

University of São Paulo  
“Luiz de Queiroz” College of Agriculture

Rice performance, water and nitrogen efficiency in different irrigation  
regimes in tropical lowland

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Thesis presented to obtain the degree of Doctor in  
Science. Area: Crop Science

Piracicaba  
2017

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Rice performance, water and nitrogen efficiency in different irrigation regimes in tropical  
lowland

versão revisada de acordo com a resolução CoPGr 6018 de 2011

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Thesis presented to obtain the degree of Doctor in  
Science. Area: Crop Science

Piracicaba  
2017

**Dados Internacionais de Catalogação na Publicação**  
**DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP**

Reis, André Fróes de Borja

Rice performance, water and nitrogen efficiency in different irrigation regimes in tropical lowland / André Fróes de Borja Reis. - - versão revisada de acordo com a resolução CoPGr 6018 de 2011. - - Piracicaba, 2017.

77 p.

Tese (Doutorado) - - USP / Escola Superior de Agricultura “Luiz de Queiroz”

1. *Oryza sativa* L. 2. Eficiência isotópica de nitrogênio 3. Evapotranspiração  
4. Arroz aeróbico 5. Molhamento e secagem alternados. 6. Plintossolo I. Título

## DEDICATION

*For agronomists, technicians, growers, researchers or whoever spends the day thinking about rational agriculture and environmental conservation as me. I hope the ideas here set out will help you to answer your questions*

## ACKNOWLEDGMENTS

This PhD thesis is result of efforts and collaboration of many people, and in order to avoid the mistake of forgetting someone I prefer express my gratitude for the institutions which each person was involved rather than individually.

I am grateful to “Luiz de Queiroz” College of Agriculture for the bachelor’s degree of agronomic engineer I got here back in 2007 and the opportunity to proceed my doctoral degree 6 years later. Agronomic engineer is how I define myself and its being in the core of my actions since then.

My sincere thanks for Two Rivers Farms who believed in this research idea since very beginning and hosted the field experiment, granted partially the research funds and let me use their impressive production apparatus as a field laboratory.

I recognize the importance of following public funding agencies: CNPq for granted the scholarship (140193/2013-8), FAPESP for funding the infra-structure of Laboratório Multiusuário em Produção Vegetal, and CAPES for Programa de Doutorado Sanduíche no Exterior – PDSE (10810/14-5). The public investment in research is a worthwhile step for this country development.

For Crop Science graduate program, thanks for the commitment and kindly help every time I needed.

I also would like to thank Embrapa - Tocantins to help me with pre-processing samples and support equipment for field measurements.

In Lagoa da Confusão city, I cannot forget the helpful support of Foliar Aviação Agrícola, Impar consultoria, Plantar representações, Cultivar representações, Evidência agrícola, SINDIATO and Brazeiro Sementes. My sincere respect for the pioneers.

All the folks from Laboratório Multiusuário em Produção Vegetal, GEA Grupo de Experimentação Agrícola, Laboratório de Isótopos Estáveis CENA / USP, Laboratório de Química Analítica – LCE. Thanks for the help, you guys were awesome.

I also appreciate all the help from Agroecosystems lab from UC Davis that gave meu thoughtful suggestion to clarify the manuscript and English review.

And the most important gratitude is for my family. Without their support and love I never would have made it.

Thank you all

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## RESUMO

**Performance do arroz, eficiência de água e nitrogênio em diferentes regimes de irrigação em planícies de inundação tropical**

A cultura do arroz (*Oryza sativa* L.) tem importante papel em fornecer alimento à crescente população mundial, e o incremento da produção virá não só do ganho de eficiência de áreas em uso, mas também da incorporação de novas áreas. As planícies tropicais do norte do Brasil já estão sendo convertidas para cultivo de arroz, apesar da falta de conhecimento científico e práticas agrícolas apropriadas às condições locais. Essa região apresenta solos com minerais de argila intemperizados e propriedades distintas daqueles de regiões tradicionais de cultivo, e a transferência das práticas agrícolas desenvolvidas para outros agroecossistemas têm resultado em produtividades variáveis e uso inadequado de insumos. A água é principal recurso no cultivo do arroz, e a irrigação com inundação constante é largamente disseminada pelo mundo. Regimes alternativos de irrigação a fim de economizar água tem sido propostos em razão da ameaça de escassez de água, e resultados promissores estimulam a disseminação dessa técnica, apesar de prejuízos à produtividade em razão do estresse por seca e/ou menor disponibilidade de nitrogênio (N) do solo. A umidade do solo e a quantidade de oxigênio interferem na dinâmica de N e podem favorecer processos de perdas e alterar a resposta do cultivo à adubação nitrogenada. Nas planícies tropicais do Brasil a disponibilidade de água e N são limitadas, e a inundação contínua não é sustentável em razão da quantidade de água requerida associada a baixa produtividade da cultura. Portanto, esta pesquisa foi realizada com o objetivo de estudar de forma integrada o regime de irrigação e o uso de N, e performance da cultura em várzea tropical. O experimento foi realizado durante três anos em Plintossolo no estado do Tocantins. Na primeira etapa da pesquisa procurou-se identificar o regime de irrigação que proporciona maior eficiência de uso da água (WUE), eficiência de uso de nitrogênio (NUE) e performance de cultivo com N nativo do solo e quando aplicado N fertilizante. Na segunda fase comparou-se o regime de irrigação que proporcionou melhores índices de eficiência de água e N ao regime de lâmina contínua com a hipótese que a relação entre aplicação de fertilizante N e componentes de produção seriam diferentes entre os regimes. Na primeira fase adotou-se delineamento de parcela subdividida com 5 regimes de irrigação (IR): lâmina alternada de inundação e drenagem em ciclo curto (AWDS); lâmina alternada de inundação e drenagem em ciclo longo (AWDL); lâmina contínua (CF); sem formação de lâmina e solo aeróbico (NF); e sem formação de lâmina e solo saturado (SS). Nas subparcelas avaliou-se as doses de N fertilizante: 0 e 150 kg ha<sup>-1</sup>. A performance de cultivo foi afetada pelo IR e dose de N. Na média dos 3 anos, o acúmulo de nitrogênio na parte aérea (NU) foi 32% maior em NF do que em todos os outros regimes de irrigação. A produtividade de grãos média entre as duas doses de N foi 7,2, 8,8 e 7,5 Mg ha<sup>-1</sup> em NF e 5,6, 8,2, 6,9 Mg ha<sup>-1</sup> in CF, respectivamente em 2014, 2015 e 2016. A NUE foi de 81,5% de recuperação de <sup>15</sup>N em NF e 62, 61, 56 e 26% em SS, AWDS, AWDL e CF, respectivamente. A média de WUE em relação a água aplicada foi 0,7 kg grão m<sup>-3</sup> em NF e 0,47, 0,40, 0,35, 0,32 kg grão m<sup>-3</sup> água em SS, AWDS, AWDL e CF, respectivamente. Na segunda fase adotou-se o delineamento experimental de parcelas subdivididas com inundação contínua (CF) e sem formação de lâmina e solo aeróbico (NF) na parcela principal e aplicação de N fertilizante (0, 50, 150, 250 kg ha<sup>-1</sup>) nas safras 2014, 2015, 2016. Biomassa (AGB) acúmulo de nitrogênio (NU) e índice de área foliar (LAI) foram avaliados ao longo do desenvolvimento da cultura nas doses de 0 e 150 kg ha<sup>-1</sup> nas safras de 2015 e 2016. Assim como a relação entre AGB, NU densidade de panícula (PD), número de espiguetas (NS) e produtividade de grãos (GY) na maturidade fisiológica a condição aeróbica proporcionou maior NU ao longo do desenvolvimento e LAI maior ou igual a CF. Na colheita, NU e PD tiveram diferentes repostas ao nitrogênio fertilizante quando em NF ou CF, mostrando NU 18% maior em NF assim como PD 27% maior em NF do que em CF na média de anos e dose de N. A resposta da GY ao nitrogênio fertilizante também foi diferente quando em NF ou CF, e na médias de dose de N fertilizante, NF proporcionou produtividade 20% e 12% maior do que CF em 2014 e 2015, respectivamente. Conclui-se nessa pesquisa que nesse agroecossistema, desde de que que não ocorra estresse por seca, o arroz cultivado em sistema de irrigação sem formação de lâmina e solo aeróbico é mais eficiente no uso de água e nitrogênio. E ainda, com melhor desempenho de produtividade e menor

dependência de N fertilizante em comparação ao cultivado em lâmina contínua ou qualquer outro regime alternativo que economize água.

Palavras-chave: *Oryza sativa* L.; Eficiência isotópica de nitrogênio; Evapotranspiração; Arroz aeróbico; Molhamento e secagem alternados; Plintossol

## ABSTRACT

### Rice performance, water and nitrogen efficiency in different irrigation regimes in tropical lowland

Rice (*Oryza sativa* L.) crop has an important role to attend the food demand of a growing world population, and the production increment will come not just from increasing efficiency in the current cropland, but also from expansion to new areas. The tropical lowland plains of northern Brazil are already being converted to rice production, although the lack of scientific knowledge and agricultural practices suited local conditions. This region presents soils with weathered clays with distinct properties from those grown in traditional rice regions, and deploying agricultural practices developed to others agroecosystem lead to instable crop yield inadequate uses of agricultural inputs. Water is the greatest resource that rice crop relies on, and worldwide continuous flooding is broadly used. Alternative irrigations regimes with purpose to save water have been proposed due the threatens of water scarcity, and promising results stimulate this approach, despite grain yield penalties due drought stress and/or decrease of soil N availability. The soil moisture and oxygen amount interfere on nitrogen dynamics, thus can enhance loss process and change crop response to N fertilizer. In tropical lowland region of Brazil water and nitrogen are limited and continuous flooding irrigation is not sustainable due huge water amount used associated to low grain yields. Therefore, this research was performed aiming an integrated assessment of irrigation regimes, nitrogen use and crop performance in rice crop in Brazilian tropical lowland. The field experiment was done during three years in a Plinthaquults soil at Tocantins. The first stage was aimed to identify the irrigation regime which provides the best water use efficiency (WUE), nitrogen use efficiency (NUE) and crop performance with indigenous N soil supply and nitrogen fertilizer. The second stage compared the best observed irrigation regime to continuous flooding, and the nitrogen relationship to crop components along their development and harvest. The first experiment was a split-plot design with 5 irrigation regime (IR) in main plot: alternate wet and dry in short cycle (AWDS); alternate wet and dry in long cycle (AWDL); Continuous flooding (CF); Non-flooding aerobic (NF); saturated soil without ponding water (SS). And subplot was N fertilizer rate: 0 and 150 kg ha<sup>-1</sup> of N. Crop performance was affected by IR and N level. In the average of three years nitrogen uptake (NU) was 32% higher in NF than any other irrigation regime. Grain yield across N level was 7.2, 8.8 and 7.5 Mg ha<sup>-1</sup> in NF and in CF were 5.6, 8.2 and 6.9 Mg ha<sup>-1</sup>, respectively in 2014, 2015 and 2016. The isotopic NUE showed total recovery of 81.5 % of <sup>15</sup>N in NF and 62, 61, 56, 56% in SS, AWDS, AWDL, CF respectively. The average WUE of delivered water was 0.7 kg grain m<sup>-3</sup> in NF, and 0.47, 0.40, 0.35, 0.32 kg grain m<sup>-3</sup> water in SS, AWDS, AWDL and CF, respectively. The second experiment was a split-plot design with: continuous flooding (CF) and non-flooding aerobic (NF) in the main plot and fertilizer nitrogen (0, 50, 150 and 250 kg ha<sup>-1</sup>) in the subplot during the 2014, 2015 and 2016. Biomass (AGB) nitrogen uptake (NU) and leaf area index (LAI) were observed along crop development in the 0 and 150 subplots in 2015 and 2016 growing seasons. As was the relationship between AGB, NU, panicle density (PD), spikelet number (NS) and grain yield (GY) at physiological maturity. The aerobic rice provided higher NU throughout rice development and LAI equal or superior to CF. At harvesting, NU and PD had different relationships to N rates among CF and NF, with NF showing 18% higher NU as 27% PD higher than CF across years and N rate. The grain GY relationship to N rates was also distinct in 2014 and 2015, and GY across N rates in NF was 20% and 12% higher than CF for 2014 and 2015, respectively. This research concludes for such agroecosystem, as long there is no drought stress, rice crop in non-flooding aerobic irrigation regime performs higher efficiency of water and N use, and moreover presents better overall crop performance with less need to nitrogen fertilizer than traditional continuous flooding or any other water saving irrigation regime.

Keywords: *Oryza sativa* L.; Nitrogen isotopic efficiency; Evapotranspiration; Aerobic rice; Alternate wet and dry; Ultisol

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## 1. INTRODUCTION

The world produces 700 million tons of rice grain per year, and rice crop (*Oryza sativa* L.) occupies 160 million hectares (Sharkey et al., 2016). Most of this production occurs in a flooded irrigation system that consumes 35-45% of all water intended for crop irrigation, which in turn consumes 70% of all the water used by all human activity (Bouman, 2016). This extraordinary consumption is not in vain, and rice production worldwide provides the daily carbohydrate requirement to 3 billion people (Maclean et al., 2013). In Brazil, rice is the sixth crop in planted area, with annual production of 10.6 million tons (IBGE, 2017) and average water consumption of 6,000 m<sup>3</sup> ha<sup>-1</sup> (Machado et al., 2006).

Despite impressive numbers, production needs to grow. It is estimated that in 2050, the demand for this cereal will be 830 million tons (20% increase) (Thomson, 2003). For this, the productive processes in the areas currently in use need to have their efficiency increased, in addition to the incorporation of new areas into the production system. In Brazil, there are approximately 15 million hectares of tropical lowlands suitable for the production of rice not yet used in the sedimentation plains of the north and northeast regions (Coelho et al., 2006).

These potential areas have soil and climate properties different from the traditional production areas in southern Brazil or even Asia, and lack information on crop performance and agronomic practices suited to local conditions. In traditional cropping regions, irrigation management with continuous flooding is widely disseminated and recommended (Bouman et al., 2007), although since the beginning of the 2000s, the global threat of water scarcity has stimulated the development of alternative irrigation management that proposes water saving (Tuong et al., 2005). Among the proposed alternatives that are still under discussion, none of them is widely adequate for the Brazilian tropical lowland, due to its different characteristics, mainly regarding soil.

In Brazil, floodplains show predominance of hydromorphic soils, of the Gleisol and Ultisol orders, with shallow subsurface horizon or temporary elevation of the water table (Stone, 2005). Although they were young soils, they have developed from alluvial deposition of highly weathered sediments from Oxisols coming from older geomorphological surfaces of the Cerrado (Sanchez, 1976). The clay fraction of these sediments contains weathered clays of the 1: 1 type (kaolinite and Fe and Al oxides) that form stable microstructure of the size of sand fraction (Tomasella et al., 2000), and which do not easily disperse even after tillage. In addition, the saturation of Al in CEC provides behavior of flocculation rather than dispersion making inefficient puddling (Sharma and De Datta, 1985; Stone, 2005). The results are soils with saturated hydraulic conductivity ( $K_{sat}$ ) above 1m d<sup>-1</sup> (Martins, 2004), and potential loss of water by percolation (Aimrun et al., 2004).

And how much does this percolation loss represent in the demand for water by the rice crop? Evapotranspiration (ET) of the crop is 400 to 800 mm water per crop cycle (Bouman et al., 2005; Guerra et al., 1998; Linqvist et al., 2015) and the efficiency of conversion of ET water into grain is strongly related to genetic factors, although agricultural management also influences (Foulkes et al., 2009; Tuong et al., 2005). To meet the demand for ET, there are water losses in the irrigation process, with the most important being percolation, runoff and direct evaporation from the ponding water. These losses cause the total water supplied to the crop to be up to 2.5 times higher than the demand for ET (Wickham and Singh, 1978).

The volume of water used unrelated to ET is the main target of research that proposes water saving at field basis, and innovative rice production system such alternate wetting and drying cycles, saturated soil culture, aerobic rice and sprinkler irrigation are the most important approaches (Tuong et al., 2005). Some of these

techniques are promising with increments of 5-30% in water use efficiency (WUE) (Cabangon et al., 2004; de Vries et al., 2010; Lu et al., 2000; Patel et al., 2010). However, the penalization in crop productivity is also reported, and depreciation between 0% and 38% leads adoption to proceed with caution and require site-specific adaptation. The lower productivity is attributed to the direct consequence of inadequate water supply to meet ET (Belder et al., 2005a; Kato et al., 2009; Patel et al., 2010; Singh et al., 2001), to genotypes not adapted to soil water tension around field capacity (Yao et al., 2012), or the indirect influence of irrigation on the lower availability of nitrogen in the soil (Dong et al., 2012; Kadiyala et al., 2015; Zhou et al., 2011).

The dynamics of N in the soil is controlled by soil moisture (Van Cleemput et al., 2007) and by the presence of oxygen (Bouldin, 1986). The substitution of oxygen by water in the soil pores establishes an anaerobic environment, with reduction of the redox potential up to +200 mV (Fageria et al., 2003; Ponnampereuma, 1972). In this reducing condition, N coming from the mineralization of the organic matter or applied in the form of ammonia fertilizers remains in its form of maximum reduction ( $\text{NH}_4^+$ ) presenting NOX -3. Nitrogen in this form is retained in soil CEC, immobilized by microbial activity or by plant uptake (Reddy et al., 1984). Loss pathways such as leaching, volatilization and denitrification are disadvantaged (Borin et al., 2016). And as a result, the efficiency of N use by the plant is high (Pan et al., 2017; Wang et al., 2016). Therefore, research centers and rice manuals recommend continuous flooding in order to obtain high productivity due to better use of nitrogen (Maclean et al., 2013; SOSBAI, 2014).

On the other hand, in alternative irrigation regimes, as the ponding water is withdrawn and the soil is drained, the soil pores are filled with air again. Aerobic condition is established and the redox potential reaches values above + 400 mV (Ponnampereuma, 1972). Ammonium ( $\text{N-NH}_4^+$ ) is oxidized to nitrate ( $\text{N-NO}_3^-$ ) by respiration of chemoautotrophic bacteria of the genera *Nitrosomonas* and *Nitrobacter*, which use the N-H bonds of ammonia as energy source (Mosier et al., 1996; Subbarao et al., 2006). The result is that nitrogen is taken to its maximum oxidation and has the NOX of +5. Once in the form of nitrate, N can be lost by leaching if the soil properties are favorable, because nitrate is an anion and is not retained in the CEC (Akkal-Corfini et al., 2010; Powlson, 1993). Moreover, as nitrate, nitrogen can be denitrified, which is the most important process of nitrogen loss in flooded soils (Bouman et al., 2007). According to Stevens and Laughlin (1998), the loss of N through  $\text{N}_2$  in soils may reach  $125 \text{ kg ha}^{-1}$ , and  $\text{N}_2\text{O}$  up to  $8 \text{ kg ha}^{-1}$ . The potential loss of N due to alternative irrigation regimes can lead to reduction in rice performance (Belder et al., 2005b; Pan et al., 2017; Wang et al., 2016).

Nitrogen is the nutrient most demanded by irrigated rice crops and the availability to plant uptake during development is one of the most limiting factors of productivity (Fageria and Baligar, 2005). Nitrogen is related to tillering, leaf area index (LAI), number of spikelet, panicle sterility and grain weight (Fageria and Baligar, 2001; Wang et al., 2016; Yao et al., 2012; Ye et al., 2013), which are the parameters that compose crop productivity. Moreover, nitrogen interferes with plant height, LAI and biomass and they are related to evapotranspiration and water use (Alberto et al., 2011; Cabangon et al., 2004; Tan et al., 2015). Therefore, water management, nitrogen use efficiency and rice productivity are related issues that should be addressed in an integrated manner.

The pioneer growers on the agricultural frontier of the Cerrado irrigation plains already are aware of this. Nitrogen fertilizer consumption rarely meet the crop planning, and additional N topdressing is required throughout the growing season. The continuous ponding water reduces its productive capacity and increases the pumping time in the rivers, which does not always have enough water. The result is variable productivities and economic instability. The management of irrigation and nitrogen is made in trial and error, harvest after harvest,

and the extension agronomist does not have the answer as well. The solution of how to produce rice in this productive environment with rational use of water and nitrogen fertilizer is 10 years behind. The goal of this work was to propose at a field basis, the irrigation regime that provides the highest water use efficiency, nitrogen use efficiency, and support the highest productivity in weathered tropical lowlands.

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## 2. NON-FLOODING REGIME IMPROVES RICE YIELD AND WATER USE EFFICIENCY IN LOWLAND TROPICAL ENVIRONMENT

### Abstract

World rice production is facing the challenge of produce more grains with less water input. Due that water saving irrigation regimes are being proposed along different regions. However, a promising new agroecosystem in tropical weathered lowland, which has fare distinct soil properties from those in traditional grown regions, still have the most adequate irrigation regimes (IR) and Nitrogen management unknown. A three-year field experiment was done in a lowland Plinthtaquults in order to investigate crop performance, water use efficiency (WUE) and nitrogen efficiency in 5 IR: alternate wet and dry in short cycle (AWDS); alternate wet and dry in long cycle (AWDL); Continuous flooding (CF); Non-flooding aerobic (NF); saturated soil without ponding water (SS). The experimental design was split-plot with IR in main plot and nitrogen fertilizer (0 and 150 kg ha<sup>-1</sup> of N) in subplot. Crop performance was affected by IR and N level. In the average of three years Nitrogen uptake (NU) at 150 kg ha<sup>-1</sup> of N fertilizer was 32% higher in NF than any other IR. Grain yield (GY) across N level was 7.2, 8.8 and 7.5 Mg ha<sup>-1</sup> in NF and in CF were 5.6, 8.2 and 6.9 Mg ha<sup>-1</sup> respectively in 2014, 2015 and 2016. The isotopic efficiency of N fertilizer showed total recovery of 81.5 % of <sup>15</sup>N in NF and 62, 61, 56, 56% in SS, AWDS, AWDL, CF respectively. The WUE of water input was three-fold lower than WUE of evapotranspiration. WUE of input water was 0.7 grain m<sup>-3</sup> in NF, and 0.47, 0.40, 0.35 and 0.32 grain m<sup>-3</sup> in SS, CF, AWDS, AWDL and CF. This research shows for such environment, as long there is no drought stress, rice performs better and has higher efficiency of water and N use when cultivated in non-flooding irrigation regime

Keywords: Nitrogen; Isotopic recovery; Apparent use efficiency; Evapotranspiration; Ultisol

### 2.1. Introduction

Rice (*Oryza sativa*) has an important role to provide affordable carbohydrate for a fast-growing world population in the coming decades (Maclean et al., 2013). By 2050 rice production is expected to increase by 20% to meet the world demand, as well as avoid price spikes and social negative impacts (Sharkey et al., 2016; Thomson, 2003).

To meet such a high demand, productivity must increase in the current cropland, as well as expand to new areas suitable to grow rice. Despite the potential for rice growth in under developed regions such as South America and West Africa (Balasubramanian et al., 2007; Coelho et al., 2006; Guerra et al., 1998), lack of irrigation water is often the primary limitation in these places. Yet, even in regions where water is available there is increasing pressure to demonstrate efficient use of water resources, thus highlighting the need to continually develop new agronomic practices to improve crop production in an increasingly resource limited world.

One approach for water saving is to develop alternative irrigation strategies that can reduce water use, but maintain minimal effect on crop yield (Bouman et al., 2007). Some alternative irrigation strategies for rice production in tropical regions include: a) aerobic rice in which fields are not flooded and soil is kept unsaturated throughout most of the season, usually being rain fed or sprinkler-irrigated (Alberto et al., 2011; Belder et al., 2005a; Bouman et al., 2005; M. D. M. Kadiyala et al., 2015; Lampayan et al., 2010); b) alternate wetting and drying, in which the crop is subjected to intermittent periods of flooding and drying (Awio et al., 2015; Belder et al., 2005b; de Vries et al., 2010; Dong et al., 2012); and c) saturated soil systems where soil pores are kept

saturated, but without ponding water (Bouman et al., 2007; Bouman and Tuong, 2001; Lu et al., 2000). All these strategies resulted in increased water use efficiency (WUE), but yields were often impaired. Therefore, to achieve maximum rice yields in the diversity of tropical environments, onsite soil properties must be understood to propose an efficient water management strategy that maintains crop performance.

