EFFECTS OF NITROGEN FERTILIZATION ON ORGANIC RICE PRODUCTION

A Thesis

by

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ABSTRACT

The increased demand for organic rice has led to a quick expansion of the industry; however, there has been little research conducted on organic rice systems that are relevant to the unique flooded paddy system that is used to produce organic rice. A critical issue for organic rice production is nitrogen management.

A laboratory trial was conducted to better understand the N mineralization rates and dynamics. The specific objectives were to examine the role of cover crop and soil amendment on nitrogen mineralization under aerobic and anaerobic conditions, as well as determine which combination of cover crop and organic amendment is optimum for a maximum N mineralization. Total mineralized nitrogen over time under aerobic and anaerobic incubations of soil – amended with Durana clover and Nature Safe (13-0-0) – seemed to be dictated by the amount of available nitrate and nitrite, since a linear increase with time was observed for the ammonium content. Of the two factors analyzed – amount of biomass and nitrogen rate added – enough statistical evidence was found to determine that the amount of N added via organic soil amendment has the greatest impact on the total amount of mineralized N. Finally, the combination of 100% cover crop plus 200 kg N/ha was determined as the optimum combination of cover crop and organic amendment because it mineralized the most N during the incubation period and presented only positive mineralization rates.

Complementary to the previous experiment, a greenhouse trial in Beaumont, TX, was conducted from May to August 2015 to study the effects that organic soil amendment

(Nature Safe 13-0-0) with different rates of application (0, 50, 100, 150, 200, and 250 kg N/ha) had on the yield components in comparison with conventional rice production (urea fertilizer 46-0-0). For the organic treatment, the highest yield and nitrogen use efficiency (NUE) was reached at 200 kg N ha^{-1} and not 250 kg N ha^{-1}, which stated a quadratic function of added N and yield. Similar results were found for the conventional treatment, however, the NUE and highest yield were achieved at 150 kg N ha^{-1} and 250 kg N ha^{-1}, respectively.

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CHAPTER I

INTRODUCTION

The demand for organic food has increased in recent years, especially in North America (FAO, 2016; Mason et al., 2007; Snyder and Spaner, 2010). The increased demand reflects growing consumer awareness of the environmental impacts of conventional agronomic systems and concerns of human health (Huang et al., 2016; Snyder and Spaner, 2010; Thuithaisong et al., 2011).

In the United States, the number of acres used for organic rice production has slowly increased – reaching almost 20 thousand hectares in 2011 (USDA ERS:, 2013). This amount cannot meet the market's demand, in part because organic crops have a lower yield than crops under conventional management (Wild et al., 2011). The restricted availability of nutrients, particularly nitrogen (N), is one of the main reasons for low yields in organic farming (Berry et al., 2002; Wild et al., 2011). In order to address this problem, the objectives of this research were to determine 1) the impact that different amounts of organic soil amendment and cover crops have on nitrogen supply in the soil collected from an organic rice field, and 2) the impact of soil amendment on the grain yield and yield components of organic rice using a greenhouse trial.

Organic farming and organic soil amendments

Conventional or intensive agriculture has increased crop yield but has also posed severe environmental problems (Mäder et al., 2002). Reduced use of organic fertilizers has created deficiencies of secondary and micronutrients such as Zn, Fe and S; soil organic matter (SOM) has been depleted from the soil, leading to a decline in soil microorganisms and soil structure (Quyen and Sharma, 2003; Thakur and Sharma, 2005; Yadav et al., 2000). Soils are showing signs of fatigue as judged by decline in the yields of rice as well as a lower response to applied chemical fertilizers (Bejbaruha et al., 2009; Quyen and Sharma, 2003; Thakur and Sharma, 2005). As a result, farmers have resorted to the application of fertilizers in higher rates than those recommended to keep producing high yields (Yadav et al., 2000). Over application of nitrogen can lead to groundwater contamination, eutrophication of fresh water bodies and coastal marine ecosystems (Golterman et al., 1988; Tashi and Wangchuk, 2015). In a three-decade-long *in situ* tracer experiment, Sebilo et al. (2013) found that after 25 years, 12-15% of the applied N fertilizer was still residing in the SOM; 8-12% of which had leached into the hydrosphere and continual leaching was expected for at least another five decades.

Organic farming has surged as an alternative to conventional farming methods (Thuithaisong et al., 2011), as it considers the medium- and long- term effects of agricultural interventions on the agro-ecosystem (Jahanban and Davari, 2013; Thakur and Sharma, 2005). Nonetheless, there are challenges associated with organic agriculture, such as the lack of effective products for use in fertilization and soil amendments, the lack of products for pest control and the lack of effective equipment for the specific needs in organic agriculture (i.e., for compost and weed management) (Jahanban and Davari, 2013; Sullivan, 2014). Furthermore, there is an increase in weed pressure and soil nutrient deficiency associated to organic farming systems, which may lead to reduced crop yields in comparison to conventional farming systems (Berry et al., 2002; Mason et al., 2007; Snyder and Spaner, 2010; Wild et al., 2011; Yadav et al., 2000). However, studies have proven that weed control may be accomplished by using crop rotations and intercrops (Mason et al., 2007; Snyder and Spaner, 2010).

Fertility management in organic farming relies on long-term integrated approaches rather than the more short-term, much targeted solutions common in conventional agriculture (Marinari et al., 2006; Thakur and Sharma, 2005). For example, organic farming relies on crop residues, green manures, and animal wastes for soil fertility management (Snyder and Spaner, 2010; Thuithaisong et al., 2011). Poultry litter has become a popular option amongst farmers (López-Mosquera et al., 2008; Ranatunga et al., 2013), being a good source of nitrogen. Pelletized fertilizers from poultry litter present higher efficiencies than fresh poultry litter in supplying N to the crop under continuously flooded since it is easier to transport and apply and has more uniform nutrient characteristics, less fecal bacteria and no odor (López-Mosquera et al., 2008; Wild et al., 2011). Furthermore, pelletizing poultry litter increases its bulk density and particle size uniformity (McMullen et al., 2005). However, to certain soils, this practice may cause an excessive supply of phosphorus (P) (Abdala et al., 2012; Ranatunga et al., 2013; Wild et al., 2011). After sustained periods of time, the continual application of poultry litter to meet N requirement for rice can lead to high soil P contents, which subsequently may reduce the ability of organic rice fields to retain P and thus increase runoff of the nutrient (Abdala et al., 2012; Linquist et al., 2010; Ranatunga et al., 2013; Xie and Zhao, 2016).

The commercial availability of pelletized amendments from non-ruminant animal proteins (such as feather meal, pork meal and blood meal) without phosphorus (such as Nature Safe 13-0-0), allows farmers to meet N requirements while minimizing negative environmental impacts, such as eutrophication, due to the continual application of fresh poultry litter and P buildup. Investigation of appropriate rates for pelletized fertilizers would allow an improved N management strategy for organic rice production (Wild et al., 2011).

Nitrogen and organic rice production

After wheat, rice is the most important cereal crop for human consumption (Fageria et al., 2011). Rice is the staple food for more than half of the world's population (USDA ERS, 2012), and in North America the demand for the organic rice has increased in the past years (FAO, 2016; Snyder and Spaner, 2010; Texas A&M AgriLife, 20-Oct-2015). Even though organic rice is produced on 20,000 ha in the U.S. (USDA ERS:, 2013), U.S. rice imports have increased in the past decades, up to 15% by 2009 (USDA ERS, 2014); organic farmers haven't been able to keep up with the domestic demand. This is due partly to the fact that organic rice production has lower yields than the conventional production (Texas A&M AgriLife, 20-Oct-2015; Thuithaisong et al., 2011; Wild et al., 2011). One of the reasons for a lower yield is due to the lack of N in the organic system. Nitrogen is the most limiting nutrient in rice (*Oryza sativa L.)* production (Djaman et al., 2016; Fageria et al., 2011; Fageria and Baligar, 2001), especially under organic production (Hazra et al., 2014) since it mainly relies on the use of crop residues, animal manures and legumes – amongst others – to meet the plant N requirements and maintain the soil fertility through time (Sullivan, 2014; Thuithaisong et al., 2011). However, if the P and N supplies for the crop are sufficient, it is feasible to increase rice yields under organic management (Mäder et al., 2002; Wild et al., 2011). If applied for long-term periods, organic manures can increase SOM content and thus enhance soil fertility (Li et al., 2010; Zhang et al., 2015). Studies have reported that it takes a period from two to five years to start building up the soil fertility (Mäder et al., 2002; Surekha et al., 2013).

The release of nutrients in a balanced way and buildup of soil fertility over time, tends to stabilize organic rice productivity and its yields (Tamaki et al., 2002; Thuithaisong et al., 2011). Mäder et al. (2002) and Surekha et al. (2013) concluded that organic crops based on legume-crop rotations are a feasible alternative to conventional farming systems, since it has the potential to increase yields with this management over time.

The maintenance of soil fertility is important for sustainable land use. A fertile soil provides essential nutrients for crop growth, supports a diverse and active biotic community, exhibits a typical soil structure, and allows for an undisturbed decomposition (Mäder et al., 2002). Organic farming practices are reported to have a positive effect on SOM and other soil quality parameters such as total nitrogen (TN), microbial biomass carbon (MBC) and cation exchange capacity (CEC) (Ali et al., 2014; Jahanban and Davari, 2013; Shahid et al., 2013; Sun et al., 2015). In the studies conducted by Bi et al. (2009) and Marinari et al. (2006), organic management affected soil microbiological and chemical properties by increasing soil nutrient availability, microbial biomass and microbial activity, which represent a set of sensitive indicators of soil quality. Supporting this result, Mäder at al. (2002) found that organically managed soils exhibited greater biological activity than the conventionally managed soils. Furthermore, an increased soil fertility and quality has been reported under organic management by Surekha et al. (2013) and Tashi and Wangchuk (2015). Regarding rice, biological fertilizers can improve the heading rate of organic rice significantly, enhance the N accumulation in different stages and increase the grain yield (Huang et al., 2016). These benefits can be attributed to the increased SOC and soil nutrient capacity due to the long-term application of organic amendments (Bi et al., 2009). Dynamics of organic C storage in agricultural soils strongly affects soil N supply and thus crop productivity since most of soil N is in an organic form. Also, there have been reports of positive effects on soil aggregate stability under organic agriculture (Hao et al., 2008; Jahanban and Davari, 2013; Mekuria et al., 2016; Ofori et al., 2005; Surekha et al., 2013; Zhou et al., 2016). Zhou et al. (2016) evaluated the effect of long-term inorganic and organic fertilization on the soil micro and macro structures in rice production. These authors concluded that as SOC increased, bulk density decreased and total porosity increased; the long-term application of organic matter (OM) supported the development of intra-aggregates of the pore system mainly due to the development of biopores. Therefore, the use of organic amendments in agricultural soil could have a positive impact in improving soil quality, both physical and biochemical properties.

Organic matter decomposition and N supply under anaerobic conditions

Rice in the United States is grown mostly under flooding practices, therefore it is important to note that decomposition of SOM may vary compared to that under aerobic conditions. In a flooded rice field, oxygen (O_2) is displaced from the soil pores by water, creating anaerobic conditions and hence supporting anaerobic respiration (International Rice Research Insitute, 2009). In the absence of O_2, nitrate (NO_3^--N) is utilized by facultative anaerobes as an electron acceptor in order to decompose SOM (Reddy and Patrick, 1986; Sutton-Grier et al., 2011). As organic matter – whether added or native to the soil – decomposes, CO_2 is formed, which is capable of releasing certain forms of fixed P that can be either taken-up by rice plants or form compounds with iron (Fe) and manganese (Mn) (Hesse, 1984).

Organic matter decomposition under anoxic conditions leads to the release of formed metabolites into the flooding water, increasing the concentration of dissolved organic carbon (DOC) (Hanke et al., 2013) and accumulating volatile fatty acids (Tsutsuki and Ponnamperuma, 1987; Watanabe, 1984). The most common metabolites formed in these environments are methane (CH_4), sulfide (S_2^-), ethylene (C_2H_4), molecular hydrogen (H_2), amines, alcohols, phenolic acids, and ammonium - which is considered a stable product of the nitrogen metabolism (Pearsall and Mortimer, 1939; Toerien and Hattingh, 1969; Tsutsuki and Ponnamperuma, 1987; Wolin, 1979). Ammonium, an inorganic form of nitrogen available to plants, is the final product of the anaerobic decomposition of organic nitrogen compounds, specifically the deamination of amino-compounds and the hydrolysis of urea (Takai et al., 1963; Watanabe, 1984). Several studies have reported that inorganic N is released in greater quantities from OM under anaerobic conditions than under aerobic conditions (Broadbent and Reyes, 1971; Takai and Kamura, 1966), as well as less microbial need of N for population growth under anaerobic conditions (Acharya, 1935).

Despite flooded conditions, rice plants can transport atmospheric oxygen from the stem to the roots, and part of this oxygen is further diffused from the roots to the soil layer adjoined to them, creating an area in the rhizosphere that can foster aerobic microbial populations (Reddy and Patrick, 1986), therefore, some of the above mentioned anaerobic metabolites can be oxidized in these areas as well as in the soil surface (Watanabe, 1984). Included in the previous statement is ammonium, which can be oxidized to nitrate or nitrite and then be subject to denitrification (Fillery and Vlek, 1982; Katyal et al., 1988; Reddy and Patrick, 1986). Denitrification leads to a loss of N in the soil-water-plant system, which can impact rice production, since less mineral N is available. Another N loss pathway from the system is via ammonia (NH_3) volatilization, which is a complex process influenced by several environmental, chemical and biological factors, being pH, ammonia concentration of the floodwater and temperature the most influencing ones (Vlek and Craswell, 1981).

Nitrogen fertilization effect on rice yield components

Rice fields are usually covered with floodwater during most parts of the growing season, and this ecosystem can be divided into (1) floodwater, (2) plowing layer, and (3) subsoil layer beneath the plow layer (Kimura et al., 2004). The water interphase of the rice ecosystem is rich in primary production not only because of rice, but also because of phytoplankton (Minzoni et al., 1988). Although the floodwater is rich in metabolites and DOC (Hanke et al., 2013), the loss of N in this part of the system occurs rapidly (Cassman et al., 1996; Peng et al., 2006) through three main pathways: (1) ammonia volatilization, (2) denitrification and (3) nitrate leaching (Artacho et al., 2009; Do and Nishida, 2014; Fageria and Baligar, 2001). The supply of mineral N to the soil in organic systems is the sum of direct inputs of mineral N through atmospheric deposition and amendment applications, plus mineralization of SOM (Berry et al., 2002). The organic nitrogen pool in soils provides an important part of the N metabolized by rice (Bonetto et al., 1988). The net amount of mineralized N and its timing is dependent upon several factors, such as soil moisture, aeration, temperature, the nature of the OM and the microbial activity present in the soil (Berry et al., 2002). Nitrate (NO_3^--N) and ammonium (NH_4^+-N) ions in the soil form the pool of N immediately available for plant uptake (Berry et al., 2002).

Agronomically, accumulation of NH_4^+-N supports about 60% of the N requirements of rice (Reddy and Patrick, 1986). Net release of NH_4^+-N in paddy soils systems is determined by the ammonification and immobilization balance which is dictated by the N requirements of the microorganisms involved, nature of the OM, soil and environmental factors (Reddy and Patrick, 1986). This idea is supported by Thuithaisong et al. (2011), who found a high correlation between microbial decomposition of SOM and gradual releases of nutrients, which then become available to rice plants.

Furthermore, they found that organic farming may have greater soil N mineralization compared to conventional farming systems. N availability and the rate of N release from different organic amendments can be an important factor in the development of plants (Claassen and Carey, 2007) and it is directly correlated with the microbial activity present in the soil (Thuithaisong et al., 2011). However, microbial preference for different nitrogen sources that are readily available, equal to plants, can lead to a greater uptake of N rather than mineralization (Moran et al., 2005), leading to N uptake competition with plants. Supporting those results, Bowen and Harper (1990) found that by adding wheat straw as an organic amendment, soil microbial biomass (SMB) increased and there was an increase in microbial activity. However, the increase in SMB and microbial activity also accelerated the decay of the added straw, overall increasing the nutrient content of the soil (Bowen and Harper, 1990; Zhang et al., 2015).

