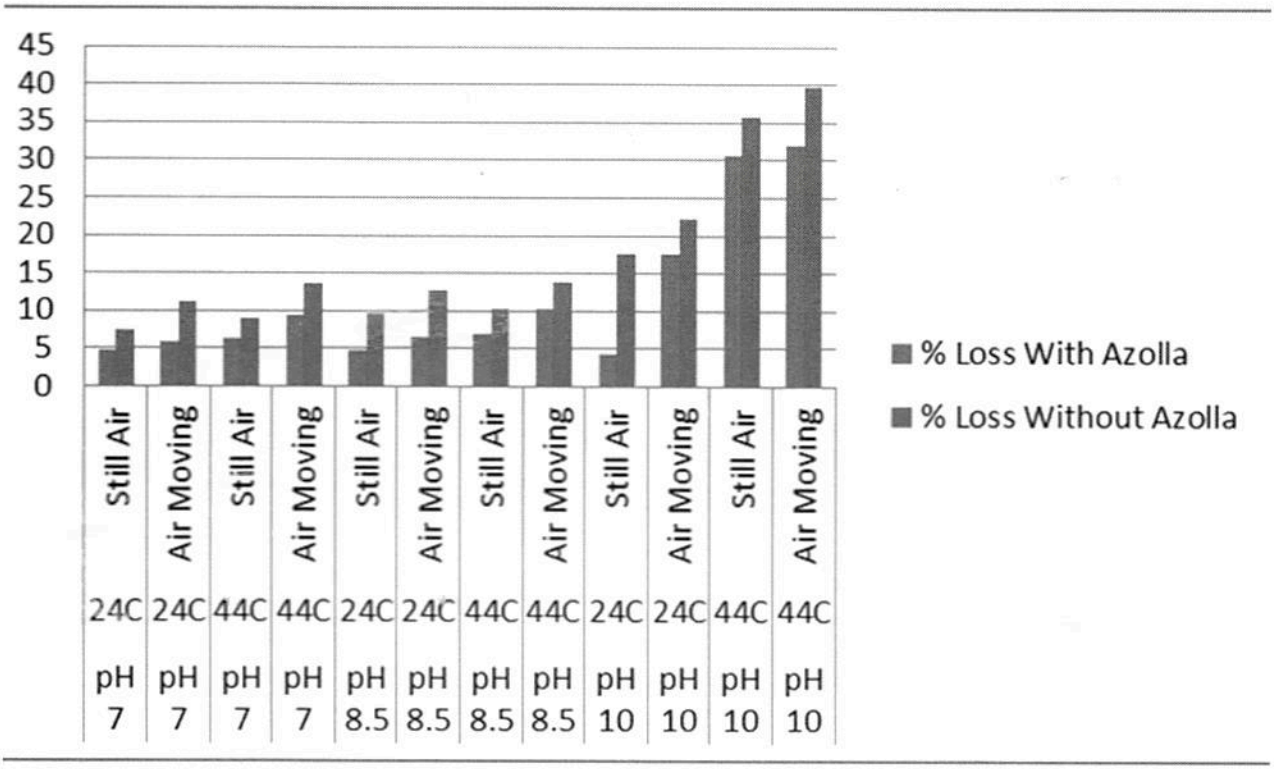


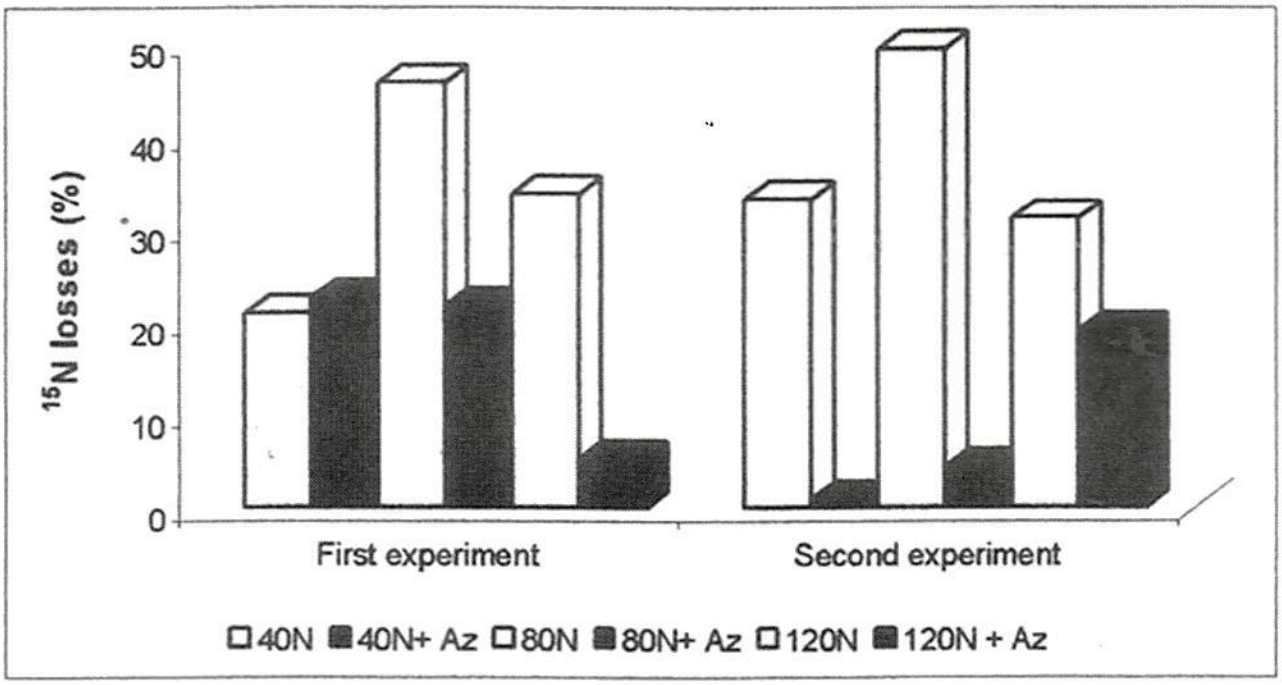
Figure 3.
Effect of pH, temperature, wind, and Azolla cover on volatilization of NH_3 (Data from Villegas and San Valentin, 1989).



full Azolla cover on floodwater surface (100%-150%) upon broadcasting Urea was the most effective as physical barrier for reducing ammonia nitrogen volatilization.
The higher NH_3-N retained with Azolla cover had caused higher pH of the culture solution as against the no Azolla treatment.
Using ^{15}N-labelled Urea, losses of N from fertilizer applied at different rates were smaller in the presence of Azolla cover than without Azolla cover (Figure 4).

This is consistent with observation on pH and NH_3 in the floodwater (Figure 1 and 2). Lower losses of N from Azolla covered plots in the 2nd dry season trial (Figure 4) also suggested that temperature contributed to thegreater losses of N from the uncovered plots.

Figure 4.
Loss of Urea-N (based on ^{15}N) at different rates with and without Azolla cover. Wet and dry season 1998-1999. (Data from de Macale, 2002)



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ISOLATION OF METHANOGENS AND METHANOTROPHS FROM A TROPICAL LOWLAND RICE SOIL

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ABSTRACT

The simultaneous activities of methanogens and methanotrophs are responsible for the net emissions of methane (CH_4) from the lowland soils. In a pot experiment conducted to determine the populations of methanogens and methanotrophs in a lowland rice soil, nine methanogens and 18 methanotrophs were isolated. Isolates were observed to be Gram negative and had rod and spherical shapes. Methane oxidation ranging from 3% to 80% was observed for methanotrophs. Six methanotrophs were observed to produce ethylene from acetylene, as indicator of N_2-fixation ability, ranging from 21.2 to 49.5 nmol C_2H_4 in an hour per tube. Burkholderia and Vibrio were identified to be the most significant taxa related to the methanotroph isolates.

KEYWORDS

methane, methanogens, methanotrophs, lowland soil

INTRODUCTION

The irrigated rice ecosystem has the highest potential to produce methane (CH_4) because of flooding and intensity of rice cultivation especially from the Asian region with 90% of the total world rice harvested area (Gowariker et al., 2009). Methane emissions are net results of simultaneous CH_4 production by methanogens and CH_4 oxidation by methanotrophs. The simultaneous biological activities of methanogens and methanotrophs are responsible for the net emissions of CH_4 from the wetland soils to the atmosphere.

The anaerobic environment in a lowland rice field favors the production of CH_4 by methanogenic archaea. Two distinct metabolic pathways of biological CH_4 formation are the CO_2 reduction that uses hydrogen gas, fatty acids or alcohols as hydrogen donor and the transmethylation of acetic acid or methyl alcohol, which does not involve CO_2 as an intermediate (Wang et al., 1995). Methane production is a reduction process, which depends on the source, quality, and quantity of organic matter (Ranganathan et al., 1995). Methane production and emission were observed to be correlated with methanogenic potential and the carbon (C) content and the carbon to nitrogen (C/N) ratio of the incorporated organic materials (Le Mer and Roger, 2001).

On the other hand, biological oxidation of CH_4 is mediated by autotrophic aerobic microbes known as methanotrophs in the oxidized layers of the paddy soil. Because of the supply of O_2 in the rhizosphere, the population and activity of methanotrophs are enhanced leading to utilization of CH_4 produced by methanogens in the anaerobic layers before it is emitted (Wang et al., 1995). Methanotrophs, which use CH_4 as the only C and energy source, oxidize as much as 60% to 90% of the CH_4 produced in the soil (Sass et al., 1990). Methane oxidation in the soil surface of a flooded soil consumes about 80% potential diffusive CH_4 flux. Although the rhizospheric soil is considered most important internal sink for CH_4 produced in the soil profile, it is relatively less efficient (10-30%) because of the greater competition with alternative O_2 sinks (Wang et al., 1995). Some methanotrophs were also observed to be capable of fixing nitrogen while utilizing CH_4 as carbon (C) source (Davis et al., 1964). Nitrogen-fixing methanotrophs are among the large proportion of the total microflora in the rice rhizosphere and have the potential to contribute to the total soil N made available for rice while mitigating CH_4 emissions. This paper presents some of methanogens and methanotrophs isolated from a tropical lowland rice soil. Yet, further studies on these microorganisms – their characterization, populations in the soil and rice rhizosphere, as well as their interaction with rice and other soil microorganisms – are needed to better understand their beneficial roles in the chemistry of lowland soil.

MATERIALS AND METHODS

In a pot experiment conducted to determine the population of methanogens and methanotrophs that influence CH_4 dynamics in a lowland rice soil, cultures of these microorganisms were isolated for characterization. Methanotroph isolates were also tested for CH_4 oxidation and N_2-fixing abilities.

Establishment of pot experiment. The experiment was laid out in Randomized Complete Block Design (RCBD) with four treatments, replicated six times: No Fertilizer (T1); 120-30-60 fertilizer recommended rate for dry season (T2); 5 t/ha rice straw (T3); and 20 bags (1 t) ha^{-1} organic fertilizer (T4). Collection of lowland soil was done from a rice field in Brgy. Puypuy, Bay Laguna, with the soil properties shown in Table 1.

Table 1
Some physical and chemical properties of Calumpang soil series used in the study.

Soil Properties	Values/Characteristics
Soil Taxonomy	*Aeric Vertic Epiaqualf* *
Location (coordinates)	14°09'43.21" N 121°15'52.69" E
Elevation (ft asl)	54
Soil pH	6.40
% Organic Matter	3.18
Total N (%)	0.15
Available P (ppm)	33.0
Available K ($cmol_{(+)}$ kg^{-1} soil)	0.86
Cation exchange Capacity (CEC $cmol_{(+)}$ kg^{-1} soil)	32.3
Soil textural class	Silty Clay
% sand	18
% silt	47
% clay	35

* Source: Dr. Rodrigo B. Badayos, personal communication, March 2014

Plastic pails were filled with 10 kg of homogenized and puddled soil kept flooded for four weeks before transplanting. The rice straws, air-dried and cut into 0.5-cm length, had 33.23% organic carbon and 0.74% N when applied at 3 weeks before transplanting. The organic fertilizer, applied a day before transplanting, consisted of coir dust and chicken manure (30:70 v/v) and contained 14.17 % organic carbon, 1.17% N, 3.28% P_2O_5, and 1.09% K_2O. Chemical fertilizer treatment was also applied a day before transplanting. Twenty-one-day old rice seedlings (PSB Rc82) were transplanted into each pot. The pails were placed in a screen house and a floodwater layer of about 5 cm was maintained throughout the experiment until two weeks before the expected maturity.

Population count of methanogens and methanotrophs. Most probable number (MPN) method was conducted to determine microbial count of microorganisms in soils with different nutrient sources. Basal medium (LBPM) with a headspace of H_2-CO_2 mixture was used as growing culture for methanogens while nitrate mineral salt medium (NMS) with 18% CH_4 headspace was used for methanotrophs (Zeikus, 1977; Espiritu et al., 1997). Incubation was done in the dark at room temperature for 14 days, for methanogen cultures, and 30 days, for methanotrophs cultures.

Isolation of methanogens. Isolation of the methanogens from the soil samples was done from the MPN tubes with positive growth. Bacterial growth was streaked on plates of the basal (LPBM) medium with 1.5% agar. The streaked LPBM agar medium plates were reincubated under H_2-CO_2 mixture in the dark at room temperature with same incubation period as the MPN tubes. Colonies were purified by several restreakings and culture upon LPBM agar medium.

Isolation of methanotrophs. Isolation of the methanotrophs from the soil samples was done from the MPN tubes with positive growth. Bacterial growth was streaked on plates of the NMS medium with 1.5% agar. The streaked NMS agar medium plates were reincubated under pure-grade methane air in the dark at room temperature with the same incubation period as the MPN tubes. Colonies were purified by several restreakings and culture upon NMS agar medium (Davis et al., 1964).

Detection of methane oxidation and nitrogen-fixation abilities of isolated methanotrophs. Purified isolates of methanotrophs were re-grown on 5 ml of NMS media in 25-ml tubes by inoculation of a loopful of culture. The tubes were immediately closed with butyl rubber stopper. Tubes were injected with pure CH_4 at about 2.5% (v/v) of tube headspace and incubated in the dark at room temperature with shaking for 5 days. The CH_4 concentrations of the air in the head space were analyzed with a gas chromatograph. The decrease in the CH_4 solution during incubation was calculated as the amount of CH_4 oxidized by the methanotrophs in a given time. Acetylene reduction assay (ARA) was used to determine N_2-fixation. Pure isolates were grown in 5 ml N-free NMS medium in 25-ml tubes. Acetylene measuring 2 ml and 50 µL of 1 mol/l sodium formate were added to the tubes aseptically and were incubated for 2 hours at room temperature with shaking. Gas samples from the tube headspace were analyzed for ethylene production by gas chromatography (Dianou et al, 1997).

RESULTS

Populations of methanogens and methanotrophs in lowland soil. The most probable number of

Table 2
Populations of methanogens and methanotrophs at different growth stages of rice (PSB Rc82).*

Nutrient source	14 Days after Transplanting (DAT)	42 DAT	70 DAT	98 DAT
	Population of Methanogens (MPN g-1 dry soil)			
No fertilizer	5.83×10^3 a	1.03×10^4 a	2.77×10^4 a	7.63×10^3 a
Chemical fertilizer (120-30-60)	1.49×10^4 a	3.70×10^4 a	3.66×10^4 a	2.47×10^4 a
Rice straw (5 t/ha)	1.91×10^4 a	3.60×10^4 a	6.48×10^4 a	3.34×10^4 a
Organic fertilizer (1 t/ha)	5.29×10^3 a	5.15×10^3 a	2.59×10^4 a	1.65×10^4 a
	Population of Methanotrophs (MPN g^{-1} dry soil)			
No fertilizer	1.45×10^7 a	8.14×10^4 b	6.82×10^6 a	7.92×10^6 a
Chemical fertilizer (120-30-60)	1.32×10^7 a	3.27×10^4 b	1.38×10^7 a	9.83×10^6 a
Rice straw (5 t/ha)	1.48×10^7 a	1.13×10^5 b	2.05×10^7 a	1.76×10^7 a
Organic fertilizer (1 t/ha)	3.72×10^6 a	1.64×10^6 a	3.66×10^6 a	3.66×10^6 a

*Values within a column having the same letter are not significantly different at 5% level of significance (LSD).

methanogens and methanotrophs in lowland soil during the growing season of rice is shown in Table 2.

The populations of these microorganisms were observed to be higher in soils with chemical fertilizer and rice straw. Moreover, the highest population numbers in soils, regardless of the kinds of nutrient source, were observed at flowering stage (70 DAT). Figure 1 shows the organic matter, nitrogen, phosphorus, and potassium contents of soil during sampling periods.

ISOLATION OF METHANOGENS AND METHANOTROPHS IN LOWLAND SOIL

Nine cultures of methanogens and 18 cultures of methanotrophs were isolated and purified. Microscopic images of the methanogen and methanotroph isolates showed cells to have short rod, rod and long rod shapes, with Gram negative reaction (Figures 2 and 3).

Spore-like structures were present together with the vegetative cells of some isolates of methanotrophs. Table 3 shows the properties of isolated methanotrophs as well as the abilities to oxidize CH_4 and fix N_2. All of the isolates showed varying CH_4-utilizing abilities based on their differences in the amount of CH_4 they were able to utilize. However, only 6 of the isolates were able to produce ethylene from acetylene ranging from 21.2 to 49.5 nmol C_2H_4 in an hour per tube.

Figure 1
Changes in some soil chemical properties during sampling periods.

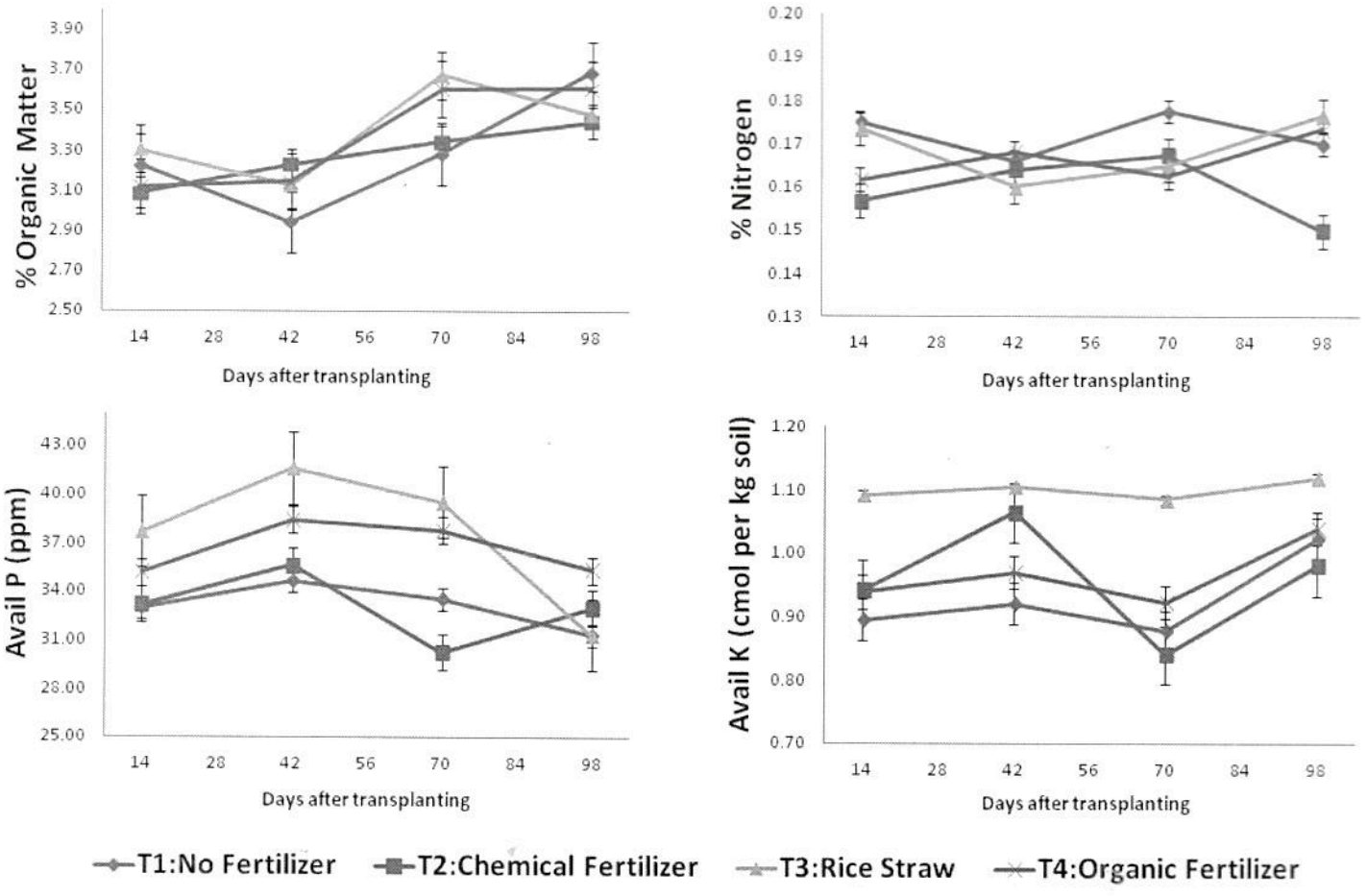

Figure 2
Microscopic observations of some methanogen isolates under oil immersion objective (100x magnification) using electric scanning microscope.