In Brazil, one of the most promising regions for rice cropland to expand is mostly settled on alluvial soils with high hydraulic conductivity, very stable micro-structure, clay fraction rich in Fe-Al oxides and kaolinite, low CEC and pH, and high contents of Al. (Buol et al., 2011; Sanchez, 1976). The upper horizons of these soils have a very stable micro-structure that does not disperse when subjected to tillage, and due to the lack of 2:1 clays, is relatively ineffective for puddling (Balasubramanian et al., 2007; Sharma and De Datta, 1985; Stone, 2005). Such features evidence the stark contrast of these soils relative to common expected characteristics for rice production. According to Bouldin (1986), an ideal lowland soil for rice production presents a high-density layer at a depth of few centimeters that restricts the percolation of water. Additionally, there is homogenous soil bulk for uniform root development and nitrogen stabilization in the form of ammonium.

Nitrogen is the most essential nutrient in rice production, and irrigation management directly affects its availability for rice uptake and loss pathways (Fageria and Baligar, 2005). Under continuous flooding the soil remains in a reduced anaerobic state and ammonium nitrification is restricted to a few oxidation sites, thus becoming negligible (Reddy et al., 1984; Van Cleemput et al., 2007). However, varying water regimes or high percolation rates can trigger nitrification and expose N to loss pathways such as denitrification and leaching (Aulakh and Bijay-Singh, 1996; Bouldin, 1986). Therefore, any water management strategy in lowland rice must take in to account N loss pathways in soil to avoid decreased availability for crop uptake and increased losses to environment. This study evaluated grain yield, crop growth, water use efficiency, and nitrogen use efficiency of lowland rice subjected to varying irrigation and nitrogen regimes in a tropical weathered plain of central Brazil.

## 2.2. Material and Methods

### 2.2.1. Site Description

On farm experiments were established the region of Lagoa da Confusão, State of Tocantins, in Northern Brazil (10°46'39.80"S; 49°55'20.94"W and 190 m ASL) during the rainy summer seasons of 2014, 2015 and 2016. The local climate is classified as Aw – Tropical wet and dry climate (Koeppen) - with average rainfall of 1800 mm, mostly from September to May and average temperature of 26.7. Figure 1 shows rainfall and daily temperature for the three-year period of this study.

The soil is classified as a Plinthaquults (US Taxonomy) with a Plinthic horizon within 60 cm of depth which has limited water percolation, without an impermeable layer. The physiochemical properties are shown in Table 1. The saturated theta ( $\theta_s$ ) at 0-0.1m depth was 0.606 and 0.639  $\text{cm}^3 \text{cm}^{-3}$ , in 2015 and 2014/2016 sites, respectively. And  $\theta_s$  to B plinthic (0.6 m and bellow) was 0.485 and 0.458, in 2015 and 2014/2016, respectively. The macro, meso and micro porous size distribution for A horizon are 45%, 15%, 39% and in B plinthic are: 31.9%, 4.5%, 63.6%, respectively.

Table 1. Chemical and physical properties of field experiment soil, Plinthaquults. Values describes 0-15 cm depth

	pH	Organic Matter	P	S	K	Ca	Mg	Al	H	CEC	Clay	Silt	Sand	Ki <sup>1</sup>	Bulk Density
Year		g dm <sup>-3</sup>	-mg dm <sup>-3</sup> -	-----mmolc dm <sup>-3</sup> -----						---- (%) ----			g cm <sup>-3</sup>		
2015	5.5	55	27	12	5.3	47	9	0	34	95	34	28	39	1.67	0.88
2014 & 2016	5.7	50	16	7	3.0	27	15	0	34	79	42	23	35	1.8	0.89

<sup>1</sup> Ki = Weathering coefficient. It is related to weathering degree of clay fraction: SiO<sub>2</sub> Al<sub>2</sub>O<sub>3</sub><sup>-1</sup>.

The area was converted to agricultural use around 6 years before the start of the experiment under crop rotation of rice during rainy season and soybean irrigated by sub -irrigation during the dry season (May to September).

### 2.2.2. Field Experiment

The experimental design was a split-plot randomized complete block with four replications in three consecutive years. Main plot treatment was irrigation regime (IR): Continuous Flooding (CF); Alternate Wet and Dry in a Short cycle (seven days flooded and 7 days non-flooded) (AWDS); Alternate Wet and Dry in Long cycle (21 days flooded and 7 days non- flooded) (AWDL); saturated soil without flooding (SS); and Non-flooded rainfed aerobic (NF). Unless NF, all treatments had water input from rain and irrigation. Each main plot was further split into sub plots of two N treatments: 0 and 150 kg N ha<sup>-1</sup>.

The main plots consisted of a 105 m<sup>2</sup> hydrologically independent plots due to 50 cm high bund and 60 cm deep drain in whole plot perimeter. Irrigation water was applied through a pressurized system with an independent inlet per plot. Initial irrigation took place roughly 25 days after seeds emergence and the water level was maintained at 5-7 cm high in CF, AWDS and AWDL. A 25-day delay to trigger irrigation is common practice in Lagoa da Confusao due to lack of water reservoirs. Water is pumped into rice basins from rivers upon the onset of the rainy season and rise in water level.

The treatments of fertilizer levels of 0 and 150 kg N ha<sup>-1</sup> were randomized within each main plot. N was applied as pearled urea (46% N) and split in four equal rates at the following developmental stages: sowing, tillering (V5-V6), panicle initiation (R0), and collar formation of flag leaf (R2) (Counce et al., 2000). A 5 mm of water was flushed after urea application to promote incorporation into the soil.

Plots were sowed with IRGA 424 rice cultivar with a cycle of approximately 116 days in the tropical region of Brazil. Crop was established in a dry seed sowing with row distance of 17 cm and plant density after emergence of 150 plants m<sup>-2</sup>. Phosphorus and Potassium fertilizer was applied at the rate of 60 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> as superphosphate and 140 kg ha<sup>-1</sup> K<sub>2</sub>O as KCl.

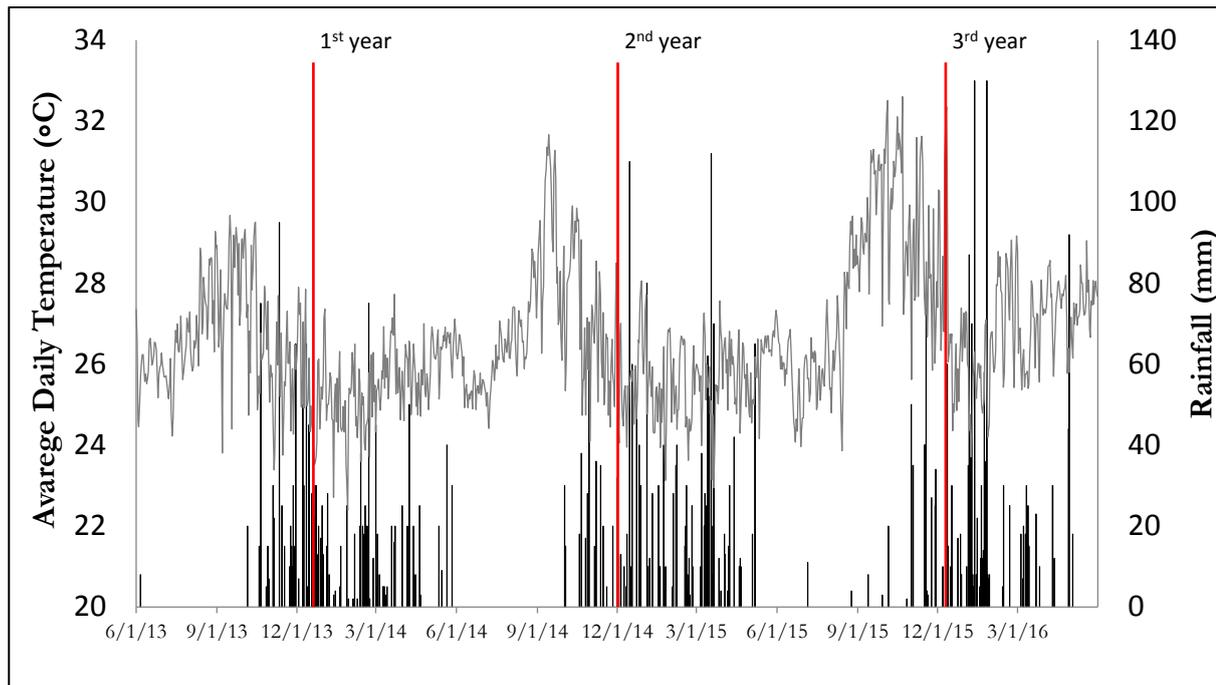


Figure 1. Mean daily temperature and rainfall along the years of field measurements. Black bars refer to rainfall, and gray line to temperature. Vertical red lines indicate the sowing date in the 3 years: 12/07/2013; 11/18/2014; 12/09/2015.

### 2.2.3. Crop Performance

Crop above ground biomass (AGB) and nitrogen content were determined by destructively sampling rice plants from 0.5 m<sup>2</sup> at physiological maturity. Plants were cut at stem base and chopped green. A representative subsample was dried to constant weight in 65 °C oven and water content was extrapolated for the entire sample. Nitrogen concentration was determined by Kjeldahl method after acid digestion and total nitrogen uptake (NU) was calculated as the product of N content (g kg<sup>-1</sup>) and plant biomass (g m<sup>-2</sup>).

Also at physiological maturity, five plants per plot were randomly sampled to determine spikelet number (NS) and thousand grain weight. Grains were manually detached, from rachis and weighed. Filled and unfilled grains were separated by air blowing, counted and reweighed. Grain yield (GY) was determined by collecting 3 m<sup>2</sup> of rice plants at 19-23% of field moisture and grains were separated from plants using an experimental mechanical thresher (SB Maquinas, Cambé, PR). Impurities were separated in a sample cleaner (Mediza, Panambi, RS) and moisture corrected to 13% in unhusked grains.

### 2.2.4. Isotopic Nitrogen Use Efficiency

The Isotopic efficiency was performed only in 2015 and 2016 years. Labeled <sup>15</sup>N nitrogen was applied to microplots settled inside the 150 kg ha<sup>-1</sup> N subplot following this same equivalent dose and topdress splitting performed on the subplot. Microplots were 0.245 m<sup>2</sup> and comprised of 4 sowing rows. Nitrogen was applied as

urea with abundance of 3.04 %  $^{15}\text{N}$ . Microplots were isolated by a metal frame with bottom and top free, 12 cm above and 18 cm deep into the soil.  $^{15}\text{N}$ -enriched fertilizer was diluted in 2.5 l of water and immediately applied uniformly onto the microplots with a sprinkling can. Water regime inside microplots was the same for each main plot and had an independent water inlet to avoid  $^{14}\text{N}/^{15}\text{N}$  contamination. Topdressing was applied on dry soil and flooded shortly thereafter, thus allowing urea incorporation in the soil and making the volatilization losses negligible.

At physiological maturity, 0.68 m<sup>2</sup> of above ground biomass was sampled from two central rows of each microplot. Each plant was cut at the stem and separated in to two groups: vegetative (leaves and stems) and reproductive (panicle). Roots were sampled from a cube of soil measuring 0.34 x 0.2 x 0.2 m, and root separation from soil was made by water sieving. Biomass material was dried at 65 °C oven to constant weight.

A soil core of 0.05 m diameter was sampled using a Dutch auger for remaining nitrogen analysis in soil from 0 to 1 m depth, and divided in sections of 20 cm representing the soil layers. Soil samples were oven dried at 40°C to constant weight.

The total amount of N and  $^{15}\text{N}$  abundance (%  $^{15}\text{N}$  atoms) was performed in an automated mass spectrometer coupled to an ANCA-GSL N analyzer (Sercon Co., UK). The total N concentrations and  $^{15}\text{N}/^{14}\text{N}$  isotope ratio were calculated according to Barrie and Prosser (1996) and dilution calculation adapted from Cabrera and Kissel (1989).

$$NDFF = \frac{(^{15}\text{Np} - ^{15}\text{Nn})}{(^{15}\text{Nf} - ^{15}\text{Nn})} \times Nu \quad \text{eq. (1)}$$

where NDFF is the amount of N in the plant derived from the fertilizer (kg ha<sup>-1</sup>),  $^{15}\text{Np}$  is the amount of  $^{15}\text{N}$  in plants (% atoms),  $^{15}\text{Nn}$  is the natural  $^{15}\text{N}$  abundance (% atoms),  $^{15}\text{Nf}$  is the quantity of  $^{15}\text{N}$  in the fertilizer (3.04 % atoms) and Nu is the N uptake from the plant (kg ha<sup>-1</sup>). The Isotope Nitrogen Use efficiency was calculated using equation 2:

$$INUE = \frac{NDFF}{N \text{ rate}} \times 100 \quad \text{eq. (2)}$$

where INUE is the ratio of N recovery by the plant from the fertilizer. And N rate is nitrogen applied as urea to the crop (150 kg ha<sup>-1</sup>).

## 2.2.5. Nitrogen Use Efficiency Indexes

The Apparent Nitrogen Use Efficiency (ANUE, kg of grain kg N applied<sup>-1</sup>) is the unit of yield increasing due unit of N fertilizer applied. It is calculated as the yield difference divided by nitrogen rate applied as fertilizer (Cassman et al., 1996).

$$ANUE = \frac{(GY \text{ fert} - GY \text{ unfert})}{N \text{ applied}} \quad \text{eq. (3)}$$

The N partial factor productivity (PPF, kg kg<sup>-1</sup>) was also used because it describes in an integrate way the efficiency of fertilizer input and indigenous soil nutrient (Cassman et al., 1996; Ciampitti and Vyn, 2012). It is the ratio between grain yield in fertilizer plot (GY fert) divided by nitrogen rate applied as fertilizer

$$PPF = \frac{GY \text{ fert}}{N \text{ applied}} \quad \text{eq. (4)}$$

The last N efficiency parameter used was the apparent N fertilizer recovery efficiency (ANR, kg of N uptake kg N applied<sup>-1</sup>), which is defined as the difference of N uptake in plant biomass among fertilized and unfertilized plots divided by N applied (Ciampitti and Vyn, 2012).

$$ANR = \frac{(N \text{ uptake fert} - N \text{ uptake unfert})}{N \text{ applied}} \quad \text{eq. (5)}$$

For all indexes, the grain yield is in 13% moisture basis, N uptake is the amount of N in above ground biomass at physiological maturity and N applied is 150 kg ha<sup>-1</sup> of Nitrogen.

## 2.2.6. Evapotranspiration and water measurements

Rainfall, radiation, air humidity and temperature data was measured hourly by a weather station (Davis Vantage Pro2, Davis, Inc., USA). Amount of irrigation water input (WI) was monitored by flow hydrometer installed in all water inlets. Fields were not drained, unless by percolation into soil profile. The soil volumetric water content was measured by dielectric conductivity probe (GS1 - Decagon, WA, USA) in a 3-15 cm depth and recorded every 30 minutes for two replicate blocks.

To express soil water content as water tension, a soil water retention curve was built from undisturbed samples collected from 0-0.1 m from 2015 and 2016/2014 experimental sites. Samples were submitted to pressures of -2, -4, -6, -8, -10, -30, -50, -70, -100 and -1500 kPa using the Richards pressure plate extractors. A soil water retention curve was established by fitting the pressure and theta values to van Genuchten model and parameters were estimated using RETC software (Van Genuchten et al., 1991). The RETC also estimated Ksat of top (0-10) and in the less impermeable layer (60-80) being 1.35 and 0.16 m d<sup>-1</sup>, respectively.

The leaf area index (LAI) and the light extinction coefficient was indirectly determined with optical hemisphere sensor (LI-cor 2200, Li-cor, Inc., USA). Measurements began 15 days after emergence and were repeated every 15 days, thereafter LAI was sampled on 7 dates each season. All data collection occurred at approximately 09:00 am hours or on cloudy days. One reading was taken over the canopy and 5 readings at soil (or water) surface. Crop height was measured at time of LAI sampling as distance from soil surface to last opened leaf.

Evapotranspiration (ETC) was estimated by the Penman–Monteith equation, using the measured crop parameters. Zero plane displacement (d) and roughness length for momentum (Zom) and vapor (Zoh) were estimated based on crop height (Allen, 1998). Crop surface resistance (rs) was assumed as 80 sm<sup>-1</sup> of (rl) (Dingkuhn et al., 1999; Turner et al., 1986). The partitioning of transpiration and evaporation was done according to Bouguer-Lambert law, which partitions radiation intercepted by crop canopy or soil surface (Van Laar et al., 1997):

$$T = ET_C (1 - e^{(-kLAI)}) \quad \text{eq. (6)}$$

$$E = ET_C e^{(-kLAI)} \quad \text{eq. (7)}$$

where k is extinction coefficient (assumed as 0.5 based on average of measured data). For CF, SS, and AWDS, AWDL during flooding time there was no water shortage, thus ET actual always met the ETC. For NF and AWDS, AWDL during non-flooding periods and no-rainy days, the actual soil evaporation was assumed to drop proportionally to square root of drying time as predicted by Ritchie (1972).

Water use efficiency was calculated in relation to ET (WUE<sub>ET</sub>) and water input (WUE<sub>Ia</sub>) as the ratio of dry grain mass per unit of water evapotranspiration (WUE<sub>ET</sub>, kg m<sup>-3</sup>), and yield divided by irrigation (I) + rainfall (R) (WUE<sub>Ia</sub>, kg m<sup>-3</sup>), respectively. The water application efficiency (EA) (Bouman et al., 2005) is

$$EA = 100 \times \frac{\Sigma(T+E)}{\Sigma(I+R)} \quad \text{eq. (8)}$$

### 2.2.7. Statistical Analysis

The dataset was submitted to statistical procedures using Statistical Analysis System, version 9.2 (SAS Institute, 2009). The assumptions of homogeneity of variance and error normality were tested by PROC TRANSREG Boxcox statement. If assumptions were violated, the variable was transformed by convenient lambda. ANOVA was performed by PROC GLM based on irrigation, N level, and year as well as its interactions as fixed effect model. Replicates were considered random effects to main plot and (replicate x main plot) as the random effect for subplot. When F probability was  $<0,05$  the means were separated by Least Significance Difference (LSD).

## 2.3. Results

### 2.3.1. Weather data

Rainfall patterns varied significantly across growing seasons. Rainfall during the rice growing season was 886, 1262, and 1064 mm during the 2014, 2015 and 2016 season, respectively (Table 2). Mean temperatures across all seasons was 25.8°C and only varied 1.3°C between 2014 and 2016. In 2016, mean temperature was 1.4 and 1.5 °C warmer at panicle initiation and grain filling compared to 2015.

Table 2. Rainfall and temperature per growing season during rice development in 2014, 2015 and 2016.

Year	Rainfall (mm)				Average Temperature (°C)			
	Vegetative	R0-R4	R5-R9	Total	Vegetative	R0-R4	R5-R9	Total
2014	354	358	174	886	24.8	25.1	25.9	25.3
2015	513	230	519	1262	25.8	25.3	25.4	25.5
2016	823	75	166	1064	25.8	27.2	26.8	26.6

### 2.3.2. Soil Water Potential in Root Zone

Differences in root zone soil water potential were observed due to differences in rainfall patterns between 2015 and 2016 (Figure 2). In 2015, rains began in November and were greater in volume thus providing adequate water replenishment to the soil before crop sowing. However, in 2016, there was a lack of water accumulation and the rice was established with lower water availability in soil. Thus, in 2015 the crop was submitted to a minimum water tension of only -70 kPa in the soil before onset of irrigation (22 DAE). However, in 2016, the tension reached approximately -950 kPa around 15-20 DAE. As a result, leaf curling occurred and crop development was delayed.

After the initiation of the irrigation treatments, soil water potential in CF remained zero kPa and in SS at zero or slightly below that (at least 96% of the maximum theta or -5kPa of tension). As expected, no restrictions were placed on rice development by these irrigation regimes. The AWDS and AWDL had a greater amplitude of water potential, beginning at zero in the flooding cycles and reaching a minimum of -21kPa and -34kPa in the drying cycles of 2015 and 2016 respectively. The lowest soil water potential was achieved by NF short after PI in 2015 (-35kPa) and flowering in 2016 (-58kPa). These negative thresholds were maintained at most for three

days long until the following rainfall. Averages of soil water tension from 25 DAE to physiological maturity were -20kPa in 2015 and -28kPa in 2016.

### 2.3.3. Crop Performance

Irrigation regimes and N treatments significantly affected AGB, NU, GY, and NS, across years (Table 3). Year had also a significant effect on all variables, except to AGB. Without N application, AGB averaged 14.3 Mg ha<sup>-1</sup> in NF, AWDS, AWDL, and 11 Mg ha<sup>-1</sup> in CF and SS. However, when N was applied at 150 kg ha<sup>-1</sup>, NF provided the highest biomass (19 Mg ha<sup>-1</sup>) among all IR. Overall AGB ranged from 8 to 22 Mg ha<sup>-1</sup>, and was increased in response to N application in AWDS, CF, NF and SS.

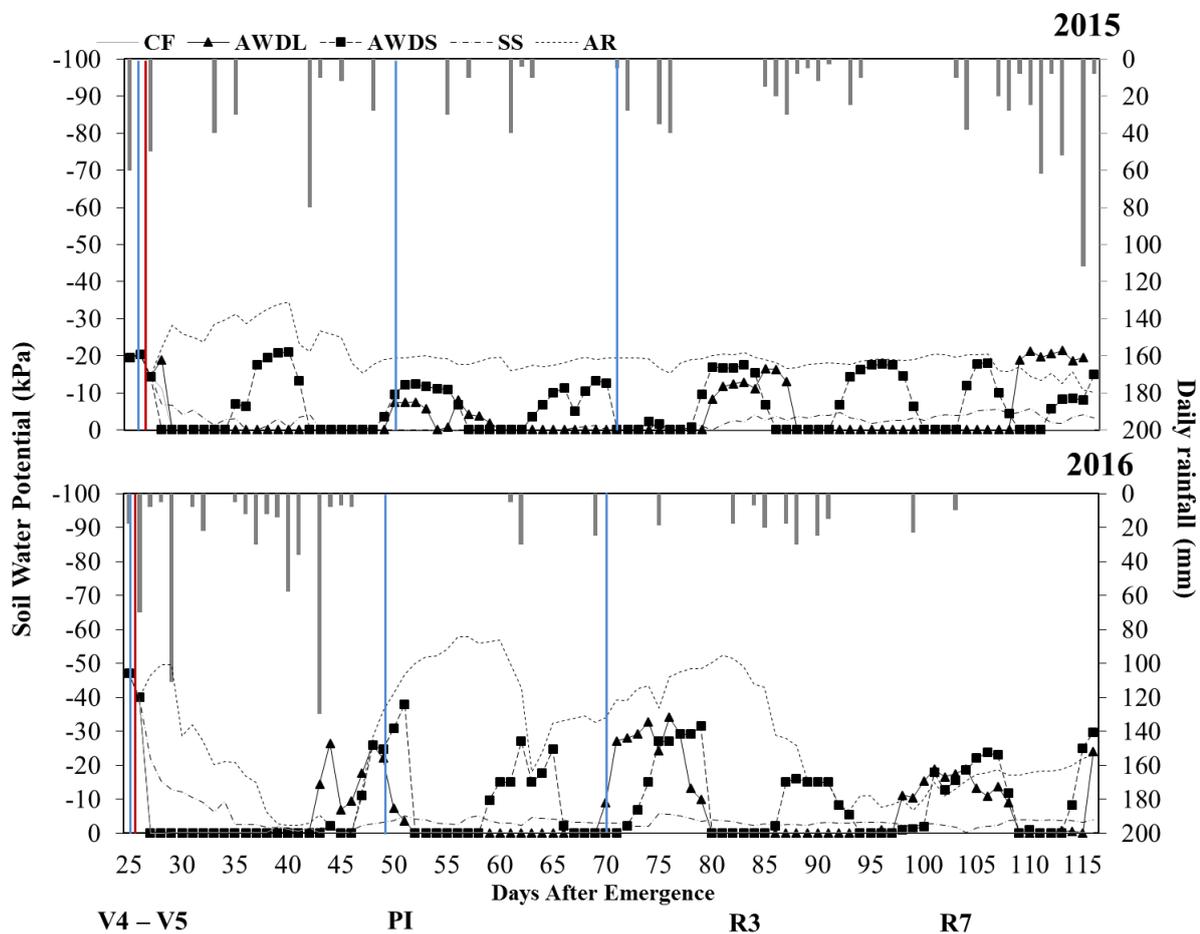


Figure 2. Soil water potential in root zone (0-0.1m) in 2015 and 2016 in left axis (values are shown as lines), and daily rainfall (mm) in right axis (values are shown on inverse scale as bars) throughout crop season. The treatments are: continuous flooding (CF), AWD long (AWDL), AWD short (AWDS), saturated soil (SS), and aerobic (AR). The acronyms below X-axis indicates phenological stage: active tillering (V4-V5); panicle initiation (PI); panicle exertion (R3); end of grain filling (R7). The red bar indicates when irrigation initiated and blue bars the nitrogen topdressing.