One way to measure grain production efficiency in relation to the applied N fertilizer, is the nitrogen use efficiency (NUE) index, which is the ratio of grain yield to N applied; this index serves the purpose of quantifying the total economic output in relation to the utilization of all nitrogen present in the system, including fertilizer and indigenous soil nitrogen (Cassman et al., 1998). Nitrogen recovery efficiency for lowland rice variates depending on several factors, including geographical location, but is usually low under the conditions of flooded soil typically used in rice cropping systems (Fageria and Baligar, 2001). In the tropics there is a typical recovery from 30-50% of applied N (Bond et al., 2008; Fageria and Baligar, 2001), while in the southern United States recovery rates are reported to be from 17-61% (Bond et al., 2008).

Rice plants require N during their vegetative stage to prime growth and tillering, which will determine the potential number of panicles (Artacho et al., 2009; Djaman et al., 2016; Fageria and Baligar, 2001; Hirzel et al., 2011). In their study, Artacho et al. (2009) found an increase in rice yield, panicle density, spikelet sterility and dry matter production, in relation with increased N fertilization; these results are consistent with the findings by several other studies (Djaman et al., 2016; Fageria et al., 2011; Fageria and Baligar, 1999; Fageria and Baligar, 2001). However, with higher N rates, the nitrogen use efficiency (NUE) diminished (Artacho et al., 2009; Fageria et al., 2011; Tong et al., 2011). This trend is followed by both conventional and organic rice production, however, it has been found that the quality of rice yield components is increased under organic management (Quyen and Sharma, 2003; Surekha et al., 2013). As Vlek (1979) stated, the desired increase in grain yield through improved N fertilization is a function of N absorption and efficiency, which is translated into grain production. Dry matter as well as grain yield depend on N accumulation in rice plant but only up to a certain limit (Fageria and Baligar, 2001), incurring in the aforementioned reduced NUE. Another useful index for improving rice yield is the grain harvest index (Donald, 1962; Fageria and Baligar, 2001). The term was introduced by Donald (1962), and it is defined as the ratio of grain yield in a dry basis to aerial dry matter yield; the purpose of this ratio is to quantify the crop dry matter partitioning into economic yield components, and can be used as an important trait for improving rice yield (Fageria et al., 2011; Howell, 1990). Better practices on N fertilization are needed to mitigate environmental impacts and increase economic benefits of N fertilization (Fageria and Baligar, 2001), as well as encourage systems of sustainable agriculture that prevent ecosystem damage and even mitigate climate change effects.

To further understand the dynamics that nitrogen has on the soils of rice fields, two experiments were conducted. A laboratory trial was conducted for better understanding the N mineralization rates and dynamics under a laboratory setting. The intent of this trial was to determine the amount and quality of cover crop and soil amendment mineralization during an aerobic and anaerobic incubation, mimicking the environment of paddy soils during rice production. To achieve this, 25 microcosms were placed under incubation for six weeks, with different treatments composed of organic amendments and cover crop. The specific objectives of the trial were to examine the role of cover crop and soil amendment on nitrogen mineralization under aerobic and anaerobic conditions, as well as determine which combination of cover crop and organic amendment is optimum for a maximum N mineralization.

As well, a greenhouse trial was conducted in order to study the effects that organic soil amendment had on rice yield components in comparison with conventional rice production urea fertilizer. The treatments (organic, conventional and control) were applied to a complete randomized block design of 96 pots with soil collected from an organic certified field and planted with a rice variety, RiceTech XL753. Analysis was conducted for interactions of 100-grain weight, panicles, height, tillers, chlorophyll content, and inorganic nitrogen in the soil.

CHAPTER II

EFFECT OF SOIL ORGANIC AMENDMENT AND COVER CROP ON SOIL NITROGEN MINERALIZATION

Overview

A trial was conducted for better understanding the nitrogen (N) mineralization rates and dynamics of paddy rice fields under a laboratory setting. The objectives of the trial were to examine the role of cover crop and soil amendment on nitrogen mineralization under aerobic and anaerobic conditions, as well as determine which combination of cover crop and organic amendment is optimum for a maximum N mineralization. To achieve this, 25 microcosms were placed under incubation for six weeks, with different treatments composed of organic amendments and cover crop.

After an initial microbial flush due to the soil re-wetting, total available mineralized N increased over time under aerobic and anaerobic incubations of soil and seemed to be dictated by the amount of available nitrate and nitrite, since a linear increase with time was observed for the ammonium content. The amount of N added via organic amendment had the greatest impact on the amount of mineralized N over time, whereas no significant evidence was found to support that with higher amounts of cover crop there is an increased N immobilization in a six week incubation period. Of the treatments evaluated, the one composed of 6,000 kg cover crop biomass ha^{-1} plus 200 kg N via organic amendment was determined as the optimum combination of cover crop and amendment, since it had positive mineralization rates throughout the whole incubation period.

Introduction

The restricted availability of nutrients, particularly nitrogen (N), is one of the main reasons for low yields in organic farming (Berry et al., 2002; Wild et al., 2011). Organic farming has surged as an alternative to conventional farming methods (Thuithaisong et al., 2011), as it considers the medium- and long- term effect of agricultural interventions on the agro-ecosystem (Jahanban and Davari, 2013; Thakur and Sharma, 2005). Nonetheless, there are challenges associated with organic agriculture, such as the lack of products for use in fertilization and soil amendments, the lack of products for pest control and the lack of effective equipment for the specific needs in organic agriculture (i.e., for compost and weed management) (Jahanban and Davari, 2013; Sullivan, 2014). Furthermore, increased weed pressure and soil nutrient deficiencies, particularly N and P, are more common in organic management systems, which may lead to crop yield reductions in comparison to conventional farming systems (Berry et al., 2002; Mason et al., 2007; Snyder and Spaner, 2010; Wild et al., 2011; Yadav et al., 2000). However, studies have proven that weed control may be accomplished by using crop rotations and intercrops (Mason et al., 2007; Snyder and Spaner, 2010). Fertility management in organic farming relies on long-term integrated approaches rather than the more short-term, much targeted solutions common in conventional agriculture (Marinari et al., 2006; Thakur and Sharma, 2005).

Rice in the United States is grown mostly under flooding practices, therefore it is important to note that decomposition of SOM may vary compared to that under aerobic conditions. In a flooded rice field, oxygen (O_2) is displaced from the soil pores by water, creating anaerobic conditions and hence supporting anaerobic respiration (International Rice Research Insitute, 2009). Organic matter decomposition under anoxic conditions leads to the release of formed metabolites into the flooding water, increasing the concentration of dissolved organic carbon (DOC) (Hanke et al., 2013) and accumulating volatile fatty acids (Tsutsuki and Ponnamperuma, 1987; Watanabe, 1984). The most common metabolites formed in these environments are methane (CH_4), sulfide (S_2^-), ethylene (C_2H_4), molecular hydrogen (H_2), amines, alcohols, phenolic acids, and ammonium - which is considered a stable product of the nitrogen metabolism (Pearsall and Mortimer, 1939; Toerien and Hattingh, 1969; Tsutsuki and Ponnamperuma, 1987; Wolin, 1979).

Ammonium, an inorganic form of nitrogen available to plants, is the final product of the anaerobic decomposition of organic nitrogen compounds (Takai et al., 1963; Watanabe, 1984). In several studies, it has been found that inorganic N is released in greater quantities from OM under anaerobic conditions than under aerobic conditions (Broadbent and Reyes, 1971; Takai and Kamura, 1966). The supply of mineral N to the soil in organic systems is the sum of direct inputs of mineral N through atmospheric deposition and amendment applications, plus mineralization of SOM (Berry et al., 2002), symbiotic N fixation (Herridge et al., 2008) and inputs through irrigation water. The organic nitrogen pool in soils provides an important part of the N metabolized by rice (Bonetto et al., 1988). The net amount of mineralized N and its timing is dependent upon several factors, such as soil moisture, aeration, temperature, the nature of the OM and the microbial activity present in the soil (Berry et al., 2002). Nitrate (NO_3^--N) and ammonium (NH_4^+-N) ions in the soil form the pool of N immediately available for plant uptake (Berry et al., 2002). From an agronomic point of view, the accumulated amount of NH_4^+-N in the soil provides about 60% of the N requirements of rice (Reddy and Patrick, 1986).

The hypotheses to be tested with this trial are: *i)* there is an optimum combination of cover crop and organic amendment that has a significant increase in the total N mineralization at the end of the six week incubation when compared to other treatments; *ii)* there will be a notable increase in N mineralization from time zero to week one, due to a bacterial flush after wetting the soil; *iii)* the mineralization dynamic will be different under aerobic and anaerobic incubation; *iv)* in presence of a greater amount of cover crop than that of organic amendment, there will be a notable N immobilization; and *v)* N mineralization will be greater in soils with larger amounts of added N via a soil organic amendment.

Materials and methods

To determine the effects of soil amendment and winter cover crop on soil N mineralization, a six-week laboratory incubation study using a randomized factorial design with two factors was conducted under aerobic and subsequent anaerobic conditions – three weeks under each condition. The two factors were soil amendment (Nature Safe, 13-0-0) with five N levels (0, 50, 100, 150 and 200 kg N/ha) and winter cover crop (Durana White Clover (*Trifolium repens* L.) with five rates (0, 50%, 75%, 100% and 125% of 6,000 kg ha^{-1} - the average biomass yield in 2012 and 2013 at the Beaumont, TX Research Center) with three replications. Microcosmos were composed of 10 g of soil, and the corresponding mix of soil amendment and cover crop, thoroughly mixed and placed on a 50 mL centrifuge tube; the total number of treatment combinations can be seen in Table 1.1.

The soil used for the incubations was a Morey silt loam (16.2% sand and 15.6% clay) with a pH of 6.4, collected from an organic certified field at the Texas A&M AgriLife Research and Extension Center in Beaumont, TX. Soil was air-dried, ground and sieved to a 2mm particle size. Chemical analysis of the soil showed values of pH, EC, 2 *M* KCl extractable NH_4^+-N, NO_3^--N, and NO_2^--N of 6.42, 12,63 µs cm^{-1}, 3.32 mg kg^{-1}, 0.31 mg kg^{-1}, and 0.22 mg kg^{-1}, respectively.

The cover crop used was Durana White Clover (*Trifolium repens* L.), an intermediate type white clover intended for use as a renovation legume for grass pastures in the southeast of the United States (Bouton et al., 2005); clover was oven dried and ground to pass a 2 mm sieve, and had a total nitrogen content of 31 g kg^{-1}. Nature Safe (13-0-0) was used as an organic soil amendment. Nature Safe 13-0-0 is derived entirely from non-ruminant animal proteins and includes feather meal, pork meal and blood meal (Geise, 2016).

For the stage of aerobic incubation, soil moisture was adjusted to 60% of field water holding capacity by adding deionized water (Paul et al., 2011). Samples were incubated for 3 weeks at 20 °C, with weekly adjustment of soil moisture. Anaerobic incubation was established after the corresponding 3 weeks of aerobic incubation. For the anaerobic incubation, samples were flooded with 6 mL of deionized water and flushed with mixed air (95% N_2, 5% CO_2) in anaerobic chambers.

Inorganic N extraction and quantification

The microcosms were sampled weekly and analyzed for inorganic nitrogen (IN) (NH_4^+-N, NO_3^--N, and NO_2^--N) content. IN was extracted by using 40 mL of a 2 mol L^{-1} KCl solution, shaken for 30 minutes in a reciprocal shaker, centrifuged for 15 minutes at 4000 rpm and filtered using a vacuum system (Franzluebbers et al., 1994). Extraction solution was analyzed for IN using the following colorimetric assays (Technicon Industrial Systems, 1977) with a microplate reader:

Ammonium

An ammonium calibration curve (0 - 25 mg L^{-1} NH_4^+-N) was prepared and used to convert absorbance readings from the extractions into ammonium concentration. A new calibration curve and its respective regression equation were made for each plate analyzed.

To determine the ammonium content, 80 μL of buffer solution, a 30 μL aliquot of sample (or calibration curve solution), 60 μL of sodium salicylate and 90 μL of NaOCl were added to each well of a 96-well plate, mixing well between every addition. The plate was incubated in the dark for 30 min and then measured for absorbance in a PowerWave

X Microplate Scanning Spectrophotometer (BioTek Instruments, Inc.) at 660 nm (Technicon Industrial Systems, 1977).

Nitrate

A nitrate calibration curve (0 – 1.0 mg/L NO_3^--N) was prepared and used to convert absorbance readings from the extractions into nitrate concentration. A new calibration curve and its respective regression equation was made for each plate analyzed.

To determine the nitrate content, 30 μL of NaOH, a 140 μL aliquot of sample (or calibration curve solution), and 40 μL of Hydrazine were added to each well of a 96-well plate reader, mixing well between every addition. The plate was incubated in the dark for 15 min and then 40 μL of color reagent were added into each well and the plate shaken to mix. The plate was incubated in the dark for additional 15 minutes and then measured for absorbance in a PowerWave X Microplate Scanning Spectrophotometer (BioTek Instruments, Inc.) at 550 nm (Technicon Industrial Systems, 1977).

Nitrite

A nitrite calibration curve (0 – 1.0 mg/L NO_2^--N) was prepared and used to convert absorbance readings from the extractions into nitrite concentration. A new calibration curve and its respective regression equation was made for each plate analyzed.

To determine the nitrite content, 30 μL of NaOH, a 140 μL aliquot of sample (or calibration curve solution), 40 μL of water and 40 μL of color reagent were added to each well of a of a 96-well plate reader, mixing well between every addition. The plate was incubated in the dark for 10 minutes and then measured for absorbance in a PowerWave X Microplate Scanning Spectrophotometer (BioTek Instruments, Inc.) at 550 nm (Technicon Industrial Systems, 1977).

Statistical analysis

The data obtained was analyzed for normality using JMP® Pro v.12.2 (SAS Institute, 2015). If data was not normal, a log-transformation was conducted to achieve normality. A Kolmogorov's D test was performed to ensure a good fit of the data. Outliers were identified using the Grubb's test with a significance of 0.05 and a total of 27 data points were removed from the data set. From this point forward, all data analysis presented was conducted with the normalized sub-data values without outliers.

Results and discussion

The measured amounts of mineralized nitrogen can be better described by the rates of mineralization under aerobic and anaerobic conditions (Table 1.2). The N mineralization rates can be identified on three main phases after the initial flush from week 0 to week 1: a net immobilization phase from week 1 to week 3, a net mineralization phase on weeks 4 and 5, and finally another net immobilization phase on week 6. All treatments presented an immobilization phase at certain point, except for the treatment composed of 100% biomass and 200 kg N/ha of organic soil amendment.

The initial flush on N mineralization was present on every treatment, and is most likely due to a first response of microorganisms to the soil being re-wetted; under laboratory conditions, this response often lasts from 2 – 5 days (Mikha et al., 2005;

Sponseller, 2007; Sugihara et al., 2015). Under every treatment, there was a significant drop in the amount of N mineralized on week 3, assumingly related to the immobilization of N after the first response to wetting and an intent of microorganisms to decompose the SOM; this phenomenon was also present in the incubation studies from Mikha et al. (2005). The different slopes observed suggest that different fractions of the added substrates are being mineralized at different times under aerobic and anaerobic conditions, as suggested by Gale and Gilmour (1988). Another possible explanation for the different visible stages in the incubation period, is the remineralization of immobilized N (Wang et al., 2001). Net release of ammonium in paddy systems is dictated by the balance between ammonification and immobilization, which is determined by the nitrogen requirements of the microorganisms involved, nature of the OM, soil properties and other environmental factors (Reddy and Patrick, 1986).

From all the treatment analyzed, six treatments presented the most N mineralized on average after the six-week incubation period (Figure 1.1):

50% Cover Crop – 150 kg N

50 % Cover Crop – 200 kg N

75% Cover Crop – 200 kg N

100 % Cover Crop – 200 kg N

125 % Cover Crop – 150 kg N

125 % Cover Crop – 200 kg N

ANOVA and Least Square Analysis were conducted to see the effects that added N, cover crop and time factors had on the amount of mineralized N, the hierarchy of effects interaction can be seen in Table 1.3. The treatments with most N mineralized at the end of the incubation period (Figure 1.1) support the finding that the amount of nitrogen added has the greatest impact – besides time – on the amount of mineralized N at the end of the incubation period ($P<0.001$). The negligible visible effect of the cover crop can be related to the use of cover crop as an enhancer of soil fertility over long periods of time (Mäder et al., 2002; Tamaki et al., 2002; Thuithaisong et al., 2011).