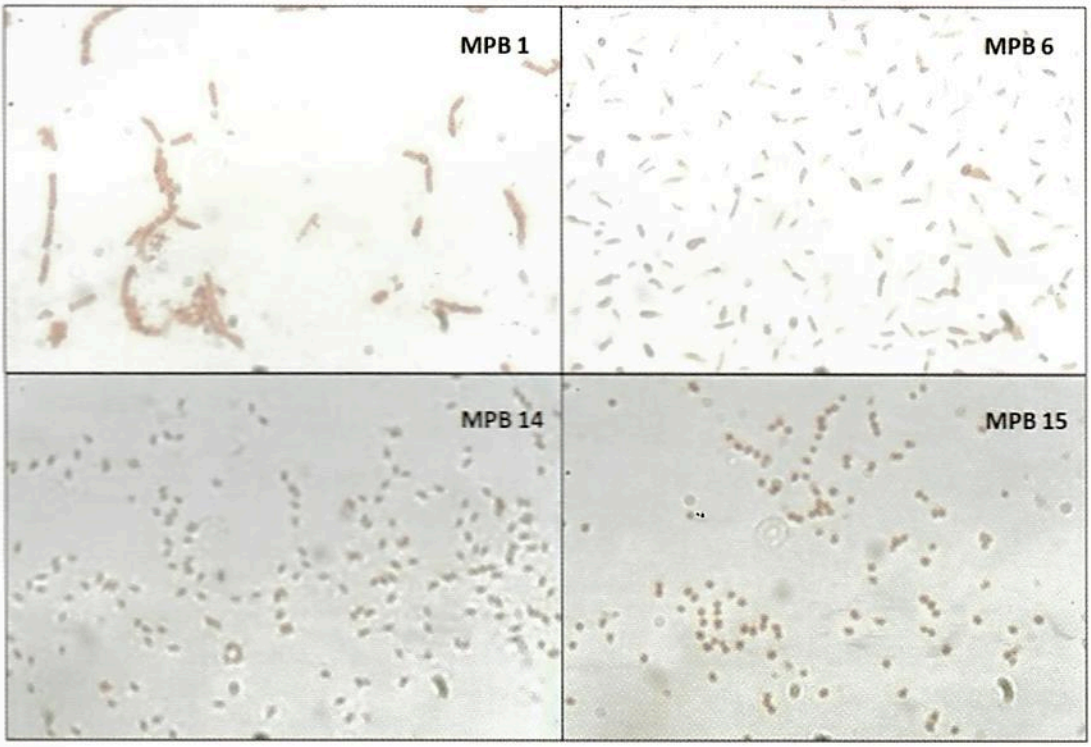

Figure 3
Microscopic observations of some methanotroph isolates from bulk soil and rhizosphere under oil immersion objective (100x magnification) using electric scanning microscope.

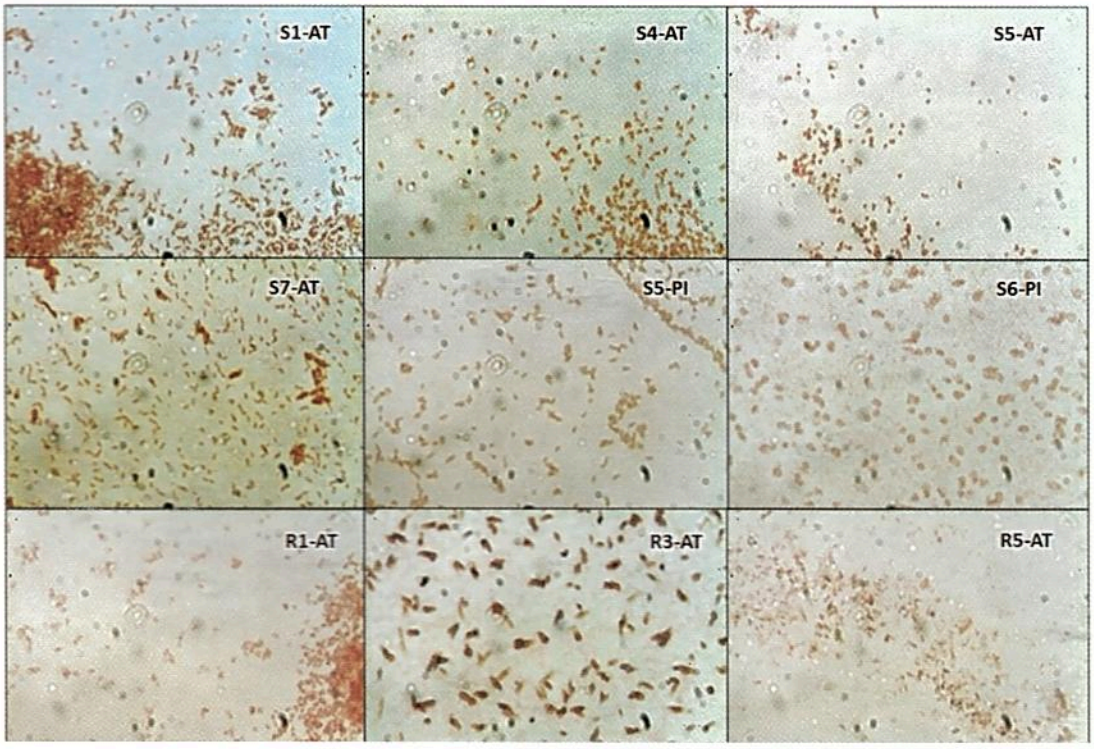

Table 3
Characteristics of isolated methanotrophs from rice rhizosphere and bulk soil with their CH_4-oxidation and N_2-fixing activities.

Isolate	Morphology*	CH_4 Oxidation after 5 Days Per Tube (%CH_4 oxidized)	N_2 Fixation: C_2H_4 Produced after 5 Days (nmol C_2H_4 in an hr per tube)
		From rhizosphere	
R1-AT	short rods, G-	20.79	21.2
R3-AT	rods, G-	23.40	0.0
R4-AT	short rods, G-	19.62	0.0
R5-AT	short rods, G-	50.20	0.0
R6-AT	short rods, G-	30.07	21.2
R8-AT	short rods, G-	3.27	0.0
R1-PI	short rods, G-	9.81	21.2
R2-PI	short rods, G-	13.60	0.0
		From soil	
S1-AT	rods, G-	13.38	0.0
S2-AT	rods, G-	81.15	0.0
S3-AT	short rods, G-	30.32	0.0
S4-AT	short rods, G-	22.89	0.0
S5-AT	coccus, G-	24.30	0.0
S6-AT	rods, G-	42.67	0.0
S3-PI	rods, G-	26.37	35.4
S4-PI	rods, G-	34.33	0.0
S5-PI	rods, G-	9.81	49.5
S6-PI	rods, G-	39.16	42.4

*Gram stain: (G-) Gram negative

DISCUSSIONS

Population and activities of methanogens and methanotrophs are influenced by carbon and nutrient sources. Between the use of rice straw (44.9 C/N ratio) and organic fertilizer (12.1 C/N ratio), results showed that population growths of methanogens and methanotrophs in lowland soils are stimulated by higher C/N ratio. Higher organic matter, phosphorus, and potassium, generally observed in soils with rice straws, may have also influenced the growth of these microorganisms. However, the effect of chemical fertilizer on methanogens and methanotrophs in this study was unclear. Based on levels of CH_4 produced from the treated soils in the study conducted by Zheng et al. (2007), it was inferred that there were significant differences in methanogen and methanotroph communities between anaerobic soils with chemical fertilizer alone, without fertilizer and with combined chemical fertilizer and manure. However, different kinds of fertilizers, particularly N-fertilizers, were observed by other workers to have different stimulation and inhibition effects on the growth and activities of methanogens and methanotrophs. On the other hand, higher numbers observed in treatments during the flowering stage of rice, could be attributed to the amount of organic matter content of the soils. According to Dubey (2005), root exudation can result to up to 50% CH_4 emission during this growth stage of rice. Future studies on the mechanisms and timing of application of different nutrient sources that influence the metabolisms of the microorganisms are recommended.

Methanogen and methanotroph species were reported to have diverse structures and have no unique features by which all species can be characterized. Cell morphologies of these microorganisms were reported to be rods, cocci to spiral and sarcina. All methanogen species isolated and characterized in the past were reported to have Gram-positive-type of cell envelop structure, yet many species were found to be Gram-negative or Gram-variable (Zeikus, 1977). For methanotrophs, many species may produce spore-like structures together with the vegetative cells of some isolates, which were observed from the isolates. The same was reported by

Dianou et al. (1997) in their study using a subtropical paddy field. Exospore- or cyst-forming abilities of methanotroph may be used in the identification of methanotroph species as members of taxonomic categories as Type I, Type II, or Type X.

Many members of Archaea and Bacteria have the ability to fix atmospheric nitrogen. Nitrogen fixation is an important phenotypic trait in most currently known methanotrophic bacteria. Most N_2-fixing types belong to Type II methanotrophs while Type I includes only some N_2-fixing species. Nitrogen fixation was said to be one cause for the dominance of Type II species in rice paddies (Kravchenco and Yu, 2006). However, it was observed in this study that isolates have different abilities in acetylene reduction in terms of amounts on ethylene produced. The N_2-fixing ability is influenced by microorganisms' differences in the structural organization of the *nif* genes, which encode for the components of the nitrogenase enzyme that catalyzes N_2- fixation (Dedysh et al., 2004). Moreover, because the nitrogenase is sensitive to levels of oxygen, the activity of the enzyme in response to the oxygen present during the incubation period may have affected the acetylene reduction by each isolate. This study was limited only to the detection of N_2-fixing ability of isolated methanotrophs. Future studies, related to this trait of methanotrophs, should include genetic analyses for complete genetic characterization and quantification of N_2-fixation.

Methanotroph isolates with N_2-fixing ability were identified to be significantly related to *Burkholderia* and *Vibrio* species. The relationships of the isolated methanotrophs with *Burkholderia* and *Vibrio* were cited by some authors based on the classifications of bacteria. Chistoserdova et al. (2009) reported that some methylotrophs, like *Methylibium petroleiphilum*, have been described to belong to Order *Burkholderiales,* which belongs to phylum Proteobacteria. On the other hand, *Vibrio* is not necessarily a methanotroph, but has been cited as an example of organisms classified as methylotrophs. Stocks and McClesky (1964) identified *Vibrio extroquens* as a pink-pigmented methanol-oxidizing bacterium. Methanotrophs were classified under methylotrophs belonging to a major phylum of the Bacteria domain called Proteobacteria. Proteobacteria constitute the majority of known bacteria of medical, industrial, and agricultural significance. On the other hand, methylotrophs are bacteria that utilize one-carbon organic compounds as electron donor and carbon source (Todar, 2011).

CONCLUSIONS AND RECOMMENDATIONS

Studies on these microorganisms are limited in tropical areas, such as in the Philippines, probably because of the difficulty of isolation and enrichment of the microorganisms. However, it is important to better understand this biological aspect of methane fluxes in rice soils to be able to develop more potential mitigation practices. Moreover, the detection of nitrogen-fixing ability of some isolated methanotrophs from a tropical lowland soil could also be a potential nutrient management options for rice farmers. Thus, further studies on enrichment and activities of methanogens and methanotrophs are recommended. Future researches on the methane oxidation and nitrogen-fixation activities of methanotrophs can lead to the development of a new biofertilizer that fixes atmospheric nitrogen for rice while mitigating methane emissions from the lowland soil.

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VERMICOMPOSTING:
A REVIEW OF ITS POTENTIAL USE IN RICE-BASED FARMING SYSTEM

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ABSTRACT
At the advent of organic agriculture, many approaches had been introduced to interested farmers. One of them is the use of vermicompost. The practice of vermicomposting became popular for the disposal of different biodegradable wastes such as kitchen wastes, farm wastes, weeds, municipal solid wastes, and sludge. They are primarily substrates or food for earthworms which are the agents of composting. The process, as compared to conventional composting, is faster, and gives a better end-product due to enzymatic and microbial activity during vermicomposting. The products of vermicomposting are vermicompost, vermiwash, vermitea, and the earthworm. Based on the nutrient composition of the vermicompost presented by several studies, different substrates fed to the earthworms produced different nutritional composition. Likewise, its application into the soils showed increasing soil mineral elements while some decreases. The use of vermicompost in the long term showed increasing concentration of both heavy and light metals, which may cause nutrient imbalances and may impair normal growth and yield potential of crops. As vermicompost application had improved growth and yield of crops, higher production efficiency and quality of vermicomposts, and its economic impact should be an interesting focus of future research and development agenda.

KEYWORDS
Vermicomposting, vermicompost, sustainable farming system, farm waste management

INTRODUCTION

With solid waste generation in world cities as high as 1.3 billion tons per year, a large portion of which is organic (Hoornweg and Bhada-Tata, 2012), a sustainable waste management is a necessity for the society. Plant and animal materials, such as plant stalks, vegetable peelings, leaves, and litters or yard waste, naturally decompose into garden soil through a process called composting. In the process, these materials break down into humus by the naturally occurring soil microbes. Vermicomposting is another method of composting using red worms (*Eisenia foetida)* or red wigglers *(Lumbricus rubellus).* It is also tested for disposal and potential recycling of otherwise environmentally hazardous wastes (Hoornweg and Bhada-Tata, 2012). Such role in waste management has been widely studied across Asia. Aside from the safe disposal and stabilization of these wastes, the process also enhances the properties of the original waste materials (Munnoli, 2010), in a sense performing an alchemy transforming them into gold (Nagavallema, 2004). Its product, vermicompost, has been studied by many authors for its potential as fertilizer and as soil conditioners (Adhikary, 2012; Sinha et al., 2010a). These positive impacts to the environment make vermicomposting a potent part of a sustainable system, as a turning point in a materials' cycle where it is put back to being an input, rather than an output. Later, it was explored to control pests in crops and as mulch to suppress weeds and to maintain soil moisture.

The benefits of vermicomposting demonstrate its capacity to support farming systems in the Philippines. On the context of integrating vermicomposting in crop production and animal industry, there poses some questions of its sustainability in the system, its practicality, doability, repeatability, and affordability in smallscale and in large-scale operations. Questions on the quality of vermicompost and vermicast and its actual contribution to each and every production management component within the farming system were likewise raised. Hence, this paper presents a review on basic information, results of studies or experimentation, and actual practices or experiences with vermiculture and vermicomposting that can serve as basis for decision making of the end-users and stakeholders, farmers, environmentalists, and agriculturists as far as the potential use of vermicomposting toward sustainable rice-based farming system is concerned.

THE EARTHWORMS: AGENT OF VERMICOMPOSTING

The earthworms are major components in the soil fauna and definitely support other organisms necessary for maintaining soil health (NIIR Board, 2004). They are involved in various soil processes either directly through weathering by their burrowing activities (Munnoli et al., 2010), or indirectly by fragmenting and conditioning of substrates for biodegradation by microorganisms (Dominguez, 2004). They are known to improve the soil they inhabit by modifying the soil physical properties including its aeration, porosity (NIIR Board, 2004; Jayakumar, 2011), drainage, bulk density, and structural stability (McColl et al., 1982), and water-holding capacity (Jayakumar, 2011). Due to their eating habits in which they practically eat their way through the soil (anecics and endogeics) with some organic materials, Aristotle called them the 'Intestines of the earth' (Garg, 2010), while Lavelle (1996) called them "ecological engineers" of the soil. They are the organisms positioned at the beginning of a station in the nature's cycle that brings consumed materials back to a form to be used again; from being an output (waste) to an input (fertilizer).

Earthworms are invertebrates belonging to the Phylum Annelida, Class Chaetopoda, and Order Oligochaeta (Kale, 2010). There are more than 4200 species under Oligochaeta, about 3200 of which belongs to megadrili or are earthworms (Munnoli, 2010). Munnoli (2010) further listed 21 species belonging to Lumbricidae, Eudrilidae, and Megascolecidae, which are used for vermicomposting.

The most studied species for the purpose of vermiculture and vermicomposting, however, are *Eisenia foetida, E. andrei, Perionyx excavatus, P. ceylanensis, P. sansibaricus, Eudrilus euginae, Lampito mauritii, Lumbricus terrestris, L. rubellus, and Drawida willsi.* They are classified by Bouche (1977) into three groups: epigeics, anecics, and endogeics.

Epigeics are generally pigmented, phytophagous (they eat organic residues), and are surface dwellers that live above the mineral soil layer. Nagavallema (2004) described them as non-burrowing type that eats 10% soil and 90% organic materials. They convert organic wastes into vermicompost faster than burrowing types and live for maximum of 28 months. They are small in size with rapid reproduction and maturation (Bouche, 1977). *Eudrilus euginae, Eisenia foetida,* and *Perionyx excavatus* are epigeic earthworms.

Anecics are geophytophagous (they eat soil and organic matter) that live in mineral soil layers, and they come to the surface to feed on organic wastes at night (Gajalakshmi and Abbasi, 2004). They drag organic matter from the surface through the vertical tunnels they create (Gajalakshmi and Abbasi, 2004) in the process modifying the soil structure and mixing organic matter deeper into the soil layers (Aalok, 2008). They also create a drilosphere aside from compost production. Bouche (1977) defined drilosphere as a 2 mm-zone around earthworm burrows, which was found by Andriuzzi (2013) to be richer in residue-derived nitrogen and carbon with

natural *Lumbricus terrestris* as the burrow earthworm. *Lampito mauritii, Lumbricus terrestris, L. rubellus,* and *Drawida willsi* are anecic earthworms.

Endogeics are geophagous (they eat soil enriched with organic matter) having little or no pigmentation (Gajalakshmi and Abbasi, 2004) that construct horizontal branching burrows (Abbasi, et al., 2009). They dwell in deeper mineral soil layers in burrows that are eventually abandoned (Lee and Smettem, 1994).

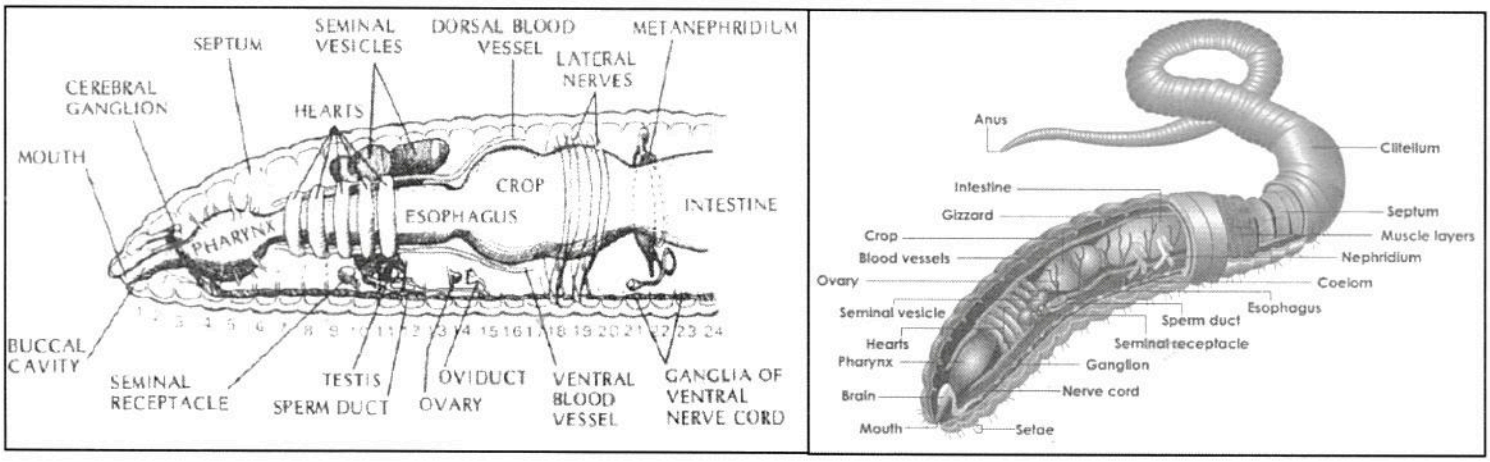
Earthworms are terrestrial organisms, but some species are also semi aquatic (Darwin, 1892). They thrive in most parts of the world except in areas with extreme drought and constant snow and ice (Edwards, 2004). Earthworm communities are dominated by different families in temperate and tropical regions. However, there are species that are extremely widely distributed. They are termed peregrine (Edwards, 2004).