The N uptake was 30% higher in NF than in CF, AWDS, AWDL, and SS, across years and N level. There was no interaction between IR and N level or year with respect to NU; therefore, IR had similar effects on N uptake unregard level of N fertilizer. The NU ranged from 69 kg ha<sup>-1</sup> in SS 0 in 2014 and 2015 to 210 kg ha<sup>-1</sup> in NF 150 in

2015. The N application increased N uptake by 60 kg ha<sup>-1</sup> across IR and years, and N uptake was higher in 2015 than in 2014 and 2016.

The NS per panicle followed the same trend as that for N uptake. NF had 11% higher NS per panicle than any other IR, averaging across years and N levels 105 spikelets. The N fertilizer applying resulted in an increase of 16% in NS, and the corresponding value was higher in 2015 than in 2014 and 2016. The interaction between N level and year significantly affected the spikelet number per panicle, which was 46% higher in 2016 than in 2014 and 2015. Grain weight was only significantly affected by year, being 1.92 g heavier in 2016 than in 2015, being unaffected by IR and N level.

Table 3. Above Ground Biomass (AGB), Total N uptake at crop maturity (NU), number of Spikelet per panicle (NS) and 1000 grains weight during 2014, 2015 and 2016. The irrigation regimes are: alternate wetting drying short (AWDS), alternate wetting and drying long (AWDL), continuous flooding (CF), non-flooding (NF), and saturated soil (SS) and each of them with 0 and 150 kg ha<sup>-1</sup> of Nitrogen.

N level	Plant Biomass (Mg ha <sup>-1</sup> )			N uptake (Kg ha <sup>-1</sup> )			Spikelet (panicle <sup>-1</sup> )			Grain Weight (g)			Grain Yield (Mg ha <sup>-1</sup> )		
	0	150	mean	0	150	mean	0	150	mean	0	150	mean	0	150	mean
<b>Year / Irrigation</b>															
<b>2014</b>															
AWDS	15	15	15b	91	111	101	79	94	87	-	-	-	5.1	7.8	6.4b
AWDL	13	15	14a	89	113	101	75	86	80	-	-	-	5.5	7.7	6.6b
CF	9	14	12c	75	122	98	72	92	82	-	-	-	4.1	7.1	5.6b
NF	16	22	19a	120	195	158	93	112	103	-	-	-	6.0	8.3	7.2a
SS	8	15	12b	69	119	94	78	101	89	-	-	-	5.0	7.3	6.2b
<b>mean</b>	<b>12</b>	<b>16</b>	<b>14</b>	<b>88</b>	<b>133</b>	<b>111B</b>	<b>79A</b>	<b>97B</b>	<b>88</b>	-	-	-	<b>5.1B</b>	<b>7.6A</b>	<b>6.4</b>
<b>2015</b>															
AWDS	15	19	17a	88	162	125	92	107	100	25	25	25	5.9	10.5	8.2 b
AWDL	14	15	15a	96	139	118	100	110	105	25	25	25	6.2	8.9	7.5 c
CF	12	16	14a	83	153	118	103	110	106	25	25	25	6.0	10.5	8.2 b
NF	14	19	17a	116	210	163	107	109	108	26	26	26	7.1	10.6	8.8 a
SS	9	19	14a	69	149	109	93	103	98	25	26	26	5.8	9.7	7.8 b
<b>mean</b>	<b>13</b>	<b>18</b>	<b>15</b>	<b>90</b>	<b>163</b>	<b>127A</b>	<b>98A</b>	<b>107B</b>	<b>103</b>	<b>25</b>	<b>25</b>	<b>25b</b>	<b>6.2B</b>	<b>10A</b>	<b>8.1</b>
<b>2016</b>															
AWDS	11	16	13a	75	127	101	75	104	89	27	27	27	5.0	7.9	6.5 b
AWDL	14	16	15a	63	128	96	79	106	93	28	27	28	5.4	8.3	6.9ab
CF	15	15	15a	105	140	123	88	103	95	27	27	27	5.4	8.4	6.9ab
NF	14	17	16a	118	195	157	98	112	105	28	27	27	6.4	8.5	7.5 a
SS	11	16	13a	67	133	100	75	98	86	27	27	27	5.6	7.7	6.7 b
<b>mean</b>	<b>13</b>	<b>16</b>	<b>14</b>	<b>85</b>	<b>145</b>	<b>115B</b>	<b>83B</b>	<b>105A</b>	<b>94</b>	<b>27</b>	<b>27</b>	<b>27a</b>	<b>5.6B</b>	<b>8.2A</b>	<b>6.9</b>
<b>Across Years</b>															
<b>AWDS</b>	14abB 17bA			85	133	115b	82	102	92 b	26	26	26	5.3	8.7	7.2
<b>AWDL</b>	14abA 16bA			83	127	110b	84	101	92 b	27	26	26	5.7	8.2	6.9
<b>CF</b>	12bB 15bA			88	138	120b	87	102	93 a	26	26	26	5.1	8.7	7.0
<b>NF</b>	15aB 19aA			118	200	168a	100	111	105 a	27	26	27	6.5	9.2	7.9
<b>SS</b>	10cB 16bA			68	134	107b	82	101	91 b	26	26	26	5.5	8.2	6.9
<b>mean</b>				<b>87B</b>	<b>147A</b>					<b>26</b>	<b>26</b>				
<b>ANOVA</b>															
<b>Irrigation (I)</b>	*			*			*			<i>ns</i>			**		
<b>N level (N)</b>	**			**			**			<i>ns</i>			**		
<b>Year (Y)</b>	<i>ns</i>			*			**			**			**		
<b>I*N</b>	**			<i>ns</i>			<i>ns</i>			<i>ns</i>			<i>ns</i>		
<b>I*Y</b>	*			<i>ns</i>			<i>ns</i>			<i>ns</i>			*		
<b>N*Y</b>	<i>ns</i>			<i>ns</i>			**			<i>ns</i>			**		
<b>I*N*Y</b>	<i>ns</i>			<i>ns</i>			<i>ns</i>			<i>ns</i>			<i>ns</i>		
<b>CV (%)</b>	21.52			25.76			11.14			2.12			9.17		

ns: not significant, \* significant at 5%, \*\* significant at less than 1% of probability of error by F test. Values followed by the same letter are not significantly different by LSD test at  $p < 0.05$  Lower-case compares among rows in the same year and upper-case compares among columns.

The grain yield ranged from 4.1 to 10.6 Mg ha<sup>-1</sup> and was affected by IR, N level, and year. NF showed the highest grain yield across unfertilized and fertilized plots in 2014 (7.2 Mg ha<sup>-1</sup>) and 2015 (8.8 Mg ha<sup>-1</sup>). In 2016, although NF presented the highest yield (7.5 Mg ha<sup>-1</sup>), it was not significantly higher than the yield in AWDL (6.9 Mg ha<sup>-1</sup>) and CF (6.9 Mg ha<sup>-1</sup>). N application showed the same effect on crop yield regardless of irrigation regime. An interaction between year and N level was observed because in 2015, yield increment due to N application was on average 34% higher than in 2014 and 2016.

#### 2.3.4. Isotopic Nitrogen Efficiency

The total plant recovery of isotopic <sup>15</sup>N ranged from 37.8 to 65.1% across IR and year (Table 4). NF had the highest N-fertilizer uptake (64.7%), followed by that in AWDS, AWDL, CF, and SS, which averaged 47.3%. With respect to plant partitioning, IR did not affect grain and root recovery. However, shoot recovery was significantly higher in NF than others (average value decreased in the order: CF > AWDS > AWDL). SS had the lowest shoot recovery across years. Grain, shoot, and root recovery, plant biomass, 0-20 cm soil layer recovery, and total soil recovery were affected by year; however, no interaction between year and irrigation was observed.

The <sup>15</sup>N recovery from the 0-20 cm soil layer was 15.9% in NF and 13.7% in SS across years, followed by the values in AWDS, AWDL, and CF. It was 45% higher in NF than in AWDL and CF. The <sup>15</sup>N recovery from the 20-40 and 40-60 cm soil layers was not affected by irrigation, and 1.54% and 0.47% labeled fertilizer was detected in the 20-40 and 40-60 cm layers, respectively, across years. Because the <sup>15</sup>N content in the 40-60 cm layer was close to the detection threshold of the method used, deeper layers were assumed to contain negligible amounts of <sup>15</sup>N.

For total recovery, which is the sum of recovery from all plant parts and soil layers, NF showed the highest value with 81.5% of labeled N. The results indicate that a 19% N-fertilizer loss occurred in NF via unidentified means, whereas an average loss of 41% occurred in AWDS, AWDL, CF, and SS. There was no difference in total labeled fertilizer recovery between 2015 and 2016 because N recovery in plant was complementary to N recovery in soil. That is, in 2015 the plant recovery was high and less <sup>15</sup>N was left in the soil. In 2016, as the crop development was limited due climatic conditions <sup>15</sup>N recovery in plant biomass and higher amount of <sup>15</sup>N was left into the soil, therefore an equivalent proportion of N was not recovered in both years.

#### 2.3.5. Nitrogen Indices

Apparent N recovery (ANR), agronomic N use efficiency (ANUE), and partial factor productivity (PFP) were affected by IR and year (Table 5). The year 2015 provided better climatic conditions for rice development and showed higher ANUE and PFP than did 2014 and 2016. ANR in 2015 was higher than that in 2014 but equal to that in 2016. ANUE ranged from 14 to 30 kg kg<sup>-1</sup> N and its average across years in CF and AWDS (23.6 kg kg<sup>-1</sup> N) was higher than that in AWDL, SS, and NF. The ANR ranged from 0.19 to 0.61 kg kg<sup>-1</sup> N and its average across years in NF and SS was 0.57 and 0.44 kg kg<sup>-1</sup>, respectively. AWDL, AWDS, CF and SS averaged 0.35 kg kg<sup>-1</sup>. The PFP was higher in NF (61 kg kg<sup>-1</sup> N) than in AWDL and SS (55.4 and 54.9 kg kg<sup>-1</sup> N, respectively), CF and AWDS were equivalent to all IR across years. Notably, the average ANR and ANUE in NF

and SS were higher and lower, respectively, than in other irrigation regimes, whereas the ANR and ANUE values in CF showed the opposite trend.

Table 4. Alternate wetting drying short (AWDS), alternate wetting and drying long (AWDL), continuous flooding (CF), non-flooding (NF), and saturated soil (SS) in 2015 e 2016. The N rate was 150 kg ha<sup>-1</sup>.

Year / Irrigation	Plant Recovery				Soil Recovery				Total Recovery
	Grain	Shoot	Root	Total Plant	0-20 cm	20-40 cm	40-60 cm	Total soil	
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
<b>2015</b>									
AWDS	35.9	13.7	0.41	50.0	8.5	1.7	na	10.3	60.3
AWDL	41.7	13.5	0.30	55.5	2.7	0.2	na	2.9	58.4
CF	37.0	14.0	0.30	51.3	7.3	0.7	na	7.9	58.0
NF	39.1	24.8	0.35	64.3	11.5	1.1	na	12.6	76.8
SS	38.6	9.7	0.26	48.6	12.6	0.4	na	12.6	61.2
<b>mean</b>	<b>38.5 a</b>	<b>15.2 b</b>	<b>0.32 b</b>	<b>53.8 a</b>	<b>8.91 b</b>	<b>0.81</b>	<b>-</b>	<b>9.3 b</b>	<b>63.0</b>
<b>2016</b>									
AWDS	28.5	17.3	0.85	46.7	14.6	1.6	0.46	16.9	61.3
AWDL	20.5	16.4	0.88	37.8	14.6	0.7	0.18	15.4	53.2
CF	21.4	20.8	0.86	43.0	9.9	1.2	0.50	11.7	54.7
NF	32.6	31.3	1.17	65.1	20.4	1.0	0.63	22.0	87.1
SS	28.2	15.7	0.92	44.8	14.8	2.3	0.55	17.7	62.5
<b>mean</b>	<b>26.2 b</b>	<b>20.3 a</b>	<b>0.94 a</b>	<b>47.5 b</b>	<b>14.91 a</b>	<b>1.54</b>	<b>0.46</b>	<b>17.9 a</b>	<b>65.0</b>
<b>Across Years</b>									
<b>AWDS</b>	32.2	15.5 bc	0.63	48.3 b	11.1 bc	1.7	0.46	13.1 ab	60.7 b
<b>AWDL</b>	31.1	14.9 bc	0.59	46.6 b	8.6 c	0.4	0.18	9.2 c	55.8 b
<b>CF</b>	29.2	17.4 b	0.58	47.1 b	8.8 c	0.9	0.50	10.3 bc	56.3 b
<b>NF</b>	35.9	28.0 a	0.76	64.7 a	15.9 a	1.52	0.63	16.8 a	81.5 a
<b>SS</b>	33.4	12.7 c	0.59	46.7 b	13.7 ab	1.3	0.60	15.6 a	62.0 b
<b>ANOVA</b>									
<b>irrigation (I)</b>	<i>ns</i>	**	<i>ns</i>	**	*	<i>ns</i>	<i>ns</i>	*	**
<b>Year (Y)</b>	**	**	**	*	**	<i>ns</i>	-	*	<i>ns</i>
<b>I * Y</b>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	-	<i>ns</i>	<i>ns</i>
<b>CV (%)</b>	17.4	18.9	42.3	15.9	29.3	80.0	58.2	26.0	15.6

ns: not significant, \* significant at 5%, \*\* significant at less than 1% of probability of error by F test. Values followed by the same letter are not significantly different by LSD test at  $p < 0.05$ . na: data not available.

### 2.3.1. Evapotranspiration and water measurements

ET among IR and N rates varied from 496 to 566 mm in 2015 and 491 to 549 mm in 2016 (Table 6). On average, fertilized plots had 12% and 11% higher ET than unfertilized plots, respectively, in both years. With respect to irrigation method, a major difference of 32 mm was observed between CF and AWDL and between CF to NF, in 2015 and 2016, respectively. Although differences in ET among IR do not correspond to the consistent differences observed in crop performance, the isolated values of T and E showed consistency. Across irrigation regimes, 2015 had 15% higher transpiration and 16% lower evaporation than did 2016, i.e., 2015 had the best overall yield and used higher

amounts of water towards the yield (T water). The same was observed for irrigation regime; NF showed the highest values for T and the lowest values for E for both N levels and years.

The water loss through infiltration ranged from 540 to 1927 mm. Even NF, the irrigation regime with the lowest water input (738 and 569 mm in 2015 and 2016, respectively), showed a relatively high percolation rate. The mean infiltration loss in CF (which had the longest time of water pumping) was 3.5-fold higher than the corresponding mean ET in 2015 and 2016.

Table 5. Apparent Nitrogen Use Efficiency (ANUE), Nitrogen Recovery Efficiency (ANR) e Partial Factor of Productivity (PFP). The irrigation regimes are: alternate wetting drying short (AWDS), alternate wetting and drying long (AWDL), continuous flooding (CF), non-flooding (NF), and saturated soil (SS) in 2014, 2015 and 2016.

	ANUE	ANR	PFP
	(kg kg <sup>-1</sup> of N)		
<b>Year / Irrigation</b>			
<b>2014</b>			
AWDS	20.5	0.19	51.7
AWDL	15.1	0.12	51.5
CF	20.1	0.31	47.2
NF	15.0	0.50	55.7
SS	15.3	0.34	48.8
<b>mean</b>	<b>17.1 B</b>	<b>0.32 B</b>	<b>51.0 B</b>
<b>2015</b>			
AWDS	31.3	0.49	70.3
AWDL	25.3	0.31	59.2
CF	30.3	0.47	70.1
NF	20.8	0.63	70.5
SS	25.7	0.53	64.6
<b>mean</b>	<b>24.9 A</b>	<b>0.49 A</b>	<b>66.9 A</b>
<b>2016</b>			
AWDS	19.0	0.35	52.6
AWDL	20.0	0.33	55.3
CF	20.5	0.25	56.1
NF	14.7	0.61	56.9
SS	14.0	0.44	51.4
<b>mean</b>	<b>18 B</b>	<b>0.46 AB</b>	<b>55 B</b>
<b>Across Years</b>			
AWDS	23.6 a	0.37 b	58.2 ab
AWDL	17.9 b	0.24 b	55.4 b
CF	23.6 a	0.35 b	57.8 ab
NF	16.0 b	0.57 a	61.0 a
SS	18.3 b	0.44 ab	54.9 b
<b>ANOVA</b>			
<b>irrigation (I)</b>	*	*	*
<b>Year (Y)</b>	*	*	*
<b>I *Y</b>	<i>ns</i>	<i>ns</i>	<i>ns</i>
<b>CV (%)</b>	28.17	33.90	7.64

ns: not significant, \* significant at 5%, \*\* significant at less than 1% of probability of error by F test. Values followed by the same letter are not significantly different by LSD test at  $p < 0.05$ .

Table 6. Total water input (WI), crop evapotranspiration (ETc) segmented in its evaporation (E) and transpiration (T), and infiltration loss accumulated during growing season of 2015 and 2016. The irrigation regimes are: alternate wetting drying short (AWDS), alternate wetting and drying long (AWDL), continuous flooding (CF), non-flooding (NF), and saturated soil (SS) in 2015 e 2016

N level	WI	Evaporation (mm)			Transpiration (mm)			ET (mm)			Infiltration Loss (mm)		
	(mm)	0	150	mean	0	150	mean	0	150	mean	0	150	mean
<b>Year / Irrigation</b>													
<b>2015</b>													
AWDS	1895	162	110	136	334	445	390	496	555	526	1399	1340	1370
AWDL	2228	141	107	124	357	420	389	498	527	513	1730	1701	1716
CF	2459	184	119	152	348	439	394	532	558	545	1927	1901	1914
NF	1262	103	81	92	407	466	437	510	547	529	752	715	734
SS	1625	153	120	137	365	446	406	518	566	542	1107	1059	1083
<b>2016</b>													
AWDS	1748	168	138	153	335	390	363	503	528	516	1245	1220	1233
AWDL	2082	170	144	157	354	394	374	524	538	531	1558	1544	1551
CF	2401	188	151	170	346	394	370	534	545	540	1867	1856	1862
NF	1064	124	111	118	367	413	390	491	524	508	573	540	557
SS	1460	163	161	162	352	388	370	515	549	532	945	911	928

WI (water input = Rainfall + irrigation); ET (estimated evapotranspiration); Infiltration loss = WI – ET.

The ET water use efficiency ( $WUE_{ET}$ ), total water input use efficiency ( $WUE_{In}$ ), and application efficiency (EA) are given in Table 7.  $WUE_{ET}$  ranged from 1.15 to 1.85 kg grain  $m^{-3}$  water across years and, on average, increased by 31% when N was applied.  $WUE_{ET}$  in NF was the highest (1.64 kg grain  $m^{-3}$  water) across years, whereas that in SS was the lowest (1.32 kg grain  $m^{-3}$  water) across years.  $WUE_{In}$  follow the same trend of WI, due the variation of WI among IR is much greater than grain yield variation. NF showed the highest  $WUE_{In}$  (0.7 kg grain  $m^{-3}$  water) across years and N levels, followed by SS and AWDS. The CF and AWDL had the lowest efficiency of 0.35 and 0.32 kg grain  $m^{-3}$  water. N application increased the  $WUE_{In}$  by 33% and 27% in 2015 and 2016, respectively.  $WUE_{ET}$  and  $WUE_{In}$  were higher in 2015 than in 2016. The EA ranged from 20 to 47% and was higher for irrigation regimes that used less amount of water. The EA for unfertilized and fertilized plots in NF was 40 and 42%, respectively, in 2015, and 43 and 47%, respectively, in 2016. EA in AWDL was equal to that in CF in 2015 for both N levels and in 2016 for N level 0.

## 2.4. Discussion

### 2.4.1. Soil Water Potential in Root Zone

The main difficulty with alternative irrigation strategies is fulfilling the crop's water requirement so not to incur a loss of grain yield. According to Wopereis et al. (1996), rice transpiration rates remain nearly unchanged as soil water potential goes from zero to -100 kPa, with leaf curling occurring below -200kPa. The effects of drought stress in rice are related to genotype, the crop age and duration of drought stress and it starts from -10kPa to -30kpa (Bouman and Tuong, 2001). In a broad meta-analysis of alternate wet and dry irrigation, Carrijo

et al. (2017) concluded there is no significant effect on rice yield when the soil water potential does drop below -20kPa during the drying cycle, and Kato et al. (2009) observed aerobic conditions did not decrease rice yield even with soil potential frequently reaching -60kpa. For modelling purpose, the Oryza2000 crop model (Bouman et al., 2001) considers the relation described by Wopereis et al. (1996). Thus, based on the observed soil water potential during the length of this study, it is reasonable to accept that ET met crop requirements as soil water potential was within the range of 0 to -28kPa in all irrigation regimes after the onset of irrigation (Figure. 2).

Table 7. ET Water use efficiency ( $WUE_{ET}$  - kg grain  $m^{-3}$ ), water input use efficiency ( $WUE_{In}$  - kg grain  $m^{-3}$ ) and application efficiency (EA- %) in 2015 and 2016. The irrigations regimes are: alternate wetting drying short (AWDS), alternate wetting and drying long (AWDL), continuous flooding (CF), non-flooding (NF), and saturated soil (SS).