Nitrogen mineralization rates were higher under the first two weeks of anaerobic conditions (Table 1.2, Figure 1.7), possibly due to the lower metabolic efficiencies of the anaerobic microbial populations (Gale and Gilmour, 1988) and had a final decline attributed to denitrification as seen by Isirimah and Keeney (1973) as well. The N mineralization rates ranged from -0.073 to 0.125 mg N kg $soil^{-1}$ during the anaerobic incubation.

Total inorganic nitrogen in the microcosms is shown in its different species present (NH_4^+-N, NO_3^--N, NO_2^--N) in Figures 1.2-1.4. For each species' dynamic, a fitted regression was adjusted; the equation and correlation coefficient for each one of them are presented in Table 1.4.

Ammonium has a consistent linear increase throughout the incubation period, regardless of aerobic or anaerobic conditions. This result is consistent with that reported by Kirchmann and Witter (1989), in which they found increasing ammonium concentrations over time under aerobic and anaerobic incubations of manure decomposition amended with oat straw. Furthermore, studies have reported that under anaerobic conditions, the loss of N through ammonia are almost negligible when compared to the loss under aerobic conditions (Kirchmann and Witter, 1989; Mahimairaja et al., 1994). In their study of poultry manure decomposition, Mahimairaja et al. (1994) stated that ammonification tends to be greater under anaerobic conditions – greatly reduced by the addition of straw – and nitrification under aerobic conditions. However, the statement of increased nitrification under aerobic conditions was not reflected on the overall content of nitrate or nitrite for the aerobic stage of the incubation. The discrepancy could be due to a nitrification rate not significant enough to counteract the ongoing denitrification, consistent with the results found by Wang et al. (2013). Li et al. (2003) found in their study that NO_3^--N was the first species to disappear under aerobic incubation.

The ammonium dynamics suggested that the decrease in the overall content of IN is mainly dependent of the cycles that nitrate and nitrite were being subject to. Contrary to the results of Linquist et al. (2006), who determined that NO_3^--N was unlikely to be present in fields that are consistently flooded, we found that over the anaerobic incubation NO_3-N increased (Figures 1.5-1.11). The decrease of NO_3-N and NO_2-N at the final stage of the incubation – under anaerobic conditions – can be related to a study conducted by Wild et al. (2011) in which they found that little to no mineralization of organic fertilizer occurs after 36 days of anaerobic incubation. With a decreased mineralization and still organic matter present in the soil after this period, it is likely that microorganisms immoblized some of the available NO_3-N and NO_2-N, since it is known that there is microbial preference for the N source that is immediately available, leading to greater uptake of mineral-N than residue (Moran et al., 2005). A more probable explanation for the reduction of the aforementioned species is their loss through denitrification, as found by Isirimah and Keeney (1973), given by the soil conditions in the microcosmos.

Conclusions

Total available mineralized nitrogen over time under aerobic and anaerobic incubations of soil – amended with Durana clover and Nature Safe (13-0-0) – seems to be dictated mostly by the amount of available nitrate and nitrite, since a linear increase with time can be expected for the ammonium content. The first hypothesis declared that there would be an optimum combination of cover crop and organic amendment in regards to the total amount of mineralized N. Of the analyzed treatments, the one composed of 100 % cover crop plus 200 kg N/ha can be determined as the optimum combination of cover crop and organic amendment because it mineralized the most N during the incubation period and always had positive mineralization rates, which would provide rice plants constant availability of inorganic nitrogen.

The second hypothesis for this trial stated that there would be an increased mineralization of N after the re-wetting of the soil, which was visible throughout all the treatments, thus supporting the aforementioned statement. The different mineralization rates – three stages were identified – and dynamics of the different inorganic N species sustain the third hypothesis stated on the introduction of this article: mineralization dynamics would be different under aerobic and anaerobic conditions.

Based on the treatments that mineralized the most nitrogen at the end of the six week (Figure 1.1) incubation period and the P-values for effects and their interactions (Table 1.3), there is no significant evidence that supports that with higher amounts of cover crop there is an increased N immobilization, at least under the conditions of this trial, which was the fourth hypothesis for this study. Finally, the same data provides enough evidence to conclude that mineralization is greater in soils with larger amounts of added N via Nature Safe, indicating that the amount of N added is the limiting factor for N mineralization, rather than the amount of cover crop added.

CHAPTER III

EFFECT OF SOIL ORGANIC AMENDMENT ON ORGANIC RICE PRODUCTION

Overview

The organic rice industry has expanded rapidly due to market demand. However, there has been little research conducted that is relevant to the unique flooded paddy system that is used to produce rice. One of the critical issues identified for organic rice production is nitrogen management. A greenhouse trial in Beaumont, TX, was conducted from May to August 2015 in order to study the effects that organic soil amendment (Nature Safe 13-0-0) with six different N rates of application (0, 50, 100, 150, 200, and 250 kg ha^{-1}) had on the rice yield components in comparison with conventional rice production (urea fertilizer 46-0-0). The treatments (organic, conventional and control) were applied to a complete randomized block design of 96 pots with soil collected from an organic certified field and planted with a rice variety, RiceTech XL753. Analysis was conducted for interactions of 100-grain weight, panicles, height, tillers, and total inorganic N present in the soil.

Nitrogen Use Efficiency (NUE) was higher for most of the conventional treatments, however, at 200 kg N ha^{-1} the organic treatment had no significant difference on yield or NUE when compared to the conventional treatment. At the highest application rate of N (250 kg N ha^{-1}) the NUE decreased 32% and 10% for the organic and conventional treatments, respectively. Organic treatments tended to produce more aboveground biomass rather than grain, and therefore their harvest index was less than that of conventional treatments.

Introduction

Organic rice is increasingly desired by U.S. consumers (Texas A&M AgriLife, 20-Oct-2015), due to enhanced awareness of environmental consciousness and its recognition for lower level of chemical residues (Huang et al., 2016; Snyder and Spaner, 2010). Although there seems to be no difference in the amylose and protein contents between organically and conventionally grown rice, organic rice presents higher antioxidative activity than conventional rice (Na et al., 2007).

Rice N requirements is closely related to crop yield levels (Fageria and Baligar, 2001). Rice plants require N during vegetative stage to promote growth and tillering, which in turn, determined the potential number of panicles (Artacho et al., 2009). Several studies have demonstrated that N contributes to spikelet, grain filling, panicle and tiller numbers, as well as improving the photosynthetic capacity of the plant (Artacho et al., 2009; Djaman et al., 2016; Fageria and Baligar, 1999; Fageria and Baligar, 2001; Hirzel et al., 2011).

However, a decrease in the agronomic N use efficiency (NUE) with increasing N fertilization has been observed (Artacho et al., 2009; Djaman et al., 2016; Fageria and Baligar, 2001; Peng et al., 2006; Tong et al., 2011), which is an index that measures yield increase in relation to the amount of N fertilizer applied. To accelerate the development of the organic rice industry, it is crucial to develop effective N management techniques (Huang et al., 2016).

A greenhouse trial with a complete random block design was conducted to evaluate the effect of different sources of N on the yield and yield components of rice, as well as its effects on the nitrogen supply and uptake in rice production. The specific objectives of this trial were to *i*) evaluate the use efficiency of rice under different N rate and N sources – organic amendment or synthetic fertilizer; *ii*) compare the impact that organic soil amendment and its rate have on yield and yield components of organic rice with conventional rice; and *iii*) compare N mineralization in soils under organic and conventional rice production.

The hypothesis that will be tested are *i)* It is possible to reach the same rice yields level under organic management as in conventional management. *ii)* Mineralized N will be greater at the end of the season under organic management.

Materials and methods

Experimental design and sampling

A greenhouse experiment was conducted from May to August 2015 in Beaumont, Texas, to evaluate the effect of organic soil amendment (Nature Safe) and synthetic fertilizer (Urea) on lowland rice production. The soil used was a Morey Silt Loam (16.2% sand and 15.6% clay) with a pH of 6.4, collected from a certified organic rice field in Beaumont, TX. Three main experimental factors were present in the trial: N source (Nafe

Safe vs. Urea), N rate (0, 50, 100, 150, 200, and 250 kg N ha^{-1}), and with and without rice crop.

Nitrogen fertilizers used were Nature Safe 13-0-0 for the organic treatment and urea 46-0-0 for the conventional treatment; nitrogen rates used were 0, 50, 100, 150, 200 and 250 kg N ha^{-1}; and the cultivar was XL753, a long-grain high yielding hybrid, with four plants in each pot. The experimental design was a complete randomized block with four replications. The experiment was conducted in plastic pots with 4.5 kg of soil in each pot. At the time of sowing, organic treatment pots had the total N rate designated, while conventional treatment pots were fertilized under common practice of split application (20% - 60% - 20%), with the first application at day 14 after sowing – second and third applications were at days 42 and 64, respectively.

After 36 days of sowing, pots were flooded with 3-4 cm of water and kept that way until a week before harvest. Before flooding, soil in pots was maintained at 60% water holding capacity by weight. Tillers were counted at days 22, 29, 35 and 85. The chlorophyll content of leaves was measured with a chlorophyll meter SPAD-502 (Konica Minolta, Osaka, Japan) at days 55, 64 and 78. Rice plants were harvested at day 90 after sowing.

Soil samples were taken at four different stages of rice growth: germination, maximum tillering, heading, and after harvest of rice plants – days 8, 39, 55 and 111, respectively. Soil samples were freeze dried, ground and passed through a 500 micron sieve and kept at -20 °C until analysis.

Soil analysis

Samples were extracted using 40 mL of a 2 mol L^{-1} KCl solution, shaken for 30 minutes in a reciprocal shaker, centrifuged for 15 minutes at 4000rpm and filtered. Extraction solution was analyzed for inorganic N (NH_4^+-N, NO_3^--N, and NO_2^--N) using the following colorimetric assays with a microplate reader:

Ammonium

An ammonium calibration curve (0 - 25 mg L^{-1} NH_4^+-N) was prepared and used to convert absorbance readings from the extractions into ammonium concentration. A new calibration curve and its respective regression equation were made for each plate analyzed.

To determine the ammonium content, 80 µL of buffer solution, a 30 µL aliquot of sample (or calibration curve solution), 60 µL of sodium salicylate and 90 µL of NaOCl were added to each well of a 96-well plate, mixing well between every addition. The plate was incubated in the dark for 30 min and then measured for absorbance in a PowerWave X Microplate Scanning Spectrophotometer (BioTek Instruments, Inc.) at 660 nm.

Nitrate

A nitrate calibration curve (0 – 1.0 mg/L NO_3^--N) was prepared and used to convert absorbance readings from the extractions into nitrate concentration. A new calibration curve and its respective regression equation was made for each plate analyzed.

To determine the nitrate content, 30 µL of NaOH, a 140 µL aliquot of sample (or calibration curve solution), and 40 µL of Hydrazine were added to each well of a of a 96-well plate reader, mixing well between every addition. The plate was incubated in the dark for 15 min and then 40 μL of color reagent were added into each well and the plate shaken to mix. The plate was incubated in the dark for additional 15 minutes and then measured for absorbance in a PowerWave X Microplate Scanning Spectrophotometer (BioTek Instruments, Inc.) at 550 nm.

Nitrite

A nitrite calibration curve (0 – 1.0 mg/L NO_2^--N) was prepared and used to convert absorbance readings from the extractions into nitrite concentration. A new calibration curve and its respective regression equation was made for each plate analyzed.

To determine the nitrite content, 30 μL of NaOH, a 140 μL aliquot of sample (or calibration curve solution), 40 μL of water and 40 μL of color reagent (Technicon Industrial Systems, 1977) were added to each well of a of a 96-well plate reader, mixing well between every addition. The plate was incubated in the dark for 10 minutes and then measured for absorbance in a PowerWave X Microplate Scanning Spectrophotometer (BioTek Instruments, Inc.) at 550 nm.

The percentage of nitrogen mineralized (%N miner.) at each sampling time (t) was calculated according to the proposed equation (Eq. 1) by Wild et al. (2011), mean values for fertilized (Fert) and unfertilized (Unfert) were used for calculating this parameter.

$$\%\,N\,miner. = \left[\frac{(NH_4N+NO_2N+NO_3N)_{(Fert,\ t)} - (NH_4N+NO_2N+NO_3N)_{(Unfert,\ t)}}{N\,fertilizer\,applied}\right] \quad \text{(Eq. 1)}$$

Yield and yield components analysis

Right before harvest, tillers, panicles and aboveground height of the tallest tiller were measured. Rice was harvested after 90 days by hand, fresh weigh of the plant –

panicles included – was recorded and plants were hung to dry upside down and then oven dried at 71 degrees Celsius for 24 hours. Whole weight of the dried plant was measured, and then panicle and aboveground biomass were recorded individually, as well as total grain weight.

In order to quantify the efficiency of grain production and N usage under both treatments, the agronomic Nitrogen use efficiency (NUE) was calculated for each treatment using the formula proposed by Artacho et al. (2009), Fageria and Baligar (2001), and Ofori et al. (2005) (Eq 2):

$$NUE = \frac{G_f - G_u}{N_a} \qquad \text{(Eq. 2)}$$

where G_f is weight of grain for fertilized treatment (g grain / pot), G_u is weigh of grain for unfertilized treatment (g grain / pot) and N_a is Nitrogen applied (g N / pot). Harvest index was also calculated following the formula proposed by Fageria and Baligar (2001) (Eq. 3):

$$Harvest\ Index = \frac{grain\ yield}{grain\ plus\ straw\ yield} \qquad \text{(Eq. 3)}$$

Statistical analysis

The data obtained was analyzed for normality using JMP® Pro v.12.2 (SAS Institute, 2015). Data was not normal, and therefore log transformed to achieve normality. A Kolmogorov’s D test was performed to ensure a good fit of the data. Outliers were identified and removed from the data set.

Results and discussion

Nitrogen mineralization analysis

Table 2.1 shows the percentages of mineralized N for each treatment (variety, N rate and N source). For the first eight days, the organic treatment presented a small percentage of N mineralized, while the conventional treatment shows no mineralization since urea hadn't been applied at that time yet. Nature Safe presented scarce to no mineralization for the second sampling date (day 39), possibly indicating that the time between samplings was too large and therefore the dynamics of N mineralization was missed. For both treatments, the third and fourth measurements (day 55 and 111, respectively) had a negative amount of N mineralized for the majority of samples, indicating either loss of N from the system or immobilization of inorganic N in the fertilized soils. Inorganic N depletion from the soil could be driven by plant uptake, microbial immobilization, or losses through denitrification or ammonia volatilization. When comparing the two treatments, samples with conventional fertilizer had a greater mineralization percentage of fertilizer than samples with organic amendment, both for samples with rice plants or the control ones.

These observations can be graphically seen on Figure 2.1, which depicts the total IN present in the soil over the period of the experiment. The negative rates of mineralized N from the fertilizer can be appreciated when comparing the curves for nitrogen rate 0 to the other rates. The maximum amount of N mineralized is under the N rate 0 for most of the graphs, accordingly to the results in Table 2.1. This, however, only emphasizes the need to sample more frequently, on the following days after the application of the fertilizer, to see if it is being mineralized within the range of time that cannot be seen with the current samplings. Furthermore, the N content of the biomass and the grain should be measured to better understand the fate and distribution of N in the soil, plant and grain system.

The correlation coefficient between the forms of IN present in the soil and pH variation were analyzed, results are shown on Table 2.2. From this table we can observe that the greatest impact was caused by the conventional treatments, where pH was raised and is consistent with other studies where the addition of urea raised the pH value (Vlek and Craswell, 1979).

Yield and yield components

The mean values for the calculated NUE and harvest index and parameters, along with plant height and yield components are shown in Table 2.3, and the influence of N rate, treatment, and their interaction on each of these effects were presented on Table 2.4.