These organisms are long, thread-like cylindrical animals with lengths from a few millimeters to 2 m (Edwards, 2004) with a prostomium (mouth) in anterior end and anus on the posterior end. Without the clitellum, the earthworm's reproductive organ, only movement can tell which is the anterior end (Gajalakshmi and Abbasi, 2004). They are segmented organisms that do not possess eyes; they are deaf but are sensitive to vibrations, heat/cold, and light (Darwin, 1892). Edwards and Bohlen (1996) described them as 'bilaterally symmetrical, externally segmented with setae on all segments' and provided a detailed description of earthworm body. Earthworms also have no skeleton. Their movements are facilitated by the setae (projections for movement) on the segments of their body. They are soft-bodied with large cavities called 'coelom' containing the coelomic fluids, which have phagocytic amoebocytes, vacuolar lymphocytes, and mucocytes. Their body wall consists of cuticle and is perforated with very small pores through which fine hairs protrude for sensory purposes. They also breathe (from 'breathing cells' buried in their body wall) and keep their body moisture with release of coelomic fluid through this body wall. The coelomic fluids contain nitrogenous metabolic wastes that are removed by the nephridia of the earthworm's excretory system.

Earthworms are hermaphrodites; they have both male and female gonads, but they still need to mate to reproduce. When mature, a distinct band called the clitellum, serves as the reproductive organ of the earthworm. The clitellum contains the eggs that are released as cocoons after being fertilized by 'sperm' from another worm. The same happens for the mating partner. Generally, cocoon production starts at 6 weeks and continues up to 6 months (Sharma et al., 2005). Garg (2010) described the life cycles of *Eisenia foetida, Eudrilus euginae,* and *Perionyx excavatus* as having maturity of 40-60, 40, and 28 days, respectively. Further, the respective incubation periods for the cocoons are 23, 16, and 18 days.

As described by NIIR Board (2004), the gut is located in the center of the tube that is the earthworm body; it is basically a straight tube from the mouth to the anus with muscular pharynx, oesophagus, intestine, and associated digestive glands. One or more gizzards, depending on species (some have as many as 10), may be found in the oesophagus region. It contains mucus, and organic and mineral matter. It also contains symbiont-like microfungi, bacteria, and protozoa, most of which are found in the foregut in a gradual decrease in the mid- and hind-gut. The alimentary canal of the earthworm gut also contains the digestive enzymes amylase, cellulose, protease, lipase, and chitinase. Figure 1 shows (a), the inside of a worm, and (b), detailed digestive parts.

Figure 1
Anatomy of the earthworm gut (from http://zannr.hubpages.com/ *and* http://crescentok.com/*).*



VERMICULTURE

"Vermi" means worm and "culture" means to cultivate or grow (Merriam-Webster Online). Vermiculture means to grow earthworms, in contrast with vermicomposting, which means composting with worms. These two terms, although essentially the same in process, differ in purpose and product. Vermiculture produces the worm while vermicast is made through vermicomposting. The worm is the agent of composting.

The mostly cultured vermi are the epigeic earthworms or commonly known as red wigglers that are naturally adopted to ingest organic wastes. They can consume 75% of their body weight per day. As described by Ontario Ministry of Agriculture, Food and Rural Affair, the ideal feed for earthworms is food or animal waste, and fresh, green, plant waste, rich in nitrogen or pre-composted for up to 2 weeks to make it easier to digest. Ideally, the feed is porous, warm 25°C, moist (75%) but not wet, not too dense (640 kg/m^2), not salty (below 0.5% salinity), and devoid of toxins, detergents, cleansers, and pesticide.

Worms can digest their food more efficiently if the waste materials have a moisture content of 75% (field capacity), and not more than 85%. Elevated rearing bins with drainage or perforation where excess water or moisture will drop out and correct the moisture level of the materials are ideal. Alternatively, raised screen surfaces (4 mm) at the bottom of the container and/or reducing the thickness of the waste to less than 30 cm will keep the pile aerated and cool, which the earthworm prefer.

Although earthworms are surface dwellers, they prefer to burrow and stay at 15 cm depth. They have light sensitive skin but not to blue light; keeping them at the underground during the daylight. However, if light is shining above the rearing bin, they will stay below the surface layer.

Vermiculture gives two products: worms and vermicomposts or vermicast, which can be produced at the same time provided that optimum conditions for the growth and multiplication of worms are met.

VERMICOMPOSTING

Vermiculture and vermicasting became synonymous to vermicomposting and cannot be separated from each other as far as the process is concerned. Hence, authors used the two words interchangeably. Either way, they refer to the process by which different organic or biodegradable wastes are converted while passing through the worm-gut (Adhikary, 2012), which acts as the waste disposal and recycling facility. Moreover, Abbasi (2009) defined vermiculture (or vermicomposting) as the "only pollution control bioprocess which has a multicellular animal as the main bioagent" compared to other processes that use bacteria, fungi, and algae. The different biodegradable wastes that had been studied were kitchen wastes (Adhikary, 2012; Nair et al., 2006), farm (plant and animal) wastes (Munnoli, 2010; Adhikary, 2012; Yi-Wei, et al., 2012; Jayakumar, et al, 2011) including weeds such as water hyacinth (Gajalakshmi et al., 2001) and water lettuce (Sannigrahi, 2009), municipal solid wastes (Mishra, 2005), industrial wastes such as sludge from paper mill (Elvira, 1998), and human feces (Yadav et al., 2010). These wastes serve as the substrates or food for the earthworms.

Compared to conventional composting, vermicomposting does not involve a thermophilic phase (Edwards and Burrows, 1988); hence, the process was found to have lower heat generation (Nagavallema, 2004). This is because precomposting of biodegradable wastes involving a thermophilic phase is necessary before the earthworms are introduced. This is also done to avoid killing the earthworms and to reduce pathogen prior to vermiculture or vermicomposting.

Moreover, it is also preferred over traditional composting because it is faster (Adhikary, 2012) and gives a better end-product due to the enzymatic and microbial activity during the process (Nair et al., 2006). With vermicomposting conducted in temperatures ranging from 20-42 °C, 75% degradation of kitchen wastes was achieved in an average of 17.5 days compared to composting, which reached the same degradation state in the average of 91.16 days (Sinha et al., 2002). Similarly, Chaudhuri (2000) observed faster reduction in C-content with worms (60% reduction during the last 20 days of vermicomposting) than without worms (36% reduction within the same time), which indicated faster decomposition of organic matter. While both have similar levels of pH, organic C, total N, C:N ratio, P, K, Ca, and Mg from start of vermicomposting to 10 days (Table 1), the values gradually decreased. Vermicompost compared to ordinary compost had a markedly lower C:N ratio and nearer-to-neutral pH at 40 days. Also, all other parameters presented were lower for vermicompost at 40 days. Chaudhuri concluded that the 30-day age of vermicompost is better than 40 days due to a more suitable C:N ratio for field applications of vermicompost and a better pool of nutrients is available.

Chaudhuri (2000) further attributed the downward trend of values of vermicompost to incorporation into earthworm tissue and leaching into the soil bed beneath the vermicompost pile. The soil bed under the vermicompost pile have higher values for organic C, total N, P, K, Ca, and Mg and lower pH and C:N ratio compared to soil beds under the compost without earthworms (Table 2).

Table 1
Physicochemical analysis of composted kitchen waste with and without earthworms at different time intervals; values are mean (n=3).

Parameters	Initial	Without worms (Control) days				With worms (Experimental) days			
	(0 day)	10	20	30	40	10	20	30	40
pH	10.0	9.85	9.24	8.67	8.27	9.54	8.97	8.55	7.59
Organic C (%)	36.8	31.1	22.8	18.7	15.2	31.50	25.66	18.74	10.48
Total N (%)	3.49	3.14	2.33	2.00	1.78	3.02	2.57	2.28	1.67
C:N	10.55	9.94	9.78	9.39	8.53	10.43	9.98	8.23	6.41
Total P (%)	0.89	1.07	1.10	1.07	1.38	1.12	0.99	1.46	1.09
Total K (%)	2.18	2.27	1.85	1.52	1.22	2.02	1.46	1.16	0.85
Total Ca (%)	4.73	5.57	5.06	4.52	4.30	5.41	4.97	4.65	2.83
Total Mg (%)	0.54	0.68	0.59	0.59	0.61	0.60	0.52	0.50	0.40

Chaudhuri, 2000.

Other enzymatic and microbial activity as influenced by vermicomposting has been documented by Aira (2007) in which microbial biomass carbon, β-glucosidase, cellulase, and alkaline phosphatase activity increased, and protease activity decreased in vermicomposting of pig slurry compared to simple composting process. However, β-glucosidase, urease, protease, phosphatase, and dehydrogenase activities decreased over time (Benitez et al., 1999) in vermicomposting of sewage sludge.

Moreover, these activities by the organisms involved affect the nutrient availability in the resulting vermicast. This can be supported by the observed nitrogen transformations during vermicomposting as influenced mainly by the earthworm. Chaudhuri (2000) observed reduction of total nitrogen in the substrate over time, but still noted a higher N content in vermicompost than compost, both with 30 days of age. Earthworms apparently had enhanced nitrogen mineralization and

Table 2
Physicochemical analysis of soil bed (initially and after 40 days) with and without earthworms during kitchen-waste composting.

Parameters	Initial	After 40 days	
		Without worms	With worms
pH	5.4	8.1	7.6
Organic C	1.69	3.21	3.37
Total N	0.14	0.28	0.31
Av. P2	1.83	36.7	58.6
Av. K2	6.0	102.0	89.0
Av. Ca2	167.7	236.6	297.5
Av. Mg2	22.5	38.8	42.0
C:N	12.0	11.4	10.8

Expressed as 1%, 2 mg 100 g-1 soil. (Chaudhuri, 2000)

increased the rates of conversion of NH4-N to NO3-N (Atiyeh, et al., 2000b; Dominguez, 2004). Increase in the proliferation of phosphate-solubilizing microorganism, which caused higher phosphates activity was also observed due to vermicomposting (Saha et al., 2010).

FACTORS AFFECTING VERMICOMPOSTING

Vermicomposting is an aerobic biological process that depends on earthworms to decompose the waste materials (Garg, 2010). It is a process requiring the following conditions:

1. **Raw Materials or Substrates.** As previously mentioned, a wide range of wastes can be used as raw materials. These materials can come from within the farming system (wastes from crops, livestock, household), community, and industry (pressmud). In general, substrates with high nitrogen content are most suitable for vermicomposting (Munnoli, 2010), or a low C:N ratio has to be attained for efficiency. This is where pre-composting becomes necessary. Pre-composting helps in mass reduction, moisture management, and pathogen reduction (Nair et al., 2006). The C:N ratio is expected to decrease over time as carbon degrades (Nair, 2006). Pre-composting of substrate is necessary to allow first the stabilization of certain characteristics (moisture, pH) of rapidly biodegradable wastes that generate volatile fatty acids, which could stress or kill the worms (Abbasi, 2009; Nair, 2006). Likewise, pre-composting of biodegradable materials involved thermophilic phase (Edwards and Burrows, 1988) and in the process, lowers heat generation (Nagavallerma, 2004); making the actual vermicomposting process a non-thermophilic phase.

   A C:N ratio of 25 (Ndegwa, 2000b) or a C:N ratio of 20 is considered suitable and acceptable for vermicompost (Morais and Queda, 2003). Food wastes containing too much oil may not be suitable substrate as it can create anaerobic conditions in the pile and so with pre-composting of other fresh organic wastes (Garg, 2010). The nutrient contents, microbial population, and enzymes in the resulting vermicasts differ for each substrate (Table 1 & 2).

   However, there are wastes that are not suitable for vermicompost. These include eggshells, meat, bones, chicken droppings and *Gliricidia* ("kakawate") loppings, tobacco leaves, onion, garlic, and chili wastes (Nagavallema, 2004). Earthworm population has been reduced by *Gliricidia* and tobacco leaves, which are known to contain toxic compounds (Nagavallema, 2004). Although *Gliricidia* is used in vermicomposting because of its high nitrogen composition, it is not recommended for favorable earthworm rearing (Nagavallema, 2004). Meat, on the other hand, may putrefy and produce noxious odor on the vermicompost pit. The raw materials used will determine the qualities of the resulting vermicast, a topic further discussed under *Vermicast*.

2. Moisture. Earthworms do not thrive in dry environments (Gajalakshmi and Abbasi, 2004). This is demonstrated by Biradar et al. (2000) who reported that rainy season is more suitable for vermicomposting and earthworm multiplication than the summer season, which has higher temperatures and lower relative humidity. Moreover, excess water leading to anaerobic conditions (Garg, 2010) kills the worm. Moisture content for optimum growth and reproduction of earthworms is 50-90% (Dominguez, 2004) and should therefore be maintained by regular watering during the process.

3. pH. Gajalakshmi and Abbasi (2004) reported that earthworms prefer neutral pH. Singh et al. (2005) also reported that near neutral initial pH is optimal for vermicomposting. Due to chemical reactions within the pile, pH tends to go down over time. Substrates that are initially acidic or with low pH are therefore not suitable for vermicomposting.

4. Temperature. Earthworms can tolerate temperatures ranging from 0-40 °C (Nagavallema, 2004) but are most active at 10-32 °C (temperature within the pile), according to Adhikary (2012). The best growth and temperature can be observed at 20-25 °C (Loehr, 1985); a reduction was observed at 30 °C and worms died at 35 °C. The earthworm Eudrilus euginae were found to be very sensitive and intolerant to low temperatures, below 16 °C. More earthworms were also observed to mature at 22 °C (Viljoen and Reinecke, 1992). Apparently, this may be interrelated with maintaining the right moisture during the process.

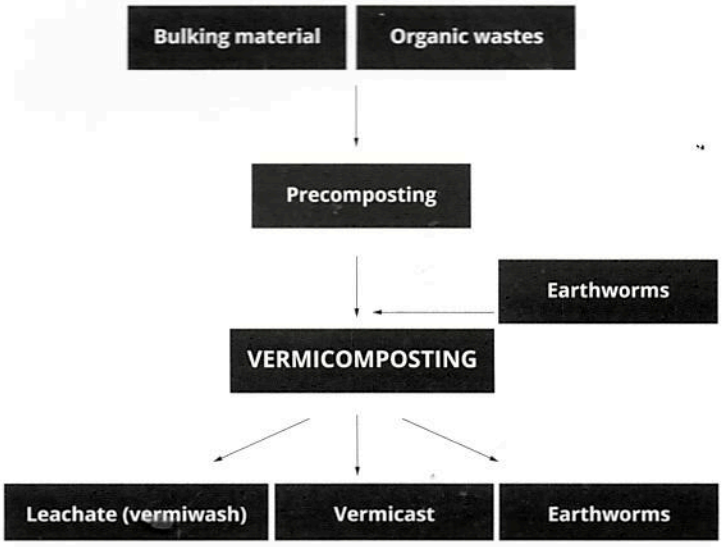
5. **Aeration**. Vermicomposting is an aerobic process. Lack of oxygen or anaerobic condition can lead to high earthworm mortality (Abbasi, 2009; Yadav, 2010). Though the earthworms are the largest aerators of the vermicompost pit through their movements within the pile, certain conditions can render it anaerobic. Too much oil in the substrate, dense beddings, and with too much water, the substrate become waterlogged (Garg, 2010) and can create anaerobic conditions. Ideally, a screen-type floor surface, with screen size of 4 mm allows drainage while keeping the substrate intact in the composting pit or bin.

METHODS OF VERMICOMPOSTING

Generally, the process of vermicomposting involves mixing and precomposting of substrate and bulking material used, introduction of earthworms (or transfer of substrate mixture to vermicompost bed), then finally, harvesting of the product. The earthworms are introduced at the rate of 1.60 kg worm/m² and fed with 0.75 kg feed/kg worm/day (Ndegwa, 2000a). A general representation is shown in Figure 2.

Figure 2

Diagram showing the general process of vermicomposting and its by-products.



Methods of vermicomposting are based on purpose and environment. The first three methods are described by Munnoli (2010) and the last method, which can be used in commercial scale vermicomposting is described by Nagavallema (2004).

- **Pit method.** Pits of different dimensions can be made below the ground level. But this method is not suitable for areas with rain intensity of 3000 mm per year. The wastes with the earthworms are placed in a pit and let to decompose. The entry of predators and exit of earthworms through this method is difficult to control.

- **Rectangular tank.** This is constructed above ground level under a full roof against heavy rains. If constructed with thick strong walls and water channel is provided around the tank, it can be permanent and sustainable for farmers.

- **Heap method.** This can be done outdoors or indoors, but is dependent on the available space. Polyethylene sheet is spread on the ground and covered with the substrate. Then, the earthworms are introduced.

- **Four-component tank.** The model was developed by ICRISAT (Nagavallema, 2004). The structure is made up of materials like hollow blocks and consists of four chambers separated by partition walls with holes to facilitate the transfer of earthworms from one chamber to another. The chambers are filled with the substrates one after another. Once the contents of the first chamber are processed, the earthworms will move to the second chamber, then to the third chamber, and so on. This also facilitates harvesting of compost from the first chambers already vacated by earthworms. The chambers of this method, however, are permanently constructed for commercial or bigger scale vermicast production.

In detail, Nagavallema (2004) described the steps of the four-component-tank as follows:

- Cover the bottom of the tank with a layer of tiles, coconut husk or polyethylene sheet.
- Spread 15-20 cm layer of waste material, then spread bulking material like cow dung. Fill the tank completely with layers as described, with cow dung on top. Allow the material to decompose for 15-20 days.
- When the temperature declines, introduce the earthworms. Occasionally sprinkle water to keep the material moist.
- Cover the tank with wire mesh or gunny bag to prevent predators.
- The vermicompost pit/tank or heap must be kept in shade to keep the temperature of the pile.
- After 45 days following earthworm introduction, the vermicast can be harvested.

THE PRODUCTS OF VERMICOMPOSTING: VERMICAST, VERMIWASH, AND VERMITEA

Vermicast

Vermicast is the casting of worms in its pure form, produced by the action of microbiological life in the digestive system of the worm. In short, vermicast is the resulting product of vermicomposting and alternatively called vermicompost. But vermicompost is a mixture of vermicast and unprocessed organic matter, which may also contain worm capsules and small worms.

Moradi (2014) described vermicast as "finely divided mature peat-like materials with a high porosity, aeration, drainage and water-holding capacity."

Vermicast is mostly humus (Adhikary, 2012) and contains high levels of humic acids (Atiyeh et al., 2002; Elvira et al., 1998) which protects plants under salt stress conditions. Indirectly, vermicast-derived humic acids are reported to relieve or protect rice from water stress by affecting the plant's metabolic reactions to stress (Garcia, 2013) and affecting its hormonal regulation and root growth pathways (Garcia et al., 2013). Likewise, vermicast-derived humic acids in increasing concentrations also improve the growth of tomato and cucumber (Atiyeh et al., 2002).

Vermicast is homogenous with reduced levels of organic contaminants while retaining most of the original nutrients of the substrates (Tajbakhsh et al., 2010). It was reported to be rich in NPK, micronutrients and growth hormones such as auxins, gibberellins, and cytokinins (Dynes, 2003). The nutrients contained in vermicast are reported to be in forms readily available to plants (Hasanuzzaman et al., 2010; Moradi et al., 2014; Sinha et al., 2013). While the nutrients are encased in mucus membranes secreted by the earthworm, they dissolve slowly; preventing immediate nutrient leaching and resulting to slow-release fertilizer (Adhikary, 2012). Sekar (2010) reported that vermicast can also be used as carrier material for biofertilizers *Azotobacter chroococcum, Bacillus megaterium,* and *Rhizobium leguminosarum* with higher survival rates in vermicast than other carriers.