N level	$WUE_{ET}$			$WUE_{In}$			EA		
	0	150	mean	0	150	mean	0	150	mean
<b>Year / Irrigation</b>									
<b>2015</b>									
AWDS	1.27	1.99	1.63	0.31	0.56	0.43	24 c	28 c	26
AWDL	1.29	1.90	1.59	0.29	0.45	0.37	22 d	24 d	23
CF	1.21	1.98	1.59	0.25	0.43	0.34	20 d	22 d	21
NF	1.47	2.02	1.74	0.56	0.84	0.70	40 a	42 a	40
SS	1.20	1.80	1.50	0.36	0.60	0.48	30 b	33 b	32
<b>mean</b>	<b>1.29 B</b>	<b>1.94 A</b>		<b>0.35 B</b>	<b>0.57 A</b>				
<b>2016</b>									
AWDS	1.07	1.59	1.33	0.29	0.45	0.37	27 c	28 d	28
AWDL	1.11	1.64	1.38	0.26	0.40	0.33	24 d	24 d	24
CF	1.09	1.64	1.37	0.23	0.36	0.29	21 d	22 c	21
NF	1.38	1.68	1.53	0.60	0.78	0.69	43 a	47 a	45
SS	1.16	1.49	1.32	0.38	0.53	0.46	33 b	36 b	34
<b>mean</b>	<b>1.16 B</b>	<b>1.61 A</b>		<b>0.35 B</b>	<b>0.50 A</b>				
<b>Across Years</b>									
AWDS	1.17	1.79	1.48 bc	0.30	0.50	0.40 c	26	28	27
AWDL	1.20	1.77	1.48 bc	0.28	0.42	0.35 d	23	24	24
CF	1.15	1.81	1.52 b	0.24	0.39	0.32 d	21	22	21
NF	1.43	1.85	1.64 a	0.58	0.81	0.70 a	41	44	42
SS	1.18	1.64	1.41 c	0.37	0.56	0.47 b	32	34	32
<b>mean</b>	<b>1.23</b>	<b>1.77</b>							
<b>ANOVA</b>									
irrigation (I)		**			**			**	
N level (N)		**			**			**	
Year (Y)		**			**			**	
I*N		<i>ns</i>			<i>ns</i>			**	
I *Y		<i>ns</i>			<i>ns</i>			**	
N*Y		**			**			**	
I*N*Y		<i>ns</i>			<i>ns</i>			<i>ns</i>	
CV (%)		9.27			9.38			2.59	

ns: not significant, \* significant at 5%, \*\* significant at less than 1% of probability of error by F test. Values followed by the same letter are not significantly different by LSD test at  $p < 0.05$ . Lower-case compares among rows in the same year and upper-case compares among columns.

### 2.4.2. Crop Performance

The grain yield was positively affected by irrigation regimes which used less water, particularly NF which obtained the highest yields in 2014 and 2015. Spikelet number and tillering (which biomass is indirectly linked to) are yield components and there is a positive correlation between them, as also reported by Clerget et al. (2016). AGB, NS and GY are related to nitrogen uptake (Fageria and Baligar, 2005, 2001; Lampayan et al., 2010), and this relationship was observed in this study (Table 3). The greatest N uptake in zero and 150 kg ha<sup>-1</sup> for NF condition indicates irrigation regime is also interfering in N availability in soil, and it could be addressing the differences in crop performance. Aerobic soil condition is related to higher rates of indigenous N mineralization than anaerobic (Aulakh et al., 2000; Dong et al., 2012; Kader et al., 2013; Updegraff et al., 1995). Also, the increase of net mineralization can deliver a higher amount of N to the rice (Dong et al., 2012). There are few results in literature that presents yield improving under aerobic conditions. Kato et al., (2009) found similar conclusion in temperate rice in Japan, and there is not such result in tropical environment. Regarding AWD strategy, result reporting best crop performance in AWD than CF are recently becoming common (LaHue et al., 2016; Wang et al., 2016), although the majority of them conclude inferior results (Belder et al., 2005a; Bouman et al., 2005; M. D. M. Kadiyala et al., 2015; Patel et al., 2010; Peng et al., 2006), or no difference among irrigations regime (Rose et al., 2016; Sánchez-Llerena et al., 2016; Yao et al., 2012). The greater N uptake for the crop under NF (168 kg ha<sup>-1</sup> of N at 150 kg ha<sup>-1</sup>) is a possible consequence of non-limiting N availability coupled with reduction of N loss. The improve in N uptake drove the better crop performance in NF.

### 2.4.3. The Isotopic Efficiency

Based on its consistently high isotopic efficiency, NF appear as the most adequate irrigation regime in tropical Plinthaquults. NF provided the most efficient fertilizer use by the crop and high amount of fertilizer N was remained in the soil. The all other irrigations treatments were inferior to NF (Table 4). The common fate of N losses from agroecosystem were not completely measured in this study, but some of them are unlikely to have occurred either because methodology or site characteristics.

Seepage was prevented due the metal frame of microplots, and ammonia volatilization directly from urea is negligible when fertilizer is incorporated into the soil right after application (Black et al., 1987; Li et al., 2008). The ammonia volatilization from soil, which is a different process from ammonium volatilization directly from urea, is a pH dependent process which turns insignificant in pH lower than 7.5 (Reddy et al., 1984). The site Plinthaquults presented an initial pH of 5.5 – 5.7 (Table 1), and even the reduction process would be able to raise soils pH due to the increase of net OH<sup>-</sup> (Ponnamperuma, 1972), an acid Ultisol did not raise its pH over 6.8 after 50 days of submergence (Borin et al., 2016). Thus, ammonia volatilization unlikely was the fate of unaccounted <sup>15</sup>N.

The N leaching is also excluded for being the fate of unaccounted N because labeled N was negligible below 40 cm for all irrigations regime (Table 4). Previous reports had no consensus if N loss potential would increase in CF or any other alternative method. When CF is adopted, N-NH<sub>4</sub><sup>+</sup> rather than N-NO<sub>3</sub><sup>-</sup> is the mineral predominant form in the soil and it is less susceptible to leaching (Devkota et al., 2013; Patrick Jr and Reddy, 1978). In aerobic irrigation, nitrification is enhanced, and N-NO<sub>3</sub><sup>-</sup> would become more likely to leach into the ground water. However, this is compensated by less water percolation carrying N downwards (Tan et al., 2015;

Zhou et al., 2011). Irrespective to IR and mineral N, leaching is able to occur when there is a discrepancy between soil supply and crop requirement (Cassman et al., 1996). The NU across years ranged from 137 to 200 kg ha<sup>-1</sup> among IR and N fertilizer applied was always 150 kg ha<sup>-1</sup>, therefore the roughly concordance between NU and fertilizer rate led to N leaching was not noticeable in the present research.

Nitrification-denitrification may provide the best explanation of the observed results. In previous studies of the fate of <sup>15</sup>N applied to lowland rice, CF is largely reported as the irrigation regime which provides superior isotopic nitrogen efficiency in relation to alternative methods (Belder et al., 2005a; Dong et al., 2012; Fillery and Vlek, 1982; M. D. M. Kadiyala et al., 2015b; Rose et al., 2016; Zhou et al., 2012, 2011). The common factor between these studies was they took place on Alfisols and/or puddled soils. In such type of soil, the water percolation rate is usually limited (Belder et al., 2007; Singh et al., 2001), and its submergence results in oxidizing zones just restricted to water/air interface and rhizosphere (Atulba et al., 2015; Bouldin, 1986). The majority part of soil bulk remains anoxic, and nitrification process is disadvantaged. As consequence, the majority of mineral N remains in the reduced form as N-NH<sub>3</sub><sup>-</sup> and losses through nitrification-denitrification are prevented (Dong et al., 2012; Reddy et al., 1984; Zhou et al., 2011). Conversely, soils with elevated infiltration rate, like the one described in this study ( $K_{sat}$  1.35 m d<sup>-1</sup>) suggests a constant downwards flux of water into the soil profile. This flux is illustrated by high volume of water percolation losses (Table 6). As a result, the N-NO<sub>3</sub><sup>-</sup> formed in oxidizing zones are spread into soil profile (Aulakh and Bijay-Singh, 1996; Bouldin, 1986) and it triggers denitrification processes due to nitrate movement into anaerobic sites (Van Cleemput et al., 2007; Zhou et al., 2011). CF, SS, AWDL, AWDS might have low <sup>15</sup>N recovery compared to NF explained by it. NF had its top 10 cm constantly aerobic and it may have transformed the labeled N to nitrate. As it should have fewer anoxic zones, the denitrification wasn't pronounced. Further studies must be done to explain if this process really occurs, however the present data showed 81.5 % recovery of applied fertilizer in NF irrigation method, while all other IR had an average of 58.7%. For such rice production environment, the method that most prevented N loss is the one that kept soil aerobic.

The results are concisely even in different weather among years (Table 2). In 2015 the weather conditions for crop development were more favorable and either in main plot and microplot the crop yielded more. The consequence is higher labeled fertilizer in plant and less remaining in the 0-20 cm soil. In 2016, the crop yielded less due unfavorable weather, and higher amount of <sup>15</sup>N was left over in the soil. The balance between soil and plant compensates each other and the total recovery (soil + plant) was equal in both years.

#### 2.4.4. N Efficiency Indexes

Data demonstrated differences in yield and N recoveries, which aligns with suggestions that irrigation regimes drive different N dynamics in the soil. The ANUE was higher in CF and AWDS in 2014, 2015 and 2016 (Table 5), thus implying these irrigation treatments had better use of N fertilizer. But as observed earlier, CF and AWDS did not provide higher yields and N Uptake, rather they were equivalent to AWDL and SS and inferior to NF. At 0 kg ha<sup>-1</sup> of N rate, NF provided the highest GY (6.5 across years) causing a lower increment than CF and AWDS in response to N fertilizer. At 150 kg ha<sup>-1</sup> of N rate, the NF provided an available amount of N which exceeded the optimum rate for this genotype and local, and other factors became limiting. Blast disease (*Magnaporthea oryzae* Cav.) increases with N-NO<sub>3</sub><sup>-</sup> availability (Osuna-Canizalez et al., 1991) and it was observed

in all irrigation regime used, although the severity NF was worst. The N rate dependence to optimum ANUE is widely reported (Cassman et al., 2002; Fageria and Baligar, 2003), and poor ANUE are commonly related to pronounced N loss (Belder et al., 2005a; M. D. M. Kadiyala et al., 2015). But for the particular case of NF in this study it can be interpreted as higher N soil surplus and thin relationship between rice crop and to N fertilizer. Therefore, if adopted NF the fertilizer rate must decrease to attend the optimum rate.

The assumption of irrigation regime interference with N dynamics in the soil also relies on the differences observed in ANR, and among the regimes NF supported higher recovery. The apparent recovery of 0.57 kg kg<sup>-1</sup> standards the ANR in NF as high as upland crops. ANR is broadly reported between 0.3 to 0.45 kg kg<sup>-1</sup> to lowland rice (Cassman et al., 2002), what is similar to the average 0.35 kg kg<sup>-1</sup> across AWDS, AWDL, CF and SS of this study. The lower ANR in lowland rice is due the intensive loss processes of volatilization and nitrification- denitrification (Ladha et al., 2005) and the lower unaccounted labeled found in isotopic technique to NF corroborates results of higher ANR in NF.

The ANR compared to isotopic recovery in plant has on average 23% lower recovery, and difference among recovery techniques has been previously reported (Belder et al., 2005a; Bouldin, 1986; Bronson et al., 2000; Schnier, 1994). The discussions usually describe “priming effect” (Hauck and Bremner, 1976) and “pool substitution” (Jenkinson et al., 1985) as being responsible for interference in plant recovery in isotopic technique. However, for unaccounted N the isotopic recovery remains valid because these does not affect leaching, ammonia volatilization and denitrification losses (Bronson et al., 2000). These studies also report isotopic recovery is lower than ANR although in our study we have observed the opposite. One explanation may be microplot metal frame disturbed plant development inside them. Thus, the lack of biomass development raised the <sup>15</sup>N content in plant. However, the differences among irrigation treatments remained similar in both techniques, as well as the conclusion: NF had the higher recovery efficiency in both techniques and all other irrigation regimes are inferior to NF and equal between them. In our study PFP for NF, CF and AWDS was 61, 57.8 and 52.8, kg grain kg<sup>-1</sup> N respectively, across years and they were statistically equal (Table 5). A high PFP implies the need to N application to production system, and PFP is interpreted as an integration of ANUE and ANR. (Cassman et al., 2003). A reference worldwide rice PFP is 61 kg grain kg<sup>-1</sup> N (Cassman et al., 1996; Ladha et al., 2005). Thus, taking in to account yield and response to N fertilizer, SS and AWDL are less recommended irrigation regimes in such agroecosystem.

#### 2.4.5. The Water use efficiency

The IR provided major differences in WUE<sub>in</sub> as grain yield ranged 15% in 2015 and 8% in 2016 among irrigation regime (Table 7) while water input ranged 105% in 2015 and 80% in 2016 (Table 6). This can be linked to the large amount of irrigation water necessary to flood the fields. At 150 kg ha<sup>-1</sup> N rate, CF and AWDL (the IR that used more water volume) presented a WUE<sub>in</sub> of 0.39 and 0.42 kg grain kg water<sup>-1</sup>, respectively. It is considerably lower efficiency compared to 0.5-1.1 kg grain kg water<sup>-1</sup> reported in similar standards of yield and N rate (Belder et al., 2005a; Borin et al., 2016; M.D.M. Kadiyala et al., 2015; Sánchez-Llerena et al., 2016; Wang et al., 2016; Yao et al., 2012). While at fertilized plot, NF had 0.81 WUE<sub>in</sub>, and it was very similar to Kato et al. (2009) which also observed higher yield to aerobic condition. The zero N fertilizer rate had inferior WUE<sub>in</sub> for irrigations regime due lower grains yields as well. This was previously reported by Belder et al. (2005a) and Ye et al. (2013) and led us to conclude that inadequate N rate and poor field practices indirectly represent a waste of water. In the present study SS had less water demand than CF,

AWDL, AWDS, but as it had a lower grain yield, the  $WUE_{ET}$  was 0.47. Thus, highlights the pursuit of water saving cannot occur at grain yield detriment.  $WUE_{in}$  is sensitive to field practices, nitrogen management and genotype once grain yield is affected by them. Moreover, the soil and hydrological characteristics dictate the water dynamics in the field, and for the present results it represented a stronger demand of irrigation volume.

The  $WUE_{ET}$  ranged from 1.8 to 1.56 kg of grain kg water<sup>-1</sup> irrigation regime at fertilized plots and 1.34 to 1.09 at unfertilized. It was a shorter interval when compared to  $WUE_{in}$ , because the estimate ET just ranged 5.9% and 2.8% in 2015 and 2016, respectively. The  $WUE_{ET}$  is lower than  $WUE_{in}$  due to the infiltration loss not being accounted for. The  $WUE_{ET}$  are similar for those found by Belder et al. (2005b) and Bouman et al. (2005) with similar methodology of ET estimation. In fertilized plots, the  $WUE_{ET}$  increased in relation to unfertilized primarily because to grain yield increment, but also due to increase in LAI as well. Other studies describe the relation of LAI and nitrogen applying (Belder et al., 2005a; Wang et al., 2016). The LAI expansion also raises the transpiration in ET (Alberto et al., 2014; Bouman et al., 2005) and that also has an important contribution for improving  $WUE_{in}$ . In regard to irrigation regimes, NF had 1.64 kg of grain kg water<sup>-1</sup>, across N levels, and had higher total transpiration. On a field scale, for a given genotype and climate conditions, good agricultural practices which favor water use through transpiration rather evaporation improve water use efficiency.

Application efficiency (EA) is an index to define irrigation systems or methods more suitable from the aspect of water use. On a macroscale, it depends on soil physical properties and is related to the amount of water needed to meet crop development, on a field scale it is sensitive to N levels and agronomic practices which ET is dependent on. The experimental site of this study has a fairly high saturated hydraulic conductivity ( $k_{sat} = 1.35$  and  $0.16$  m d<sup>-1</sup> in A and B plinthic horizons, respectively), compared to others lowland rice production areas. Usually it is in a range from 0.8 to 0.0003 m d<sup>-1</sup> in Philippines, India, China, California and Uzbekistan (Devkota et al., 2013; Liang et al., 2014; Singh et al., 2001; Tan et al., 2015; Wopereis et al., 1994). The micro-structure of this soil has a coarse sand size and it provides low bulk density and prominent macroporosity, resulting in a high hydraulic conductivity (Ks) (Buol and Eswaran, 1999). Within 0.6 to 1 m, the B textural horizon presents a clay increment content and it slows down the water infiltration causing the water table to rise during the rainy season (Fageria et al., 2002) The macroporosity is the physical soil attribute closest correlated to hydraulic conductivity (Ankeny et al., 1990) and its soil site macroporosity of 45% stands this soil way over the average of 0.24 % observed in a wide characterization of soil macroporosity in Asia (Aimrun et al., 2004). To summarize the results showed that NF has at least twice more efficiency in EA than CF, AWDS, AWDL, thus appointing to NF as the irrigation regime that lost least amount of applied water and is the most suitable irrigation regime for this system.

## 2.5. Conclusion

The irrigation regime and nitrogen fertilizing interferes in N uptake and grain yield of rice crop. Non-flooding regime provides higher yield and overall crop performance in zero and 150 kg N ha<sup>-1</sup>. The isotopic efficiency showed less unrecovered N from fertilizer in NF irrigation and apparent nitrogen recovery (ANR) was higher for NF as well. Apparent nitrogen efficiency was higher for Continuous flooding and Alternate wetting and drying short, as these irrigation regimes showed lower yields at 0 N fertilizer rate. Partial Factor Productivity demonstrated that NF, CF and AWDS are more adequate to N fertilization.

Water use efficiency was higher for non-flooding condition, in both terms of total water input and water ET, compared to all other irrigation regimes. The integrated view of crop performance, water and nitrogen

efficiency, as long there is no water shortage, the non-flooding is the more suitable irrigation regime for such agroecosystem. Further studies are necessary to understand N dynamics and loss processes in such environment.

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### 3. RELATIONSHIP OF NITROGEN AND CROP COMPONENTS IN AEROBIC RICE AND CONTINUOUS FLOODING IRRIGATION IN WEATHERED TROPICAL LOWLAND

#### Abstract

Aerobic rice is a promising rice production system which enables rice to grow in water-constrained environments and provides an alternative to continuous flooding irrigation. Although significant water savings are reported in aerobic systems in different lowland regions, a negative impact in crop performance prevents its wide deployment. The main reasons for grain yield decreases are given to be genotype and inappropriate water supply. However, agronomic practices such nitrogen management can interfere in overall crop performance of rice due to the influence of soil moisture on N availability to crop uptake. The goal of this research was to establish a relationship between nitrogen use and crop performance by comparing flooding conditions to aerobic rice in a tropical environmental with adequate water supply. A field experiment was carried out from 2014 to 2016 with an experimental design of split plot with irrigation regime (IR) in the main plot: continuous flooding (CF) and non-flooding aerobic (NF), and in the subplot fertilizer nitrogen rates: (0, 50, 150 and 250 kg ha<sup>-1</sup>). Biomass (AGB) nitrogen uptake (NU) and leaf area index (LAI) were quantified during crop development only in the 0 and 150 kg ha<sup>-1</sup> subplots, as was the relationship between AGB, NU, grain yield and its components and all nitrogen rates at harvest. The aerobic rice provided higher NU throughout rice development and LAI equal or superior to CF. At harvesting, NU and panicle density (PD) had different relationships to N rates among CF and NF, with NF showing 18% higher NU as 27% PD across years and N rate. The grain yield (GY) relationship to N rates was also distinct in 2014 and 2015, and GY across N rates in NF was 20% and 12% higher than CF for 2014 and 2015, respectively. We conclude that lowland rice managed through aerobic system improves nitrogen uptake by plants as long the water filled space remains around 60%.

Keywords: *Oryza sativa*; Plinthaquults; Grain yield; Water saving, Evapotranspiration

#### 3.1. Introduction

Aerobic rice is an evolving production system which uses less water than a traditional continuous flooding irrigation regime (Tuong et al., 2005). Aerobic rice is not submitted to ponding water, and the soil remains unsaturated. The crop can be either rainfed or irrigated, and therefore upland rice is sometimes included in the definition of aerobic rice (Kato et al., 2009). However, aerobic rice more accurately refers to lowland rice production where flooding irrigation is suitable and aerobic production serves as an alternative to save water while still reaching similar grain yield (Peng et al., 2006). The gain in water economy is due to the absence of puddling tillage and therefore water ponding. Eliminating evaporation directly from water surface allows for 29-40% less water input (Bouman et al., 2005; Jabran et al., 2015; Sharma et al., 2016). In spite of the relevant benefit of less water consumption, crop performance and grain yield are usually considered inferior to continuous flooding (Belder et al., 2005b; Peng et al., 2006; Zhou et al., 2011), although a few studies have observed equal or slightly higher yields in aerobic conditions (Kato et al., 2009; Sánchez-Llerena et al., 2016). Thus, it is unclear

if this field gap is intrinsic to the differences in the irrigation regime or if aerobic rice just does not have well-established genotypes and agronomic practices such as nitrogen management.

Nitrogen (N) is the most required and limiting nutrient to rice production in lowland environments, and it currently consumes 16% of mineral N fertilizer produced in the world (Ladha et al., 2016). In order to meet the rice demand of a fast-growing population, the N fertilizer production will need to reach 23 million tons by 2030 (Daberkow et al., 2000). The global average of N use in rice is around 120 kg ha<sup>-1</sup> year<sup>-1</sup> (Ladha and Chakraborty, 2016) although this rate can widely vary across production environments, such as aerobic system. The world leader in rice production is China, and their N rate can reach 300-400 kg ha<sup>-1</sup> (Zhao et al., 2012). The Philippines and Vietnam use 100 kg ha<sup>-1</sup> of N (Deb et al., 2016; Silva et al., 2017), and in Brazil it is 50 kg ha<sup>-1</sup> across lowland and upland rice systems (FAO, 2004).

One of the reasons for such distinctions in N rates is its interdependence to other agroecosystem factors (Fageria and Baligar, 2005). The nitrogen relationships with water management and indigenous N pools are the most relevant issues causing different responses to N fertilizer applications (Somaweera et al., 2016; Tan et al., 2015; Wang et al., 2016; Zhou et al., 2012). An integrated assessment of N and crop interaction is the nitrogen use efficiency index (NUE) (Cassman et al., 2002; Ciampitti and Vyn, 2012; Dobermann et al., 2000). A poor NUE negatively impacts the environment due to risk of nitrate leaching or runoff, air pollution and greenhouse gas emissions – N<sub>2</sub>O derived from nitrification-denitrification processes (Cassman et al., 2003). Traditionally, continuous flooding is described as the most suitable to provide a high NUE (Reddy and Patrick, 1976), although recent research has showed alternate wet and dry with moderate drying periods is able to deliver the same NUE as CF (Pan et al., 2017; Sun et al., 2012; Wang et al., 2016; Ye et al., 2013). However, aerobic irrigation regimes retain a stigma of providing low nitrogen use in rice crops (Belder et al., 2005a; Bouman et al., 2005; Sharma et al., 2016; Somaweera et al., 2016; Zhou et al., 2012).

Grain yield is the ultimate crop parameter which responds to N fertilizer, although each yield component has its own relationship to N availability. Above ground biomass (AGB) has a wide variable response to site genotype among N management (Fageria and Santos, 2013; García et al., 2003; Somaweera et al., 2016). Leaf area index (LAI) and N uptake are closely related to N site specificity (Ao et al., 2010; Murchie et al., 2002; Zhong et al., 2003), although LAI is better set throughout genotype breeding (Fageria and Baligar, 2005; Long et al., 2006). Number of spikelets, spikelet sterility and grain weight also respond to N (Kato et al., 2009; Lampayan et al., 2010; Yao et al., 2012), although they seem to indirectly depend on N and crop attributes as plant density and row spacing affect them strongly. Therefore, to propose an N strategy in aerobic rice, one must take into account the integrated effect in all yield components.

Therefore, this research intends to integrate water and nitrogen factors to address the following questions: (i) Do aerobic condition and continuous flooding influence differently crop response to N applied as fertilizer, especially in a site condition where an aerobic regime seems be more suitable? (ii) What is the impact on grain yield, given that not all yield components will interact in fertilizer nitrogen and irrigation regimes? (iii) In different irrigation regimes, where both supply water properly, the crop will performance be affected just by N availability or could the aerobic condition itself influence plant development?

## 3.2. Material and Methods

### 3.2.1. Site Description

On farm experiments were established in the region of Lagoa da Confusão, State of Tocantins, in Northern Brazil (10°46'39.80"S; 49°55'20.94"W and 190 m ASL) during the rainy season in the summers of 2014, 2015 and 2016. The local climate is classified as Aw – tropical wet and dry climate (Koeppen) - with average rainfall of 1800 mm, mostly from September to May, and with an average temperature of 26.7°C. Figure 1 (chapter 1) shows rainfall and daily temperature for the three-year period of this study.