Under organic treatment, plants produced less panicles in comparison with the conventional management (Figure 2.2), regardless of the amount of N added. From the regression lines on Figure 2.2, we can conclude that the panicle production had a strong and positive linear increase (r = 0.94) under conventional treatment, whereas the organic management presented an almost null increase in panicle production with N rates of 50, 100 and 150; however, there was a spiked panicle production with an N rate of 200 kg N/ha. The increment in panicle production didn’t hold for the following rate, 250 kg N/

ha. For the treatments with 0 and 50 kg N ha^{-1}, panicle production was the same. The amount of nitrogen added had the most influence on the production of panicles (P-value < 0.0001); however, the source of nitrogen (organic or synthetic) also had a significant impact on this parameter.

Consistent with previous studies (Texas A&M AgriLife, 20-Oct-2015; Thuithaisong et al., 2011; Wild et al., 2011), organic management had a lower yield than conventional rice (Figure 2.3). However, it is important to note that yield was significantly not different between both treatments at a rate of 200 kg N/ ha (p-value = 0.8679). Under this rate the organic treatment produced more panicles.

Panicle production is dependent upon the number of tillers, since all tillers may produce a panicle but not all do. Tillers, panicles and yield are strongly related and it can be seen in Figures 2.4 – 2.6 and P-values on Table 2.5; where the trend was similar for each treatment: conventional management had a steady and almost linear increase, whereas organic treatment presented a sudden increase for the three parameters at the 200 kg N/ ha. This means that at this rate, there was an increased tillering for organic production, which led to a higher panicle count and therefore an increase in yield. The slopes of the regression line on Figure 2.6 showed that plants under conventional treatment produced more panicles per tiller produced, ~75% of the tillers produced a panicle, while only ~30% of the plants under organic treatment did. Aboveground biomass production was higher for organic treatments (Table 2.4, Figure 2.7), but yield was lower, indicating that most of the plant resources were allocated in producing vegetation rather than grain.

This is further corroborated by the correlation equations between aboveground biomass production and yield (Figure 2.8), which show that conventional treatment produces more grain for every gram of biomass produced. Conventional treatments presented a higher NUE than the organic treatments (Figure 2.9), except for the N application rate of 200 kg N ha^{-1}. Under this rate of fertilizer, there is no significant difference between the NUE of either treatment (P-value = 0.3276). However, at a rate of 250 kg N ha^{-1} the NUE has reduced 32% and 10% for organic and conventional treatment respectively, which is consistent with the findings by Li et al. (2014).

Conclusions

Organic soil amendments have a slower release of nitrogen to the soil, and therefore a constant availability of inorganic N for the plant. However, the rates of mineralization of organic compounds may not be synched with the plants' requirements of N, and therefore the mineralized N may be lost from the system rather than up-taken by plants.

My results showing no significant difference between organic and conventional treatments at a rate of 200 kg N ha^{-1} on either NUE or grain yield, supports the hypothesis that it is possible to achieve the same yields under conventional and organic treatments. However, the yield attained at 200 kg N ha^{-1} was not the highest yield obtained, which was at 250 kg N ha^{-1} under conventional treatment – although it was not significantly different from that obtained at 200 kg N ha^{-1}. There was not enough statistical evidence to support the second hypothesis - N mineralized would be greater after harvest for the organic treatment - since the inorganic N present under both treatments was not significantly different of each other.

CHAPTER IV

CONCLUSIONS

The increase of the world's population will translate into an increased demand for rice, a staple food for over 50% of the world. For this reason, the need for increased rice yields with less inputs has become crucial. Furthermore, it has been proven that organic agriculture can be beneficial for the soil and may have less impact on the environment than conventional farming (Jahanban and Davari, 2013; Mäder et al., 2002; Marinari et al., 2006; Mason et al., 2007). An increased social conscience regarding the impact that humans and their food production have on the environment has led to a higher demand for organic products, including grains such as rice (Snyder and Spaner, 2010). However, organic systems tend to have lower yields than conventional systems, and so it has become imperative to find solutions to tackle this obstacle.

The incubation trial conducted to better understand the dynamics of N mineralization under aerobic and anaerobic conditions as well as identifying a combination of cover crop and organic soil amendment that led to higher mineralization rates and content in the soil produced the following results. Three stages of nitrogen mineralization rates could be identified: a bacterial flush after rewetting the soil, an aerobic stage and an anaerobic stage. The dynamics of the different inorganic N species sustain the hypothesis that the mineralization rates and dynamics would be different under aerobic and anaerobic conditions. Total available mineralized nitrogen over time under aerobic and anaerobic incubations of soil – amended with Durana clover and Nature Safe (13-0-

0) – seems to be dictated mostly by the amount of available nitrate and nitrite, since a linear increase with time was observed for the ammonium content.

Of the two factors analyzed – amount of biomass added and nitrogen rate added via organic soil amendment – sufficient statistical evidence was found to determine that the amount of N added via organic soil amendment had the greatest impact on the total amount of mineralized N, and the amount of cover crop added proved to have no significant impact. Finally, of the analyzed treatments, the one composed of 100 % Cover Crop – 200 kg N can be determined as the optimum combination of cover crop and organic amendment because it mineralized the most N during the incubation period and always had positive mineralization rates, which would provide rice plants constant availability of inorganic nitrogen.

From the greenhouse trial results, we can determine that it is possible to achieve the same amount of yield under conventional and organic treatments while using an organic soil amendment. It is interesting to note that for the organic treatment, the highest yield and NUE was reached at 200 kg N ha^{-1} rather than 250 kg N ha^{-1}, agreeing with the results found by a previous study by Li et al. (2014), which state a quadratic function of added N and yield. Similar results were found for the conventional treatment, however, the NUE and the highest yield were not achieved at the same N rate, but at 150 kg N ha^{-1} and 250 kg N ha^{-1}, respectively.

The results of these trials, while not conclusive, are promising and contribute to the quest of finding high yields with organic agriculture. Further research is needed to better understand the timings of available inorganic nitrogen, and therefore be capable to synch it with the plant requirements. This would enhance the NUE, resulting in higher yields and harvest index, which would be beneficial for both meeting global rice demand and reducing the impact that rice production has on the environment.

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APPENDIX A

TABLES AND FIGURES

Table 1.1. Nomenclature and factorial design for incubation trial

Sample ID	Winter cover crop		Soil Amendment
	Percentage of biomass[†]	kg biomass/ ha	kg N/ ha
0-0	0%	0	0
0-50	0%	0	50
0-100	0%	0	100
0-150	0%	0	150
0-200	0%	0	200
50-0	50%	3000	0
50-50	50%	3000	50
50-100	50%	3000	100
50-150	50%	3000	150
50-200	50%	3000	200
75-0	75%	4500	0
75-50	75%	4500	50
75-100	75%	4500	100
75-150	75%	4500	150
75-200	75%	4500	200
100-0	100%	6000	0
100-50	100%	6000	50
100-100	100%	6000	100
100-150	100%	6000	150
100-200	100%	6000	200
125-0	125%	7500	0
125-50	125%	7500	50
125-100	125%	7500	100
125-150	125%	7500	150
125-200	125%	7500	200

[†] Percentage of 6000 kg/ha of biomass, the average produced in Beaumont, TX in 2012 and 2013.

Table 1.2. Rates of mineralization under aerobic and anaerobic conditions.

	Aerobic Incubation							
	WEEK 0		WEEK 1		WEEK 2		WEEK 3	
Treatment	Total IN (mg N kg soil^{-1})	Mineralization rate (mg N kg soil^{-1} d^{-1})	Total IN (mg N kg soil^{-1})	Mineralization rate (mg N kg soil^{-1} d^{-1})	Total IN (mg N kg soil^{-1})	Mineralization rate (mg N kg soil^{-1} d^{-1})	Total IN (mg N kg soil^{-1})	Mineralization rate (mg N kg soil^{-1} d^{-1})
0-0	1.8	-	2.4	0.078	1.5	-0.128	1.7	0.033
0-50	1.6	-	2.2	0.090	2.1	-0.019	1.8	-0.041
0-100	1.8	-	2.2	0.061	1.6	-0.081	2.0	0.053
0-150	1.5	-	2.3	0.107	2	-0.036	1.9	-0.017
0-200	1.5	-	2.4	0.126	2.1	-0.042	2.0	-0.016
50-0	1.8	-	2.2	0.054	1.6	-0.090	1.7	0.023
50-50	1.5	-	2.2	0.106	1.8	-0.052	1.8	-0.006
50-100	1.8	-	2.2	0.063	1.6	-0.081	1.8	0.022
50-150	1.5	-	2.3	0.117	2.2	-0.022	2.0	-0.030
50-200	1.6	-	2.3	0.095	2.2	-0.014	2.0	-0.034
75-0	1.6	-	2.2	0.091	2.1	-0.016	1.9	-0.024
75-50	1.6	-	2	0.058	2.1	0.010	1.9	-0.019
75-100	1.5	-	2.3	0.125	2.2	-0.017	1.9	-0.043
75-150	1.5	-	2.1	0.093	2.2	0.015	1.9	-0.048
75-200	1.3	-	2.2	0.120	2.3	0.009	2.1	-0.015
100-0	1.8	-	2	0.029	1.7	-0.048	1.7	0.001
100-50	1.5	-	2.1	0.095	2.1	0.002	1.8	-0.045
100-100	1.8	-	2.1	0.045	1.8	-0.049	1.9	0.022
100-150	1.5	-	2	0.073	2.2	0.025	2.0	-0.030
100-200	1.5	-	2.2	0.099	2.2	0.006	2.2	0.007
125-0	1.5	-	2.1	0.088	1.9	-0.030	1.8	-0.016
125-50	1.4	-	2.2	0.107	2	-0.022	1.9	-0.020
125-100	1.5	-	2.2	0.098	2.2	0.006	1.9	-0.045
125-150	1.6	-	2.3	0.101	2.3	-0.001	2.0	-0.034
125-200	1.5	-	2.2	0.104	2.1	-0.001	2.0	-0.010

Table 1.2. Continued,

	Anaerobic Incubation					
	WEEK 4		WEEK 5		WEEK 6	
Treatment	Total IN (mg N kg soil^{-1})	Mineralization rate (mg N kg soil^{-1} d^{-1})	Total IN (mg N kg soil^{-1})	Mineralization rate (mg N kg soil^{-1} d^{-1})	Total IN (mg N kg soil^{-1})	Mineralization rate (mg N kg soil^{-1} d^{-1})
0-0	1.9	0.029	2.3	0.062	2.1	-0.028
0-50	2.4	0.094	3.3	0.125	2.9	-0.053
0-100	2.2	0.024	2.4	0.029	2.3	-0.015
0-150	2.5	0.086	2.9	0.061	3.0	0.002
0-200	2.9	0.121	3.3	0.059	3.0	-0.040
50-0	1.9	0.026	2.2	0.043	2.1	-0.018
50-50	2.4	0.089	2.9	0.067	2.8	-0.019
50-100	2.2	0.054	2.3	0.011	2.0	-0.036
50-150	2.6	0.093	2.8	0.022	3.4	0.081
50-200	2.7	0.102	3.2	0.068	3.4	0.029
75-0	2.5	0.079	3.0	0.074	2.5	-0.064
75-50	2.5	0.085	2.8	0.046	2.8	-0.006
75-100	2.6	0.098	3.1	0.078	2.7	-0.061
75-150	2.6	0.096	2.8	0.027	2.8	0.003
75-200	2.6	0.071	3.1	0.067	3.1	0.002
100-0	1.9	0.028	2.2	0.043	2.1	-0.005
100-50	2.5	0.097	2.9	0.058	2.8	-0.016
100-100	2.2	0.037	2.3	0.019	2.4	0.008
100-150	2.6	0.093	3.2	0.079	2.6	-0.073
100-200	2.6	0.046	2.9	0.054	3.3	0.047
125-0	2.4	0.094	3.0	0.075	2.7	-0.040
125-50	2.5	0.091	2.9	0.045	2.5	-0.057
125-100	2.5	0.09	2.9	0.049	2.8	-0.004
125-150	2.6	0.084	2.9	0.039	3.3	0.053
125-200	2.6	0.078	3.0	0.057	3.2	0.026

Table 1.3. Analysis of variance for N mineralization as affected by cover crop, soil amendment and their interactions for an incubation experiment during 2015 (n=3).

Effect	N mineralization
	P value
Time	< 0.0001
Nitrogen	< 0.0001
Cover Crop	0.3536
Nitrogen * Time	< 0.0001
Cover Crop * Time	0.0593
Cover Crop * Nitrogen	0.1623
Cover Crop * Nitrogen * Time	0.1914

Table 1.4. Equations for regressions of figures 2-4.

Corresponding coefficients to the equation form				$y = ax^4 + bx^3 + cx^2 + dx + e$			Correlation Coefficient
Treatment	y =	a	b	c	d	e	R^2
0-0	NH_4^+				0.09	0.61	0.81
	NO_3^-	-0.00	0.05	- 0.18	0.13	0.42	0.67
	NO_2^-	0.01	0.11	- 0.39	0.29	0.79	0.57
0-50	NH_4^+				0.13	0.55	0.92
	NO_3^-	-0.01	0.09	- 0.33	0.34	0.39	0.99
	NO_2^-	-0.01	0.13	- 0.48	0.52	0.70	0.86
0-100	NH_4^+				0.10	0.65	0.84
	NO_3^-	-0.00	0.05	- 0.18	0.14	0.42	0.72
	NO_2^-	-0.00	0.01	- 0.02	-0.07	0.78	0.27
0-150	NH_4^+				0.16	0.50	0.85
	NO_3^-	-0.01	0.07	- 0.26	0.25	0.40	0.97
	NO_2^-	-0.01	0.12	- 0.41	0.43	0.70	0.68
0-200	NH_4^+				0.19	0.55	0.98
	NO_3^-	-0.01	0.08	- 0.29	0.30	0.40	0.81
	NO_2^-	-0.02	0.23	- 0.82	0.89	0.70	0.87
50-0	NH_4^+				0.09	0.61	0.85
	NO_3^-	0.00	0.05	- 0.16	0.12	0.42	0.76
	NO_2^-	-0.01	0.07	- 0.23	0.14	0.77	0.52
50-50	NH_4^+				0.14	0.50	0.91
	NO_3^-	-0.01	0.07	- 0.24	0.23	0.37	0.98
	NO_2^-	-0.01	0.15	- 0.54	0.59	0.67	0.93
50-100	NH_4^+				0.09	0.64	0.84
	NO_3^-	0.00	0.04	- 0.13	0.08	0.42	0.60
	NO_2^-	-0.01	0.08	- 0.24	0.12	0.78	0.41
50-150	NH_4^+				0.20	0.50	0.95
	NO_3^-	-0.01	0.06	- 0.21	0.19	0.40	0.70
	NO_2^-	-0.01	0.08	- 0.33	0.47	0.69	0.68
50-200	NH_4^+				0.22	0.50	0.95
	NO_3^-	-0.01	0.07	- 0.24	0.23	0.40	0.98
	NO_2^-	-0.01	0.14	- 0.51	0.58	0.72	0.82

Table 1.4. Continued,

Corresponding coefficients to the equation form				$y = ax^4 + bx^3 + cx^2 + dx + e$			Correlation Coefficient
Treatment	y =	a	b	c	d	e	R^2
75-0	NH_4^+				0.11	0.58	0.80
	NO_3^-	-0.01	0.07	-0.24	0.24	0.38	0.94
	NO_2^-	-0.01	0.11	-0.37	0.43	0.71	0.83
75-50	NH_4^+				0.14	0.52	0.92
	NO_3^-	-0.01	0.06	-0.19	0.16	0.39	0.96
	NO_2^-	0.00	0.02	-0.06	0.09	0.72	0.61
75-100	NH_4^+				0.14	0.55	0.94
	NO_3^-	-0.01	0.07	-0.23	0.23	0.37	0.92
	NO_2^-	-0.02	0.23	-0.83	0.95	0.63	0.80
75-150	NH_4^+				0.16	0.59	0.88
	NO_3^-	0.00	0.05	-0.17	0.16	0.39	0.93
	NO_2^-	0.00	0.06	-0.23	0.32	0.67	0.41
75-200	NH_4^+				0.25	0.43	0.94
	NO_3^-	0.00	0.06	-0.20	0.21	0.36	0.94
	NO_2^-	-0.01	0.10	-0.37	0.52	0.61	0.79
100-0	NH_4^+				0.08	0.60	0.81
	NO_3^-	0.00	0.02	-0.07	0.03	0.41	0.79
	NO_2^-	0.00	0.03	-0.11	0.03	0.77	0.64
100-50	NH_4^+				0.12	0.56	0.91
	NO_3^-	-0.01	0.07	-0.23	0.22	0.37	0.98
	NO_2^-	-0.01	0.11	-0.40	0.52	0.67	0.80
100-100	NH_4^+				0.10	0.62	0.91
	NO_3^-	0.00	0.01	-0.04	0.00	0.41	0.74
	NO_2^-	0.00	0.03	-0.13	0.09	0.77	0.62
100-150	NH_4^+				0.17	0.54	0.85
	NO_3^-	-0.01	0.07	-0.22	0.22	0.37	0.98
	NO_2^-	-0.01	0.11	-0.37	0.41	0.67	0.84
100-200	NH_4^+				0.21	0.50	0.93
	NO_3^-	0.00	0.05	-0.18	0.19	0.37	0.97
	NO_2^-	0.00	0.04	-0.19	0.34	0.67	0.83

Table 1.4. Continued,

Corresponding coefficients to the equation form		$y = ax^4 + bx^3 + cx^2 + dx + e$					Correlation Coefficient
Treatment	y =	a	b	c	d	e	R^2
125-0	NH_4^+				0.11	0.53	0.78
	NO_3^-	0.00	0.06	-0.19	0.18	0.38	0.99
	NO_2^-	-0.01	0.15	-0.52	0.57	0.67	0.91
125-50	NH_4^+				0.12	0.54	0.82
	NO_3^-	-0.01	0.06	-0.21	0.22	0.37	0.97
	NO_2^-	-0.01	0.13	-0.45	0.54	0.67	0.70
125-100	NH_4^+				0.13	0.59	0.86
	NO_3^-	0.00	0.05	-0.18	0.18	0.38	0.97
	NO_2^-	-0.01	0.10	-0.38	0.52	0.65	0.62
125-150	NH_4^+				0.17	0.55	0.85
	NO_3^-	0.00	0.05	-0.16	0.15	0.39	0.98
	NO_2^-	0.00	0.04	-0.22	0.38	0.70	0.61
125-200	NH_4^+				0.20	0.54	0.95
	NO_3^-	0.00	0.04	-0.15	0.15	0.37	0.93
	NO_2^-	-0.01	0.11	-0.44	0.54	0.69	0.94

Table 2.1. Percentage of fertilizer N mineralized over four time periods.