They also have a healthy collection of microorganisms like micorrhizal fungi, actinomycetes, and nitrogen-fixing and "phosphate solubilizing" bacteria (Sinha, 2013; Adhikary, 2012), as well as enzymes like amylase, lipase, cellulose, and chitinase (Adhikary, 2012). Likewise, Yadav (2010) reported that coliforms were completely inactivated in the vermicasts produced in his study. Although there are unpublished reports that some vermicasts contain *E. coli* (Ang-Lopez, personal communication, 2014), this has to be reviewed if such situation occurs under Philippine condition.

As mentioned above, vermicomposting can be done with a wide variety of substrates and the resulting vermicast from the different sources also have different chemical properties. Summarized in Table 3 are the N,P,K content of vermicasts produced from substrates used by researchers (perhaps using different procedures) in different years and countries.

Tomato fruit wastes (Fernandez-Gomez, 2010), goat manure (Loh, 2005), coffee pulp (Orozco, 1996), water lettuce or *Pistia stratiotes* (Sannigrahi, 2009), sugar mill effluent (Cynthia, 2012), oyster shell (Kwon, 2009), and biosolids (Ndegwa, 2000b) are not included in the substrates list.

Futher, Perialde (2012) of PhilRice Midsayap, characterized the N, P, and K content of vermicast produced from different combinations of wastes with rice straw and duck dung as constant ingredient. The other wastes mixed were kitchen wastes, scrap paper, half-filled rice, grass clippings, carbonized rice hull, and water lily leaves. The ratio of the substrate was one part rice straw, one part other waste and two parts duck dung. Her results are shown in Table 4.

Cattle manure is commonly used as bulking material. It is commonly mixed with the main substrate to enhance the microbial diversity of the feed material and to make it, in the case of human feces and other wastes, acceptable to the earthworm (Yadav, 2010). In the case of PhilRice MRF (material recovery facility), carabao dung from the Philippine Carabao Center (PCC) is used instead of cattle manure.

AGRICULTURAL IMPORTANCE: CROP PRODUCTION MANAGEMENT

Effects on growth and yield of rice and other crops

As fertilizer, vermicast has been proven to improve the performance of several crops. When used to provide 50% of N requirement of rice crop, vermicast improved the grain yield by 12.2% in a rice legume cropping system (Jeyabal and Kuppuswamy, 2001). Similarly, the legume yield increased by 19.9%. Hasanuzzaman (2010) reported that the same level of grain yield from 80 kg inorganic N/ha can be produced with only 40 kg inorganic N/ha supplemented with 8 t/ha vermicast. Moreover, Jayakumar (2011) noted the increase in growth and yield of rice applied with vermicast from turkey litter at the rate of 40kg vermicast plus farmers' practice for a 12 m^2 plot (or 3.3 t/ha). It also increased "organic carbon," available NPK and microbial population. The treatments were applied after transplanting.

In another study by Mishra (2005), where municipal solid waste was used as substrate for vermicomposting, the resulting vermicast increased seed germination, chlorophyll contents, and rice yield. However, heavy metals (Cr, Ni, Cu, Zn, and Pb) levels also increased in soil and plant parts. In Zamboanga, the application of 50% (1 t/ha) vermicast plus 50% of the recommended NPK rate (30-7-7 kg NPK/ha) showed significant yield (3.3 t/ha in the DS; 4.66 t/ha in wet season) and return of investment or ROI (31.8% in dry season; 103.8% in wet season) similar to the application of full rate of inorganic NPK fertilizer both in the dry (3.8 to/ha and 71% ROI) and wet season (5.04 t/ha and 119.6% ROI) (Pontillas, et al., 2009). This shows the possible importance of

Table 3
Nitrogen, phosphorus and potassium content of vermicasts from different substrates fed to earthworms for composting farm wastes, domestic wastes and industrial wastes.

Substrate	Nitrogen, %	Phosphorus, % P_2O_5	Potassium, % K_2O	References
Farm wastes				
Cattle manure [a,d]	2.83	0.03	1.17	Lazcano, 2008; Karmakar, 2012
Pig manure	2.36	10.31	0.48	Atiyeh, 2001
Spent mushroom substrate (rice straw in origin)	1.01	0.32	0.22	Abbiramy, 2012
Corn pulp with cow dung	4.19	2.64	7.45	Manyuchi, 2012
Water hyacinth	1.34	4.79	1.22	Karmakar, 2012
Rice straw	1.04	4.38	1.08	Karmakar, 2012
Coconut coir	0.92	3.99	0.82	Karmakar, 2012
Sheep Manure with tomato fruit waste	1.29	0.47	2.73	Fernandez-Gomez, 2010
Turkey litter	1.66	4.06	1.87	Jayakumar, 2011
Industrial wastes				
Sewage sludge	1.31	0.69	1.54	Azizi, 2012
Pressmud [b]	3.79	4.45	2.11	Parthasarathi, 2000
Winery wastes *	2.24	2.10	1.18	Nogales, 2005
Domestic Wastes				
Municipal solid waste	0.94	1.56	0.90	John Paul, 2011
Food waste	1.05	0.66	0.23	Manyuchi, 2013
Kitchen waste	1.67	2.50	1.02	Chaudhuri, 2000
Human feces [c]	2.8	5.39	7.83	Yadav, 2010
PhilRice MRF Vermicompost[e]	0.76	0.07	0.11	Analyzed at ASPPD Laboratory

* Laboratory testing scale only

[a] commonly used as *bulking material* - material which is mixed with the substrate to enhance microbial diversity of feed material (Yadav, 2010).

[b] values obtained from 60 days vermicomposting harvest at 33-35% moisture

[c] values originally expressed in mg/g by Yadav (2010)

[d] values originally expressed in mg/kg by Lazcano (2008)

[e] vermicompost produced at PhilRice MRF (rice straw from experimental station and mixed with carabao dung from PCC)[e] mixed with other materials and wastes; analyzed by KMRuiz at ASPPD Laboratory (Sample received: December 4, 2013).

Table 4
Nitrogen, phosphorus and potassium content of vermicast from different substrate combination by Perialde (2012) (submitted to DA as unpublished report).

Description	Total N, %	P_2O_5 %	K_2O %
Rice straw + kitchen wastes + duck dung	0.60	2.85	0.19
Rice straw + scrap papers + duck dung	0.65	1.69	0.15
Rice straw + half-filled rice + duck dung	0.51	1.48	0.18
Rice straw + grass clippings + duck dung	1.46	1.57	0.21
Rice straw + carbonized rice hull + duck dung	0.69	2.22	0.51
Rice straw + carbonized rice hull + kitchen wastes + duck dung	0.63	2.47	0.15
Rice straw + water lily leaves + duck dung	0.57	2.44	0.21
Substrate composition is 25% rice straw, 25% other waste, and 50% duck dung			

vermicast in reducing the amount and cost of fertilizer applied without sacrificing grain yield. The question is: will 1 ton of vermicast be sufficient in larger scale of rice production areas? This will depend now on the rate of vermicast production without sacrificing the quality of the vermicast. Economically, it boils down to the cost of vermiculture and vermicomposting facility to increase vermicast production for large and commercial scale of rice production. The PhilRice MRF vermicomposting can only produce vermicast good for the dry season, and for less than 100 ha (unpublished data).

The application of vermicast also positively and significantly affected the growth of *Cyamopsis tetragonoloba* or guar gum (Pavithra and Prabha, 2014). Application of vermicast enriched with rock phosphate increased yield of cowpea by 28% over farmyard manure with phosphate fertilizer (Sailaja, 2002). Peyvast (2008) stated that the number of spinach leaves was increased with the application of vermicast, while an increased weight of cloves of garlic was observed when applied with vermicast substituting 50% of the recommended NPK rate (Suthar, 2009). The marketable yields of strawberry, tomato, and pepper, along with other growth parameters significantly increased with the application of combined vermicast and inorganic fertilizer compared with application of inorganic fertilizer alone (Arancon et al., 2003). Further, Arancon (2004) quantified 35% increase in marketable fruit yield of strawberry. Arancon (2003) concluded that these improvements in yields and plant growth are partially due to the increase in microbial biomass, leading to the production of hormones. In cucumber, yield was increased by 26% with application of 30 t/ha vermicast (Azarmi, 2009). Similarly, Guerrero (2011) reported a higher yield in pechay produced from 50% inorganic fertilizer with 50% vermicast. This led to 30% reduction in fertilizer input cost compared to 100% inorganic fertilizer. Application of 100% vermicast also produced comparable yield with the 50% vermicast: 50% inorganic fertilizer ratio, but gave 40% reduction in fertilizer cost.

When used as potting medium or to supplement a potting medium, vermicast improved the growth and general performance of tomato and cucumber (Atiyeh et al., 2002 and raspberry (Atiyeh, 2000). However, in ornamental plants such as pansies and primula, reduction in the growth and biomass of leaves were observed with increasing levels of vermicast: peat ratio (Lazcano and Dominguez, 2010); while about 20% mortality of the plants grown in 25% vermicast: 75% peat mixture was observed.

Effects on pest populations

Substitution of MetroMix potting medium with 20% and 40% vermicast suppressed populations of aphids and mealy bugs on peppers and mealy bugs on tomato; thereby, decreasing losses due to these pests (Arancon, 2005). Loss in cabbage leaf area due to white caterpillar infestation was also reduced. Plant parasitic nematode populations in tomato, bell pepper, strawberry, and grape fields were suppressed by the application of vermicast (Arancon, 2002). However, increases in 'fungivorous' and 'bacterivorous' nematode populations were noted in the same study. The nematode *Meloidogyne incognita* was also effectively controlled in eggplant through the use of vermicast with neem oil and garlic extract (Nath, 2011). Moreover, vermicast mixed in container media for tomato suppressed the pathogen *Fusarium oxysporum* attributed to higher population of microorganisms and antagonistic bacteria present in vermicast (Szczech, 1999).

Effects on soil properties

Given the physical properties, nutrient content, and microbiological characteristics, vermicasts affect the soil it is applied with.

Physical properties. Vermicast prevents soil erosion and compaction (Sinha, 2013) by the observed improved bulk density and total porosity (Azarmi, 2008). Tejada (2009) also reported that vermicast from beet vinasse and green waste decreased soil loss. It increases soil macropores range of 50-500 µm which is good in maintaining soil structure (Marinari, 2000). It also favorably influenced water-holding capacity and drainage (Kannan, 2005).

Chemical properties and fertility. In paddy rice soils, application of 20 t/ha and 40 t/ha vermicast for three years increased the level of available iron (Fe), Zinc (Zn), and Manganese (Mn) in a study by Mousavi ., (2010). Similarly, in tomato field, application of 15 t/ha increased total carbon, N, P, K, Calcium (Ca), Zn, and Mn (Azarmi 2008). Electric conductivity (EC) was also increased with vermicast application while soil pH decreased. In this case, it may pose the danger of salt and acid accumulation in soil.

Manyuchi (2013a) found that pH, EC, and N content had decreased; phosphorus level slightly increased; and potassium content did not change 40 days after application of vermicast to clay loam soil. Manyuchi attributed the decrease in N to denitrification and the increase in P to increase in soluble phosphates. An increase in nitrate-N 30 days after application of vermicast, followed by its decline at day 70 was observed by Chaoui (2003) while ammonium-N remained low during the entire observation time. In strawberry field studied by Arancon (2006), total N, ammonium-N, nitrate-N, and orthophosphates increased significantly. In another study by Mishra (2005), he found an increase in the levels of chromium (Cr), nickel (Ni), copper (Cu), Zn, cadmium (Cd), and lead (Pb). Generally, application of vermicast to soil improves its bulk density and porosity; decreases pH; and increases EC, P, K and other micronutrients, including some heavy metals such as Cr, Ni, Cu, Zn, Cd, and Pb. Nitrogen, however, tends to decrease due to the action of microorganisms.

Microbiological properties. Soil enzyme activity and carbon dioxide production were used as indices of microbial activity in soils amended with vermicast (Marinari, 2000). Arancon (2006) reported an increased dehydrogenase activity and microbial biomass N in vermicast-amended soil. The decrease in nitrate N as observed by Chaoui (2003) and Manyuchi (2013) demonstrated the presence of microorganisms responsible for denitrification. Moreover, Gopinath (2008) found that vermicast enhances microbial activities of dehydrogenase, β-glucosidase, urease, and phosphatase.

VERMIWASH

Vermiwash is the brown colored liquid fertilizer collected after passage of water through a column of worm culture. It is very useful as a foliar spray for all crops. It was also reported to be high in plant-available NPK (Manyuchi, et al., 2013). Vermiwash has secretions of earthworms that contains mineral nutrients (Pant et al., 2009) such as N, P, K, Ca, and the hormones auxin and cytokinin (Rai, 2008). Due to these properties, they effectively increased the chlorophyll contents and fruit quality of tomato (Tejada, 2009) and strawberry (Singh et al., 2010). Nitrogen-fixing bacteria such as *Azotobacter sp., Agrobacterium sp.,* and *Rhizobium sp.* were also found in vermiwash (Zambare et al., 2008). They can also develop resistance in crops receiving its spray. As proof, they decreased infection of tomato from late blight disease or *Phytophthora infestans* (Zaller, 2006).

Two of several processes in vermiwash production were those of Kumar (2005) and modified by Gopal (2010). The process described by Kumar (2005) was the use of a 20 l capacity elevated tank containing a layer of brick and pebbles of up to 10 cm from the bottom of the tank/container. Above the layer of bricks and pebbles, 2-3 cm of coconut fiber was placed and moistened. Then, 2 kg of worms were introduced and 4 kg of kitchen waste was spread over the worm layer. Water was added to keep the mixture moist. After 24 hours, leachate can be collected through the holes at the bottom of the tank.

On the other hand, Gopal (2010) modified and optimized the process using a 200 l plastic barrel with a tap or stopper fitted just above the barrel's base for vermiwash collection. The bottom of the barrel was filled with 10 cm layers each of clean pebbles, coarse gravel and beach sand to make a 30 cm-filtration layer and serve as accumulation or collecting compartment for vermiwash. Above the filtration layer, matured coconut leaf vermicompost was placed. Then, 100 kg of 10:1 mixture of partially decomposed coconut leaf and cow dung was spread over the vermicompost and moistened to 40% moisture content. Earthworms (500 individuals) were then introduced to the mixture of substrates. For 10 days, water was not added. Then, a 25 l pot that has perforations at the bottom inserted with cotton wicks through which water can trickle out, was hung over the barrel. After the 10th day, water was allowed to fall drop by drop from a water-filled pot hung above the barrel into the vermicomposting system. Pot was refilled with water every two days. After 30 days, clear brown

colored liquid collected at the bottom of the barrel. The accumulated vermiwash was then collected through the tap of the barrel at weekly intervals for a month. Gopal (2005) suggested that for another cycle, the content of the barrel should be removed and fresh substrate along with earthworms should be added. Farmers can make an exit hole in the tanks built for large-scale vermicompost production and collect the vermiwash regularly.

VERMITEA

In contrast to vermiwash, vermitea is produced by "brewing" or fermenting vermicast with sugar or molasses in a process described by Pothiselvi (2012). Mature vermicast should be used for making vermitea due to higher levels of microorganisms present than in incompletely decomposed vermicast. The sugar is needed to keep the microorganisms alive (Singh, 2014). Manandhar (2008) reported that this by-product of vermicast exhibited maximum control of foot rot or Bakanae caused by *Gibberella fujikuroi* in rice. The rice seeds were soaked in vermicast tea for pre-germination treatment, followed by soil drenching of vermi tea at the rate of 1 l/sqm at seven-days interval starting from germination (Manandhar, 2008). Like vermiwash, vermitea also has suppressive properties to insect pests. The application of 10% and 20% vermitea can effectively suppress and even kill green peach aphid, citrus mealy bug, and two-spotted spider mite, which attacks tomato and cucumber, (Edwards et al., 2009).

Pothiselvi (2012) described the following process in making vermicast tea. Forty grams of brown sugar were added to 1 l tap water in a container. The power head pump was used to keep the mixture aerated. The outlet was submerged just right below the water's surface. Forty grams of the vermicast was put in cheese cloth bag tied with a string. The string was long enough to facilitate drawing out the bag from the liquid after fermentation. The bag containing vermicast was submerged in the water. A lid was put on to prevent contaminants.

ESTIMATED COSTS AND RETURNS OF VERMICOMPOSTING

One of the considerations in vermicomposting is the needed structures for its establishment. In simple composting that can be done in the backyard, simple coverings like the plastic sheets can serve as protection from animals and excessive water from rains. Vermicomposting requires strong roofs and adequate protection from entry of predators and exit of earthworms.

The cost of vermicompost ranges from less than P1,000 to P1M with an average of P57,198 from a survey of 24 adopters. From his survey, Adorada (2007) also computed the net benefit of small-scale vermicomposting system to be P78,123 using the technique of Hufschmidt et al. (1983). The highest profits were generated by adopters who have the capacity for high production and linkage to stable markets.

Vermicomposting in smaller scale, however, is still doable if the objective is just to maintain soil health and productivity. However, the varying quality of the vermicompost, depending on the substrates used, can also affect the needed nutrient input to soils for rice and other crops' production. The poor quality of vermicompost may also require higher quantity needed to supply such nutrient demand per target yield. Substrates to be used, on the other hand, also depend on the farm wastes generated within the system; thus, they dictate their sustainability in and outside the farming system.

SUMMARY: CAN VERMICOMPOSTING BE AN INTEGRAL PART OF SUSTAINABLE RICE FARMING SYSTEM?

A sustainable farming system is a long-term goal and will take time to achieve depending on the farming innovation employed. It also involves the use of inputs that are environment-friendly and are accessible for farm use. Usually, these inputs come from the wastes within the farming system. In the farm, crops are produced by the tons; thus, organic wastes are also produced by the hundreds of tons. The same situation will be for the animal wastes. These inevitable wastes are either discarded or left to decay. Some are disposed of by the atmosphere-offending process of burning, as in the case of rice straw. Nature is equipped with processes that eventually turn most of its cycles' wastes into forms that will be used by another. Unfortunately, though, manmade systems did not include waste disposal. A waste disposal mechanism for these wastes is needed for a sustainable system.

The positive impacts to environment make vermicomposting a potent part of a sustainable system, as a turning point in a materials' cycle, in which it is put back to being an input, rather than an output.

Moreover, the reported benefits of applying vermicompost as substitute to reduce the recommended rate of chemical fertilizer by half, with yields similar or even higher than the full application of the recommended rate by chemical fertilizer for rice and other crops, showed the economic advantage of using vermicompost. Reduced inputs will mean reduced cost on crop production. The additional cost of vermicomposting can be compensated by the savings on fertilizer and additional income from the wastes. This is feasible in view of small scale rice farming where the advantages

of vermicomposting can be achieved. It will both solve the problems of disposal of farm wastes while providing the farm with organic inputs as substitute to expensive chemical fertilizers and pesticides.

Yield gaps between the experimental field and the actual yield of adaptors in the tropics was not considered in this review as most of the papers reviewed were conducted in smaller scales or in experimental areas. Moreover, publications on the basics and practices of vermicomposting in the Philippines are limited. However, extrapolating the results of few researchers on the input and output of the experiments such as the application of 1 to 2 t of vermicompost to a hectare into larger scale (10 ha), it can be implied that producing large amount of compost while buying or sourcing out organic materials might not be sustainable. This is in consideration to the needed quantity to supply the nutrient demand of rice and other crops to optimize their potential yield. Otherwise, the need to buy extra vermicompost outside the system becomes inevitable, which most of the rice farmers may not afford. Therefore, an internal source of the compost should be build-up within the farming system for a sustainable source of nutrient supplements and pest control for the rice and rice-based cropping system.

The production of high quality and quantity of vermicompost, its by products, and vermi/earthworm should be standardized, be it for backyard gardening or for commercial purposes by focusing on the different substrates or wastes surrounding the farm. Their application can also be optimized in larger scale rice farming by innovations on the production system to include the optimized use of tools and/or machineries. Likewise, feasibility or socio-economic analysis should still be given importance as it will be the basis for recommendation to the Philippine rice farmers as far as their income and profit are concerned.