The soil is classified as a Plinthaquults (US Taxonomy) with a Plinthic horizon within 60 cm of depth which has limited water percolation, without an impermeable layer. The physiochemical properties are shown in Table 1 (chapter 1) The saturated theta ( $\theta_s$ ) at 0-0.1m depth is 0.606 and 0.639  $\text{cm}^3 \text{cm}^{-3}$  in the 2015 and 2014/2016 sites, respectively.  $\theta_s$  to B plinthic (0.6 m and bellow) is 0.485 and 0.458, in 2015 and 2014/2016, respectively. The macro, meso and micro porous size distribution for A horizon are 45%, 15% and 39% and in B plinthic are 31.9%, 4.5% and 63.6%, respectively.

The area was converted to agricultural use around 6 years before the start of the experiment under crop rotation of rice during rainy season and soybean irrigated by sub-irrigation during the dry season (May to September).

### 3.2.2. Field Experiment

The experimental design was a split-plot randomized complete block design with four replications during 2014, 2015 and 2016 crop seasons. The main plot treatments were irrigation regime (IR): continuous flooding (CF) and non-flooded rainfed aerobic (NF). CF had water input from rain and irrigation and NF just from rain. Each main plot was further split into sub plots of four N fertilizer levels: 0, 50, 150 and 250 kg N ha<sup>-1</sup>.

The main plots consisted of 105 m<sup>2</sup> hydrologically independent plots with 50 cm high bunds and a 60 cm deep drain in the whole plot perimeter. Irrigation water was applied through a pressurized system with an independent inlet for each plot. Initial irrigation took place roughly 25 days after seed emergence, and the water level was maintained at 5-7 cm high in CF. A 25-day delay to trigger irrigation is common practice in Lagoa da Confusao due to lack of water reservoirs. Water is pumped into rice basins from rivers at the onset of the rainy season and rise in water level.

Nitrogen subplot treatments were randomized within each main plot. N was applied as pearled urea (46% N) and split in four equal rates at the following developmental stages: sowing, tillering (V5-V6), panicle initiation (R0) and collar formation of flag leaf (R2) (Counce et al. (2000)). 5 mm of water was flushed after urea application to promote incorporation into the soil.

Plots were sowed with IRGA 424 rice cultivar with a cycle of approximately 116 days in the tropical region of Brazil. The crop was established in a dry seed sowing with row distance of 17 cm and plant density

after emergence of 150 plants m<sup>-2</sup>. Phosphorus and Potassium fertilizer was applied at the rate of 60 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> (as superphosphate) and 140 kg ha<sup>-1</sup> K<sub>2</sub>O (as KCl).

### 3.2.3. Crop performance along seasons' development

In the subplots of 0 and 150 kg N ha<sup>-1</sup> in both irrigation regimes, measurements began 15 days after emergence and were repeated every 15 days thereafter, completing 7 samplings each season. Plant above ground biomass (AGB) and nitrogen content (NC) were determined by destructively sampling rice plants from 0.5 m<sup>2</sup> collected in roughly 15-day intervals. Plants were cut at the stem base and chopped green. A representative subsample was dried to constant weight in a 65°C oven, and water content was extrapolated for the entire sample. Nitrogen concentration was determined by the Kjeldahl method after acid digestion and total nitrogen uptake (NU) was calculated as the product of N content (g kg<sup>-1</sup>) and plant biomass (g m<sup>-2</sup>).

The leaf area index (LAI) and the light extinction coefficient were indirectly determined with an optical hemisphere sensor (LI-cor 2200, Li-cor, Inc., USA). All data collection occurred at approximately 09:00 am or on cloudy days. One reading was taken over the canopy and 5 readings at the soil (or water) surface. Crop height was measured at time of LAI sampling as the distance from soil surface to the last opened leaf.

### 3.2.4. Crop performance at harvest

At maturity, five plants per plot were randomly sampled to determine the number of spikelets per panicle. Grains were manually detached from the rachis and weighed. Filled and unfilled grains were separated by air blowing, counted and reweighed. Panicle density was counted inside a 0.5 m<sup>2</sup> quadrat randomly settled inside the subplot. AGB, NC and NU were determined the same as described during crop development. Grain yield was determined by collecting 3 m<sup>2</sup> of rice plants at 19-23% field moisture, and grains were separated from plants using an experimental mechanical thresher (SB Maquinas, Cambé, PR). Impurities were separated in a sample cleaner (Mediza, Panambi, RS) and moisture corrected to 13% in unhusked grains.

### 3.2.5. Evapotranspiration and water filled porous

Rainfall, radiation, air humidity and temperature data were measured hourly by a weather station (Davis Vantage Pro2, Davis, Inc., USA). Irrigation water input (WI) was monitored by a flow hydrometer installed in all water inlets. Fields were not drained, unless by percolation into the soil profile. Evapotranspiration (ETC) was estimated by the Penman–Monteith equation, using the measured LAI and crop height. Zero plane displacement (d) and roughness length for momentum (Z<sub>om</sub>) and vapor (Z<sub>oh</sub>) were estimated based on crop height (Allen, 1998). Crop surface resistance (r<sub>s</sub>) was assumed as 80 sm<sup>-1</sup> of (r<sub>l</sub>) (Dingkuhn et al., 1999; Turner et al., 1986). The proportion of transpiration and evaporation was determined according to the Bouguer-Lambert law, which measures radiation intercepted by the crop canopy or soil surface (van Laar et al., 1997):

$$T = ET_c (1 - e^{(-kLAI)}) \quad \text{eq. (6)}$$

$$E = ET_c e^{(-kLAI)} \quad \text{eq. (7)}$$

where  $k$  is the extinction coefficient (assumed to be 0.5 based on the average of measured data). For CF and NF during rainy days ET actual was assumed to meet the ETCrop. For NF in non-rainy days, the actual soil evaporation was assumed to drop proportionally to the square root of the drying time as predicted by Ritchie (1972). Water-filled pore space (WFPS) was calculated from the volumetric moisture content of soil measured at 0.03 to 0.15 cm depth by dielectric conductivity probe (GS1 - Decagon, WA, USA) recorded every 30 minutes and transformed in daily averages in two of four replicate blocks. The output of dielectric probe was  $\theta_v$  ( $\text{g g}^{-1}$ ) and de WFPS calculations was made according to Linn and Doran (1984) as follow:

$$WFPS (\%) = \frac{\theta_v}{TP} \times 100 \quad \text{eq. (9)}$$

$$TP (\%) = \left(1 - \frac{P_b}{P_p}\right) \times 100 \quad \text{eq. (10)}$$

$$\theta_v (\%) = \theta_g \times P_b \quad \text{eq. (11)}$$

where  $\theta_v$  is volumetric water content (%); TP is total soil porosity (%);  $P_b$  is soil bulk density measured in undisturbed samples ( $\text{Mg m}^{-3}$ );  $P_p$  is particle density assumed as  $2.65 \text{ Mg m}^{-3}$ .

### 3.2.6. Exchangeable nitrogen content in soil

Soil samples for mineral Nitrogen content analysis was performed in  $150 \text{ kg ha}^{-1}$  of N fertilizer subplot for both IR in the depth of 0.15 m, during the 2015 and 2016 seasons. The first sampling was done one day before rice sowing and after that following a roughly interval of 15 days until physiological maturity in a total of 8 sampling per season.

The samples were composed by 3 subsamples collected with a Dutch auger and kept refrigerated until laboratory extraction. The extraction was made homogenizing the samples, and in a fresh aliquot of 20g was added 100 ml of 2M KCl. After 1 hour shaking the extracts were filtered in filter paper (Keeney and Nelson, 1982). A mirror subsample was sent to dry oven for moisture correction. The Nitrate and ammonium determination was done through Flow Injection Analyzer - FIA (ASIA, Ismatec, Switzerland) using Bromocresol Purple as pH indicator and zinc column to nitrate reduction for ammonium (Kamogawa and Teixeira, 2009).

### 3.2.7. Statistical Analysis:

The dataset was submitted to statistical procedures using Statistical Analysis System, version 9.2 (SAS Institute, 2009). Data related to harvest measurements were tested against assumptions of homogeneity of variance and error normality by PROC TRANSREG Boxcox statement. If assumptions were violated, the variable was transformed by the convenient lambda. Analysis of variance was performed by PROC GLM based on irrigation, N level and year, as well as its interactions as a fixed effect model. Block replicates were considered random effects to the main plot and block x main plot as the random effect for the subplot. When F probability was  $<0,05$  the means were fitted to linear regression using PROC REG, and model parameters compared through confidence interval. The data related to on season development were submitted to multivariate analysis of variance by contrast command. The treatments mean profiles were compared among years using repeated time statement.

### 3.3. Results

#### 3.3.1. Crop performance along seasons' development

Mauchly's Test of Sphericity for repeated measurements in time was performed to validate the assumptions of multivariate normality and homogeneity of variance for aboveground biomass (AGB), nitrogen uptake (NU), and nitrogen content (NC). The  $p$ -value was  $<0.05$  for all three observed variables, therefore profile analyses were performed.

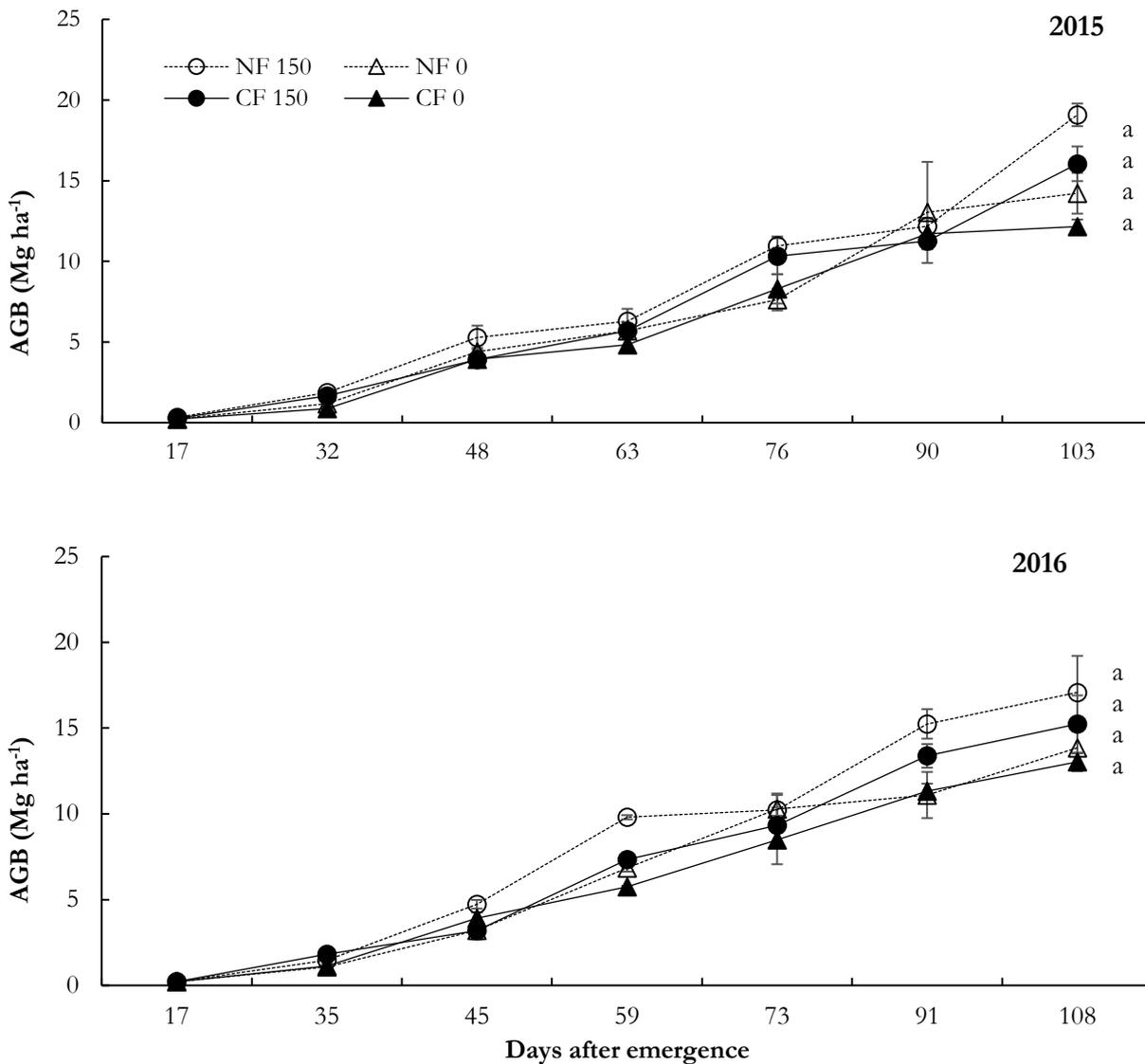


Figure 3. Graphical profile of mean above ground biomass (AGB) (kg ha<sup>-1</sup>) during 2015 and 2016 growing seasons. Standard errors are given in each data mean. Different lowercase letters at end of mean profile refers to significant difference ( $p < 0.05$ ) according to MANOVA.

In 2015 and 2016 the mean profile of AGB among treatments CF 150, NF 150, CF 0 and NF 0 were not significantly different (Figure 3) This indicates that the biomass and its increments among the growing seasons of 2015 and 2016 were not affected by different IR and N levels. If biomass is taken into account just at physiological maturity, in 2015 and 2016 the F test shows significance ( $p < 0.05$ ). It is reasonable to observe the

difference only in final AGB due to grain yield participation on it. The mean AGB along the growing season was 0.3, 1.4, 4.4, 5.6, 9.3, 12.3 and 15.4 Mg ha<sup>-1</sup> in 2015 and 0.2, 1.4, 3.8, 7.4, 9.6, 12.8 and 14.8 Mg ha<sup>-1</sup> in 2016.

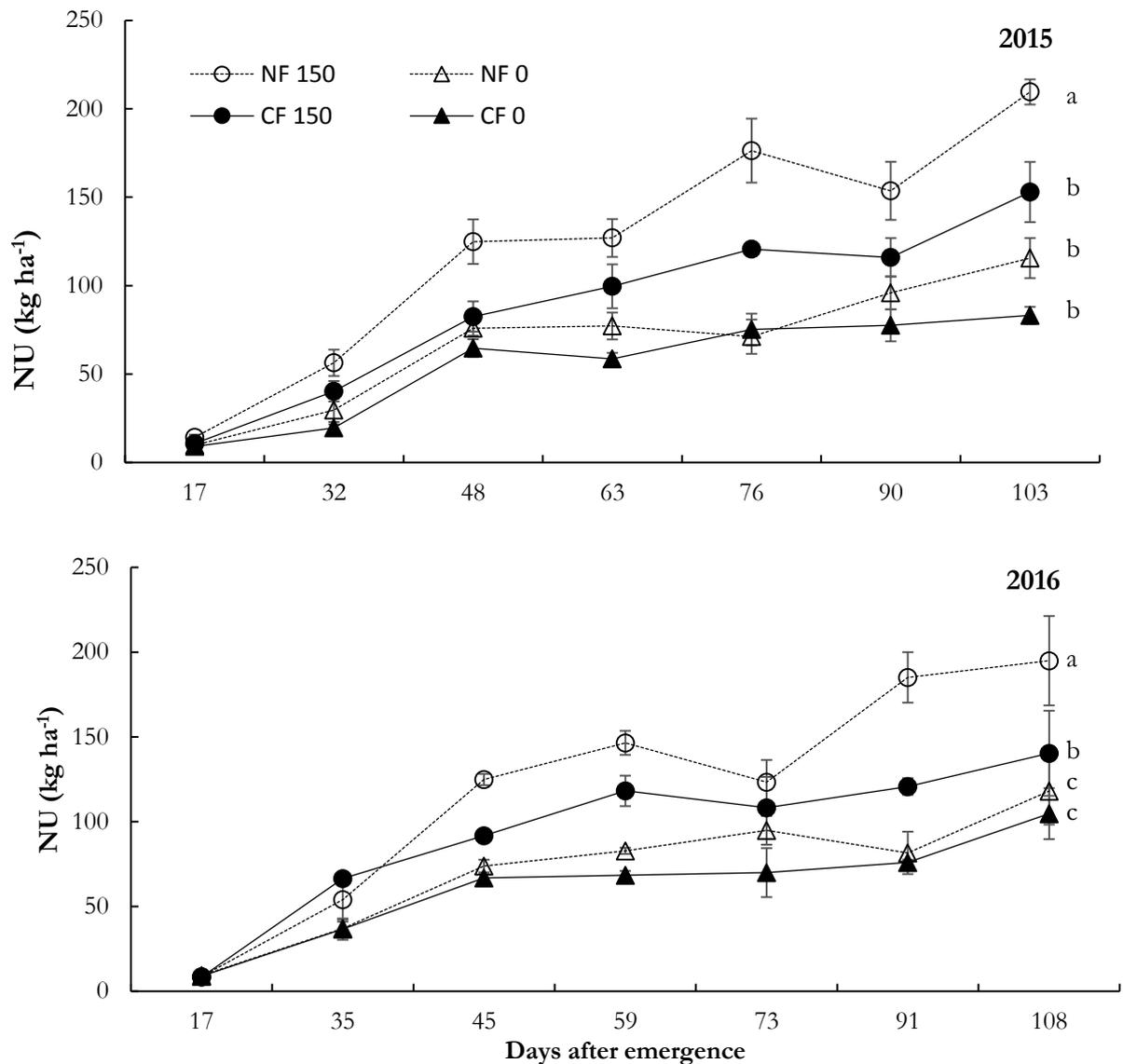


Figure 4. Graphical profile of mean plant nitrogen uptake (kg ha<sup>-1</sup>) during 2015 and 2016 growing seasons. Standard errors are given in each data mean. Different lowercase letters at end of mean profile refers to significant difference ( $p < 0.05$ ) according to MANOVA.

In 2015 the profile analysis of NU showed that NF 150 kg ha<sup>-1</sup> had a higher overall uptake than CF 150, NF 0, and CF 0 across all sampling dates, and there was no significant difference between CF 150, NF 0, and CF 0 (Figure 4). At floral differentiation occurred at 48 DAE (panicle initiation -R0) where NF 150 had accumulated in its above ground biomass 127 kg ha<sup>-1</sup> of N, while CF 0, CF 150 and NF 0 had, on mean, 78 kg ha<sup>-1</sup>. At 90 DAE rice blast disease (*Magnaporthea oryzae*) impacted all experiment treatments, although NF 150 and CF 150 were most affected. This caused NU reduction. At crop maturity, the total NU was 210 kg ha<sup>-1</sup> for NF 150 kg<sup>-1</sup> of N, followed by 153, 116 and 83 kg ha<sup>-1</sup> for CF 150, NF 0 and CF 0, respectively.

Similarly, in 2016, the NF 150 treatment had a higher overall uptake than CF 150, CF 0 and NF 0 across all sampling dates ( $p < 0.05$ ) (Figure 4). Also like 2015, the N uptake distinction among IR and N levels

occurred roughly in panicle initiation (45 DAE). However, a difference from 2015 was that CF 150 had the next highest N uptake, and was significantly higher than CF 0 and NF 0. In 59, 91 and 108 DAE the NF 150 remained with a NU higher than all other treatments, although at 73 DAE there was no difference among IR and N levels. In 2016 the rice blast hit the crop later than 2015, causing loss of biomass mainly in NF 150 and CF 150 and leading to the absence of a difference in NU at 73 DAE. The final N uptake was 190, 140, 118 and 105 kg ha<sup>-1</sup> in NF 150, CF 150, NF 0 and CF 0 respectively.

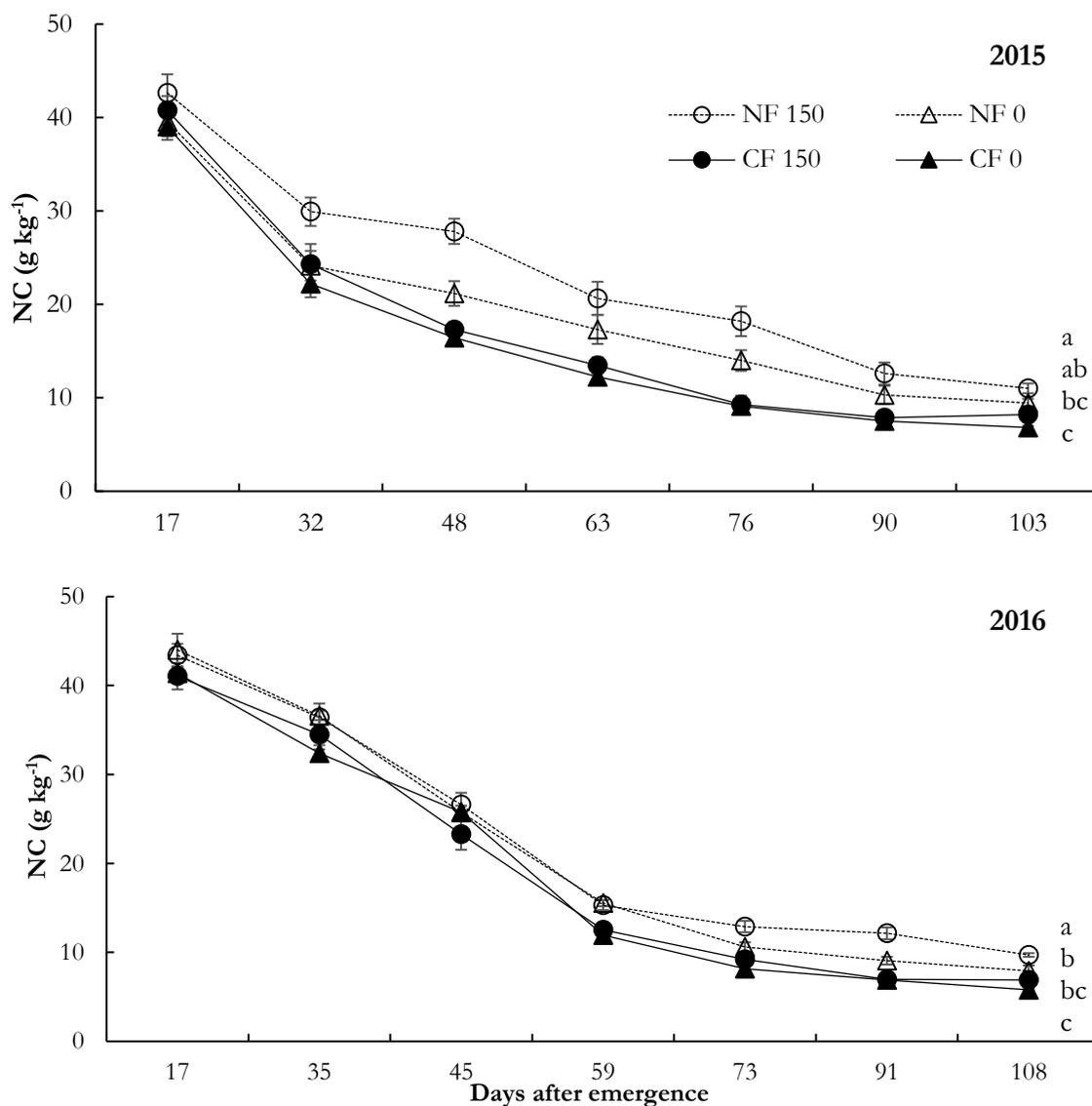


Figure 5. Graphical profile of nitrogen content in plant tissue (g kg<sup>-1</sup>) during 2015 and 2016 growing seasons. Standard errors are given in each data mean. Different lowercase letters at end of mean profile refers to significant difference ( $p < 0.05$ ) according MANOVA.