Treatment	8 d	39 d	55 d	111 d
Organic				
50	0.05	-0.02	-0.13	-0.20
100	0.02	0.03	-0.11	-0.16
150	0.21	0.02	-0.05	-0.02
200	0.31	0.03	-0.06	-0.06
250	0.06	0.04	-0.02	-0.09
Ogranic + XL753				
50	0.06	-0.05	-0.44	-0.46
100	0.27	-0.03	-0.21	-0.23
150	0.14	-0.02	-0.14	-0.15
200	0.11	-0.01	-0.11	-0.12
250	0.29	-0.01	-0.09	-0.09
Conventional				
50	0.00	-0.18	0.50	-0.09
100	0.00	0.00	0.21	-0.01
150	0.00	-0.03	0.44	0.29
200	0.00	-0.03	0.46	0.20
250	0.00	0.25	0.40	0.05
Conventional + XL753				
50	0.00	-0.54	-0.52	-0.12
100	0.00	-0.32	-0.27	-0.07
150	0.00	-0.18	-0.18	-0.04
200	0.00	-0.15	-0.14	-0.03
250	0.00	-0.07	-0.11	-0.03

Table 2.2. Correlation coefficients (R-values) between pH and inorganic nitrogen forms.

	Correlation Coefficient r			
Inorganic N	Organic	Organic + XL753	Conventional	Conventional + XL753
Nitrate	-0.05	-0.03	-0.17	0.17
Nitrite	-0.11	-0.03	-0.08	0.27*
Ammonium	-0.03	0.07	0.54*	0.40*
Total IN	-0.05	0.08	0.56*	0.46*

* Significant at the 0.05 probability level

Table 2.3. Plant height and yield components under different treatments.

Treatment	N rate (kg ha^{-1})	Fresh weight (g)	Weight of stem - dry (g)	Filled Grain Weight (g)	Tillers	Panicle count	Plant height (cm)	NUE	HI
Organic	0	86	14.5	18.15	12	9	91.75	-	0.55
	50	117	20	24.03	19	11	34.75	41.59	0.55
	100	129	22.5	27.45	21	12	96.25	32.89	0.55
	150	155.25	27.75	31.27	29	13	103	30.91	0.53
	200	198.5	36.25	43.55	40	17	105.25	44.88	0.53
	250	179.25	32.25	39.59	31	15	105.75	31.31	0.54
Conventional	0	86.75	14.5	18.79	13	9	91.5	-	0.57
	50	113.75	19.25	25.93	17	11	102	50.49	0.56
	100	132	22.75	30.03	20	13	101.5	39.73	0.57
	150	160.25	25.75	37.64	21	15	106.75	44.41	0.58
	200	183.75	29.75	43.77	24	19	108.25	41.13	0.57

NUE and HI stand for Nitrogen Use Effiency and Harvest Index, respectively.

Table 2.4. Significance of the main effects of nitrogen rate (N rate) and treatment and interaction among the measured soil characteristics and yield components.

Effect	pH	Fresh weight	Weight of stem	Panicle count	Filled Grain Weight	Plant height	Tillers	NUE	HI
	P value								
N rate	0.4199	<0.0001**	< 0.0001**	< 0.0001**	<0.0001**	<0.0001**	< 0.0001**	0.0015**	0.3731
Treatment	< 0.0001**	0.6414	0.2885	0.0006**	0.0125*	0.0164*	0.0003**	0.2080	0.0028**
N rate * Treatment	0.1233	0.4301	0.7170	0.0006**	0.2052	0.8086	0.0050**	0.8077	0.7730

*, ** Significant at the 0.05 and 0.01 probability level, respectively.

NUE and HI stand for Nitrogen Use Effiency and Harvest Index, respectively.

Table 2.5. Significance of the main effects yield components on grain yield.

Effect	Treatment	
	Organic	Conventional
Panicle	<0.0001**	<0.0001**
Tillers	<0.0001**	<0.0001**
Aboveground biomass	<0.0001**	<0.0001**
Stem dry weight	<0.0001**	<0.0001**
Plant height	<0.0001**	<0.0001**

** Significant at the 0.01 probability level

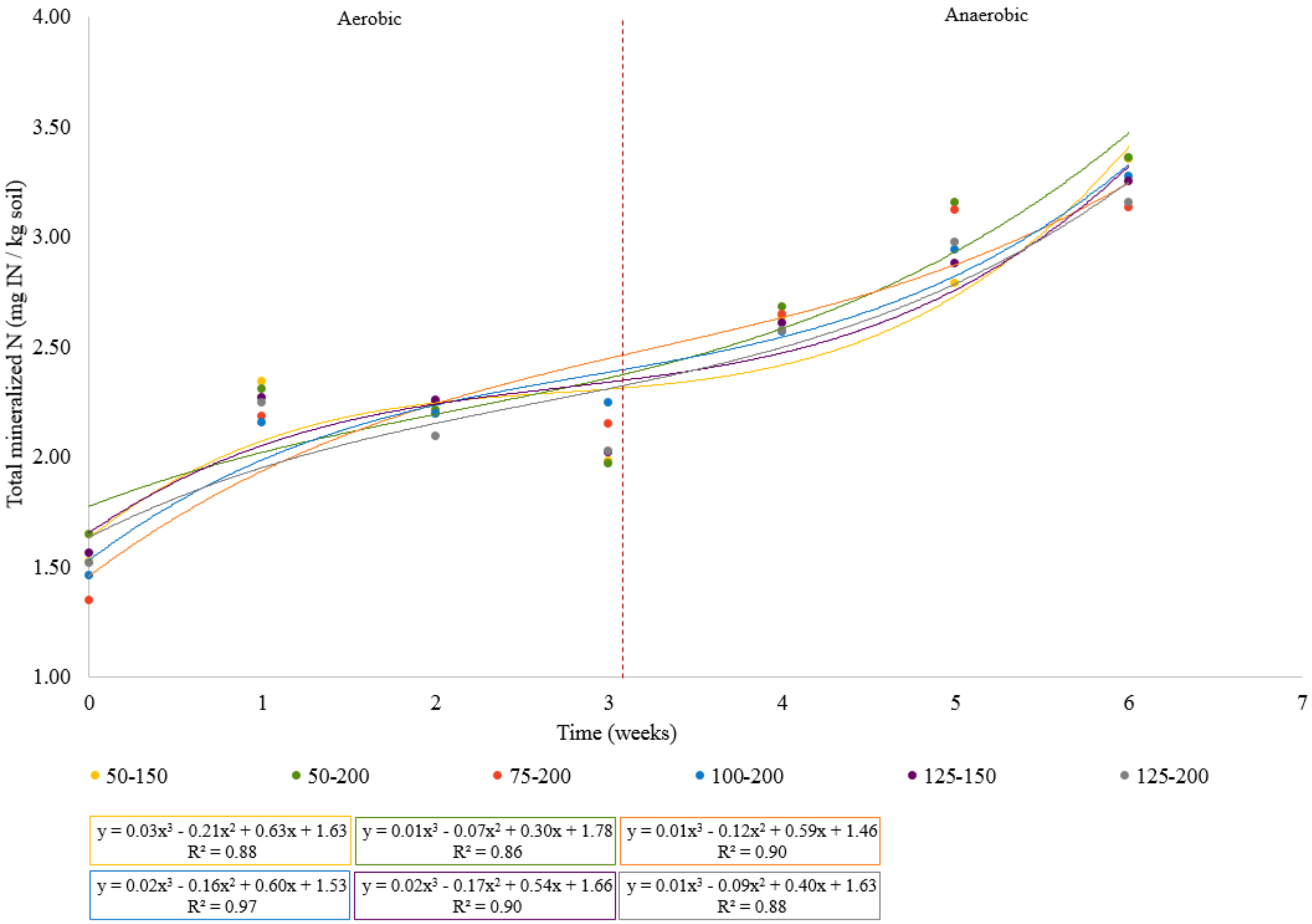


Figure 1.1. Treatments with maximum mineralization at the end of the incubation period.

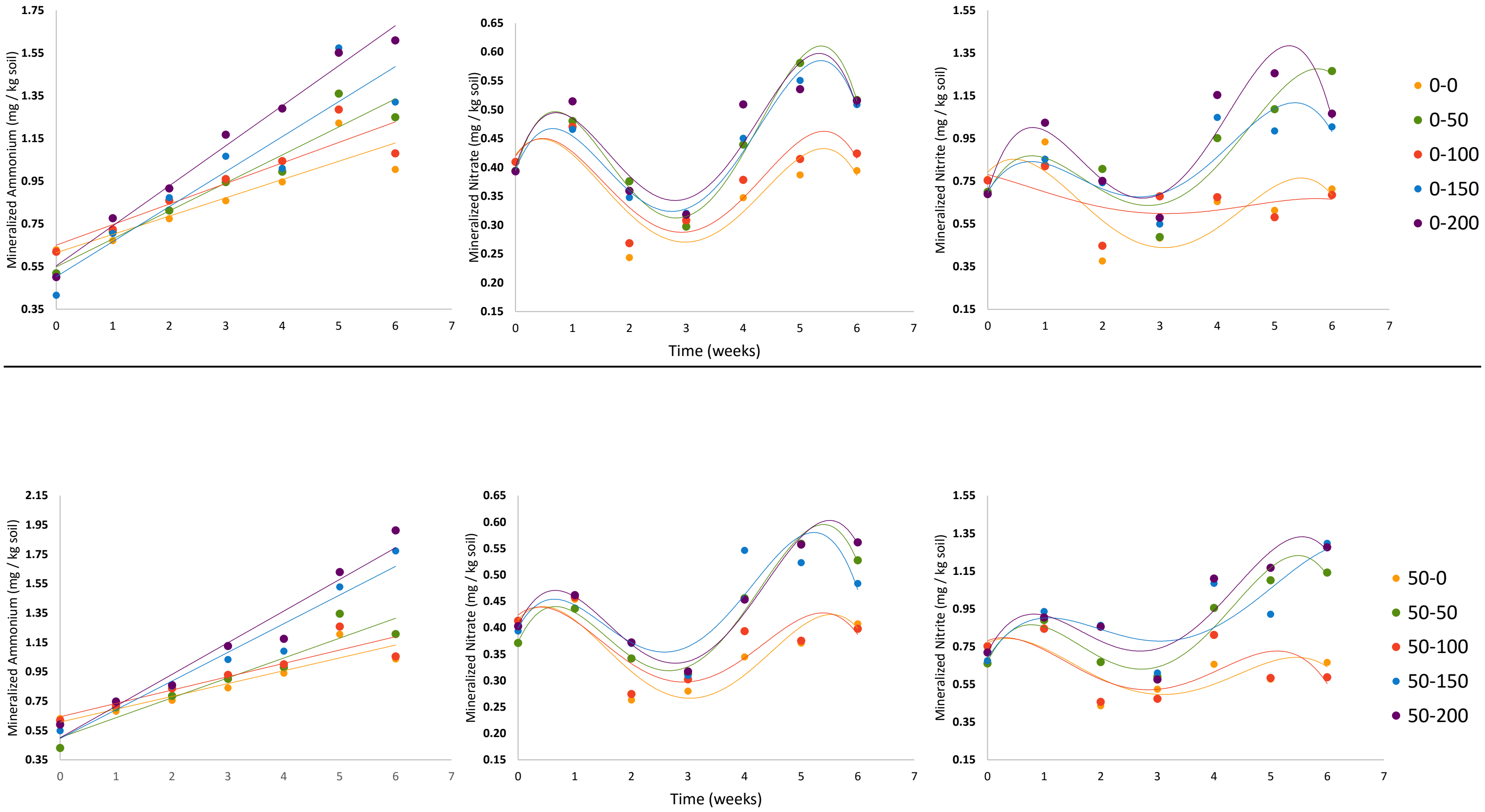


Figure 1.2. Mineralized N partition over incubation period for treatments with 0% and 50% cover crop.

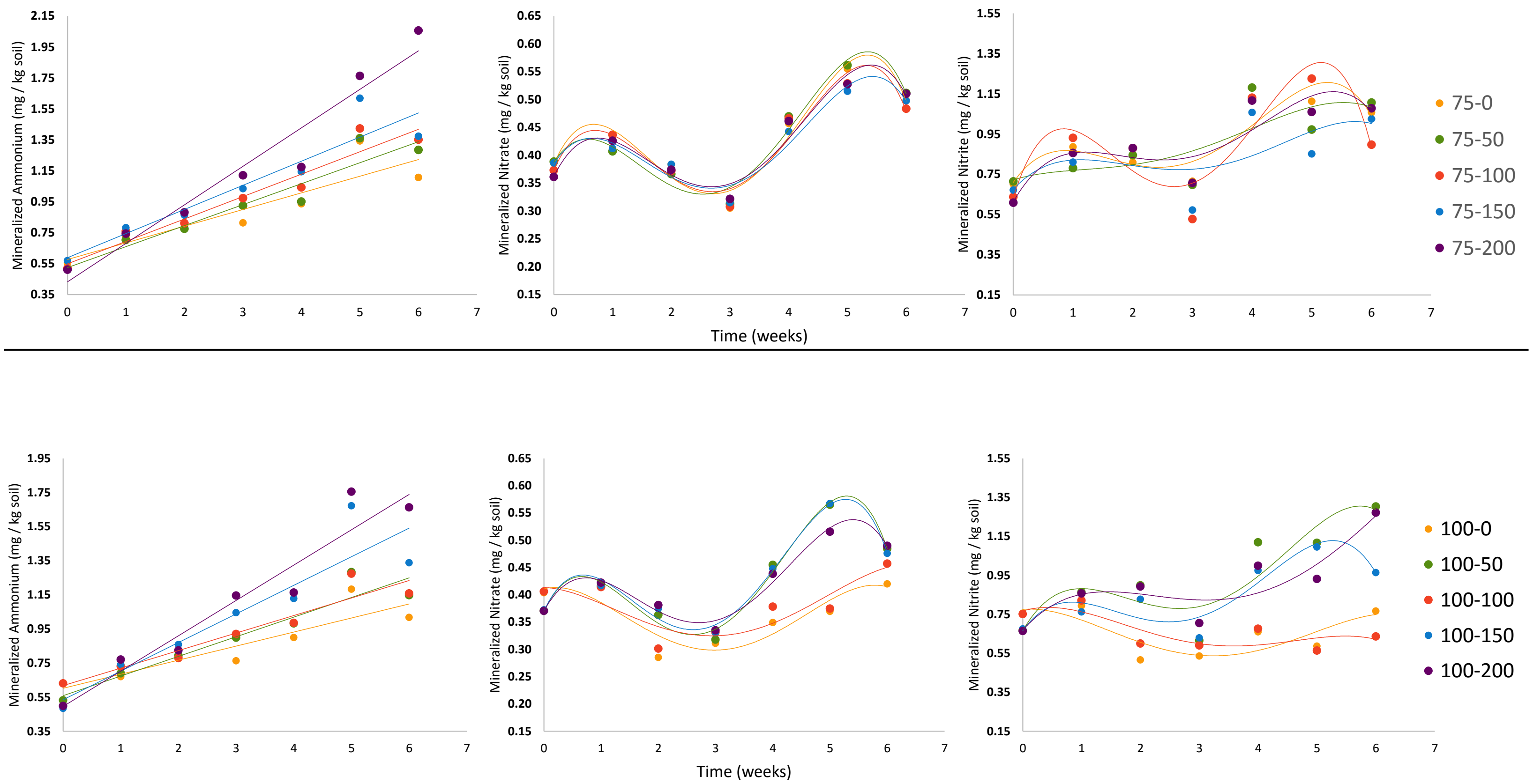


Figure 1.3. Mineralized N partition over incubation period for treatments with 75% and 100% cover crop.