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RAPID GENERATION ADVANCE IN DEVELOPING RECOMBINANT AND BACKCROSSED INBRED LINES FOR HIGH TEMPERATURE TOLERANCE IN RICE *(ORYZA SATIVA L.)*

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ABSTRACT

High temperatures of 38°C to 39°C during flowering and early grain filling stages of rice can reduce yield by 10-14%. Thus, there is a need to integrate high temperature tolerance in the rice breeding program. The rapid generation advance (RGA) using modified single seed descent (SSD) is a breeding technique where four filial generations in rice are possible in a year instead of two under normal field conditions. Through RGA, we aimed to generate homozygous breeding populations for high temperature tolerance. An RGA facility was constructed at Philippine Rice Research Institute (PhilRice) to accommodate 7,920 rice plants. Sixteen F_1 populations derived from heat tolerant and mega varieties from Southeast Asian country partners were advanced until 3 populations remained in the F_9 generation to form RILs (recombinant inbred lines) and 4 BILs (backcrossed inbred lines). Rice plants were subjected to short-day treatment (i.e., 9 h) or long dark period (i.e., 15 h) per day one month after sowing to induce early heading and flowering. One generation was completed in approximately 90 days; thus, four generations in a year. During RGA, selection for plant type and phenotyping were not possible due to the miniature, single-tiller plants produced. Selection for plant type was done in the field after generation advance in RGA. In 2014 dry season, 2 populations of Gayabyeo/N22 and AS 996-9/N22 selected from the RGA-derived heat-tolerant RILs and 4 populations of Hanareumbyeo*3/N22, Gayabyeo*3/N22, Hanareumbyeo*3/Dular, and NSIC Rc160*3/Dular, displayed low percent sterility compared to the check varieties under high temperature conditions.

KEYWORDS

High temperature or heat tolerance, rapid generation advance, rice, single seed descent

INTRODUCTION

Rice has been grown for centuries under wide range of environmental conditions in Asia and 50% of the world population depends on rice for food (Wassmann et al., 2009). With the changing world climatic events especially rising temperatures, rice production is threatened. Temperatures exceeding 35°C at daytime and >29°C at night can cause physiological abnormalities in rice that result in sterility. The development of new heat-tolerant rice varieties is among the best approaches in breeding programs to address changing climatic conditions in the affected farming communities (Manigbas et al., 2014).

Rapid generation advance (RGA) is a method which combines close plant spacing in pots, and artificially short days to minimize growth duration (Mackill et al., 1996). The strategy which typically partnered with RGA procedure is the single seed-descent (SSD) method, a concept proposed by Goulden in 1939 and subsequently modified by Brim in 1960. It is a modification of bulk method in which each plant is given chance to survive until homozygosity of the population is attained. SSD is a method that rapidly fixes genes in the population and attains a genetically stable generation in a shorter time. Using only one or two seeds per plant per generation and sown at closer spacing, this procedure can be applied to increase efficiency in breeding because 4 generations can be attained in a year compared to 2 generations using conventional breeding methods. This is possible to accelerate generation turnover every year so that within 1½-year period, homozygosity of the breeding materials can be achieved. This strategy is very important for developing populations for heat tolerance because screening for high temperature tolerance is only done once a year during the dry season where temperature can reach 39°C. Thus, after a year in RGA treatment (F_2-F_6 generations), uniform populations or lines can be screened for high temperature tolerance and selection for plant type can be performed in the field simultaneously.

Maruyama (1987) discussed the advantages of RGA with SSD. It shortened the breeding cycle, increased the number of favorable genotypes, and reduced breeding costs. The principle of RGA shortening the breeding cycle is fundamentally based on the rice plant's response to photoperiod. Growth of rice, a short-day plant, is accelerated by a short-term light exposure alternated with long, uninterrupted dark period of 10-14 h. Photoperiod sensitivity in rice occurs during the vegetative phase, which is divided further into two phases: the basic vegetative phase (BVP) and the photoperiod-sensitive phase (PSP). As the name suggests, the PSP of rice, which happens toward the end of the vegetative phase, is the stage of the plant which highly responds to light stimulus (Vergara & Chang, 1985).

Photoperiod sensitivity has been reported to increase as the plant ages up to 28 days after sowing (DAS), then decreases at 35 DAS and above, indicating an optimum age of responsiveness to photoperiod. Flowering of rice depends on two interrelated factors: temperature and day length. High temperature generally hastens panicle development and heading, but to an optimum value depending on the cultivar (Vergara & Chang, 1985).

Selection during early generations may cause genetic drift or loss of favorable genotypes because of high heterozygosis. Heterozygosis masks useful characters. Harvesting all seeds may cause random drift and means having to maintain a large population to achieve maximum recombination. In SSD, a minimum population size with maximum genetic diversity is kept.

In Korea, breeders practiced growing their F_1s in greenhouses during winter and their early segregating generations were grown in IRRI. In the 1970s, they adopted and established RGA facilities in their breeding stations, resulting to the recommended release of many of their cultivars within 5 to 7 years from hybridization (Heu et al., 1982). For high temperature tolerance breeding in which field screening can only be done in high temperature months of the year (e.g., April-May), RGA is one of the best methods to be applied because essentially one screening is done in a year. The rest of the time can be devoted in advancing the breeding populations in RGA especially to pre-selected crosses.

The International Rice Research Institute (IRRI) has practiced RGA in advancing bulk populations from crosses made in IRRI and the National Programs of collaborating countries such as Japan, Korea, and India. F_1 seeds (from single cross, backcross, or topcross) are created and grown in their respective countries. The F_2 seeds are then sent to IRRI to be subjected to RGA until the F_4 generation (Vergara et al., 1982), and then returned to their respective breeding centers for subsequent pedigree selection and protocols of their National Programs. These countries have also been reported to have released varieties which have undergone RGA.

RGA with SSD can also function as a modified system of pedigree selection, but to a limited extent. In IRRI, RGA with SSD has been used in screening for physiological stress tolerance such as cold tolerance (Heu et al., 1982), salinity at seedling stage, submergence at seedling stage, drought, photoperiod sensitivity, short growth duration, and bacterial leaf blight screening (Vergara et al., 1982).

SSD is not only applicable to rice breeding. In fact, the SSD concept has been first suggested by Goulden in 1939 in wheat breeding. Other crop improvement practices in annual species, like yardlong bean (Sarutayophat

& Nualsri, 2010), soybean [(Glycine max L. (Merr)] (Macchiavelli & Beaver, 2001), and small cereal grains, have been utilizing SSD to advance the generation of the crop in little amount of time (i.e., F_6 generation in 14 months) to attain homozygosity of the population. In corn (Zea mays L.), a modified SSD, called MSSD, was used to compare with other breeding methods such as conventional pedigree and double haploid (DH) breeding (Jumbo et al., 2011). It was found out that MSSD can be an efficient alternative to both pedigree and DH methods for developing high performing lines from the Germplasm Enhancement of Maize (GEM) breeding because it requires fewer resources.

Bradshaw et al. (2009) used SSD method for swedes (Brassica napus L. var. napobrassica Peterm) in Scottish Crop Research Institute, UK. They developed swedes population from F_2 to F_6 selfing generations in the glasshouse because it was not possible to grow two or three generations a year due to vernalization requirements of swedes. Also, it was not possible to grow a large number of plants at high density because the inflorescence needs to be covered by glycine bag to prevent cross pollination. Another important advantage of SSD method was mentioned in a study conducted in barley (*Hordeum vulgare* L.) breeding for quality improvement in Tunisia (Medimagh et al., 2012). The study was initiated to assess the effectiveness of pedigree selection method, bulk selection, and single seed descent selection. The results showed that pedigree selection was more efficient in high input environment. The bulk method was very effective for selection of kernel weight in the target environment, while single seed descent selection was good for protein content and husk percentage in harsh and difficult environments like high temperature tolerance breeding in this study.

At PhilRice in Science City of Muñoz, Nueva Ecija, Philippines, RGA with SSD has been successfully applied in developing RILs and BILs for high temperature or heat tolerance in a short period of time. Field evaluation for high temperature can only be done once a year during the dry season; thus, developing homozygous populations with genes for high temperature tolerance through RGA is an efficient way to save time and resources. An unused room was converted into a rapid generation advance (RGA) facility where hundreds of plants could be accommodated in one treatment. RGA is simply a darkroom where plants can be treated under short-day or long-night periods to induce and hasten flowering. It can also be used for long-day and short-night treatment with the use of several light bulbs that can be lighted for prolong periods. RGA is also used to synchronize flowering of photoperiod sensitive and non-sensitive rice plants for crossing work. The turn-over period from sowing to harvesting is fast when RGA or SSD method is used. It takes only 1 week after harvesting before the next generation of sowing is done. Flowering of rice plants is induced because of controlled light and dark periods. There are no prolonged lag periods so that 4 generations is possible in one year. Under field conditions, only 2 generations is possible in one year because of the cropping season. One limitation, however, is the number of crosses for SSD method. Only few selected crosses can be subjected to this method because the population size is restricted to small space in the RGA facility and glasshouse. The F_1s are planted in the field to get appropriate number of F_2 seeds and thereon advanced up to F_6 generation using SSD method. This method is very useful in developing RILs and BILs for selection for heat tolerance in the field because it shortens the breeding cycle by 1.5-2.0 years. Considering that field evaluation for high temperature tolerance in the Philippines or in the tropics is only done once during the dry season (April-May), RGA technique becomes very efficient because the breeder can devote some of the time in advancing rice populations in the RGA facility.

Japan was the first country reported to have successfully applied rapid generation advance in varietal development and release. In 1932, breeders in Aichi Agricultural Experiment Station grew their F_1s in the greenhouse during winter. Breeders in the Nagasaki Agricultural Experiment Station subjected their F_1 and F_2 under short-day treatment. The practicality and efficacy of rapid generation advance were recognized, resulting in increased use of RGA procedure and installation of RGA facilities. The first variety produced from RGA with SSD was Nipponbare, which was released in 1963. The breeder Nishio used RGA in advancing F_1 to F_4 generation and was able to release the variety after six years. Nipponbare became the top-ranking variety in Japan in terms of land-area cultivated (Heu et al., 1982).

The main objective of the study was to generate homozygous breeding populations for high temperature tolerance within a short period of time through RGA.

MATERIALS AND METHODS

The experiment was conducted at PhilRice in Science City of Muñoz, Nueva Ecija, Philippines in 2010.

Initial crosses between heat-tolerant donors (N22, Dular, and Nipponbare) and mega varieties from the Philippines, Korea, and Vietnam were made during 2009 wet season. New germplasm was introduced from South Korea and Vietnam as a result of ASEAN collaborative project for breeding heat-tolerant rice. Sixteen cross combinations of F_2 population were subjected to RGA treatment from F_3 to F_5 generation (2010-2011) (Table 1). They were designated as HTKR when the cross was made in Korea, HTVN in Vietnam, and HT in the Philippines.

Table 1
Cross combination of entries with respective 50% flowering dates during RGA

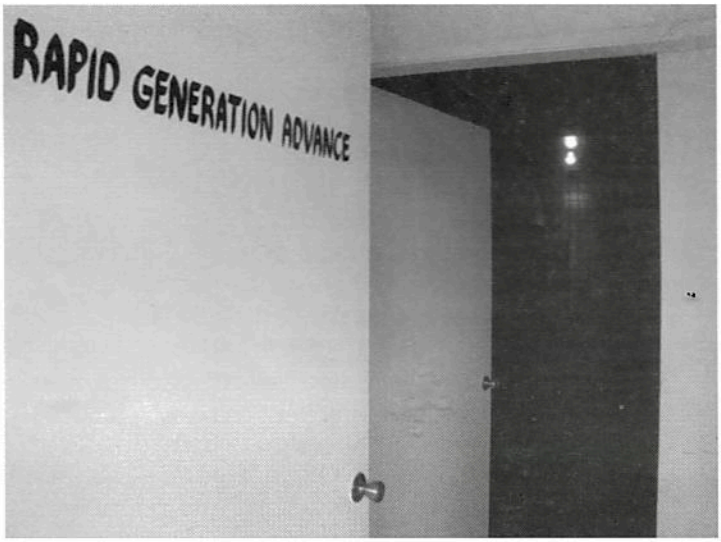
Index	Plot Number	Cross	Hybridization was made	Days to 50% Flowering		
				F3	F4	F5
1	HT109	AS 996-9 x N22	Philippines	61	59	51
2	HTKR110	AS 996-9 x N22	Korea	55	57	51
3	HTVN106	AS 996-9 x N22	Vietnam	61	59	51
4	HT110	AS 996-9 x Dular	Philippines	66	57	57
5	HTKR109	AS 996-9 x Dular	Korea	69	59	58
6	HTVN105	AS 996-9 x Dular	Vietnam	67	57	52
7	HT116	Gayabyeo x N22(46459)	Philippines	65	60	59
8	HTKR102	Gayabyeo x N22	Korea	55	54	49
9	HTVN102	Gayabyeo x N22	Vietnam	50	46	49
10	HT101	Gayabyeo x Dular	Philippines	69	58	55
11	HTKR101	Gayabyeo x Dular	Korea	67	58	57
12	HTVN101	Gayabyeo x Dular	Vietnam	69	59	52
13	HT118	Hanareumbyeo x N22(46459)	Philippines	74	65	57
14	HTKR104	Hanareumbyeo x N22	Korea	61	60	51
15	HT102	Hanareumbyeo x Dular	Philippines	61	55	63
16	HTKR103	Hanareumbyeo x Dular	Korea	58	56	65
17	HT114	IR 66 x Dular	Philippines	68	59	57
18	HTVN109	IR 66 x Dular	Vietnam	68	59	59
19	HT117	Jinmibyeo x N22(46459)	Philippines	68	56	51
20	HTKR106	Jinmibyeo x N22	Korea	55	54	51
21	HTVN103	Jinmibyeo x N22	Vietnam	60	55	52
22	HT103	Jinmibyeo x Dular	Philippines	68	discarded	
23	HTKR108	Junambyeo x N22	Korea	49	48	44
24	HT105	Junambyeo x Dular	Philippines	54	64	50
25	HTKR107	Junambyeo x Dular	Korea	54	55	49
26	HT113	NSIC Rc160 x Dular	Philippines	66	60	61
27	HTVN108	OM5930 x N22	Vietnam	54	55	50

The F_1 seeds were distributed to collaborative member-partner countries in Southeast Asia for generation advance and further selection in high temperature location in their respective areas. In succeeding RGA cycles, sixteen populations proceeded to F_5 generation and harvested as bulk (F_6 seeds). The seeds were planted in the field nursery as individual plant and selected for desirable agronomic traits. Sowing was done based on flowering date so that the plants were exposed to high temperature during their reproductive stage in which plants are most susceptible. In 2014 dry season, 3 RILs and 4 BILs were selected and further evaluated for yield and resistance to pests and diseases in 2014 wet season.

PHILRICE RGA FACILITY

An unused 4.25m x 2.80m room was converted to a dark room by painting all walls and floors with black to ensure zero light penetration during short-day treatment (Figure 1).

Figure 1
Rapid generation advance facility



Five steel pushcarts measuring 1.41m x 0.74m x 0.23m were fabricated for planting and for transporting the plants from the glasshouse to the dark room. Each pushcart can hold 264 (12 cups by 22 cups) small cups at a time. The RGA facility can accommodate a maximum of 7,920 plants per planting. This number requires around 320 m^2 space in the field, while only 5.2 m^2 (in the 5 carts) or 11.9 m^2 (RGA dark room).

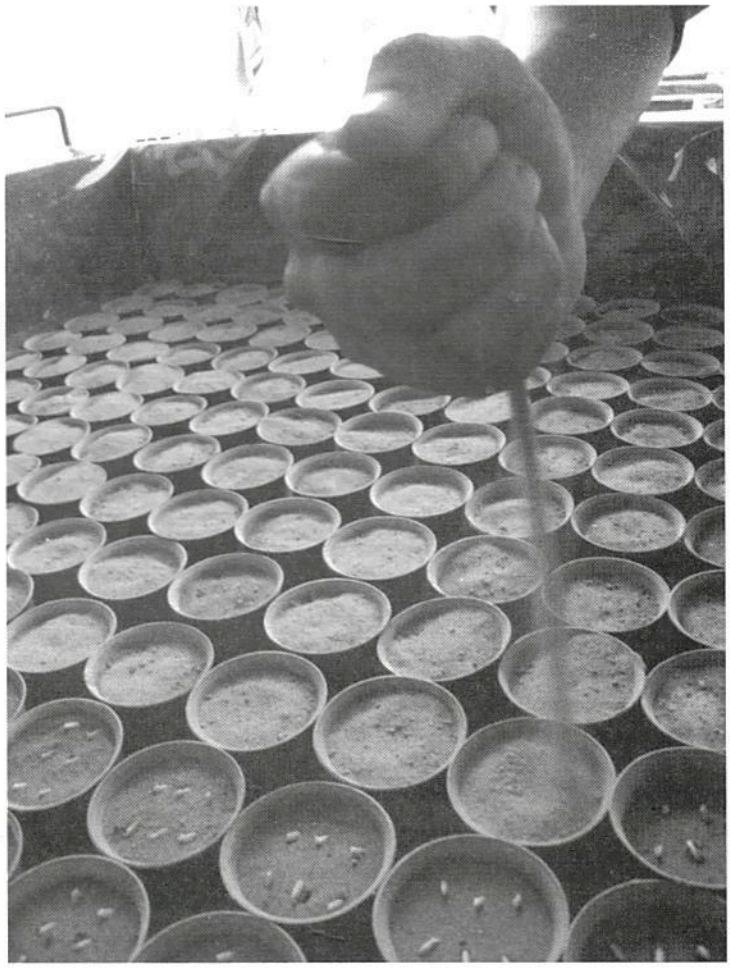
Sowing

To break dormancy, seeds were oven dried in 50°C for 48 hours. These were then pre-germinated (soaked for 24 hours and incubated for another 24 hours) in properly labeled Petri dishes. With the use of forceps, six uniformly pre-germinated seeds were evenly sown in each cup with around 1cm spacing between seeds. These were then covered with about 1 cm fine garden soil and watered (Figure 2). Carts were then covered with fine net to protect them against rats and birds, since the plants were allowed to grow in open air up until 28 DAS.

Taking care of rice plants

One week after sowing, carts were irrigated by misting them and saturating the cups (Figure 3). After seedling emergence, the carts were continuously flooded of about 1-2 cm water depth until dough stage.

FIGURE 2
Covering the seeds with fine garden soil with 6 seeds per cup



Complete fertilizer (14-14-14) was used as basal fertilizer, applied 10 DAS at a rate of 10-20 gm^{-2}. Urea (46-0-0) was used as top-dress at the rate of 15gm^{-2}.

When the plants became excessively vegetative, leaf pruning was done to decrease shading and prevent the

Figure 3
Water is maintained for good growth

plants from growing too tall and cause premature lodging.

Selection pressure during RGA

Due to the miniature plants produced during RGA, selection becomes very limited compared to pedigree method. Panicles were short and had few seeds required for the next generation (Figure 4). Therefore, observation of the crosses after RGA was done in the field for high temperature screening during the dry season. Maruyama (1987) reported that some highly heritable traits can be selected during RGA treatment such as reaction to pests and diseases, physiological responses, grain characters, morphological characters, and some traits linked to marker genes. While in RGA treatment,

Figure 4
Short panicles and less seeds are produced when plants are in RGA

plants with poor phenotype (i.e., prominent tall types, grassy habit), disease infection (i.e., BLB, blast susceptibility), and unacceptable grain traits (colored lemma, palea, and endosperm); and awns were routinely removed to increase the number of desirable genotypes in succeeding generations. Since we wanted to maintain the overall population size sown every cycle, the number of seeds harvested per panicle was increased to ensure survival of entries and cross combinations.

Start and Duration of Treatment

Since only one seed was required in RGA, seeds were harvested when at least 50% of the panicle had matured. Short-day treatment commenced at 28 DAS. Pushcarts were wheeled into the dark room at 1600 h for a continuous dark treatment, then wheeled out into the glasshouse at 0700 h, completing a 15-hour dark period and 9-hour day period.

When 50% of the plants per cart had flowered, carts were permanently placed inside the glasshouse until maturity. Average day temperature inside the glasshouse can reach up to 38-40 °C during dry season and 36-38°C during wet season. High temperature speeds up maturity of grains. However, since temperature cannot be controlled in the glasshouse, intense heat contributed to increased spikelet sterility of heat-intolerant plants.