Regarding N content (NC) in above ground biomass, the standard error showed a smaller interval than those observed in NU and AGB. Therefore, profile analysis could better differentiate NC among treatments (Figure 5). In 2015, the NC profile was the same between NF 150 and CF 150 being superior to CF 0. The differentiation among profiles occurred at 32 DAE, and similar to NU, NF 150 had the highest NC from that stage onward. At panicle initiation, NC was 27.8 g kg<sup>-1</sup> in NF 150, 21.2 g kg<sup>-1</sup> in CF 150 and 13 g kg<sup>-1</sup> in CF 0 and

NF 0. At crop maturity, NC was 11, 9.5, 8.2 and 6.8 g kg<sup>-1</sup> for NF 150, CF 150, NF 0 and CF 0, respectively. In 2016, the mean NC profile of NF 150 was the highest over the growing season, followed by CF 150 and NF 0, however CF 150, NF 0 and CF 0 were all the same. The differentiation among profiles occurred at 59 DAE, which is 27 days later than 2015, and just between 0 and 150 N levels. At panicle initiation, mean NC for all treatments was 25.3 g kg<sup>-1</sup>. At physiological maturity mean NC was 9.7, 7.9, 6.9 and 5.8 g kg<sup>-1</sup> for NF 150, CF 150, NF 0 and CF 0, respectively. In both years, leaf tissue NC decreased as crop development progressed.

LAI also was affected by N level and IR. The mean profile of LAI throughout the 2015 growing season was highest for NF 150, followed by CF 150, NF 0 and CF 150 (figure 6). At panicle initiation NF LAI was 5.2 m<sup>2</sup> m<sup>-2</sup> and was higher than all other treatments, which had a mean of 4.4 (m<sup>2</sup> m<sup>-2</sup>) without distinction among them. In the sampling dates after PI, CF 150, NF 0 and CF 150 became significantly different, with this trend continuing until harvest. The final LAI was 8.3, 7.4, 5.1 and 3.8 m<sup>2</sup> m<sup>-2</sup> in NF 150, CF 150, NF 0 and CF 0, respectively. In 2016, the differences between mean profiles was less pronounced than 2015. At panicle initiation, all treatments had an equivalent LAI of 3.05 m<sup>2</sup> m<sup>-2</sup>. The distinction occurred at 73 DAE and just among N levels. NF 150 and CF 150 were 4.8, and NF 0 and CF 0 were 3.8 m<sup>2</sup> m<sup>-2</sup>, on average. At harvest the LAI in NF 150 was 6.1 m<sup>2</sup> m<sup>-2</sup>, and it was statistically similar to CF 150, which had a mean of 5.43 m<sup>2</sup> m<sup>-2</sup>. NF 0 and CF 0 had an LAI of 4.3 and 3.6 m<sup>2</sup> m<sup>-2</sup> respectively. At 73 DAE the decrease of LAI was related to blast disease, as well as NU. In 2015 the effects of the fungus were not visible in the profile due to fewer sampling dates.

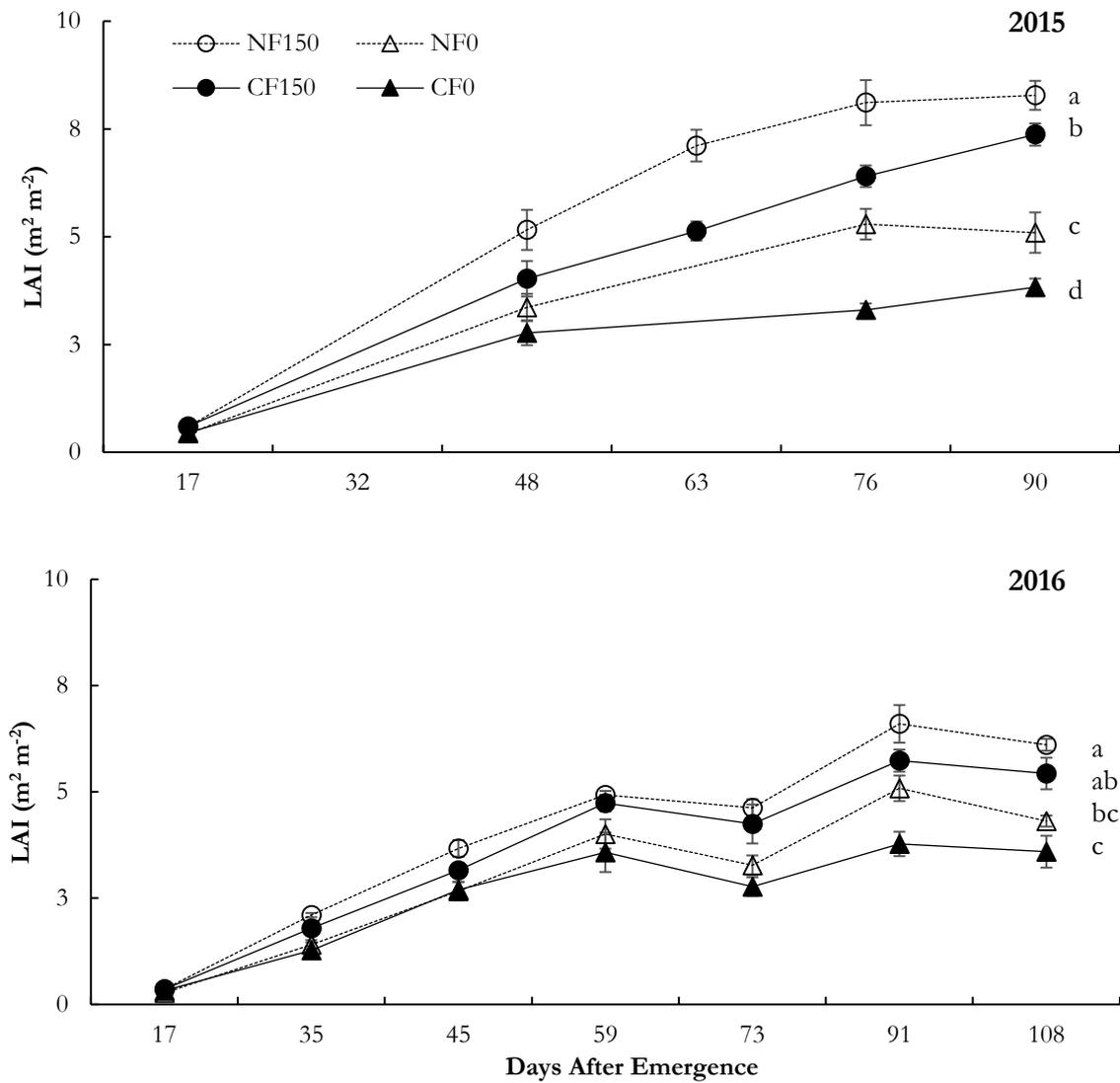


Figure 6. Graphical profile of leaf area index (LAI) (m<sup>2</sup> m<sup>-2</sup>) during 2015 (A) and 2016 (B) growing seasons. Standard errors are given in each data mean. Different lowercase letters at end of mean profile refers to significant difference ( $p > 0.05$ ) according to MANOVA.

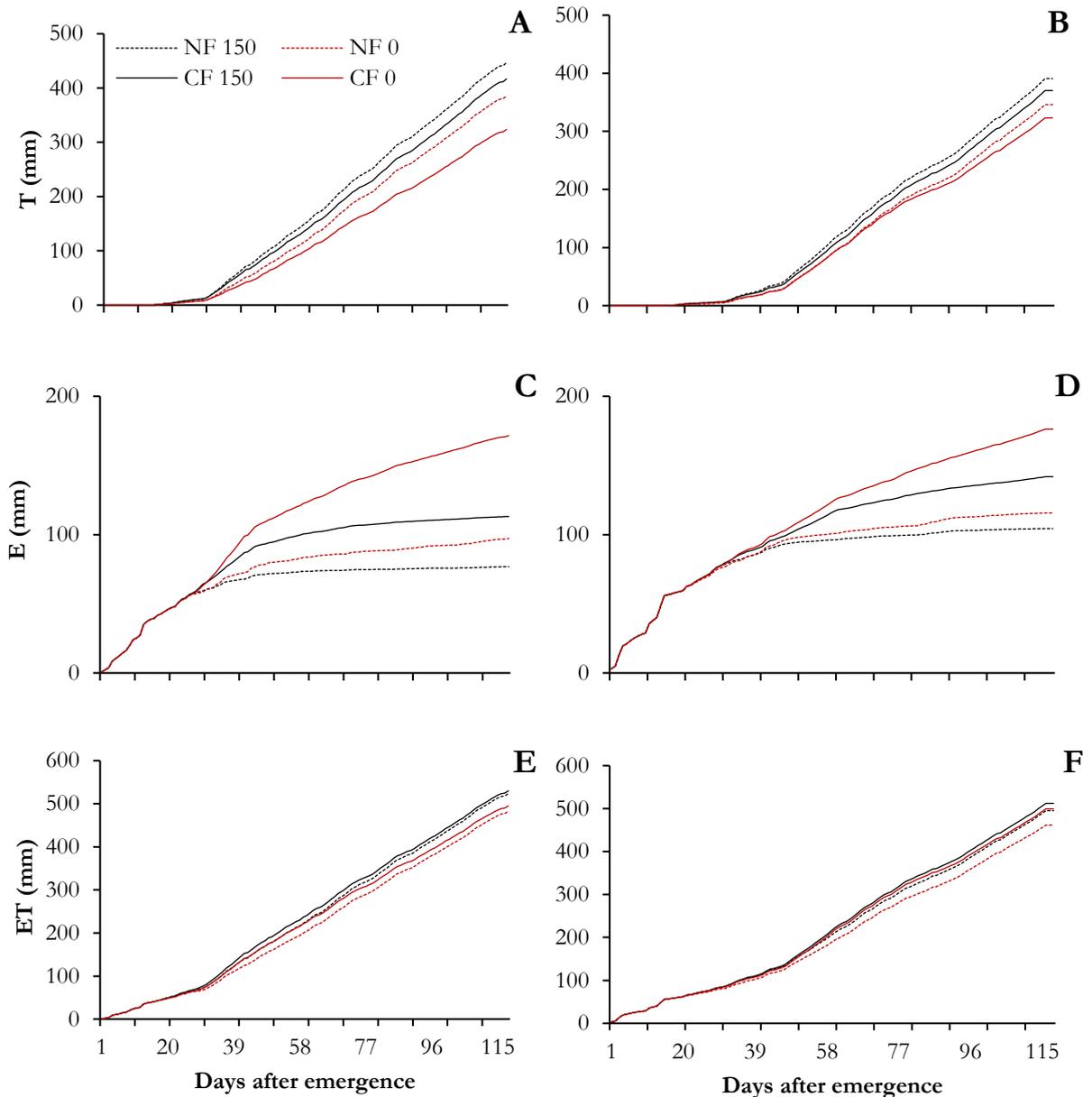


Figure 7. Estimated accumulative Transpiration (T), Evaporation (E) and Evapotranspiration (ET –mm day<sup>-1</sup>) during the 2015 (A; C; E) and 2016 (B; D; F) growing seasons.

The accumulative transpiration and evaporation rates began to differentiate among treatments shortly after irrigation treatments started (figure 7). This differentiation was around 31 DAE in 2015 and 39 DAE in 2016. The main crop attribute that was affected by IR and nitrogen level was LAI (Figure 6) which is one parameter of ET estimation. In 2015, the daily mean T was 3.6, 3.8, 2.7 and 3.3 mm day<sup>-1</sup> for CF 150, NF 150, CF 0 and NF 0, respectively. In 2016 T was 3.2, 3.4, 2.8 and 3.0 mm day<sup>-1</sup> for CF 150, NF 150, CF 0 and NF 0 respectively. Water evaporation was also affected by IR and N levels, with the highest estimated E in CF 0 in both years, with a mean of 1.5 mm day<sup>-1</sup>. CF 150 was next highest with 1.0 and 1.2 mm day<sup>-1</sup> in 2015 and 2016, respectively. Evaporation in NF 0 and NF 150 was 0.8 and 1.0 mm day<sup>-1</sup> in 2015 and 1.0 and 0.9 mm day<sup>-1</sup> in 2016. Despite having a higher IAF than NF 0 (figure 7), the higher evaporation in CF 150 was due to the influence of ponding water in the ET estimation model. The consolidation of T and E in ET shows a daily mean ET of

4.4, 4.5, 4.1 and 4.2 respectively to NF 150, CF 150, NF 0 and CF 0 in 2015 and 4.2, 4.3, 4.2 and 3.9 to NF 150, CF 150, NF 0 and CF 0 in 2016.

The WFP ranged from 24 to 100% across IR and growing seasons. The lowest values of WFP were observed in 2015 before the CF irrigation started in an uncommonly long rainfall interval. Both IR were equally exposed to it and crop showed signs of drought stress. In 2015 after IR started the WFP in CF remained at 100% as expected, while NF ranged from 58 to 65%, averaging 62%. In 2016 the rainfall was poorly distributed and the WFP was widely ranged in NF, from 43 to 71%, averaging 59% from beginning of irrigation in CF to harvest.

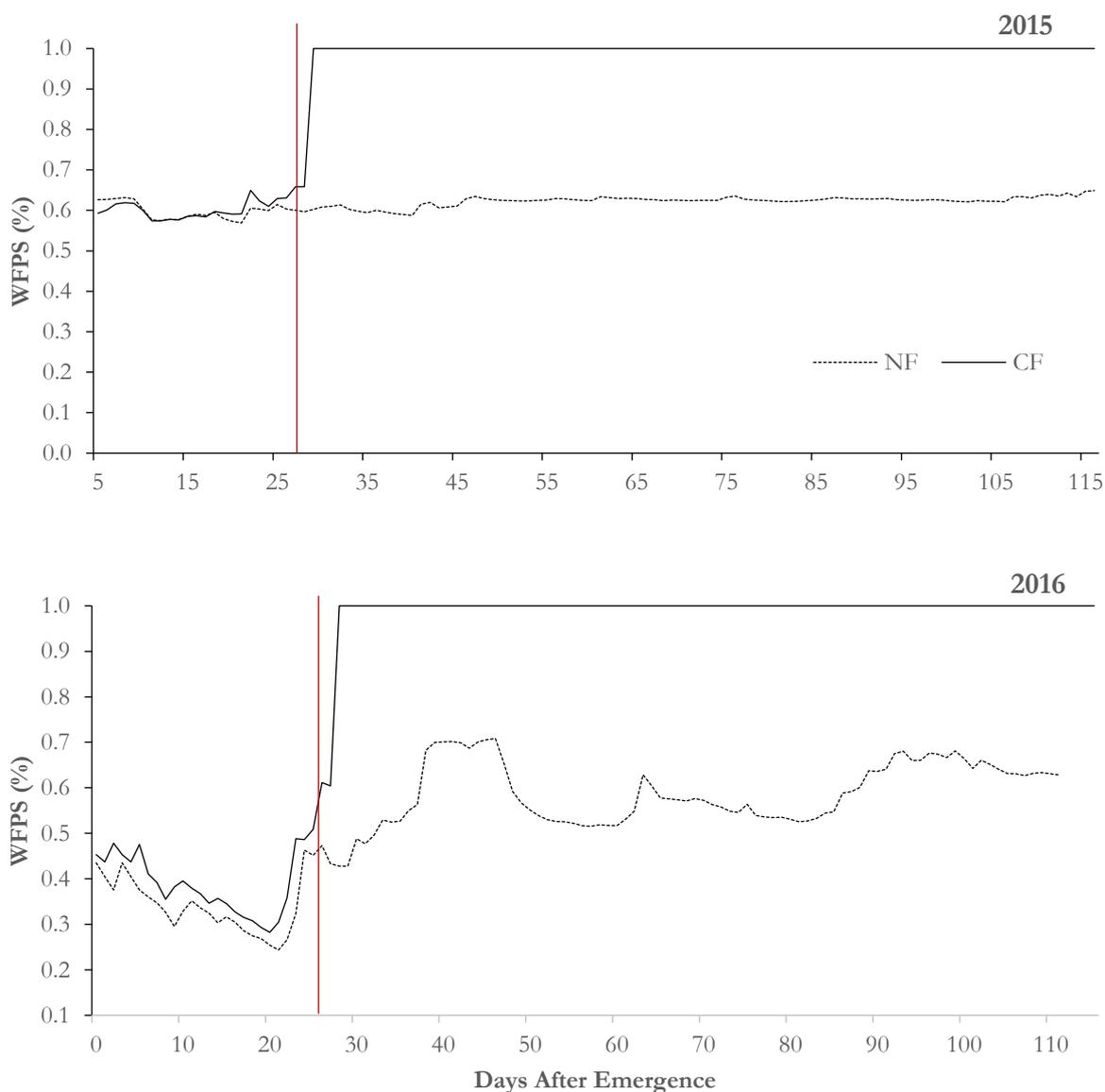


Figure 8. Water-filled pore spaces (% - WFPS) at 0.0 – 0.15 m in a Plinthaquults. The treatments are: continuous flooding (CF); non-flooding aerobic (NF) along 2015 and 2016 seasons. The red bars indicate when irrigation started.

There were differences among IR in availability of N exchangeable in soil (0-0.15 depth) only at 45/46 DAE. In 2015, there was 48 kg ha<sup>-1</sup> of ammonium in NF while in CF it was 34 kg ha<sup>-1</sup>. In 2016, there was 46 kg ha<sup>-1</sup> of ammonium in NF and CF 36 kg ha<sup>-1</sup>. The same trend was observed for nitrate. In 2015, it was also higher in NF (26 kg ha<sup>-1</sup>) than CF (16 kg ha<sup>-1</sup>) whereas in 2016 for NF was 39 kg ha<sup>-1</sup> and CF 24 kg ha<sup>-1</sup>. After 59 DAE onwards the

ammonium and nitrate content ranged from 2-5 kg ha<sup>-1</sup> and were not different among them. In 2015 nitrate and ammonium presented similar values in soil before the experiment was installed, roughly 10 kg<sup>-1</sup> for both of them, and in 2016 nitrate was 23 kg ha<sup>-1</sup> and ammonium 10 kg ha<sup>-1</sup>.

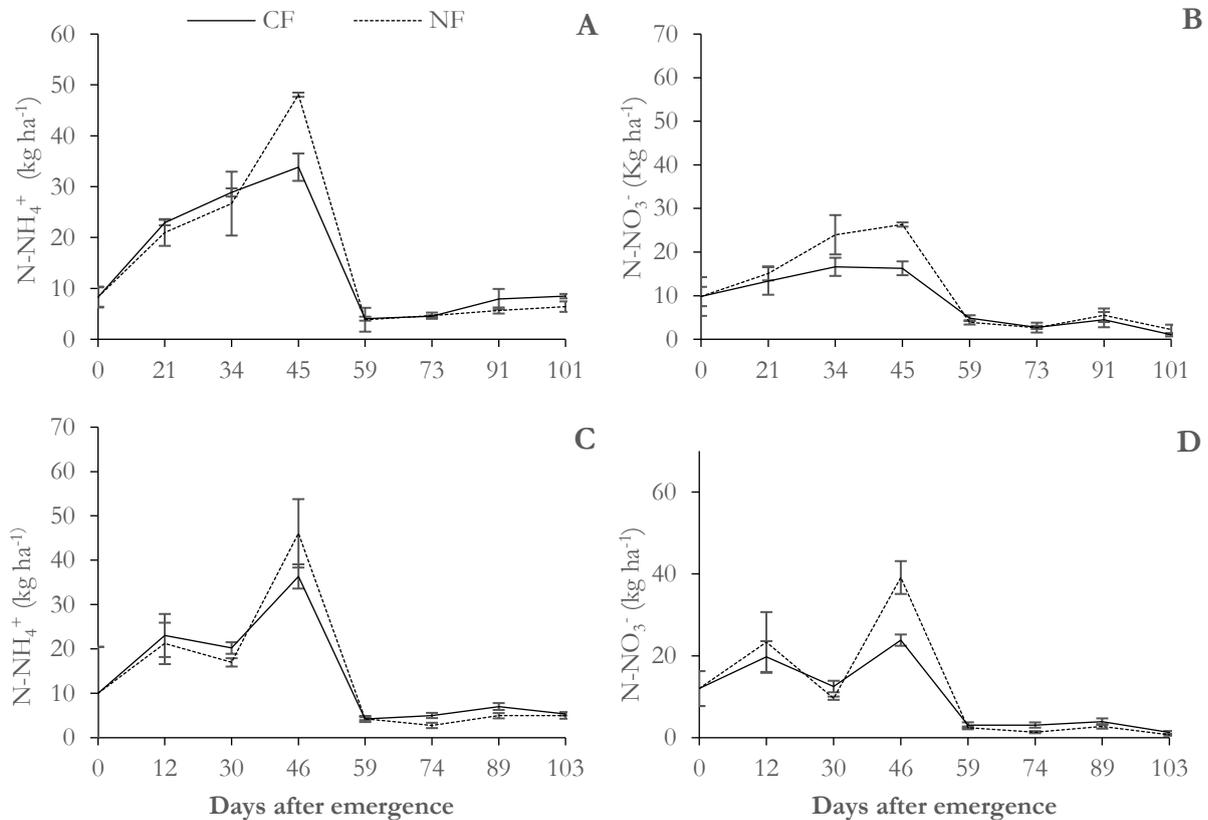


Figure 9. Exchangeable mineral nitrogen among rice growing seasons (Kg ha<sup>-1</sup>). A) Ammonium N in 2015; B) Nitrate in 2015; C) Ammonium in 2016; D) Nitrate in 2016.

### 3.3.2. Crop performance at harvest

Table 8. Analysis of Variance probability for above ground biomass, N uptake and N content in above ground biomass, number of spikelets per panicle, panicle density (panicle m<sup>-2</sup>) and grain yield of rice. The fixed effects are irrigation regimes (continuous flooding and non-flooding); nitrogen levels (0, 50, 150 and 250 kg ha<sup>-1</sup>) and growing seasons (2014, 2015 and 2016).

Source of variation	Biomass	N uptake	N content	Spikelet	Panicle	Grain Yield
Irrigation regime (I)	0.04*	<0.01**	0.03*	0.23 <sup>ns</sup>	<0.01**	0.03*
Nitrogen level (N)	<0.01**	<0.01**	<0.01**	<0.01**	<0.01**	<0.01**
Year (Y)	<0.01**	0.05*	<0.01**	<0.01**	<0.01**	<0.01**
I x N	0.04*	0.26 <sup>ns</sup>	0.12 <sup>ns</sup>	0.22 <sup>ns</sup>	0.03*	<0.01**
Y x I	<0.01**	0.12 <sup>ns</sup>	0.45 <sup>ns</sup>	0.02*	0.13 <sup>ns</sup>	0.03*
Y x N	0.04*	0.09 <sup>ns</sup>	<0.01**	0.71 <sup>ns</sup>	0.66 <sup>ns</sup>	<0.01**
Y x I x N	0.46 <sup>ns</sup>	0.78 <sup>ns</sup>	0.56 <sup>ns</sup>	0.61 <sup>ns</sup>	0.66 <sup>ns</sup>	0.32 <sup>ns</sup>

ns: not significant; \* Significant at  $p \leq 0.05$ ; \*\* Significant at  $p \leq 0.01$  by ANOVA F test.

Table 8 shows the analysis of variance for rice above ground biomass (AGB), N uptake (NU) and N content in above ground biomass (NC), number of spikelets per panicle (NS), panicle density (panicle  $m^{-2}$ ) (PD) and grain yield (GY). Nitrogen level and year showed significant effects in all observed crop parameters, and IR also affected these parameters across years with the exception of NS. The AGB, NC, NS, PD and GY showed at least one significant interaction between two of three fixed effects (IR, N level and year) and will have their results unfolded in linear models by year. NU was the only parameter that didn't have any interactions, and none of all observed variables had a triple significant interaction.