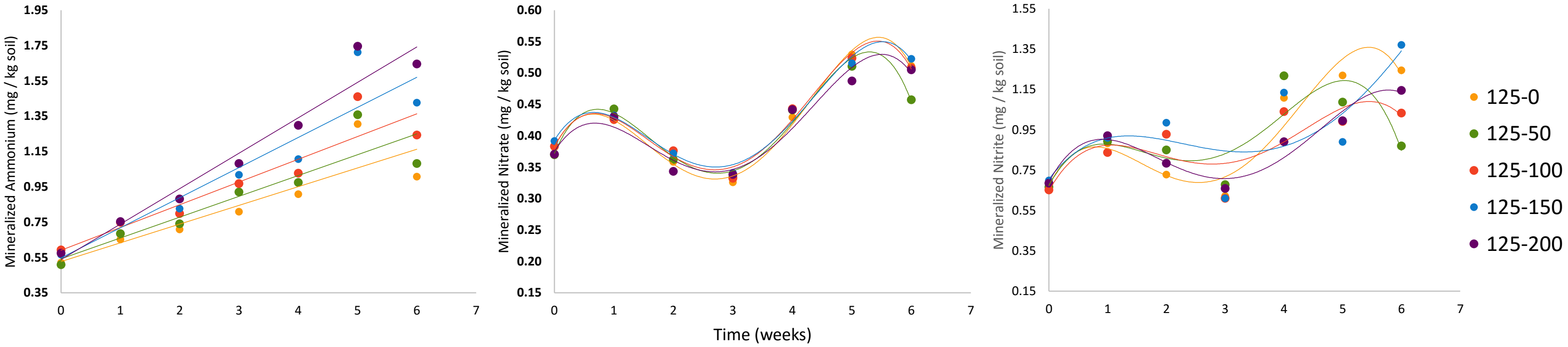


Figure 1.4. Mineralized N partition over incubation period for treatments with 125% cover crop

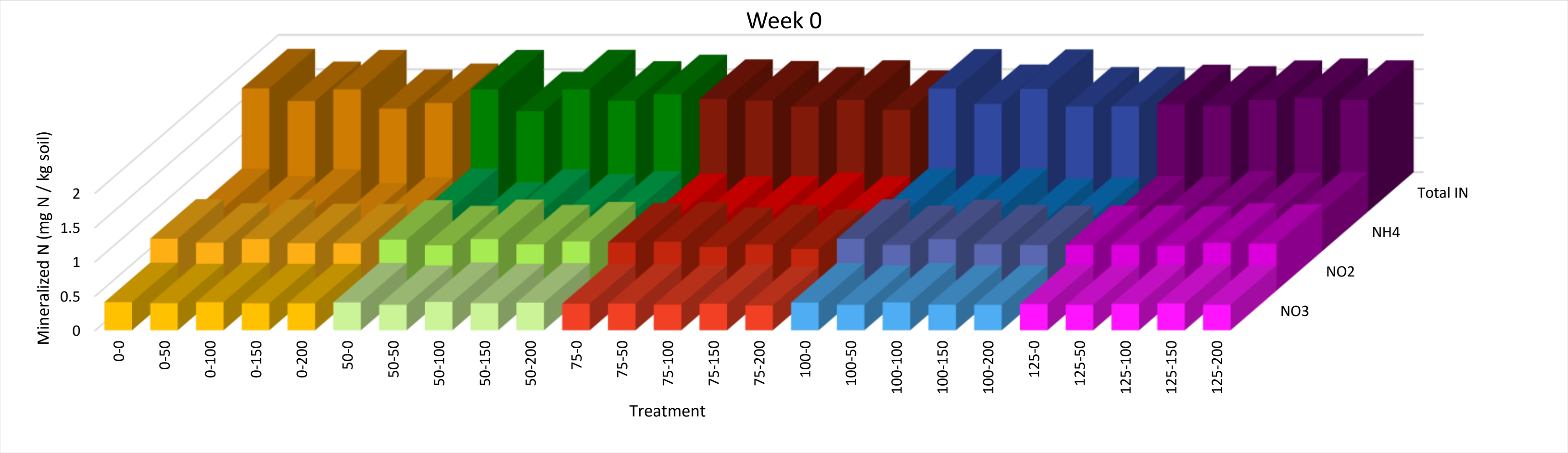


Figure 1.5. Distribution of inorganic N species in the different treatments at week 0.

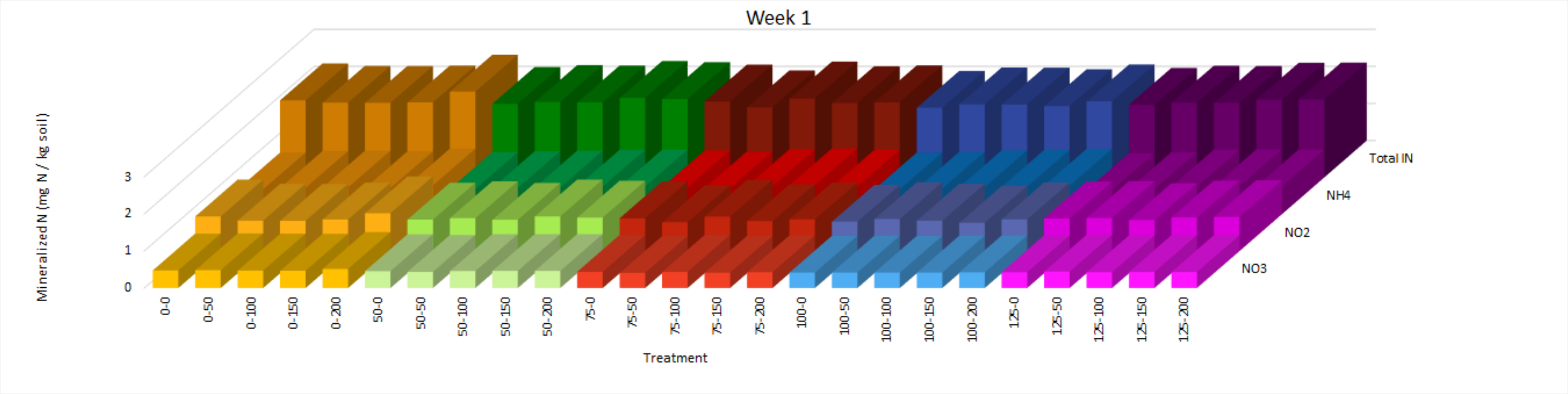


Figure 1.6. Distribution of inorganic N species in the different treatments at week 1.

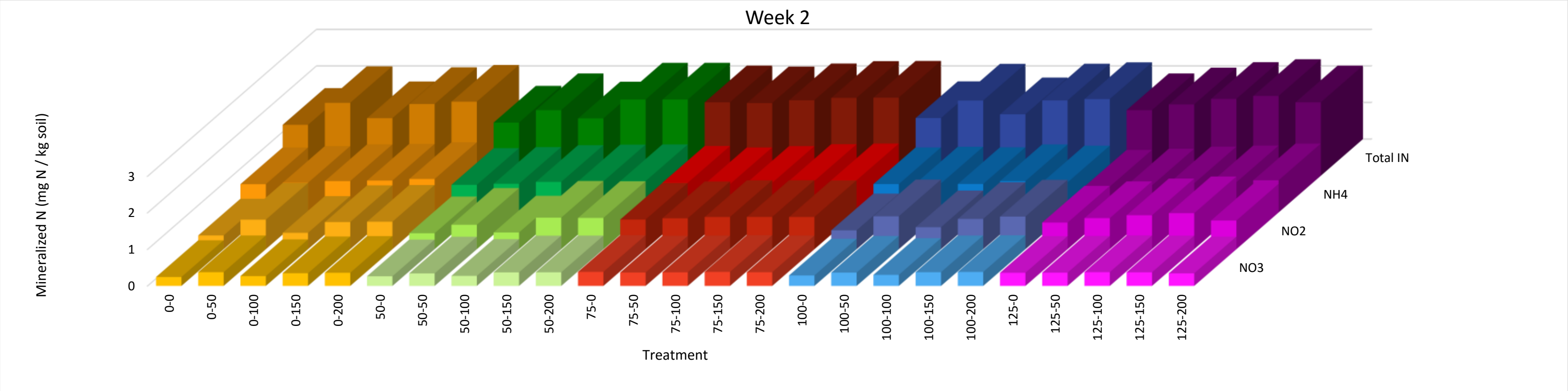


Figure 1.7. Distribution of inorganic N species in the different treatments at week 2.

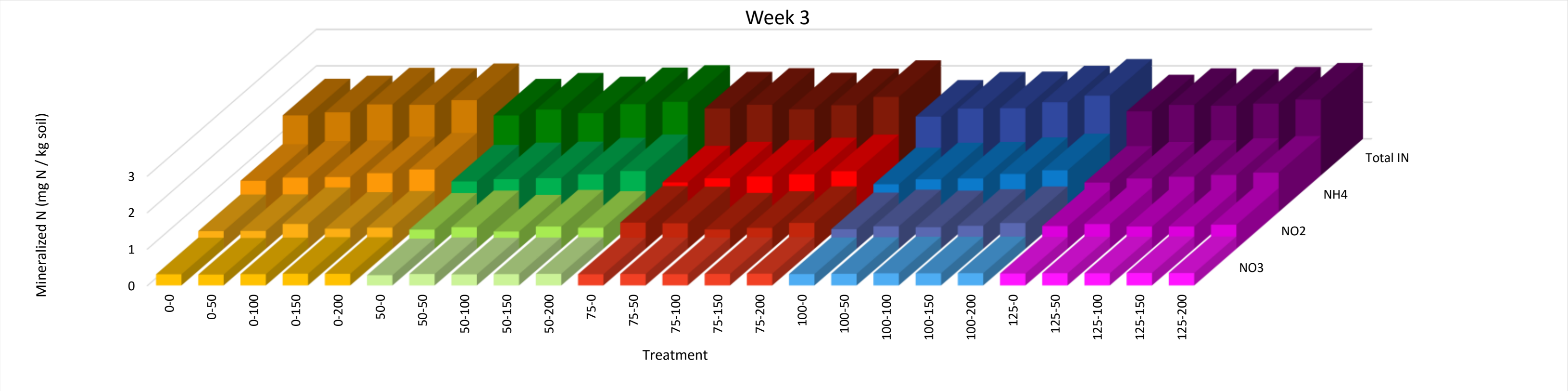


Figure 1.8. Distribution of inorganic N species in the different treatments at week 3.

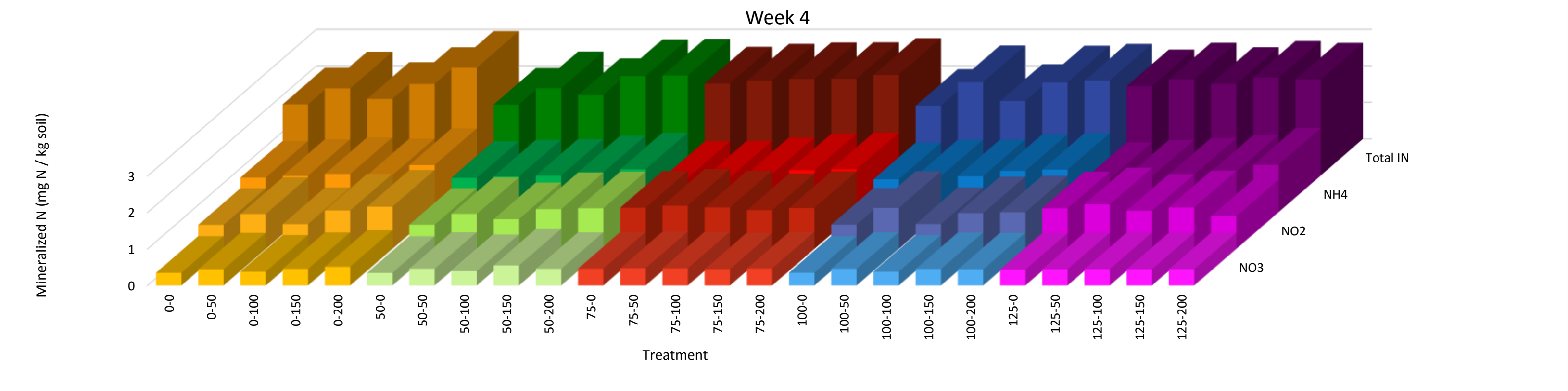


Figure 1.9. Distribution of inorganic N species in the different treatments at week 4.

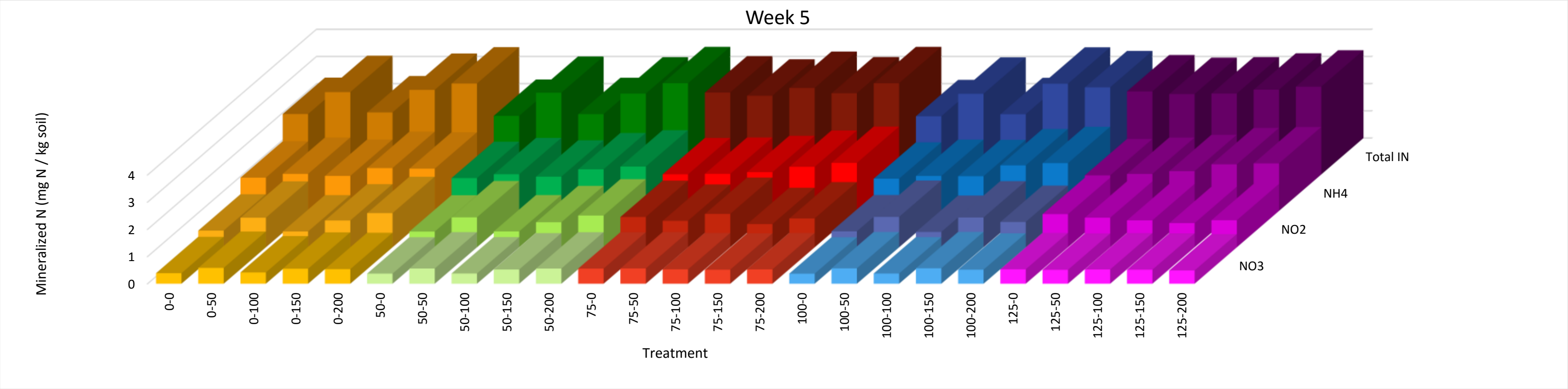


Figure 1.10. Distribution of inorganic N species in the different treatments at week 5.