Harvesting and Turn-over Period

Panicles were harvested individually. Two seeds were taken from each plant and bulked together with seeds from the same entry. (As mentioned earlier, number of seeds harvested per panicle increase when plant types are discarded to maintain population size). The rest of the panicles were bulked, threshed, and kept as reserved seeds. Freshly harvested seeds were oven-dried and pre-germinated in a week

Field Evaluation of RILs

In 2012 Dry Season, the F_6 generation (59 entries, 16 populations) produced through RGA were planted in the field to test and assess their performance, while synchronizing flowering to the hottest day of the year (April-May) for heat tolerance evaluation. Heat tolerance was evaluated based on the percent sterility/fertility of the sampled panicles during harvest. The main panicle was collected from each of the 10 sampled plants from the selected desirable phenotypes per advanced/uniform breeding line and check varieties. The high temperature-tolerant breeding lines were selected and classified based on the sterility/fertility data that were generated (Table 2). Under normal plant spacing, irrigation, fertilizer application, and solar exposure, the plants were able to express their actual phenotype. Selection was made and entries from two RILs Gayabyeo/N22 and AS 996-9/N22 and four BILs Hanareumbyeo*3/N22, Gayabyeo*3/N22, Hanareumbyeo*3/Dular, and NSIC Rc160*3/Dular expressed desirable agro-morphological characteristics and disease reaction.

RESULTS AND DISCUSSION

Thirty-seven entries or cross combination of different rice genotypes from various collaborative member-

Table 2
Recombinant and backcrossed inbred lines selected in 2014 dry season under high temperature (i.e., 37-39°C) conditions in the field at PhilRice, Nueva Ecija. Percent sterility was recorded in 2012 dry season glasshouse experiment under high temperature condition.

Breeding line / Variety	Cross Combination	Classification	Percent Sterility	Reaction
HTKR102-RGA5-1-B-3-B	Gayabyeo/N22	RIL	20.1	I
HTKR110-RGA5-2-B-1-B	AS996-9/N22	RIL	17.3	T
PR42224-RGA4	Hanareumbyeo*3/N22	BIL	14.0	T
PR42222-RGA4	Gayabyeo*3/N22	BIL	23.2	I
PR42223-RGA4	Hanareumbyeo*3/Dular	BIL	16.0	T
PR42219-RGA4	NSIC Rc160*3/Dular	BIL	15.6	T
IR52		S check	41.5	S
N22		R check	7.3	T
AS996-9		Parent	21.8	I
Gayabyeo		Parent	46.1	S
Hanareumbyeo		Parent	42.5	S
NSIC Rc160		Parent	27.6	I

Legend:

Percent Sterility	Reaction
<17.5	Tolerant (T)
18-40	Intermediate (I)
>41	Susceptible (S)

partner countries (Vietnam: AS996-9, OM5930; South Korea: Gayabyeo, Hanareumbyeo, Jinmibyeo, Junambyeo; Philippines: NSICRc 160; and Cambodia: IR66) and heat-tolerant donors (i.e, N22, Dular, Nipponbare) were selected from the field nursery for generation advance in the RGA facility. From F_2 harvests, the plants were advanced up to F_6 generation which was equivalent to 4 generations per year with RGA technique compared to only two generations per year using the pedigree, mass selection, and backcross methods which is mostly done under field conditions. The range of days to 50% flowering under RGA treatment in the F_3 population was 50-69; F_4, 46-65; and F_5, 44-65 which is almost 20 days earlier than under field conditions (Table 1). Under normal field conditions, days to 50% flowering ranges from 62-90 days after sowing. Maturity and turn-over period during RGA treatment is very fast since plants are allowed to mature in the glasshouse in which high temperature induces faster panicle development and maturity (Vergara and Chang 1985) and only 1-2 mature seeds per panicle are needed for the next sowing.

In Table 3, the column days to harvesting refers to the number of days from sowing to the time the last entry has reached 50% panicle maturity, signaling harvesting of entries in the particular cart.

It took only 3 months and 7 days to finish one generation of the materials generated using RGA technique thus 4 generations per year is achievable. This makes breeding

Table 3
Duration of Rapid Generation Advance per breeding cycle

Filial Generation	Number of Crosses	Sowing Date	Harvesting Date	Days to Harvesting
F_2	16	27 Oct 2010	1 Feb 2011	97
F_3	13	10 Feb 2011	18 May 2011	97
F_4	13	22 Jun 2011	21 Sep 2011	91
F_5	13	3 Oct 2011	8 Jan 2012	97

and development of RILs increase in efficiency, fast in attaining homozygosity, and becomes more economical due to less land use and space requirement with more number of crosses accommodated, and few management inputs.

The 6 sampled crosses subjected to RGA treatment and their corresponding days to 50% flowering and days to maturity is shown in Table 4.

Flowering dates recorded went as early as 46 DAS to 59 DAS while maturity (at least 50% of the panicle) ranges from 68 DAS to 94 DAS. Plants of different cross combinations and within a cross have different flowering behavior and maturity period when grown in the field. But since all plants were subjected to short-day treatment in RGA at the same time, they all flowered and matured at the same time (i.e., within a cross). Generally, flowering and maturity period are enhanced compared to normal planting in the field. This is the advantage of RGA technique.

After the RGA treatment, usually F_6 generation, plants were harvested as bulk (i.e., no selection) and planted in the field nursery as individual plant with 20 x 20 cm spacing. These plants were allowed to grow under good cultural and management practices under high temperature conditions. Entries were grouped according to flowering date and planted on staggered basis so that at reproductive stage, the plants were exposed to high temperature during the dry season. Important morphological traits were taken at every growth stage of the crop, evaluated for pests and diseases resistance, and further selected for good crop stand and desirable phenotypes. At maturity, agronomic traits were measured including percent fertility or sterility as a measure of heat tolerance. Further selection was applied in the following generations in which grain yield, quality, percent sterility/fertility determined. Table 2 shows the populations with N22 and Dular as sources of heat tolerance that were selected based on phenotype and had undergone heat stress screening in the glasshouse and in the field after RGA treatment. In 2012 dry season glasshouse experiment, percent sterility was recorded under high temperature conditions. Tolerant and intermediate reactions were observed of the breeding lines. All of the RIL and BIL populations advanced in the RGA facility had intermediate to tolerant reactions to high temperature based on percent sterility. The susceptible check IR52 showed 41.5% sterility and N22 the tolerant check registered 7.3%. The parents Gayabyeo had 46.1% and Hanareumbyeo 42.5% sterility and were susceptible. AS996-9 and NSIC Rc160 showed intermediate tolerance to high temperature. They were selected to advance in the next generation for further observation. These breeding lines are being evaluated for yield and quality and heat tolerance will be confirmed using growth chamber. The combination of RGA and field selection for heat tolerance, homozygous/uniform lines can be obtained in 3.5 years starting in F_2 population thus, reducing the breeding cycle and cost of variety development.

In this study, selection of crosses or materials that will undergo RGA treatment is very important. Selected crosses of South Korean, Vietnam, and Philippine cultivars were combined with the high temperature tolerant donors N22 and Dular and initially used for RGA treatment. Through this way, one year is enough to make an F_6 generation compared to two years when grown in the field. The selection was made in later generation for these populations because of the quantitative inheritance behavior of heat tolerance. It is also recognized that RGA technique cannot be used in all crosses or as only procedure in the breeding program. It is practically useful in specific studies or special crosses that need the RGA treatment (i.e., short day treatment in less space). Normally, it is decided by the breeder what technique to use depending on the trait to improve and the selection method to be used to achieve the objectives.

Table 4
Sampled crosses, days to 50% flowering and maturity in the F_3 to F_5 generation

Cross Combination	F_3		F_4		F_5	
	Flowering	Maturity	Flowering	Maturity	Flowering	Maturity
Gayabyeo x N22	50	75	46	68	49	72
Jinmibyeo x N22	60	76	55	74	52	75
Junambyeo x Dular	54	77	55	78	49	75
Gayabyeo x Dular	69	94	59	83	52	78
IR 66 x Dular	68	88	59	85	59	82
AS996-9 x N22	61	71	59	70	51	61

CONCLUSIONS

Through well-planned, scheduled, and selected crosses for RGA, 4 generations can be achieved in a little more than a year, significantly reducing the breeding cycle compared to only two generations per year using conventional breeding such as pedigree, mass selection/ bulk, and backcross methods. Since screening for high temperature tolerance can only be done once a year during the dry season where temperature can increase up to 39°C, RGA technique merits the cause of producing homozygous lines on the first year and testing and selection is done on the second year rather than wait for 2 years before testing in high temperature environments. The miniature plants produced through dense spacing enables the breeder to advance thousands of plants to near or homozygosity and more number of populations in a minimal space, therefore saving on labor and resources. Although there is an increased chance of unfavorable genotypes appearing in latter generations due to limited selection during RGA, it is possible for some highly heritable and polygenic traits, such as grain traits, tolerance to abiotic stress like high temperature, few morphological characteristics, and physiological responses, to show up. Potentials of a basic, functional RGA facility should be further explored to optimize its applicability in other breeding activities at PhilRice such as screening for biotic and abiotic stresses, and ultimately, its applicability in varietal development. Selection is postponed in the early segregating generation until homozygosity is attained in a very short period; thus, saving time and resources. RGA technique is simple and saves more resources in terms of facilities used compared with tissue culture or double haploid breeding done under sterilized conditions using growth hormones and regulators.

Using RGA procedure, 2 homozygous populations of heat-tolerant RILs HTKR102 and HTKR110 and 4 populations of BILs PR42219, PR42222, PR42223, PR42224 were produced and being evaluated for yield and grain quality during dry seasons. These populations after one dry season screening in high temperature environment (PhilRice, Nueva Ecija) have resulted in lower percent sterility and were considered tolerant and intermediate tolerant. The development of specific or pre-selected crosses of RILs and BILs using RGA is an alternative breeding method in which selection in the field can be delayed to later generation especially for quantitative traits or polygenic characters to save money and shorten the breeding program for high temperature tolerance.

The RGA technique, although very well known to breeders, has not been extensively used because of the lost opportunity to do simultaneous selection in the early segregating generations for important traits. But for traits that are controlled by quantitative traits, like yield and heat tolerance, selection can be delayed in the later generation using the RGA technique especially that screening for heat tolerance can be done only during the dry seasons. Moreover, the breeder has to choose which among the selected populations should undergo RGA treatment because not all crosses can be subjected to this procedure to benefit the advantage of the technique.

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SIMULATING POTENTIAL YIELD OF RICE *(ORYZA SATIVA L.)* UNDER DIFFERENT NITROGEN LEVELS, CLIMATE TYPES, AND PROJECTED INCREASE IN AIR TEMPERATURE DUE TO CLIMATE CHANGE WITH DSSAT CERES-RICE MODEL

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ABSTRACT

The Decision Support System for Agrotechnology Transfer (DSSAT) CERES- Rice Model is an application program designed to simulate potential yield in response to soil-water-atmosphere environment and particular crop management. But the Model needs to be calibrated to generate the crop genetic coefficients to simulate potential yields of new or popular rice varieties like PSB Rc82 and NSIC Rc160. To generate the genetic coefficients, soil, crop management, crop growth and yield and weather data were obtained from an experimental field in PhilRice Nueva Ecija in 2012 dry and wet seasons. The genetic coefficients were used to simulate potential yields of the same rice varieties in another field in Nueva Ecija and Midsayap across nitrogen fertilizer levels, and in response to a projected increase in minimum air temperature by 1.13^{0}C and maximum air temperature by 0.35^{0}C due to potential climate change. The genetic coefficients ranged from 566.1 to 691.2 growing degree days at basic vegetative phase and from 1.19 to 1.187 temperature tolerance coefficients at reproductive stage. The simulated yields of PSB Rc82 and NSIC Rc160 were closed to the observed yields in Nueva Ecija in 2013 with highly acceptable normalized Root Mean Square Error (nRMSE) of less than 10%. However, yield simulation in Midsayap was unacceptable due to an nRMSE of more than 30%. This was attributed mainly to pest damage to the rice crop. In response to the projected increase in air temperatures in Nueva Ecija, simulated yields of PSB Rc82 and NSIC Rc160 decreased by an average of 2.37%.

KEYWORDS
Crop genetic coefficient, simulation, potential yield

INTRODUCTION

The attempt to raise rice yield has become more important than ever, now that the country struggles to produce its own rice requirement in the face of increasing population and competition for land and water, and frequent occurrences of extreme weather. Plant breeders employed various techniques to push the limits of rice genetic yield potential leading to releases of promising inbred and hybrid rice varieties. At the same time, crop physiologists and agronomists developed integrated crop management practices to attain the rice yield potential and input use efficiency of these rice varieties leading to the release of PalayCheck® System for irrigated lowland rice. PalayCheck® is a holistic, integrated and objective system of rice production that combines rice technologies with learning process to ensure farmers' understanding and sustained adoption (PhilRice-FAO, 2007). In particular, the nitrogen (N) management component of PalayCheck® is mainly based on the use of Leaf Color Chart (LCC) that can assess the "real-time" plant need for N fertilizer. In irrigated lowland rice soils, the application of N fertilizer is needed to exploit the genetic potential of modern rice varieties grown under integrated crop management or PalayCheck System. Crop growth and light interception and photosynthesis are determined by the crop nitrogen status. Five years after the series of on-farm testing and eventual release of the PalayCheck® System handbook, new inbred and hybrid varieties were put for public use and were found to have the potential to replace their predecessors. Hence, there is a need to assess the performance of these varieties under various N management levels to attain their yield potentials and improve N use efficiency. We hypothesized that the yield potential of a particular rice genotype or variety grown under optimum crop–water–nutrient–pest management can still be improved under a more favourable weather condition, i.e., stable irradiance and lower minimum and maximum air temperatures (Cruz et al., 1996). Farm yield potential can then be compared with potential yield that is usually assessed by a crop simulation model under non-limiting growing conditions to look into avenues to improve yield and resource use further like the use of better genotypes grown under optimum crop management and weather conditions. Rice crop growth and yield simulation models like ORYZA1 (Kropff et al., 1994; Cruz et al., 1996) and the DSSAT CERES-Rice (Jones et al., 2003) can be used to assess the potential rice yield and the potential impact of climate change on rice yield.

Occurrences of extreme weather, such as increasing global temperature and unpredictable rainfall patterns, are some indications that climate change is upon us. Release of greenhouse gases through burning of fossil fuels, and carbon dioxide emission as product of generation of electricity are some anthropogenic activities that contribute to increase in global temperature (Myhrvold and Caldeira, 2012). These manifestations can affect rice yields. Based on analysis of weather data from IRRI farm in Laguna, Philippines from 1979 – 2003, Peng et al. (2004) found that higher night air temperatures from global warming diminished rice yields by 10% for every centigrade increase in the minimum temperature in dry season. As projected by the Philippine Atmospheric Geophysical and Astronomical Services Administration (PAGASA) in its studies reported in 2014, mean air temperature will increase by 1^0 C in 2010 and by 2^0 C by 2050 (www.pagasa.dost.gov.ph).

Our understanding of the crop/genotype–management–environment system can be broadened and its usefulness extended to other sites when we complement our field studies with the use of DSSAT. DSSAT crop models (e.g., CERES-Rice, CERES-Maize, and CROPGRO-Soybean) encourage researchers to use them in evaluating the yield performance of new variety, seed technology or crop management to help decision-makers in transferring these technologies from one place to another where soil and weather vary (Uehara and Tsuji, 1998). DSSAT has been applied to study management options in agricultural production, climate forecasts and extension of knowledge to farmers (Timsina and Humphreys, 2006). Several studies also used this program to examine the risk in crop production due to weather variability (Rosenzweig and Iglesis, 1994; Yao et al., 2007). Thus, the objectives of this study were:

1. Calibrate the CERES-Rice Model using soil, crop growth, crop management and weather data in one test site in PhilRice Nueva Ecija (Type I Climate) to generate the crop genetic coefficients of popular inbred rice varieties PSB Rc82 and NSIC Rc160;
2. Validate the CERES-Rice Model by using the crop genetic coefficients of PSB Rc82 and NSIC Rc160 to simulate their potential grain yields in another test site in PhilRice Nueva Ecija and in PhilRice Midsayap, North Cotabato (Type III Climate) and compare the simulated and observed yields; and
3. Simulate the impact of projected increase in air temperatures on grain yields of PSB Rc82 and NSIC Rc160 due to climate change.

MATERIALS AND METHODS

Soil Property, Plant Material, N Fertilizer Treatment and Estimation of Indigenous Soil Nutrient Supply

Field experiments were conducted in PhilRice Nueva Ecija in the Science City of Muñoz, Nueva Ecija (15^0 40.3" N, 120^0 23.5" E; Maligaya clay soil) in 2012 dry and wet seasons and 2013 dry season and in PhilRice Midsayap, Bual Norte, Midsayap, North Cotabato (7^0 10.1" N, 124^0 28.58" E; Kabacan clay soil) in 2013 dry season. Table 1 describes the soil properties in PhilRice Nueva Ecija and PhilRice Midsayap.

Table 1
Pedological characteristics of Maligaya soil series in PhilRice Nueva Ecija in Muñoz, Nueva Ecija and Kabacan soil series in PhilRice Midsayap represented by Osias, Kabacan, North Cotabato (Miura et al., 1995).

	Maligaya Clay	**Kabacan Clay**
Higher Category Classification (USDA)	Ustic Epiaquert	Typic Tropaquept
Location of profiled soil	PhilRice CES, Maligaya, Muñoz, Nueva Ecija (15º 40' 23" N, 120º 53' 43" E)	Osias, Kabacan, North Cotabato (7º 06' 18" N, 124º 50" 30" E)
Elevation, meters above sea level	50	20
Landform	Flat position on back swamp between the Talavera and Chico Rivers	Flat position on flood plain of the Kabacan River
Slope	Almost flat (<2%)	Almost flat (<2%)
Land Use	Paddy field, double rice cropping	Paddy field, double rice cropping
Parent Material	Recent alluvial deposit	Recent alluvial deposit
Drainage	Imperfectly drained	Very poorly drained
Present of salt and alkali	Free	Free
Organic matter (%)	2.61 (0-8 cm); 1.08 (8-23 cm)	3.42 (0-20 cm)
Organic Carbon (%)	1.52 (0-8 cm); 0.63 (8-23 cm)	1.99 (0-20 cm)
Total N (%)	0.106 (0-8 cm); 0.040 (8-23 cm)	0.168 (0-20 cm)
C/N ratio	14.3 (0-8 cm); 15.8 (8-23 cm)	11.8 (0-20 cm)
pH (water)	5.4 (0-8 cm); 6.9 (8-23 cm)	4.4 (0-20 cm)
EC (mS/cm)	0.15 (0-23 cm)	0.23 (0-20 cm)
Available P, Olsen (ppm)	6.35 (0-23 cm)	30 (0-20 cm)
Exchangeable Potassium (cmol+/kg)	0.175 (0-23 cm)	0.37 (0-20 cm)
CEC (sum)	61.875 (0-23 cm)	48.55 (0-20 cm)

In PhilRice Nueva Ecija in 2012 dry and wet season, inbred rice varieties PSB Rc82 (110 days to maturity) and NSIC Rc160 (122 days to maturity) were tested under varying nitrogen (N) levels, and phosphorus (P) and potassium (K) ratios. PSB Rc82 and NSIC Rc160 were popular among rice farmers and consumers due to grain yield and quality. The experimental units had 4 replicates and were laid out in a strip plot design with plot dimension of 5 m x 5 m (25 m^2). The seedlings were transplanted in the test site after 21 days in the seedbed. Planting distance was 20 cm x 20 cm. Each variety was transplanted at 2-3 seedlings per hill. The Leaf Color Chart or LCC (PhilRice 2008) was used to assess the "real-time" crop need for N fertilizer. The Growth Stage-based N fertilizer application was a "fixed-time" and "fixed-dose" method usually employed by rice farmers. Fertilizer treatments were: (1) Control or zero fertilizer, (2) N omission plot with -N, +P, +K where 40 kg/ha each of P and K fertilizers were applied, (3) P omission plot with +N, -P, +K where 140 kg/ha of N and 40 kg/ha of K were applied, (4) K omission plot with +N, +P, -K where 137 kg/ha of N and 40 kg/ha of P were applied, (5) LCC based N fertilizer application with 4:2:1 NPK ratio where 35 kg urea N/ha was applied when LCC reading was below 4 in dry season, (6) LCC-based N fertilizer application with 4:1:2 NPK ratio where 35 kg urea N/ha was applied when LCC reading was below 4, and (7) Growth Stage-based N fertilizer application where N was applied in three splits: 35 kg urea N/ha each at mid-tillering, panicle initiation (EPI), and flowering stages. All P and K fertilizers were applied at 14 days after transplanting (DAT). In the wet season, P and K omission plots were not used since indigenous P and K supplies in the soil were adequate. LCC reading was done every week starting at 21 DAT until early flowering. Grain yield (t/ha) from each treatment was obtained from 5 m^2 sample area in the center of the field, oven-dried at 70°C to constant weight and adjusted to 14% grain moisture content prior to calculation of grain yield in ton per hectare (t/ha). Agronomic nitrogen use efficiency (AE_N) was estimated from grain yields in N fertilized plot and plot that did not receive N fertilizer:

$$AE_N = \frac{\text{(kg grain yield in N fertilized plot – kg grain yield in unfertilized plot)}}{\text{Total kg N fertilizer applied per hectare}}$$

For every 1 ton yield of rough rice, the estimated total plant nutrient uptakes in grain and straw were 16.1 to 17.5 kg N, 3.0 to 3.6 kg P and 17.0 to 22.0 kg K (Yoshida, 1981; Dobermann & Fairhurst, 2000). Hence, the indigenous soil N, P and K supplies in our test site were estimated by multiplying the yields (t/ha) in the N, P and K omission plots by 16.8 kg N, 3.3 kg P and 19.5 kg K.