AGB was affected differently by IR and N levels each year (Figure 10). In 2014, it was affected by N and IR, and the linear models show an AGB of 11.2  $Mg\ ha^{-1}$  in CF and 16  $Mg\ ha^{-1}$  in 0 N level (significant different intercepts). The slopes were also different among IR. CF had a higher inclination than NF, which led to the increment of biomass to not differ among IR after 50  $kg\ ha^{-1}$ . In 2015, AGB was also affected by N and IR, but in this year the slopes of CF and NF models are statistically equal. As a result, biomass starts from 12.7 and 14.53  $Mg\ ha^{-1}$  for CF and NF respectively, and the increment of biomass remains proportionally equal between IR along increments of N levels. The models predict a biomass of 15.2 and 22.2  $Mg\ ha^{-1}$  for CF and NF; respectively; at 250  $kg\ ha^{-1}$  of N. In the 2016 growing season the biomass was not significantly affected by IR or N level. The mean biomass across N levels was 15.7 and 16.5  $Mg\ ha^{-1}$  for CF and NF, respectively.  $R^2$  of all linear models varied from 0.92 to 0.01.

NU was equally affected by IR and N levels across 2014 to 2016 (Figure 11). The indigenous soil nitrogen provided higher NU when NF was adopted rather than CF, with values considered statistically distinct according to model standard errors. However, during the three years of the experiment, the slope of NU due N levels across IR did not differ. Therefore, the effect of N fertilizer was equal to CF and NF in N uptake, although in NF maintained a higher N uptake than CF. In 2014, the N uptake increased from 120 to 231  $kg\ ha^{-1}$  in NF and from 75 to 133  $kg\ ha^{-1}$  in CF, and in 2016 the N uptake increased from 118 to 200  $kg\ ha^{-1}$  in NF and from 105 to 167  $kg\ ha^{-1}$  in CF. 2014 and 2016 showed equivalent overall NU across IR and N levels. In 2015 the mean NU was higher than 2014 and 2016 and ranged from 116 to 250  $kg\ ha^{-1}$  in NF and from 83 to 220  $kg\ ha^{-1}$  in CF. The adjusted linear models show nitrogen uptake is highly predicted by N fertilizer rate ( $r^2 > 0.81$  across all models).

Regarding NC in biomass, in 2014 and 2016 the mean NC across all N levels was higher for NF than CF. In 2015, although N level affected NC, there is no difference between the IR. This is also apparent by the significant interaction between year and IR in 2016. (Table 8). In all years, there was no difference in NC among IR to 0  $kg\ N$  level and the increment rate ranged from 0.005  $g\ kg^{-1}$  to 0.024  $g\ kg^{-1}$  in biomass per unit of N applied as fertilizer (Figure 12). Thus, no pattern of N content dilution due to an increase in biomass was observed. Actually, N content increased together with biomass. The nitrogen content ranged from 7.6 to 14  $g\ kg^{-1}$  in NF and 6.8 to 12  $g\ kg^{-1}$  CF across the years.

The number of spikelets per panicle was the only observed parameter that was not affected by IR across years. It was affected only by N level and year (Table 8). The interaction between years and IR is because the relationship of N and N level was significant to CF but not for NF (Figure 13). In 2014, the NF intercept was 98.8 while CF was 74.28 grains panicle<sup>-1</sup>. In 2015 at zero N, grains panicle<sup>-1</sup> was 104 for CF and 98.8 for NF with no difference, and in 2016 it was 88 and 99.6 grains panicle<sup>-1</sup>, respectively to CF and NF, with no difference as well. The covariance matrix of MANOVA showed no significant correlation ( $p < 0.05$ ) between SN and any other observed variable. Number of grains per panicle is a yield component, but its correlation to nitrogen level is dependent on IR.

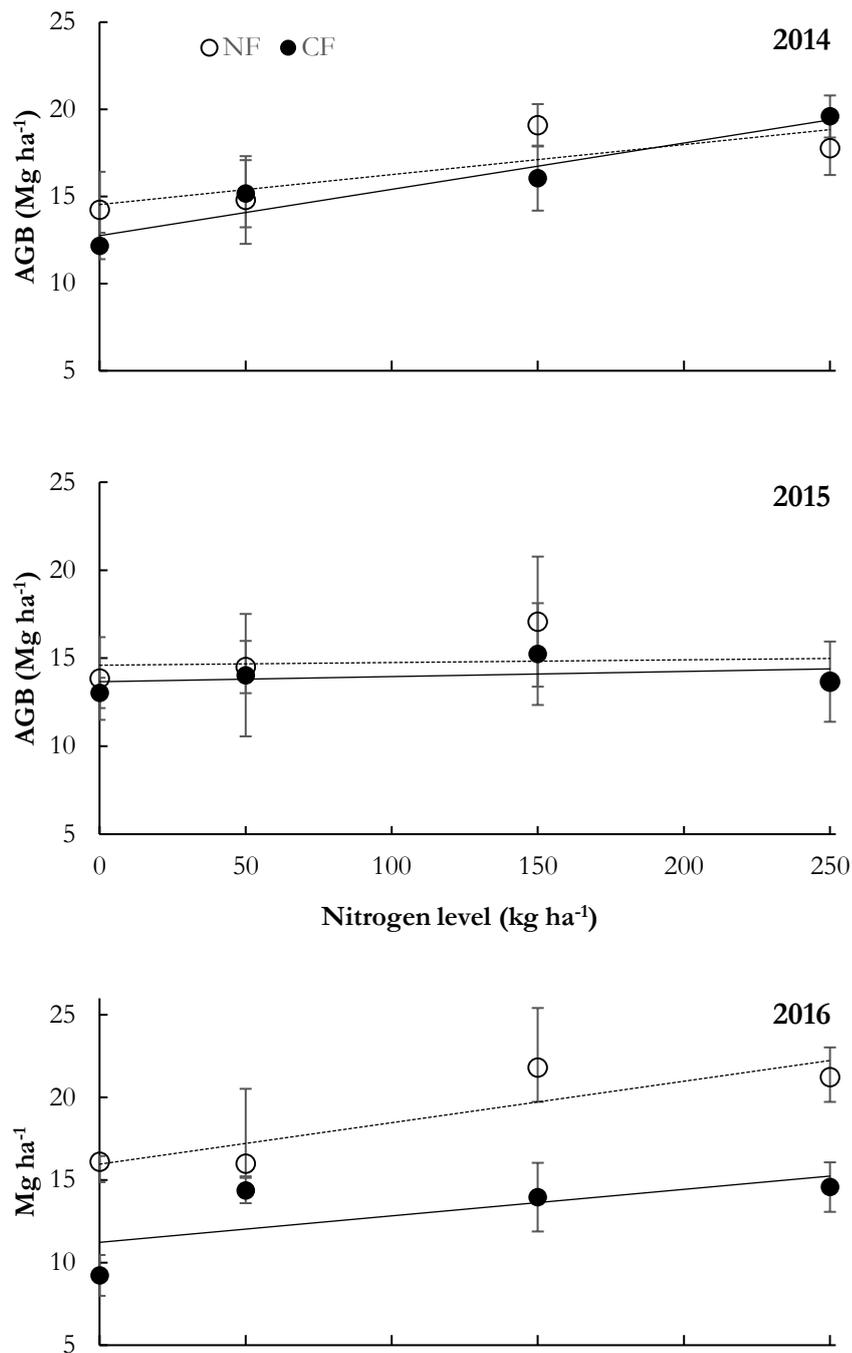


Figure 10. Relationship between above ground biomass ( $\text{Mg ha}^{-1}$ ) and N level in different irrigation regimes (IR) during 2014, 2015 and 2016 (C) growing seasons. Data points are the means of 4 observations  $\pm$  SE. The linear models are: 2014 CF  $f(y) = 0.0266x + 12.749^{**}$  ( $R^2 0.92$ ); 2014 NF  $f(y) = 0.0172x + 14.533^{**}$  ( $R^2 0.66$ ); 2015 CF  $f(y) = 0.016x + 11.224^{**}$  ( $R^2 0.49$ ); 2015 NF  $f(y) = 0.0251x + 15.959^{**}$  ( $R^2 0.77$ ); 2016 CF  $f(y) = 0.0029x + 13.662^{ns}$  ( $R^2 0.12$ ); 2016 NF  $f(y) = 0.0015x + 14.6^{ns}$  ( $R^2 0.01$ ). Ns: not significant; \* Significant at  $p \leq 0.05$ ; \*\* Significant at  $p \leq 0.01$  by ANOVA F test.

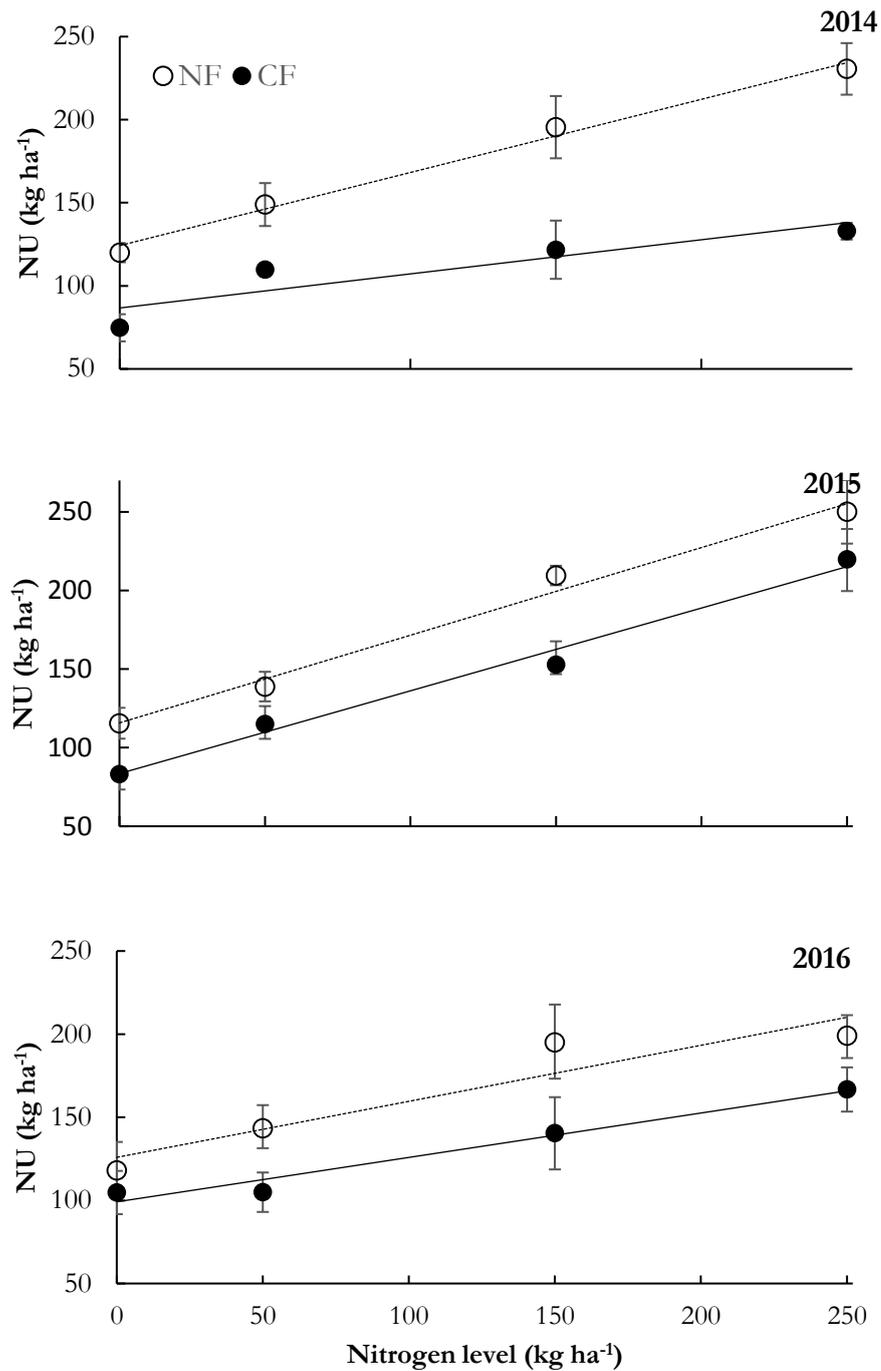


Figure 11. Relationship between nitrogen uptake (NU) and N level in different irrigation regimes (IR) during 2014, 2015 and 2016 growing seasons. Data points are the means of 4 observations  $\pm$  SE. The linear models are: 2014 CF  $f(y) = 0.2053x + 86.67^{**}$  ( $R^2$  0.82); 2014 NF  $f(y) = 0.4401x + 124.23^{**}$  ( $R^2$  0.99); 2015 CF  $f(y) = 0.5267x + 83.504^{**}$  ( $R^2$  0.99); 2015 NF  $f(y) = 0.5577x + 115.78^{**}$  ( $R^2$  0.99); 2016 CF  $f(y) = 0.2671x + 99.126^{**}$  ( $R^2$  0.97); 2016 NF  $f(y) = 0.337x + 125.88^{**}$  ( $R^2$  0.88). Ns: not significant; \* Significant at  $p \leq 0.05$ ; \*\* Significant at  $p \leq 0.01$  by ANOVA F test

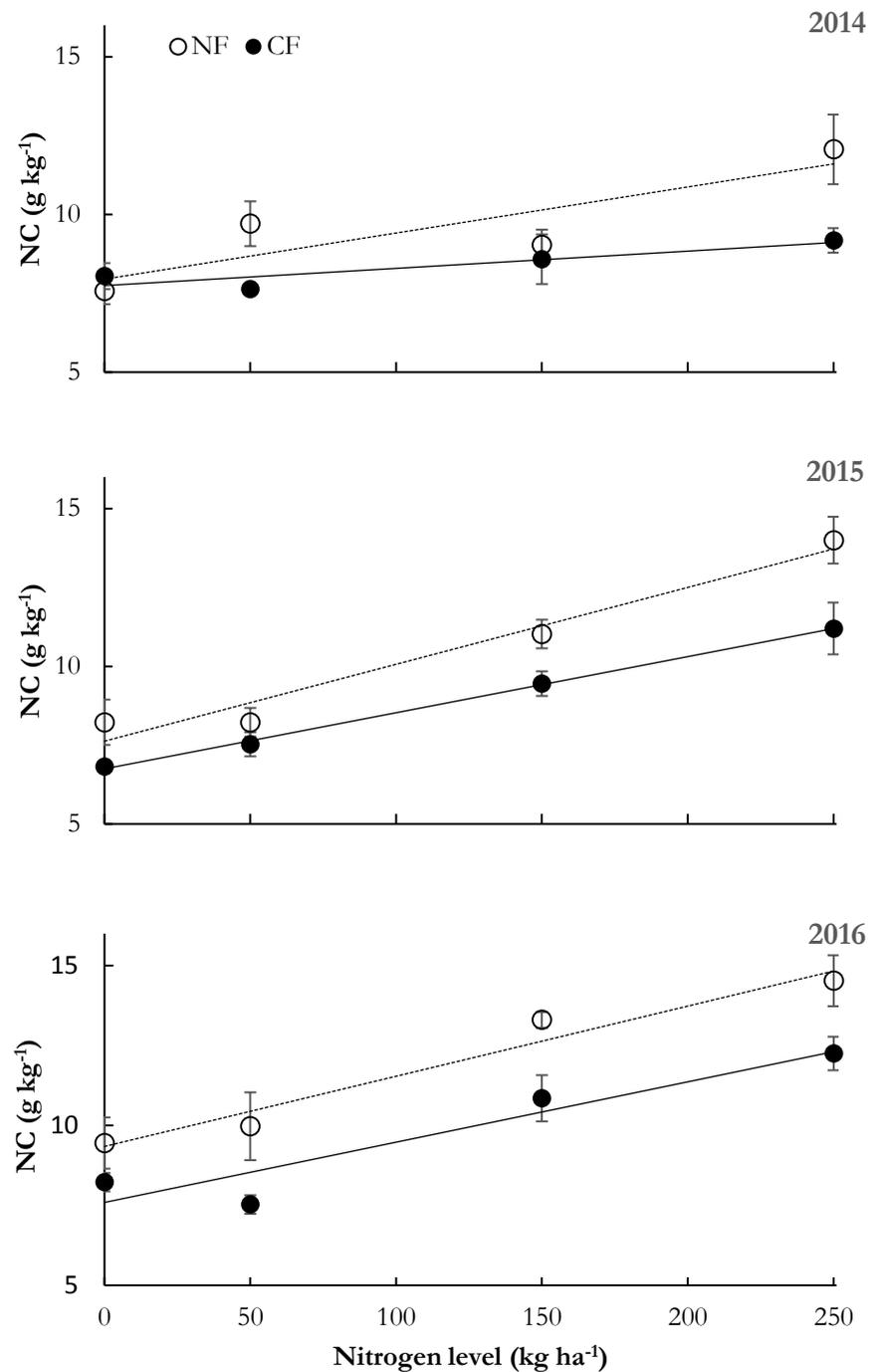


Figure 12. Relationship between nitrogen content in above ground biomass (NC) and N level in different irrigation regimes (IR) during 2014, 2015 and 2016 growing seasons. Data points are the means of 4 observations  $\pm$  SE. The linear models are: 2014 CF  $f(y) = 0.0055x + 7.7467^{**}$  ( $R^2$  0.75); 2014 NF  $f(y) = 0.0146x + 7.9517^{**}$  ( $R^2$  0.75); 2015 CF  $f(y) = 0.0178x + 6.7479^{**}$  ( $R^2$  0.99); 2015 NF  $f(y) = 0.0244x + 7.6258^{**}$  ( $R^2$  0.96); 2016 CF  $f(y) = 0.0189x + 7.5903$  ( $R^2$  0.89<sup>\*\*</sup>); 2016 NF  $f(y) = 0.0219x + 9.3432^{*s}$  ( $R^2$  0.86). Ns: not significant; \* Significant at  $p \leq 0.05$ ; \*\* Significant at  $p \leq 0.01$  by ANOVA F test.

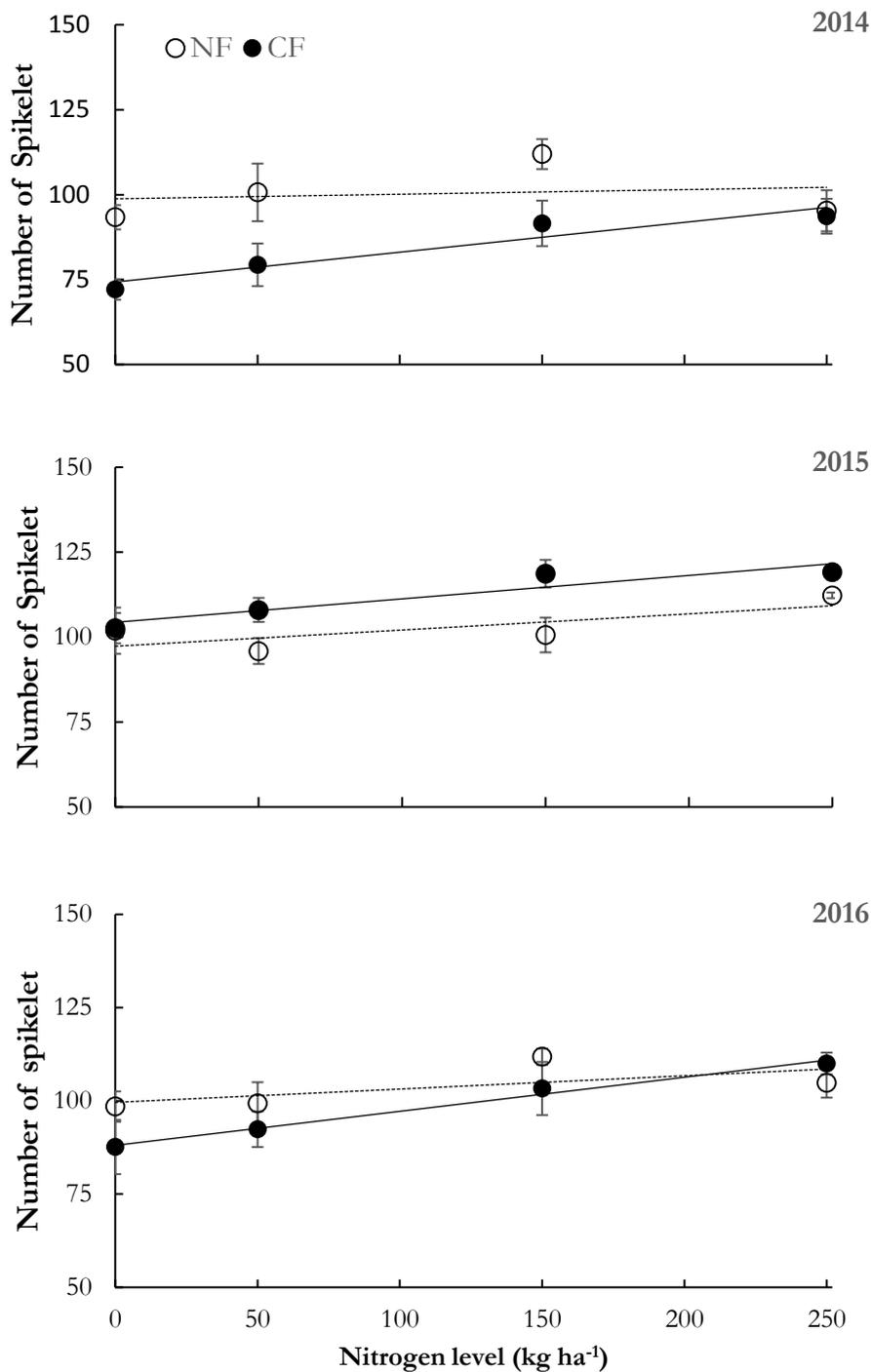


Figure 13. Relationship between number of spikelet (NS) and N level in different irrigation regimes (IR) during 2014, 2015 and 2016 growing seasons. Data points are the means of 4 observations  $\pm$  SE. The linear models are: 2014 CF  $f(y) = 0.0878x + 74.28^*$  ( $R^2 0.91$ ); 2014 NF  $f(y) = 0.0136x + 98.76^{ns}$  ( $R^2 0.03$ ); 2015 CF  $f(y) = 0.069x + 104.37^*$  ( $R^2 0.88$ ); 2015 NF  $f(y) = 0.069x + 104.37^{ns}$  ( $R^2 0.58$ ); 2016 CF  $f(y) = 0.0913x + 88.053^*$  ( $R^2 0.98$ ); 2016 NF  $f(y) = 0.0357x + 99.584^{ns}$  ( $R^2 0.41$ ). Ns: not significant; \* Significant at  $p \leq 0.05$ ; \*\* Significant at  $p \leq 0.01$  by ANOVA F test.

The panicle density of CF was significantly affected by N level across all years (Figure 14), and no influence was observed for NF in any year, although the mean PD was higher in NF than CF across N levels and years. At 0 kg N level, panicle density was 310 panicles m<sup>-2</sup> in NF and 218 panicles m<sup>-2</sup> in CF for 2014, 373 panicles m<sup>-2</sup> in NF and 245 panicles m<sup>-2</sup> in CF for 2015, and 272 panicles m<sup>-2</sup> in NF and 199 panicles m<sup>-2</sup> in CF for 2016. The model which best adjusted to PD as a function of N level was quadratic in 2014 and 2015. The

highest predicted PDs were 281 and 340 panicles  $m^{-2}$  at N levels of 153 and 175  $kg\ ha^{-1}$ , respectively for 2014 and 2015. For 2016, the data was best fit by a first order linear model with a panicle increment rate in CF irrigation of 0.29 panicle  $kg^{-1}$  of N.