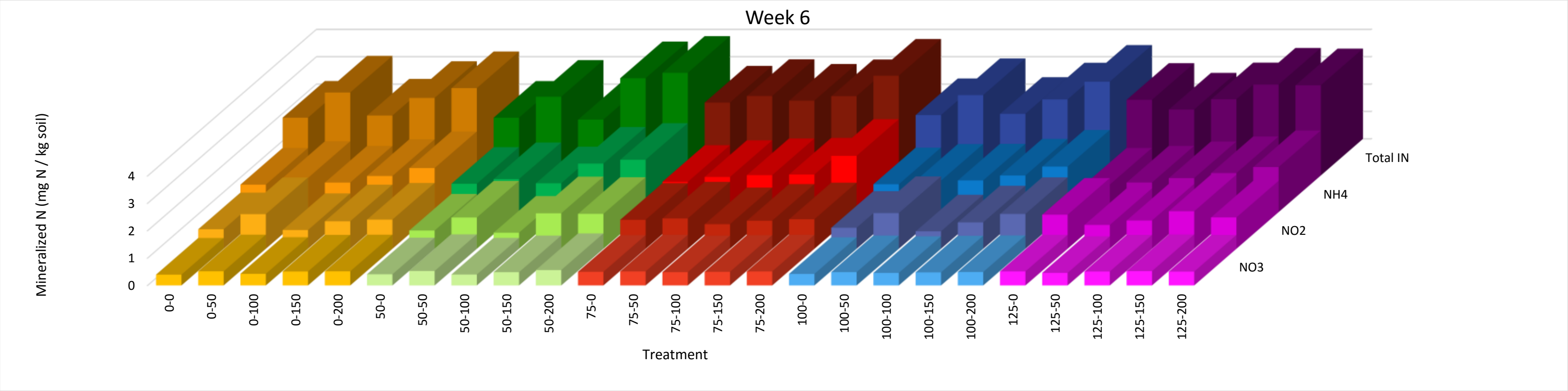


Figure 1.11. Distribution of inorganic N species in the different treatments at week 6.

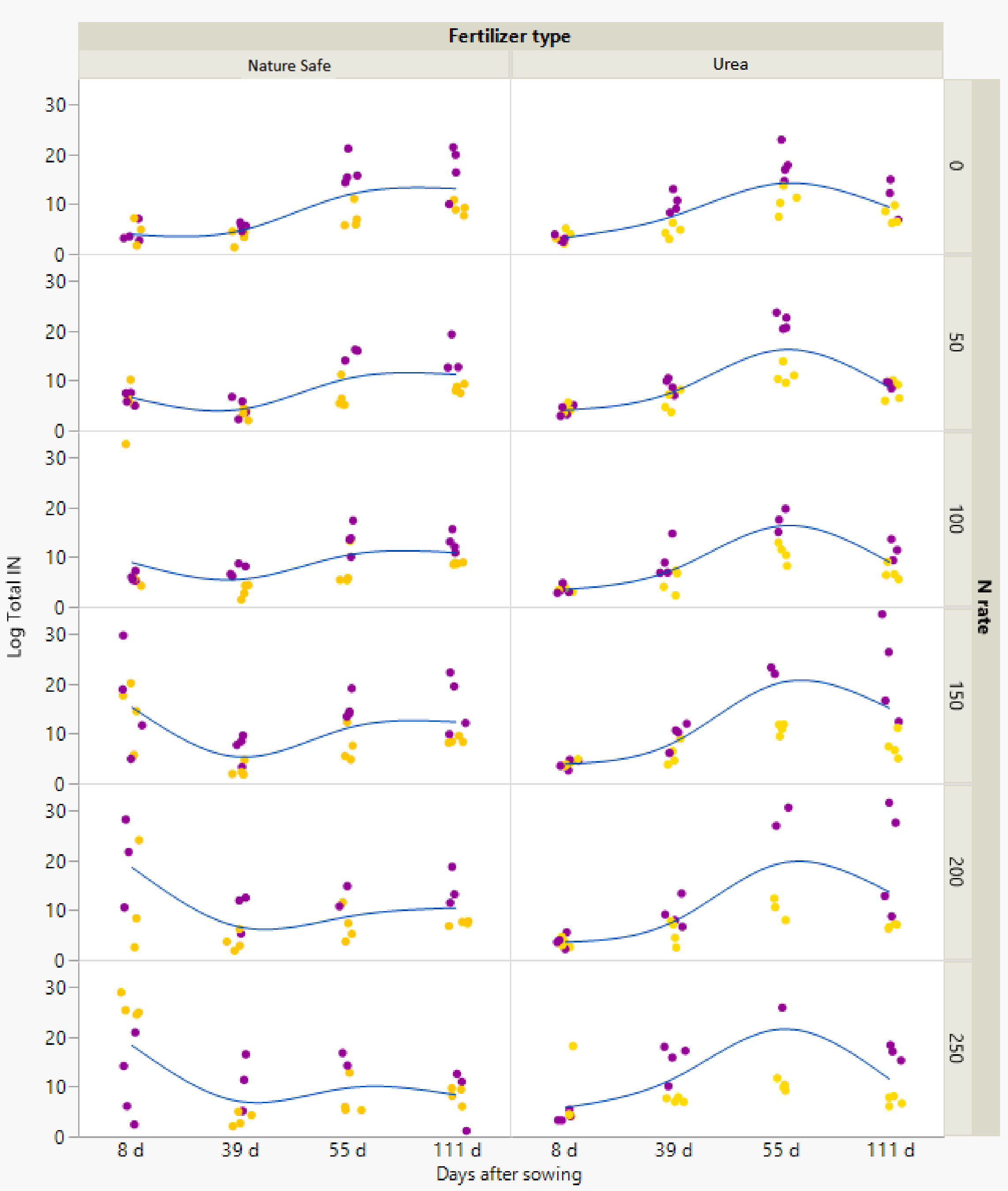


Figure 2.1. Total inorganic N (IN) over time. Yellow points represent planted samples and purple points represent samples without plants.

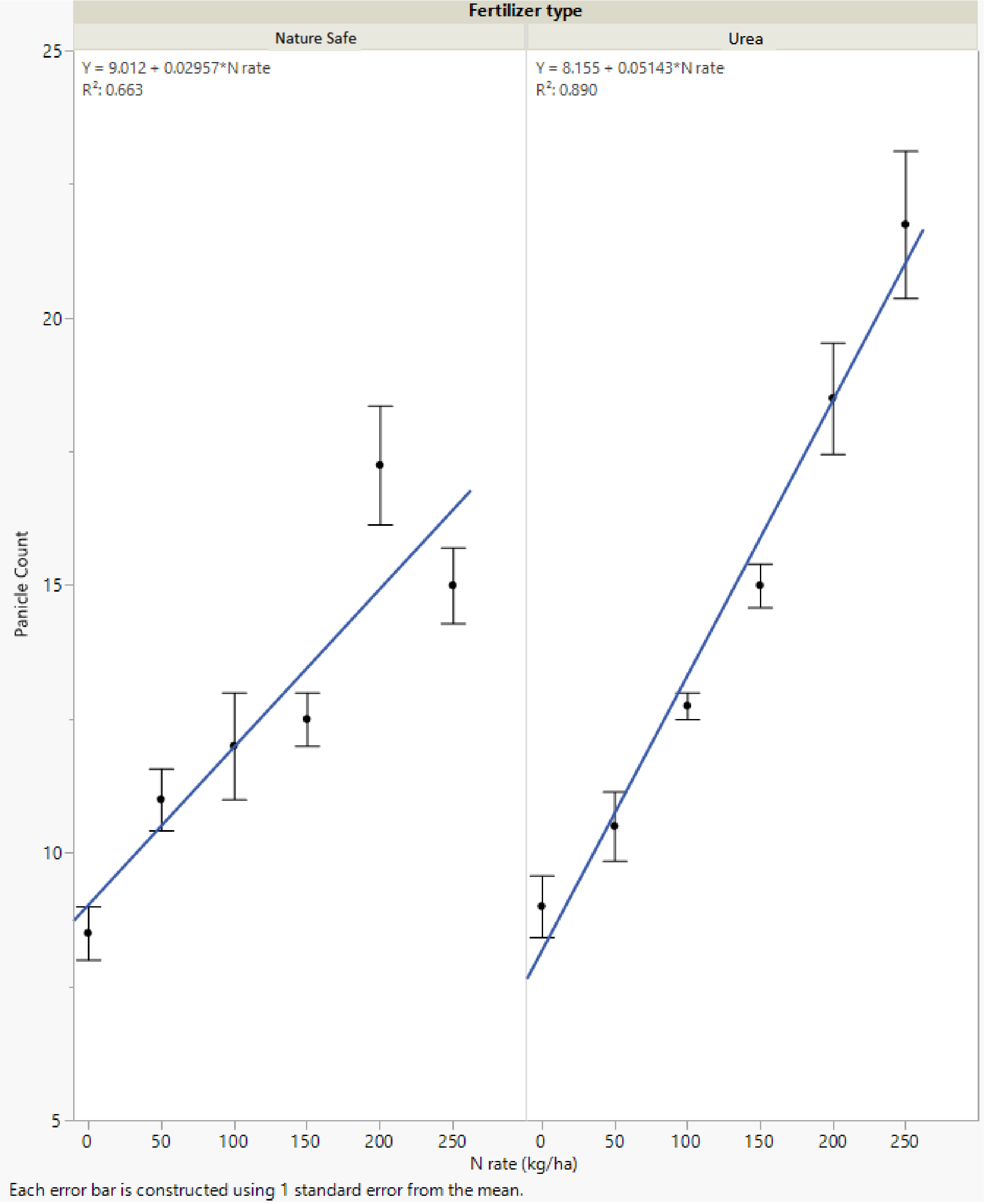


Figure 2.2. Final panicle count vs N rate applied.

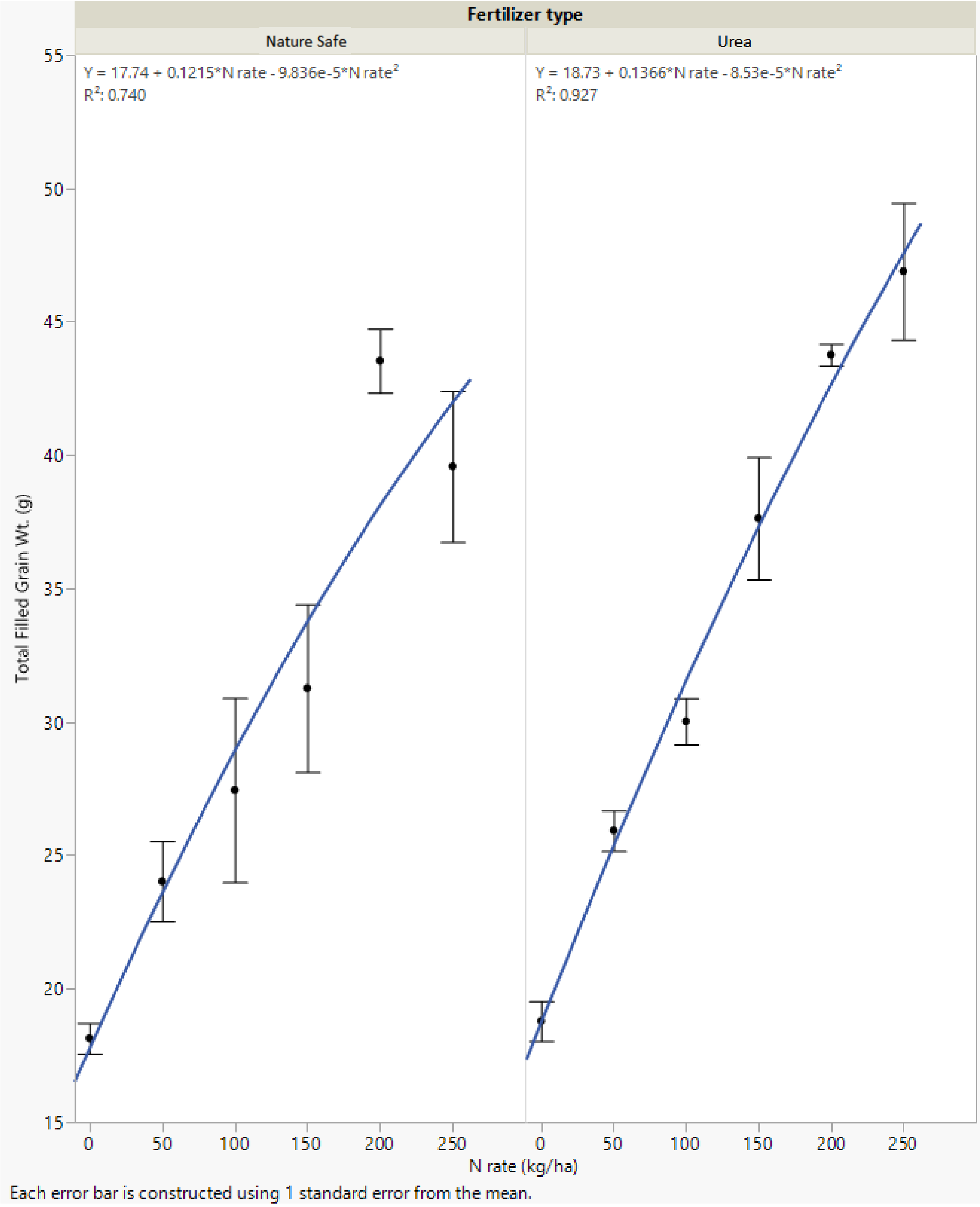


Figure 2.3. Total filled grain weight vs. N rate applied.