In the 2013 dry season, another field experiment in a different field in PhilRice Nueva Ecija was conducted to validate the CERES-Rice Model. The experimental units had 4 replicates and were laid out in a strip plot design with plot dimension of 4.8 m x 4.7 m (22.56 m^2). The seedlings were transplanted after 25 days in the seedbed. Planting distance was 20 cm x 20 cm. Each variety was transplanted at 2-3 seedlings per hill. Fertilizer treatments were: (1) fixed application of 60 kg/ha N fertilizer in which 30 kg each of P and K were applied, and N fertilizer was applied in 14 DAT, early panicle initiation (EPI) and heading, (2) fixed application of 90 kg/ha N fertilizer in which 30 kg each of P and K were applied, and N fertilizer was applied in 14 DAT, EPI and heading, and (3) fixed application of 180 kg/ha N fertilizer in which 30 kg each of P and K were applied, and N fertilizer was applied in 14 DAT, EPI and heading.

Another field experiment was conducted in PhilRice Midsayap in 2013 DS to validate the model. The experimental units were laid out in a strip plot design with a 5 m x 5 m (25 m^2) plot dimension. Each experimental unit had four replicates. After 14 days in the seedbed, seedlings were transplanted at a planting distance of 20 cm x 20 cm. Each variety was transplanted at 2-3 seedlings per hill. The fertilizer treatments were: (1) 70 kg N fertilizer/ha, (2) 105 kg N fertilizer/ha, and (3) 140 kg N fertilizer/ha. In each treatment, 40 kg P/ha and 40 kg K/ha were applied at 14 DAT. Data from all sites were analysed using LSD with 5% level of significance.

Weather Data

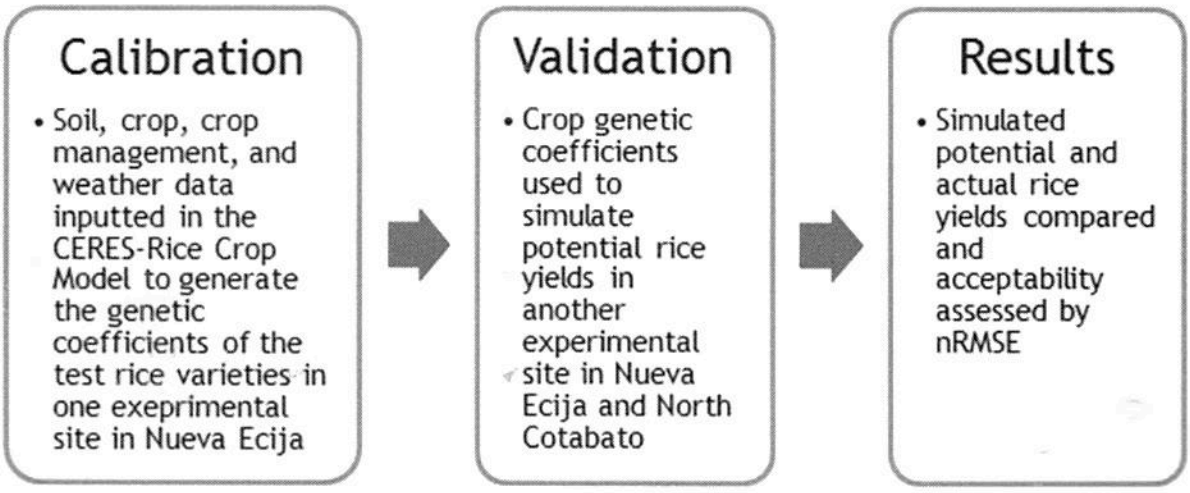
Daily solar radiation (i.e., irradiance in MJ/m^2), rainfall (mm), and minimum and maximum air temperatures (^0C) were obtained from the Automatic Weather Station, Davies Instruments, Vantage Pro 2.

Calibration and Validation of DSSAT CERES-Rice Model

To calibrate the CERES-Rice Crop Model, data on soil, weather, crop management, crop growth and grain yield were obtained from one test site in PhilRice Nueva Ecija in 2012 dry and wet seasons (Figure 1).

Data were inputted into the Rice Model to generate the crop genetic coefficients for PSB Rc82 and NSIC Rc160 using the Genetic Likelihood (GLUE) program of DSSAT. Following the recommended simulation runs of 3000, the GLUE estimated the best genotype-specific coefficients based on the experimental set-ups and observed data of the cultivar of interest (He et al., 2010). To validate the CERES-Rice Crop Model, the crop genetic coefficients were used to simulate the potential grain yield of PSB Rc82 and NSIC Rc160 in other test sites in PhilRice Nueva Ecija and PhilRice Midsayap in 2013 dry season and compared with the observed yield or yield potential under optimum crop management.

Figure 1
Schematic diagram of DSSAT CERES-Rice Model calibration to generate the crop genetic coefficients in one experimental site, validation to simulate potential rice yield in another experimental site, and comparison of simulated and observed yields and its acceptability based on normalized Root Mean Square Error (nRMSE), e.g., the predictive capability of the Rice Model is highly acceptable if nRMSE is 10% or lower, and unacceptable if more than 30%.



The Root Mean Square Error (RMSE) is used as a standard statistical metric to measure model performance in meteorology, air quality and climate research studies (Chai and Draxler 2014). The normalized Root Mean Square Error (nRMSE), on the other hand, is used to give a measure of the relative difference of simulated versus observed data (Nyangáu et al., 2014). Hence, nRMSE determines the degree of agreement between the simulated and observed yields.

The simulated and observed values are compared to validate the Rice Crop Model performance using the root mean square error (RMSE):

$$RMSE = \sqrt{\frac{\Sigma(S - M)^2}{n}}$$

Where:
S = simulated value
M = measured or actual value
n = number of observation

The normalized RMSE (nRMSE) is determined to express the mean difference as a percentage of the average of the observed values with the formula:

$$nRMSE = \frac{RMSE}{\frac{\Sigma M}{n}} * 100$$

Where:
S = simulated value
M = measured or actual value
n = number of observation

A model with an nRMSE of 10% or lower is considered excellent or highly acceptable (Loague and Green, 1991). A model is considered "good" if the nRMSE is greater than 10% but less than 20%, "fair" if the nRMSE is greater than 20% but less than 30%, and "poor" or unacceptable if the nRMSE is greater than 30%.

Simulation of potential yield in response to projected increase in air temperature

To assess the impact of projected increase in minimum air temperature (by 1.13^{0}C) and maximum air temperature (by 0.35^{0}C) on grain yield of PSB Rc82 and NSIC Rc160, simulation was done using the 2013 dry season data on soil, crop growth and development, crop management and weather from another experimental site in PhilRice Nueva Ecija. The fertilizer treatments considered were: (1) LCC-based N fertilizer application for a total 70 kg N/ha, (2) Complete fertilizer (i.e., 14-14-14-12S) applied at 14 days after transplanting followed by LCC-based N fertilizer application for a total of 112 kg N/ha, (3) Farmer's Practice of Growth Stage-based N fertilizer application for a total 185 kg N/ha, and (4) Complete fertilizer (i.e., 14-14-14-12S) applied at 14 DAT and Growth Stage-based N fertilizer application for a total of 217 kg N/ha. In each treatment, 40 kg P/ha and 40 kg K/ha were applied at 14 DAT.

The projected increases in minimum and maximum air temperatures in PhilRice Nueva Ecija were based on the studies of Peng et al. (2004) that used the International Rice Research Institute (IRRI) weather data from 1979 to 2003 (Figure 2). The simulated potential yields in response to the projected temperature increases were compared with the simulated potential yields in response to the actual minimum and maximum air temperatures from January to May 2013 for PSB Rc82 and NSIC 160.

Figure 2
Minimum and maximum air temperatures in PhilRice Nueva obtained from the Automatic Weather Station from date of transplanting to crop maturity in 2013 dry season. Based on actual air temperatures, minimum air temperature was projected to increase by 1.13^{0}C and maximum air temperature was projected to increase by 0.35^{0}C due to potential climate change.

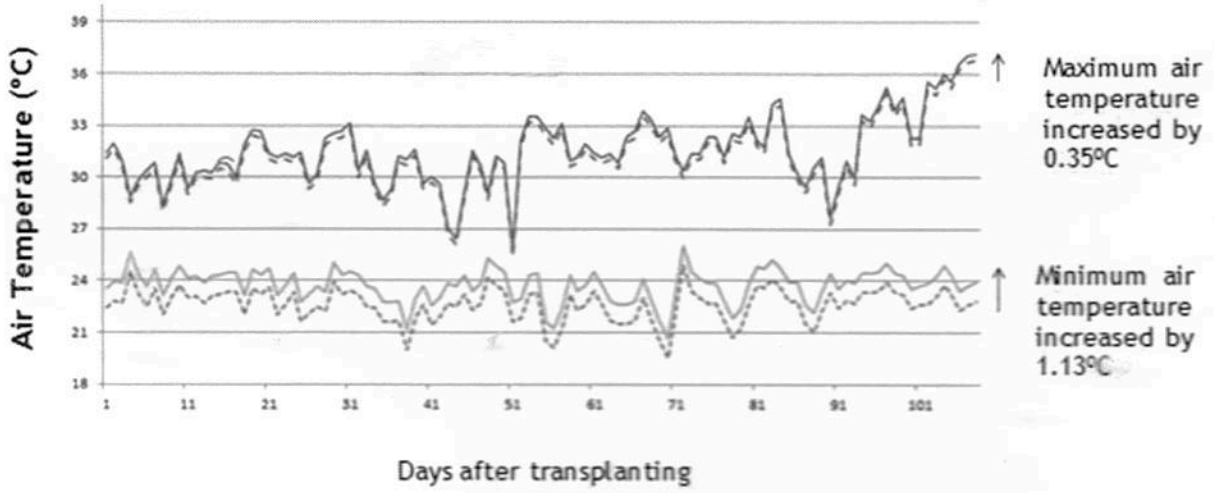


Table 2

Grain yields (t/ha) of PSB Rc82 and NSIC Rc160 in response to the following fertilizer treatments in PhilRice Nueva Ecija in 2012 dry season. (a) Control with no fertilizer. (b) Nitrogen Omission plot: –N+P+K). (c) Phosphorus Omission plot: +N-P+K). (d) Potassium Omission plot: +N+P-K). (e) LCC-based N application with 4:2:1 NPK ratio. (f) LCC-based N application with 4:1:2 NPK ratio. (g) Growth Stage-based N application with 3:1:1 NPK ratio. In a column, means followed by a common letter did not differ at 5% level of significance using LSD. When the total plant nutrient uptake in the grain and straw per ton rice yield (Yoshida, 1981; Dobermann and Fairhurst, 2000) was multiplied by the average yields of PSB Rc82 and NSIC Rc160 in the Nutrient Omission Plots, the estimated indigenous soil N, P, and K supplies were 92.4 kg N/ha, 28.9 kg P/ha and 179.4 kg K/ha.

Treatment	Total NPK applied (kg/ha)	Dry Season	
		PSB Rc82	NSIC Rc160
Control (No Fertilizer)	0	5.2 c	5.0 b
-N, +P, +K	0-40-40	5.2 c	5.8 b
+N, -P, +K	140-0-40	9.3 a	8.2 a
+N, +P, -K	137-40-0	9.5 a	8.9 a
LCC-based N (4:2:1 NPK)	131.25-52.5-26.25	8.7 a	8.2 a
LCC-based N (4:1:2 NPK)	131.25-26.25-52.5	9.2 a	8.2 a
Growth stage-based N (3:1:1 NPK)	105-35-35	7.2 b	8.0 a

RESULTS

Yield response to N fertilizer application

In PhilRice Nueva Ecija in 2012 dry season, the Control with no fertilizer had grain yields of 5.2 t/ha for PSB Rc82 and 5.0 t/ha for NSIC Rc160 (Table 2).

These yields were not significantly different than yields in the N-omission plot. In the P-omission plot, PSB Rc82 had a grain yield of 9.3 t/ha and NSIC Rc160, 8.2 t/ha. Yields in the P-omission plot were not significantly different than yields in the K-omission plot and LCC-based N fertilizer application treatments. The Growth Stage-based N fertilizer application had grain yields of 7.2 t/ha for PSB Rc82 and 8.0 t/ha for NSIC Rc160 and were lower than yields in LCC-based N fertilizer applications with varying NPK ratios and in P and K omission plots wherein N applications were based on LCC. The LCC-based N fertilizer application had agronomic N use efficiencies (ANUEs) of 26.8 to 30.9 kg grain/kg N for PSB Rc82 and 18.5 to 18.9 kg grain/kg N for NSIC Rc160. The Growth Stage-based N fertilizer application had ANUEs of 19.8 kg grain/kg N for PSB Rc82 and 21.3 kg grain/kg N for NSIC Rc160.

In PhilRice Nueva Ecija in 2012 wet season, the Control with no fertilizer had grain yields of 4.5 t/ha for PSB Rc82

Table 3

Grain yields (t/ha) of PSB Rc82 and NSIC Rc160 in response to the following fertilizer treatments in PhilRice Nueva Ecija in 2012 wet season. (a) Control with no fertilizer. (b) Nitrogen Omission plot only (i.e., –N+P+K) since indigenous soil P and K supplies were adequate in 2012 dry season. (c) LCC-based N application with 4:2:1 NPK ratio. (d) LCC-based N application with 4:1:2 NPK ratio. (e) Growth Stage-based N application with 3:1:1 NPK ratio. In a column, means followed by a common letter did not differ at 5% level of significance using LSD. When the total plant nutrient uptake in the grain and straw per ton rice yield (Yoshida, 1981; Dobermann and Fairhurst 2000) was multiplied by the average yields of PSB Rc82 and NSIC Rc160 in the Nitrogen Omission Plot, the estimated indigenous soil N supply was 80.2 kg N/ha.

Treatment	Total NPK applied (kg/ha)	Wet Season	
		PSB Rc82	NSIC Rc160
Control (No Fertilizer)	0	4.5 b	5.4 a
-N, +P, +K	0-30-30	4.6 b	4.6 b
LCC-based N (4:2:1 NPK)	57.5-23-11.5	6.3 a	6.3 a
LCC-based N (4:1:2 NPK)	57.5-11.5-23	6.4 a	6.1 a
Growth Stage-based N (3:1:1 NPK)	46-15.3-15.3	5.1 a	5.3 a

and 5.4 t/ha for NSIC Rc160 (Table 3). Yields in the Control with no fertilizer and N-omission plot were generally lower than yields with LCC-based N fertilizer application treatments (i.e., 6.1 to 6.4 t/ha) and Growth Stage-based N fertilizer (i.e., 5.1 to 5.3 t/ha) and did not differ between varieties. The LCC-based N fertilizer application had estimated ANUEs of 26.8 to 30.9 kg grain/kg N for PSB Rc82 and 18.5 to 18.9 kg grain/kg N for NSIC Rc160. The Growth Stage-based N fertilizer application had an ANUE of 19.8 kg grain/kg N for PSB Rc82 and 21.3 kg grain/kg N for NSIC Rc160.

Generally, results showed that higher yields and ANUEs were obtained with "real-time" LCC-based N fertilizer applications than with "fixed-time" and "fixed-dose" Growth Stage-based N fertilizer application. The indigenous soil P and K supplies were not limiting in PhilRice Nueva Ecija with Maligaya clay in 2012 dry and wet seasons. Likewise, indigenous soil P and K supplies were not limiting in PhilRice Midsayap with Kabacan clay (tabular data not shown). Although the estimated indigenous soil N supplies were 92.4 kg N/ha in dry season (Table 2) and 80.2 kg N/ha in wet season (Table 3), N fertilizer had to be applied at 131.25 to 140 kg N/ha to attain yield potentials of 8.2 to 9.5 t/ha in dry season (Table 2) and N fertilizer application of 57.5 kg N/ha to attain yield potentials of 6.1 to 6.4 t/ha in wet season (Table 3). Yields in dry season were higher than yields in wet season due to differences in irradiance. From 1 day after transplanting (DAT) to crop maturity at 101 DAT, the average irradiance was 19.65 MJ/day in dry season from January to May and 15.13 MJ/day in wet season from June to October.

Calibration and Validation of DSSAT CERES-Rice Model

Using the GLUE Program of the Rice Model, the genetic coefficients of PSB Rc82 and NSIC Rc160 were generated for eight crop developmental phases (Table 4). For PSB Rc82, the genetic coefficients were 566.1 at P1 for the basic vegetative phase and 1.19 at G4 for temperature tolerance coefficient. For NSIC Rc160, the genetic coefficients were 691.2 at P1 and 1.187 at G4.

To validate the Rice Model, the generated crop genetic coefficients from one experimental site in 2012 dry and wet seasons were used to simulate the potential yields of PSB Rc82 and NSIC Rc160 in another experimental site in PhilRice Nueva Ecija and PhilRice Midsayap in 2013 dry season. The simulated and observed yields in each experimental site were compared and level of acceptability was assessed using the normalized Root Mean Square Error (nRMSE). In PhilRice Nueva Ecija, the simulated potential yield of PSB Rc82 was lower than the observed yield at 0 and 60 kg N/ha and higher than the observed yield at 90 and 180 kg N/ha with highly acceptable nRMSE of 9.79% (Table 5). For NSIC Rc160, the simulated potential yields were higher than the observed yields across N fertilizer treatments with highly acceptable nRMSE of 7.50%.

Table 4

Genetic coefficients of PSB Rc82 and NSIC Rc160 generated using the GLUE program of the DSSAT CERES-Rice Model. Data used to generate the genetic coefficients were obtained from PhilRice Nueva Ecija in 2012 dry and wet seasons in Tables 2 and 3. The genetic coefficients were obtained at various crop growth and development phases. P1 is the basic vegetative phase. P2R is the extent to which the phasic development leading to panicle initiation is delayed. P5 is the beginning of grain filling to physiological maturity. P2O is the critical photoperiod or longest daylength at which the development occurs at a maximum rate. G1 is the potential spikelet number coefficient. G2 is the single grain weight in grams. G3 is the tillering coefficient. G4 is the temperature tolerance coefficient (Nyang'au, 2014).

Code for each Development Phase	Genetic Coefficient	
	PSB Rc82	NSIC Rc160
P1	566.1	691.2
P2R	171.2	91.71
P5	338.8	335.0
P2O	11.71	11.62
G1	59.09	55.64
G2	0.026	0.025
G3	0.954	0.552
G4	1.19	1.187

In PhilRice Midsayap, the simulated potential yields of PSB Rc82 were lower than the observed yields across N fertilizer treatments with an unacceptable nRMSE of 51.67% (Table 6). For NSIC Rc160, the simulated potential yields were lower than the observed yields across N fertilizer treatments with an unacceptable nRMSE of 37.63%.

SIMULATED YIELD IN RESPONSE TO PROJECTED INCREASE IN AIR TEMPERATURE DUE TO POTENTIAL CLIMATE CHANGE

From 1 day after transplanting (DAT) to crop maturity at 101 DAT, the actual minimum air temperatures ranged from 20.0°C to 24.9°C and the actual maximum air temperatures ranged from 26.8°C to 38.2°C (Figure 2). Using the crop genetic coefficients generated in PhilRice Nueva Ecija in 2012 dry and wet seasons (Table 4), the impact of projected increase in minimum air temperature by 1.13°C and maximum air temperature by 0.35°C from 1 to 101 DAT (Figure 2) on grain yield was simulated in PhilRice Nueva Ecija in 2013 dry season. The simulated potential yields in response to the projected temperature increases were compared with the simulated potential yields in response to the actual minimum and maximum air temperatures across N fertilizer treatments for PSB Rc82 and NSIC Rc160. Results showed that the simulated potential yields of PSB Rc82 (Figure 3) and NSIC Rc160 (Figure 4) generally decreased in response to the projected increase in minimum and maximum air temperatures across N fertilizer levels (i.e., 70 to 217 kg N/ha).