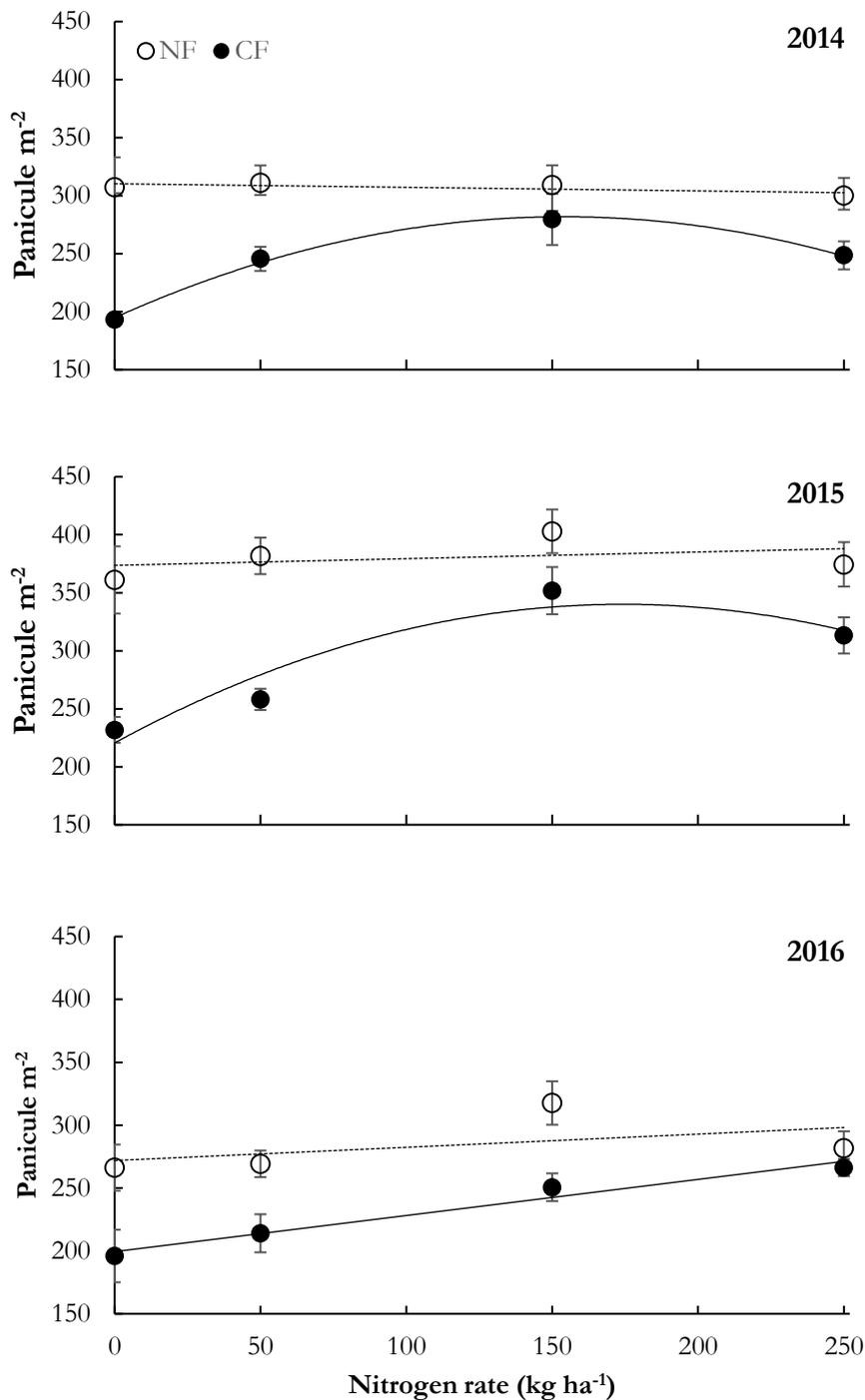


Figure 14. Relationship between panicle density (PD) and N level in different irrigation regimes (IR) during 2014, 2015 and 2016 growing seasons. Data points are the means of 4 observations  $\pm$  SE. The linear models are: 2014 CF  $f(y) = 0.0037x^2 + 1.1309x + 194.79^*$  ( $R^2 0.99$ ); 2014 NF  $f(y) = 0.0308x + 310.22^{ns}$  ( $R^2 0.03$ ); 2015 CF  $f(y) = -0.0039x^2 + 1.3705x + 220.56^*$  ( $R^2 0.61$ ); 2015 NF  $f(y) = 0.0576x + 373.56^{ns}$  ( $R^2 0.13$ ); 2016 CF  $f(y) = 0.2873x + 199.43^{**}$  ( $R^2 0.96$ ); 2016 NF  $f(y) = 0.1051x + 271.91^{ns}$  ( $R^2 0.24$ ). Ns: not significant; \* Significant at  $p \leq 0.05$ ; \*\* Significant at  $p \leq 0.01$  by ANOVA F test.

Grain yield was affected by IR and N level in all years (Figure 15). In 2014, the polynomial models showed a GY of 4.0 Mg ha<sup>-1</sup> in CF and 5.8 Mg ha<sup>-1</sup> in NF at 0 kg ha<sup>-1</sup> N. The slopes and intercepts were significantly different among IR according to model confidence intervals. The maximum point of GY occurred earlier in NF than in CF with 8.1 Mg ha<sup>-1</sup> at N level of 162 kg ha<sup>-1</sup> and 8.0 Mg ha<sup>-1</sup> at N level of 282 kg ha<sup>-1</sup>. In 2015, at zero N level GY was 7.4 Mg ha<sup>-1</sup> in NF and 5.6 Mg ha<sup>-1</sup> in CF. At 50 kg ha<sup>-1</sup> of N level the GY in NF was still higher to NF than CF, but in 150 and 250 kg ha<sup>-1</sup> CF and NF showed the same GY. In 2016, mean GY across N rates was higher in NF than CF, but there was no difference in the model parameters among IR.

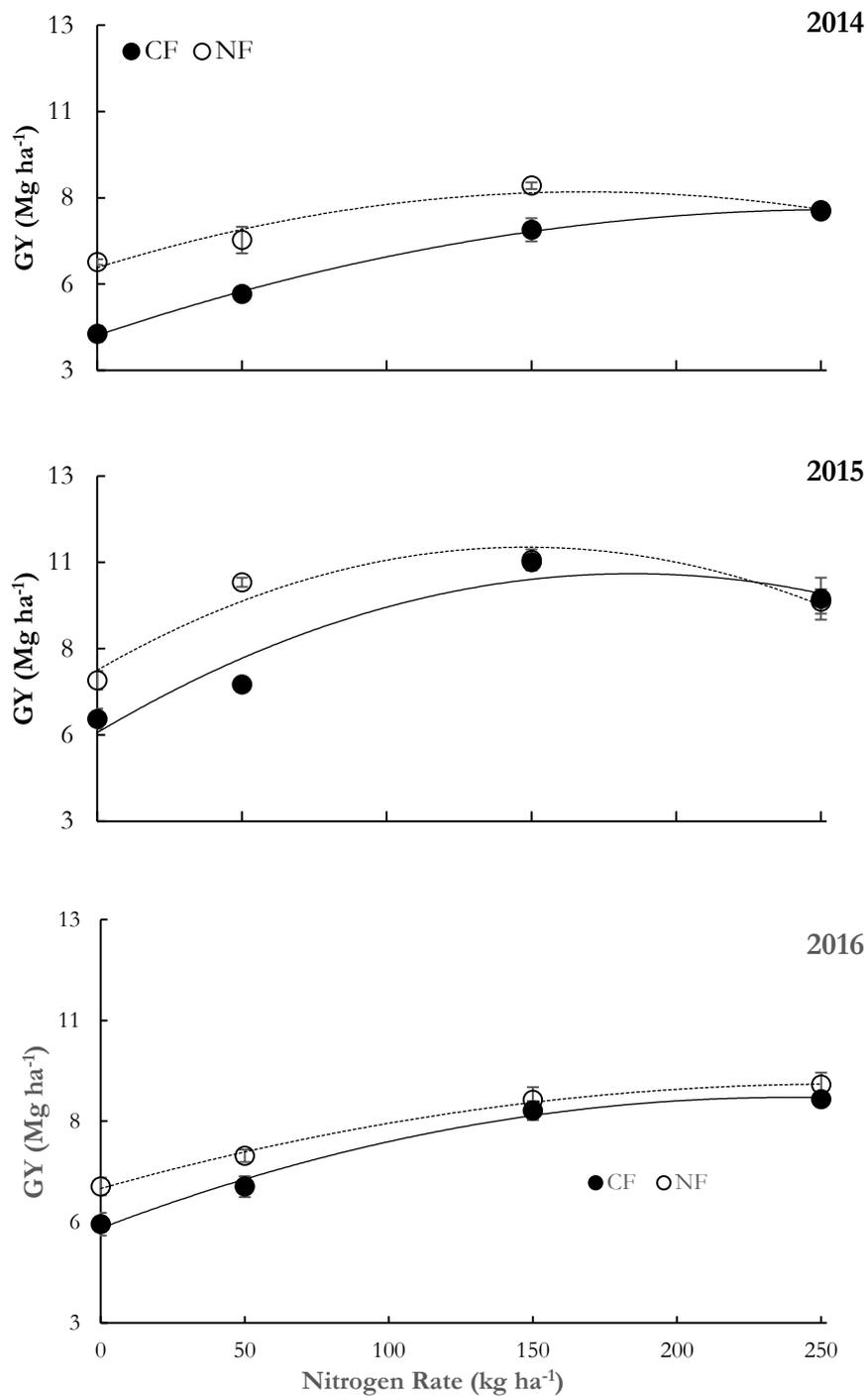


Figure 15. Relationship between grain yield (GY) and N level for continuous flooding (CF) and non-flooding (NF) in 2014, 2015 and 2016 growing seasons. Data points are the means of 4 observations  $\pm$  SE. The linear models are: 2014 CF  $f(y) = -0.00005x^2 + 0.0282x + 4.0124^*$  ( $R^2$  0.99); 2014 NF  $f(y) = -0.00008x^2 + 0.026x + 5.975^{**}$  ( $R^2$  0.94); 2015 CF  $f(y) = -0.00014x^2 + 0.0498x + 5.5673^{**}$  ( $R^2$  0.92); 2015 NF  $f(y) = -0.00016x^2 + 0.0481x + 7.3772^{**}$  ( $R^2$  0.92); 2016 CF  $f(y) = -0.00006x^2 + 0.0272x + 5.3467^*$  ( $R^2$  0.99); 2016 NF  $f(y) = -0.00004x^2 + 0.0201x + 6.3315^*$  ( $R^2$  0.98). Ns: not significant; \*Significant at  $p \leq 0.05$ ; \*\* Significant at  $p \leq 0.01$  by ANOVA f test.

The GY was highly predicted by N level in the observed interval.  $R^2$  of all models ranged from 0.92 to 0.99. The MANOVA covariance matrix showed significant correlation ( $p < 0.05$ ) between GR and PD.

The relationship between NU and GY showed two statically different linear models in each IR (CF and NF) across all fertilizer nitrogen levels in 2014, 2015 and 2016 (figure 16). For CF data, the model starts with a lower intercept than NF data (6.1 in NF versus 3.4 in CF) but has a higher slope (0.03 in CF versus 0.01 in NF). The  $R^2$  in CF is 0.5 indicating a fair predicted relation while in NF  $R^2$  is only 0.17. Both models show a trend of increasing GY over the increment of accumulate NU, but with a different gain rate.

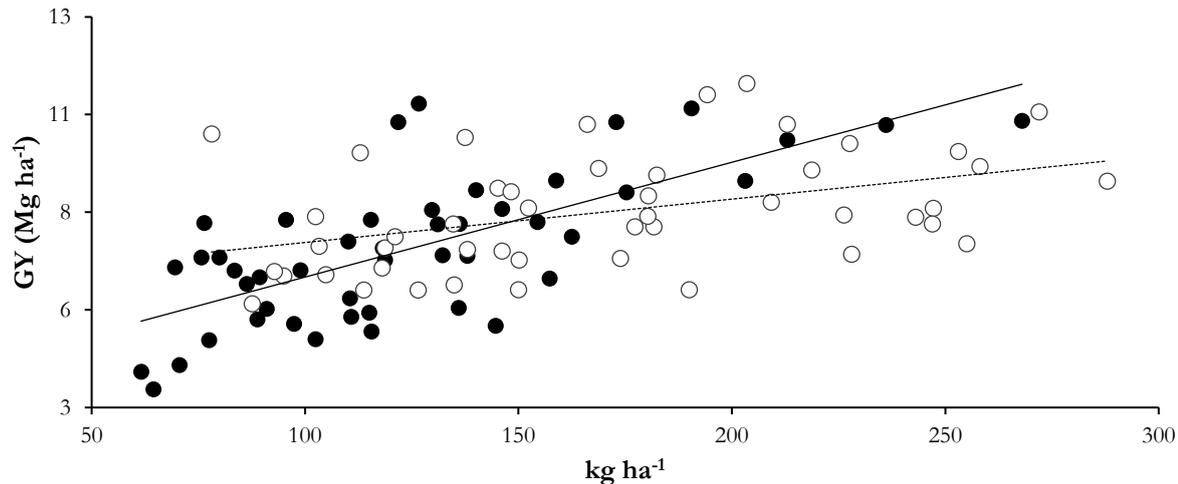


Figure 16. Relationship between nitrogen uptake in aboveground biomass (NU) at crop maturity and grain yield (GY) in 2014, 2015 and 2016 growing seasons. The linear models are: CF  $f(y) = 0.0294x + 3.4012$  \*\* ( $R^2 = 0.51$ ); NF  $f(y) = 0.0111x + 6.115$  \*\* ( $R^2 = 0.17$ ). *Ns*: not significant; \* Significant at  $p \leq 0.05$ ; \*\* Significant at  $p \leq 0.01$  by ANOVA f test

### 3.4. Discussion

#### 3.4.1. Crop performance along seasons' development

Crop components were differently affected by IR and N levels throughout the growing seasons. The lack of influence among NF, CF and N rates in AGB mean profile is because none of them limited crop development (Figure 3). Although biomass and grain yield are closely related in different genotypes (Zhang et al., 2004), for a given variety, rice GY is more closely related to post heading biomass relative to panicle and spikelet mass (Ao et al., 2010; Ye et al., 2013). As the profile analysis considers the whole cycle means, the differences during grain filling and crop maturity were not enough to drive significance to the analyses, and this is confirmed by the non-significant correlation between AGB and any other crop parameter in the MANOVA matrix. During the vegetative stages NU and LAI were the crop components significantly affected by IR and N (Figure 4 and 6).

Throughout the crop development in 2015 and 2016, the nitrogen accumulative uptake was higher in NF 150 than CF 150, and it concisely occurred after ponding water started in the CF treatment (Figure 4). N uptake is impaired in alternative irrigation methods compared to CF (Belder et al., 2005a; Peng et al., 2006; Rose et al., 2016; Tan et al., 2015; Yao et al., 2012). Poorer crop performance is related to water absence or nitrate leaching and denitrification (Devkota et al., 2013; Zhou et al., 2011). However, our results point in the opposite

direction. NU increased even with AGB remaining unaffected by IR and N levels. Some studies have recently presented data on the pattern of greater N accumulation in rice plants grown under aerobic conditions (Pan et al., 2017; Sun et al., 2012; Ye et al., 2013). The NC throughout 2015 and 2016 was affected by IR and N levels, and NF provided statistically higher NC than CF in both years at 150 to 0 kg ha<sup>-1</sup> (Figure 5). As a result, NC lead to the observed differences in NU. The increasing accumulative N uptake until crop maturity (despite the punctual N decrease due to blast strike) shows the importance in IR/N management, providing N availability to crop uptake until later stages of development as also seem in Fageria and Santos (2013); Somaweera et al. (2016). Aerobic soil condition is related to higher rates of indigenous N mineralization than anaerobic (Aulakh et al., 2000; Kader et al., 2013; Updegraff et al., 1995), and keeping soil in aerobic conditions the entire cycle contributes to the greatest NU in NF.

In our study, LAI was affected by IR and N levels, and it explains the differences estimated in transpiration and evaporation. LAI is largely reported as dependent of IR and N levels (Alberto et al., 2011; Belder et al., 2005a; Bouman and van Laar, 2006; Pan et al., 2017; Wang et al., 2016), although when crop water requirements are fully met, due to adequate supplies in IRs, (actual ET is equal to potential crop ET) there is no differences in LAI (Pan et al., 2017). The leaf blade represents an important N sink during vegetative development until grain filling (Murchie et al., 2002; Somaweera et al., 2016), therefore LAI is affected by indigenous N and fertilizer (Figure 6). The more transpiration impacts the ET, the higher the water use efficiency and crop performance will be in a given site and genotype (Bouman et al., 2007; Bouman and Tuong, 2001), since evaporated water from soil or pond water has no effect in carbon assimilation. Figure 7 showed the accumulative transpiration across years. Transpiration was 8% higher in NF than CF while evaporation was 34% lower. The higher transpiration in NF compared to CF is lined up to the overall better performance of NF in crop performance at maturity.

The WFPS in 2015 and 2016 averaged around the optimum air / water balance of 60% (62% in 2015 and 59% in 2016) which is related as maximum aerobic activity in the soil. At this WFPS soil provided no limiting oxygen and water supply for microorganism development (Linn and Doran, 1984). Around 60% of WFPS the nitrification, ammonification and organic matter decomposition are in the maximum rate, while denitrification is not pronounced until 70% to 100% of WFPS (Bouwman, 1998). Exchangeable nitrogen was higher in either ammonium and nitrate around 45 DAE in both years (Figure 09), and higher N availability in aerobic rice was also reported by Cai et al., (2001) and Devkota et al. (2013). High aerobic activity in NF led to the higher NU and the associated benefit to crop development than CF. The increasing of N availability close to crop panicle initiation, which is the phenological stage related to grain yield express its potential, was also contributed even more to the best overall crop performance in NF.

### 3.4.2. Crop performance at harvest

At crop maturity AGB showed a poor relationship to N level among years, and most of the variation was not explained by irrigation or N applied as fertilizer. In addition, the lack of covariance to any other yield component led us to conclude the AGB in a given site, year and genotype is useless to predict GY among different IR. Differently, NU is highly related to N rate, and also highly affected by IR. However, the linear response and statistically equally slopes in NF and CF does not show optimum N rate to be applied within 0 to 250 kg ha<sup>-1</sup> of

N intervals. The higher the N fertilizer, the higher NU will be in both IRs. Therefore, to use these relationships to propose a N fertilizer rate aimed to best NU under an agronomic point of view is also meaningless.

PD was the yield component more elucidative to explain the effects of IR in GY, and not just because of their positive covariance. The lack of N rate effecting PD in NF but having an effect in CF shows the first crop parameter behaving concisely differently among IRs. PD is directly related to tillering in a fixed sowing density. The active tillering period in rice comprises from the 5<sup>th</sup> leaf till just before panicle differentiation (20 to 45 DAE) (Moldenhauer et al., 2015). This is stimulated by incident radiation in the axillary bud (Hanada, 1993), and it is closely related to N content in AGB (Ahmed et al., 2016; Tanaka and Garcia, 1965; Wang et al., 2016; Zhong et al., 2003). In our study, the absence of ponding water in NF might have increased tillering due the radiation issue, but the non- response to N fertilizer is because NF provided enough N from indigenous soil sources to express tillering potential.

However, CF depended on applied fertilizer to provide PD increases. The maximum PD was supported by 153 and 175 Kg ha<sup>-1</sup> of N in 2015 and 2016, respectively. The NU had a positive correlation with PD, and PD in turn, had a positive correlation to GY in the covariance matrix of MANOVA.

The different relationship between NU and GY at crop maturity in NF and CF (Figure 16) shows us the irrigation regime can create a substantial alteration in nitrogen use by rice. Although for both IRs GY is positively related to NU, in CF the GY is better predicted by NU ( $R^2$  0.5) than NF ( $R^2$  0.17). This indicates that IR is not just ruling the availability of N in soil, leading to different NU, but it is also interfering in crop development in an indirect manner. The aerobic soil increases nitrification (Devkota et al., 2013; Dong et al., 2012; Zhou et al., 2012) and absorbing N as nitrate (N-NO<sub>3</sub><sup>-</sup>) is a signal for plant growth (Crawford, 1995). The NF might have raised the N- NO<sub>3</sub><sup>-</sup> fraction in NU, and this stimulated the plant to a higher development. Increasing N-NO<sub>3</sub><sup>-</sup> content in nitrogen supplies increases rice dry weight cultivated in nutrient solution (Holzschuh et al., 2009; Ying-Hua et al., 2007). Our data supports this assumption because the intercept of the NF model is higher than CF, which means in the lower NU threshold NF provided greater GY than CF. On the other hand, excess of N-NO<sub>3</sub><sup>-</sup> in plant tissue turns rice more susceptible to blast disease (Osuna-Canizalez et al., 1991) and indeed blast occurred in a higher severity in NF than in CF at 150-250 kg ha<sup>-1</sup> of fertilizer N, partially compromising GY.

The GY and N fertilizer relationship among IRs were very tidy (Figure 13), and their statically different model parameters in 2014 and 2015 confirm our hypothesis that fertilizer management must be adjusted for particularly IRs. The recommended fertilizer N rate commonly practiced in this region of Brazil ranges from 90 to 120 kg ha<sup>-1</sup> of N (Santos and Santiago, 2014), although growers rarely go over 100 kg ha<sup>-1</sup> as part of integrated blast management. Our data showed a GY 17%, 11% and 5% higher in NF than CF at simulated 120 kg ha<sup>-1</sup> N fertilizer in 2014, 2015 and 2016, respectively. If lower rates than 120 kg ha<sup>-1</sup> were used, greater differences between IRs would be expected.

### 3.5. Conclusion

The hypothesis that the irrigation regime is able to influence the nitrogen uptake by rice and therefore crop performance was confirmed. Throughout the growing season, the nitrogen uptake, nitrogen content in above ground biomass and leaf area index were affected by the irrigation regime and nitrogen fertilizer in 2015

and 2016. The mean profile of nitrogen uptake was highest in non-flooding at 150 kg ha<sup>-1</sup> in 2015 and 2016, and the mean profile of leaf area index and nitrogen content were higher in non-flooding 150 or equivalent to continuous flooding 150 among 2015 and 2016. The differences among crop parameters began shortly after ponding water was imposed in continuous flooding, and its negative impact continued until crop maturity. The estimated ET showed 34% higher daily evaporation in continuous flooding and 8% higher daily transpiration in NF.

In crop maturity, the nitrogen uptake, panicle density and grain yield were higher in non-flooding conditions than continuous flooding across nitrogen fertilizer rates in all years. Significant interaction among irrigation regimes and N fertilizer were observed for panicle density in all years, and for grain yield in 2014 and 2015, showing grain yield and its most related crop component has a distinct response to N fertilizer in non-flooding and continuous flooding irrigation. In general terms, as long as non-flooding aerobic rice does not suffer drought stress, this production system is able to provide better overall crop performance and grain yield than continuous flooding.

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## 4. Final considerations

This thesis concisely concluded non-flooding aerobic rice in tropical lowland in Brazil provides better efficiency of water and nitrogen use, as well as crop performance and grain yield. This information already has been disseminated during ongoing field experiments during 2015 and 2016 to extension professional, researchers and growers throughout field days and technical seminars in Tocantins. However, the application of non-flooding irrigation regime must be aware of these fundamental aspects: (i) non-flooding aerobic does not mean water absence. The aerobic condition must meet an appropriate ET for crop development. (ii) the decreasing of N fertilizer and crop performance relationship will depend of organic matter and soil nitrogen pool characteristics. (iii) The rice genotype must be observed concerning drought and Blast tolerance.

Therefore, for a wide dissemination of such irrigation regime and fulfill the environmental gain of water and nitrogen savings, it is suggested that the following topics should be addressed by further researches:

Understand the nitrogen transformation in soil, mainly nitrification-denitrification process. Assess the emissions of  $N_2O$  and  $N_2$  caused by irrigation shifts. Modelling the long-term impact in soil nitrogen pool and carbon.

Quantify the relationship between physical soil properties as porosity, texture, matric potential to water availability. There are different soil proprieties in tropical lowland and create a soil classification survey for irrigation regime would be important for regional water management.

Incorporate in regional rice breeding program the genotypes suitable for non-flooding irrigation regime and improve drought tolerance to rice tropical varieties.