For PSB Rc82, there was a 0.46% increase in simulated potential yield in response to the projected temperature increase for LCC-based N application treatment with a total of 112 kg N/ha (Table 7). But in the remaining three N fertilizer treatments, the simulated potential yields decreased by 1.44 to 3.73% in response to the projected temperature increases.

Table 5

Comparison of simulated and observed grain yields of PSB Rc82 and NSIC Rc160 in different N fertilizer treatments in 2013 at PhilRice Nueva Ecija. Note: nRMSE = Normalized Root Mean Square Error and highly acceptable at 10% or lower, and unacceptable if more than 30%. Phosphorus and potassium levels were adequate in the experimental site in PhilRice Nueva Ecija.

Variety	Grain Yield (t/ha) at Different N Fertilizer Rates								nRMSE
	0 kg N/ha		60 kg N/ha		90 kg N/ha		180 kg N/ha		
	Simulated	Observed	Simulated	Observed	Simulated	Observed	Simulated	Observed	
PSB Rc82	3.96	5.03	6.06	6.2	6.9	6.74	7.13	6.62	9.79%
NSIC Rc160	4.47	4.55	5.91	5.34	6.54	6.15	6.66	6.2	7.50%

Table 6

Comparison of simulated and observed grain yields of PSB Rc82 and NSIC Rc160 in different fertilizer treatments in 2013 at PhilRice Midsayap. Note: nRMSE = Normalized Root Mean Square and highly acceptable at 10% or lower, and unacceptable if more than 30%. Phosphorus and potassium levels were adequate in the experimental site in PhilRice Midsayap.

Variety	Grain Yield (t/ha) at Different N Fertilizer Rates								nRMSE
	0 kg N/ha		70 kg N/ha		105 kg N/ha		140 kg N/ha		
	Simulated	Observed	Simulated	Observed	Simulated	Observed	Simulated	Observed	
PSB Rc82	1.55	3.48	4.79	3.43	4.77	3.50	5.74	3.39	51.67%
NSIC Rc160	1.96	3.77	4.45	3.72	4.83	3.41	4.83	3.57	37.63%

Figure 3
Simulated potential grain yield of PSB Rc82 in response to actual air temperature and projected minimum air temperature increase of 1.130C and maximum air temperature increase of 0.35°C. The fertilizer treatments were: (1) LCC-based N fertilizer application for total N of 70 kg/ha; (2) LCC-based N fertilizer application for total N of 112 kg/ha; (3) Farmer's Practice of Growth Stage-based N application for total N of 185 kg/ha; and (4) Growth Stage-based N application for total N of 217 kg/ha. Simulation of potential yield in response to projected air temperature increases was done only in PhilRice Nueva Ecija since previous yield simulation and comparison to observed yields in that site were highly acceptable based on nRMSE.

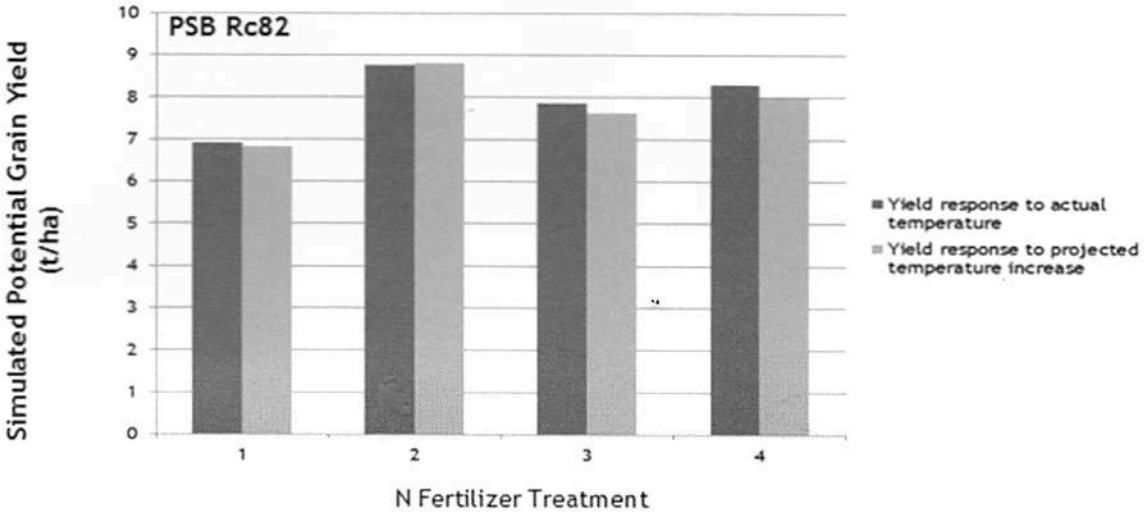
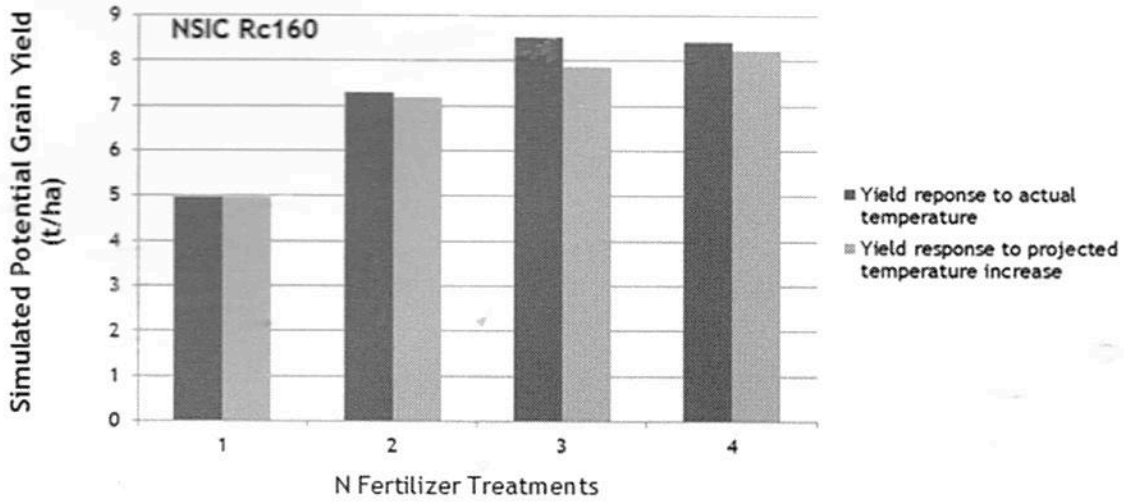


Figure 4
Simulated potential grain yields of NSIC Rc160 in response to actual air temperatures and projected minimum air temperature increase of 1.13°C and maximum air temperature increase of 0.35°C. The fertilizer treatments were: (1) LCC-based N fertilizer application for total N of 70 kg/ha; (2) LCC-based N fertilizer application for total N of 112 kg/ha; (3) Farmer's Practice of Growth Stage-based N application for total N of 185 kg/ha; and (4) Growth Stage-based N application for total N of 217 kg/ha. Simulation of potential yield in response to projected air temperature increases was done only in PhilRice Nueva Ecija since previous yield simulation and comparison to observed yields in that site were highly acceptable based on nRMSE.



For NSIC Rc160, there was a 0.2% increase in simulated potential yield in response to the projected temperature increase for LCC-based N application treatment with a total of 70 kg N/ha. Likewise, in the remaining three N fertilizer treatments, the simulated potential yields decreased by 1.63 to 7.76% in response to the projected temperature increases. Overall, for PSB Rc82 and NSIC Rc160, the simulated potential yields decreased by an average of 2.37% in response to the projected increase in minimum air temperature by 1.13°C and maximum air temperature by 0.35°C across N fertilizer levels.

DISCUSSION

Yield Response to N Fertilizer Application

The various fertilizer treatments in the present study allowed us to assess yield in response to indigenous soil N, P and K supplies through Nutrient Omission Plot Technique, and attainment of yield potential and relatively high ANUE in response to N fertilizer treatments under non-limiting indigenous soil P and K supplies. Yields of PSB Rc82 and NSIC Rc160 in the P-omission and K-omission plots did not differ but were significantly higher than yields in the Control with no fertilizer and N-omission plot. This indicated that indigenous soil P and K supplies were not as limiting as the indigenous soil N supply in PhilRice Nueva Ecija with Maligaya clay soil and in PhilRice Midsayap with Kabacan clay soil. Although the estimated indigenous soil N supply was 92.4 kg/ha in 2012 dry season (Table 2), yields of 5.0 to 5.8 t/ha in the Control with no fertilizer and N-omission plot were lowest among the N fertilizer treatments. Hence, to increase yield, additional N fertilizer had to be applied. Higher yield potentials of 8.7 to 9.5 t/ha for PSB Rc82 and 8.2 to 8.9 t/ha for NSIC Rc160 were achieved with LCC-based N fertilizer applications in dry season. Based on yield in the Control with no fertilizer or N-omission plot, the average yield increase with LCC-based N fertilizer application

Table 7

Simulated potential yields of PSB Rc82 and NSIC Rc160 in response to actual air temperature and projected increase in minimum air temperature by 1.13°C and projected increase in maximum air temperature by 0.35°C due to potential climate change in PhilRice Nueva Ecija in 2013 dry season. This was done only in PhilRice Nueva Ecija since previous comparison of simulated and observed yields in that site in 2012 dry season had highly acceptable nRMSE of less than 10%. Observed yields were highest at 8.9 t/ha for NSIC Rc160 and 9.5 t/ha for PSB Rc82 at 137 kg N/ha in 2012 dry season.

Variety	N fertilizer Treatment	Simulated Yield in Response to Actual Temperature (t/ha)	Simulated Yield in Response to Projected Temperature Increase (t/ha)	Difference (%), + or -
PSB Rc82	LCC-based N application for total of 70 kg/ha	6.92	6.82	-1.44
	LCC-based N application for total of 112 kg/ha	8.76	8.80	0.46
	Farmer's Practice Growth Stage-based N application for total of 185 kg/ha	7.86	7.64	-2.80
	Growth Stage-based N application for total of 217 kg/ha	8.30	7.99	-3.73
NSIC Rc160	LCC-based N application for total of 70 kg/ha	4.97	4.98	0.20
	LCC-based N application for total of 112 kg/ha	7.30	7.18	-1.63
	Farmer's Practice Growth Stage-based N application for total of 185 kg/ha	8.51	7.85	-7.76
	Growth Stage-based N application for total of 217 kg/ha	8.42	8.23	-2.26

was 62% for PSB Rc82 and NSIC Rc160. Yield trends in response to the different fertilizer treatments were similar in 2012 wet season. However, yields were lower in wet season due to lower irradiance.

The highest rice yield potentials of 8.9 to 9.5 t/ha in dry season and 6.1 to 6.4 t/ha in wet season attained with LCC-based N fertilizer application in our field studies were higher than the national average rice yield of 4.3 t/ha in dry season and 4.2 t/ha in wet season in irrigated lowland system (Philippine Rice Statistics, 2014). The wide yield gaps between yields in our experimental fields and on-farm national average rice yields suggested that there was a lot of scope to improve yields on-farm by improving crop management practices (e.g., use of certified seeds, appropriate water, nutrient, and pest and harvest management). The integrated crop management practices recommended to improve yield of irrigated lowland rice are written in the PalayCheck System Handbook (PhilRice, 2007).

Rice yield potential in response to N fertilizer application, other crop management practices, soil and weather data from the test site were important inputs in the calibration of the DSSAT Ceres–Rice Model.

CERES-Rice Crop Model Calibration and Validation

Following input of data on crop growth, development and yield, crop management like N fertilization and water management, general soil physical and chemical properties and weather in the CERES-Rice crop model, a set of eight crop genetic coefficients covering basic growth and development phases was generated for inbred varieties PSB Rc82 and NSIC Rc160. Genetic coefficients are sets of parameters that describe quantitatively genotype and environmental interactions (IBSNAT, 1993). The reliability of the crop genetic coefficients obtained from one experimental site can be tested by simulating the potential yield in another test site where the crop is grown under optimum water, nutrient, pest management or integrated crop management with the use of PalayCheck System for irrigated lowland rice (PhilRice, 2007). In PhilRice Nueva Ecija in 2013 dry season, the normalized Root Mean Square Errors (nRMSEs) of 9.79% for PSB Rc82 and 7.50% for NSIC Rc160 were highly acceptable. However, in PhilRice Midsayap in 2013 dry season, the nRMSEs of 51.67% for PSB Rc82 and 37.63% for NSIC Rc160 were unacceptable.

The unacceptable nRMSEs in PhilRice Midsayap can be attributed to yield loss due to rice bug and rice black bug. The yield reduction caused by rice bug (*Leptocorisa oratorius)* and rice black bug (*Scotinophara coarctata*) was estimated to be 30% to 40%. The rice bug can cause significant yield loss when there are 5-10 nymphs and adults per square meter during the milk and grain filling stages of rice (PhilRice-FAO, 2007). The rice black bug can cause significant yield loss when there are more than 20 nymphs and adults per square meter and more than 20% whiteheads during milk and grain filling stages.

Another factor that may have contributed to the high nRMSE could be the differences in soil properties (Table 1). In PhilRice Nueva Ecija where calibration was done to determine the crop genetic coefficients, the soil type was Maligaya clay with 2.61% organic matter, 0.11% total N, 6.35 ppm available P and 0.175 cmol+/kg exchangeable potassium. In PhilRice Midsayap, where validation or simulation was done, the soil type was Kabacan clay with 3.42% organic matter, 0.17% total N, 30 ppm available P and 0.37 cmol+/kg exchangeable potassium.

Simulated Yield in Response to Increased Air Temperature Due to Potential Climate Change

In response to the projected increase in minimum and maximum air temperatures due to potential climate change, the simulated yields generally decreased by an average of 2.37% for PSB Rc82 and NSIC Rc160 across N fertilizer treatments. With the use of the potential yield model ORYZA1, the predicted yields of PSB rice varieties were highest due to lower minimum and maximum air temperatures and more stable irradiance in Iguig, Cagayan, Philippines than in other rice breeding test sites in the Philippines (Cruz et al., 1996). The lower air temperature was associated with longer grain-filling duration that contributed to the high yield (Cruz et al., 2008).

Analysis of the weather data of the International Rice Research Institute in Los Baños, Laguna from 1979-2003 showed an increase of 0.35^{0}C in the annual mean maximum air temperature and an increase of 1.13^{0}C in the annual mean minimum air temperature (Peng et al., 2004). For each one-degree increase in the growing-season minimum air temperature, grain yield decreased by 10% and the influence of increased maximum temperature on yield was insignificant. Yield reduction may be due to increased plant maintenance respiration that lessens the amount of assimilates available for grain production and biomass accumulation (Monteith, 1981; Long, 1991; Amthor, 2000). But respiration is not enough to explain the reduced yields of other crops like maize, wheat, and soybeans in an increased night temperature (Peters et al., 1971).

Results of the simulated potential yields in response to increased minimum and maximum air temperatures were in agreement with previous studies on the impact of climate change on rice yields (Cruz et. al., 1996, 2008; Peng et al., 2004). To further assess the response of rice

to climate change, other weather conditions should also be considered. Changes in the pattern and amount of rainfall, coupled with rising temperature have a negative impact to worldwide agricultural productivity and yield (Ahmed and Fayyaz-ul-Hassan, 2011). High relative humidity and air temperature cause organ temperatures of rice to increase, leading to high spikelet sterility (Yan et al., 2010).

There is a need to assess the coping mechanisms of rice and other crops in response to climate change. Developing an optimum transplanting date during rice production can be a coping mechanism against climate change. Through the use of CERES v3.0 for simulation and ClimProb for predicting probable weather events, optimum transplanting dates for rice grown in rainfed condition were developed in the state of Kerala, India to coincide with the wet season (Saseendran et al., 1998). This ensures suitable supply of water during crop growth and development.

An increase in the ambient air temperature by 5°C can cause spikelet sterility in several rice cultivars by disrupting pollen production and anther dehiscence during or after anthesis (Prasad et al., 2006). Thus, developing new varieties with an onset of anthesis in the early hours of the morning is essential to lessen the damage of high air temperature during the plant's anthesis and reproduction. Looking for cultivars with low sensitivity to high temperature and studying the genes that govern this trait can help in the genotypic screening of rice against high air temperatures. However, beyond the effects of weather, we have to be cognizant of the other changes associated with climate change, i.e., increases in both abiotic stresses, such as drought, and biotic stresses such as pest and disease pressure (Boote et al., 2011), and strategies to cope with such stresses.

Climate is usually described in terms of the mean and variability of temperature, precipitation and wind over a period of time, ranging from months to millions of years (Le Treut et al., 2007). The unimpeded growth of greenhouse emissions is raising the average global temperatures (Takle et al., 2013). The consequences include changes in precipitation patterns, more extreme weather events, and shifting seasons. The overall impacts of climate change on agriculture are expected to be negative, threatening global food security (Harris, 2008; Takle et al., 2013). Climate change can affect food security through temperature increase, changing weather patterns, rising sea levels, and water scarcity. The changes in weather patterns caused by increasing rate of climate change cause shifts in growing seasons and unpredictable timing and volume of rainfall. This leads to greater uncertainty and risks for farmers, potentially eroding the value of traditional agricultural knowledge such as the planting season for a particular crop. Rising sea levels can contaminate freshwater resources, affect agricultural productivity, and increase the vulnerability of communities to typhoon surges. Contamination of freshwater resources due to rising sea levels could lead to regional tensions and open conflict between provinces for adequate water supply. If coping mechanisms will not be developed to protect crop development and productivity against the projected climate change, the threat to food security will continue to increase.

CONCLUSION

The Nutrient Omission Plot Technique employed in the experimental fields of PhilRice Nueva Ecija and PhilRice Midsayap indicated that the estimated indigenous soil P and K supplies were not as limiting as the indigenous soil N supply in dry and wet seasons.

Although the estimated indigenous soil N supplies ranged from 80.2 to 92.4 kg N/ha, additional N fertilizer of 137 kg N/ha following "real-time" LCC-based N fertilizer application was needed to achieve the highest yield potentials of 9.5 t/ha for PSB Rc82 and 8.9 t/ha for NSIC Rc160 in dry season. In wet season, LCC-based N fertilizer application of 57.5 kg N/ha was needed to achieve the highest yield potentials of 6.4 t/ha for PSB Rc82 and 6.1 t/ha for NSIC Rc160. Generally, "real-time" LCC-based N fertilizer application attained higher yield potential and agronomic N use efficiency than the "fixed-time" and "fixed-dose" Growth Stage-based N fertilizer application.

The non-limiting indigenous soil P and K supplies in our test sites allowed us to assess more directly the attainment of rice yield potential in response to different N fertilizer levels.

Crop growth, development and yield potential, soil properties, weather, and crop management data from one test site were important inputs in the DSSAT Ceres-Rice Model calibration to generate the crop genetic coefficients (i.e., parameters that measure genotype x environment interactions) that ranged from 0.026 to 566.1 for PSB Rc82 and from 0.025 to 691.2 for NSIC Rc160 for the 8 crop development phases.

The crop genetic coefficients were validated by simulating the potential yield in another test site and compared to the observed yields. The predictive capability of the Rice Model in PhilRice Nueva Ecija was highly acceptable since the normalized Root Mean Square Error (nRMSE) was less than 10%. However, the nRMSE in PhilRice Midsayap was higher than 30%, therefore unacceptable, due the pest damage to the rice crop and possibly due to differences in soil properties between PhilRice Nueva Ecija with Maligaya clay soil and PhilRice Midsayap with Kabacan clay soil.

Based on literature, the minimum air temperature and maximum air temperature were increased by 1.13^{0}C and 0.35^{0}C, respectively, due to potential climate change. In response to the projected air temperature increases, the simulated potential rice yields in PhilRice Nueva Ecija decreased by an average of 2.37% possibly due to the decrease in grain filling duration and high spikelet sterility.

In addition to the potential impact of climate change on rice crop productivity, we have to be cognizant of the increases in abiotic and biotic stresses and the threat to food security due to climate change.

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