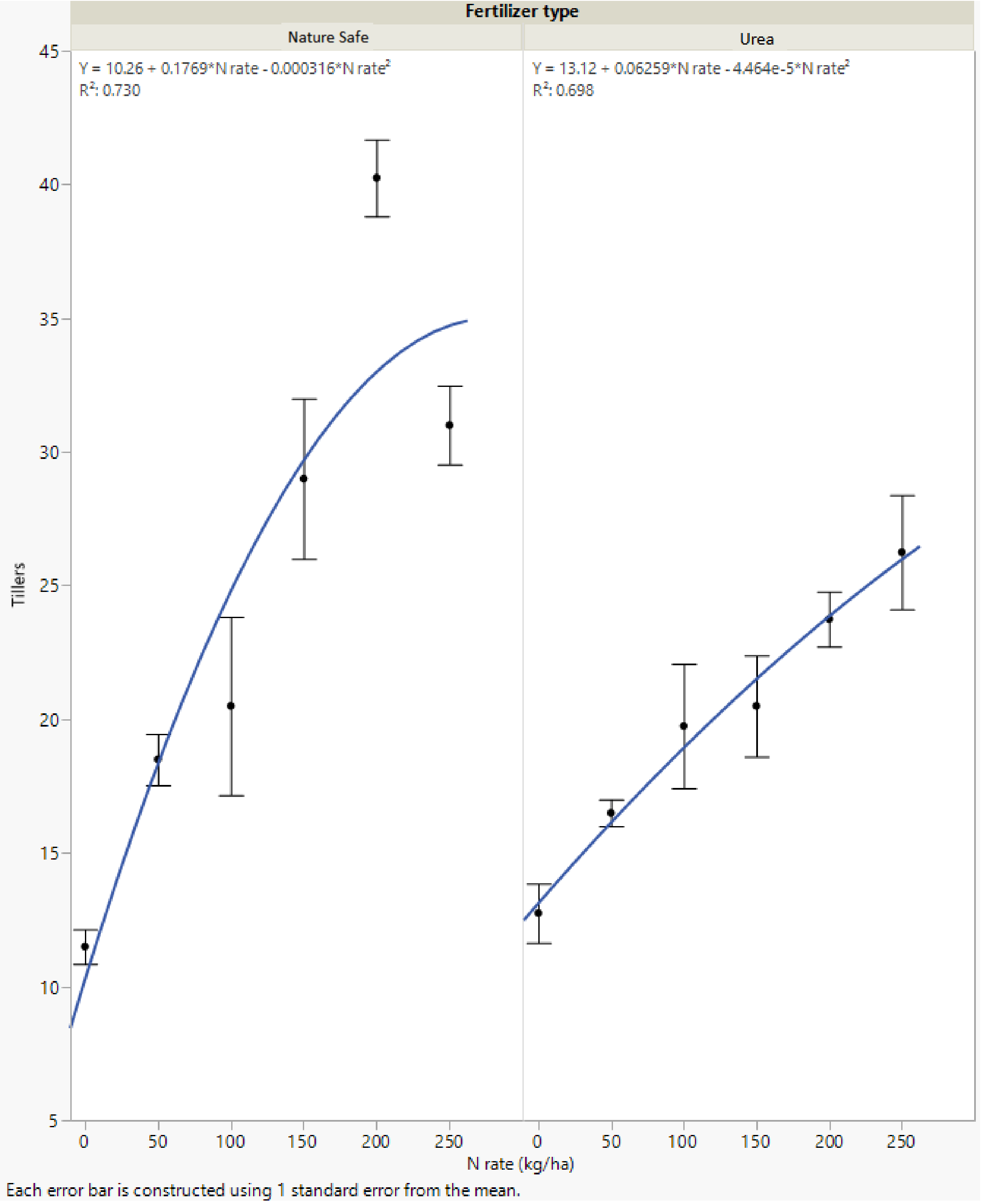
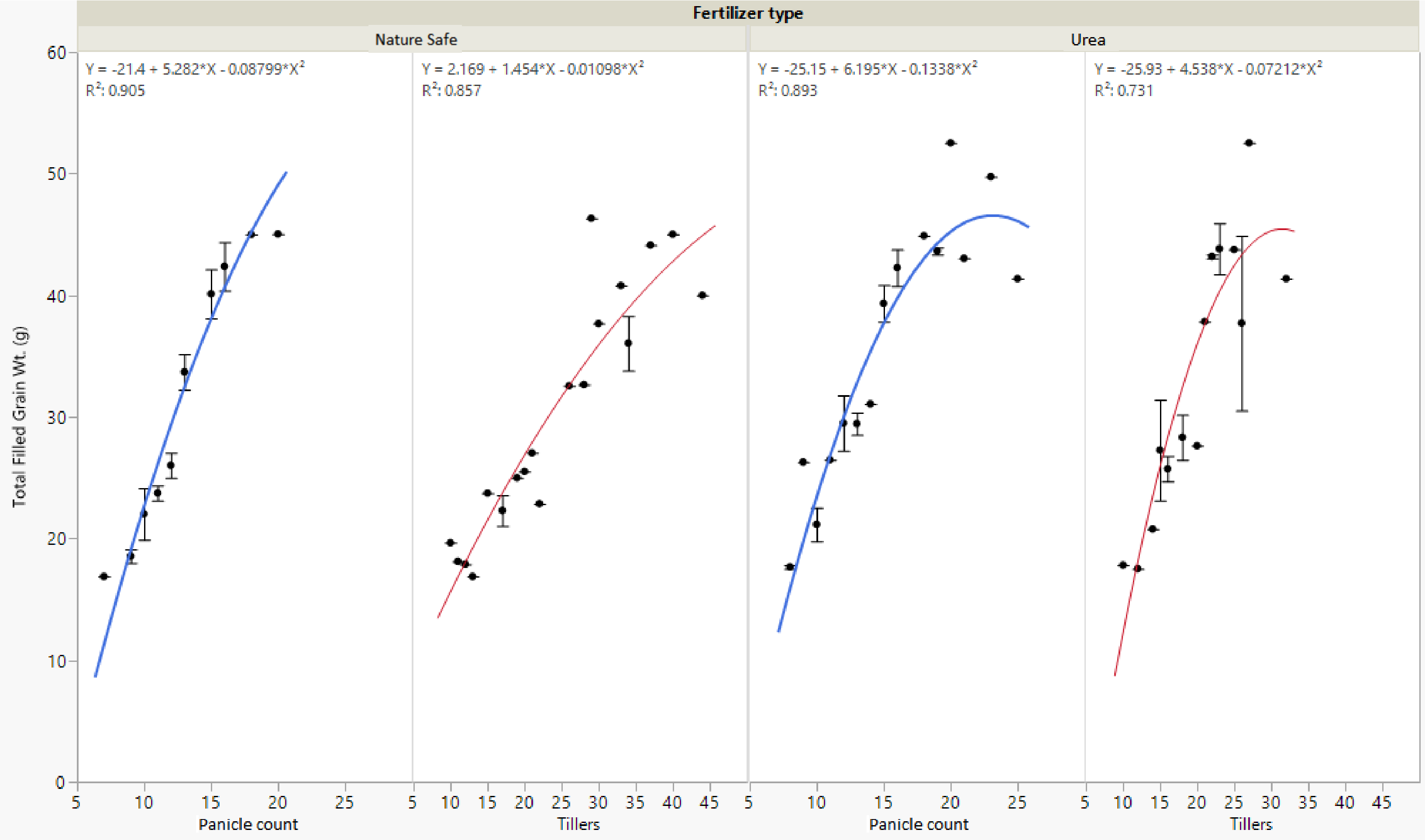
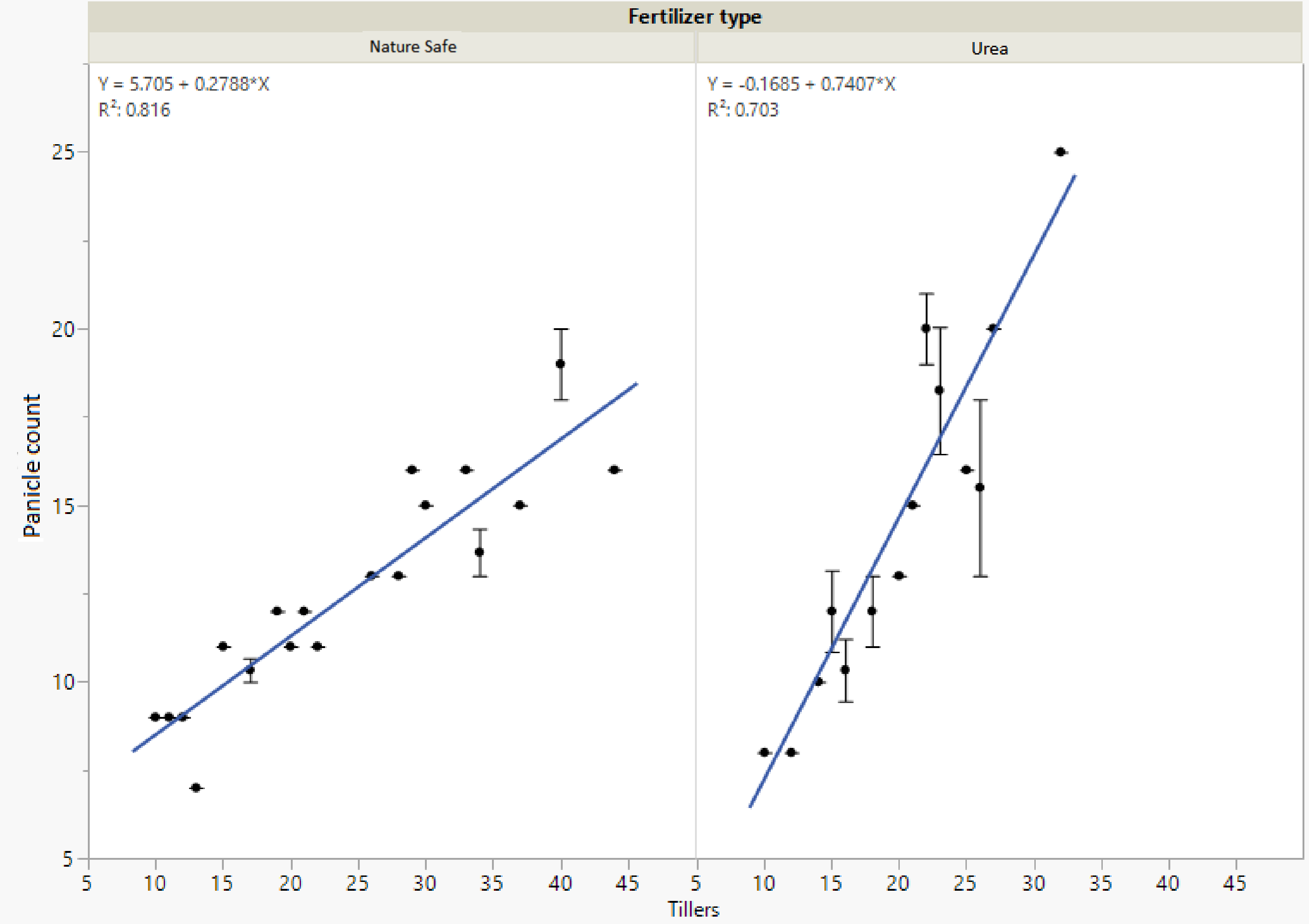


Figure 2.4. Tillers vs. N rate applied.



Each error bar is constructed using 1 standard error from the mean.

Figure 2.5. Total grain weight vs tillers and panicle numbers.



Each error bar is constructed using 1 standard error from the mean.

Figure 2.6. Relation of tillers and panicles under both treatments.

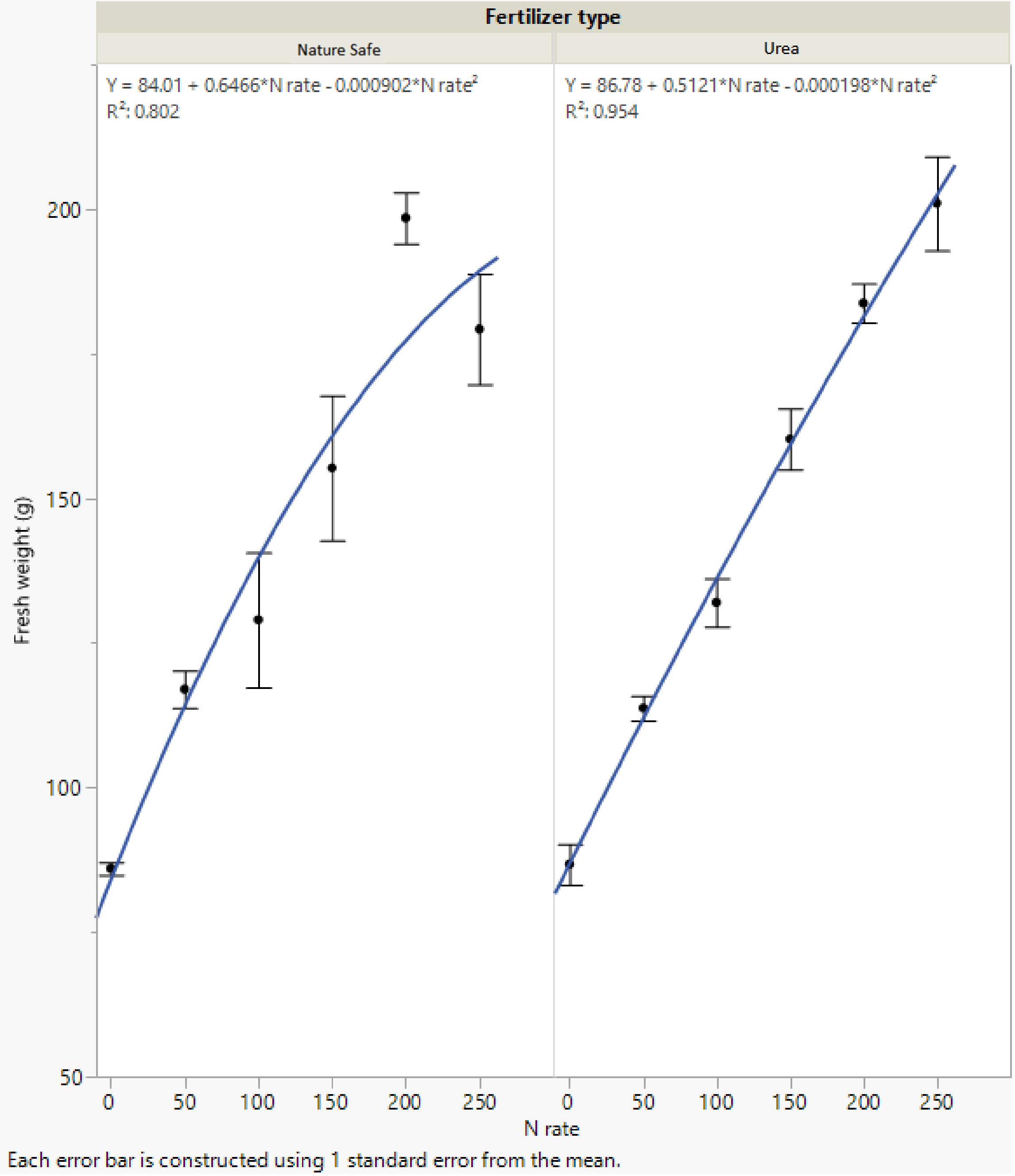


Figure 2.7. Fresh biomass weight vs N rate applied.

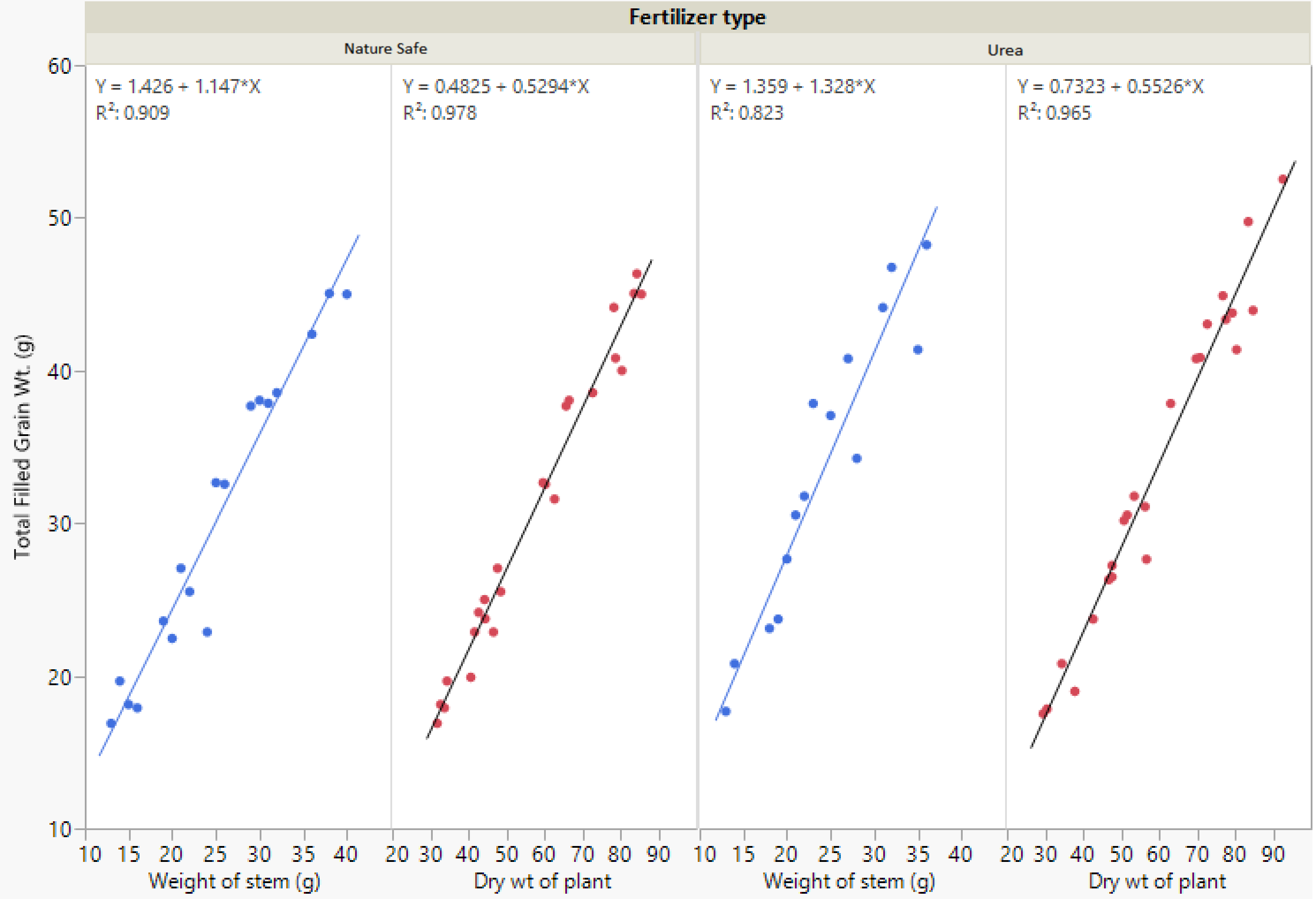


Figure 2.8. Correlation between aboveground biomass production and yield.

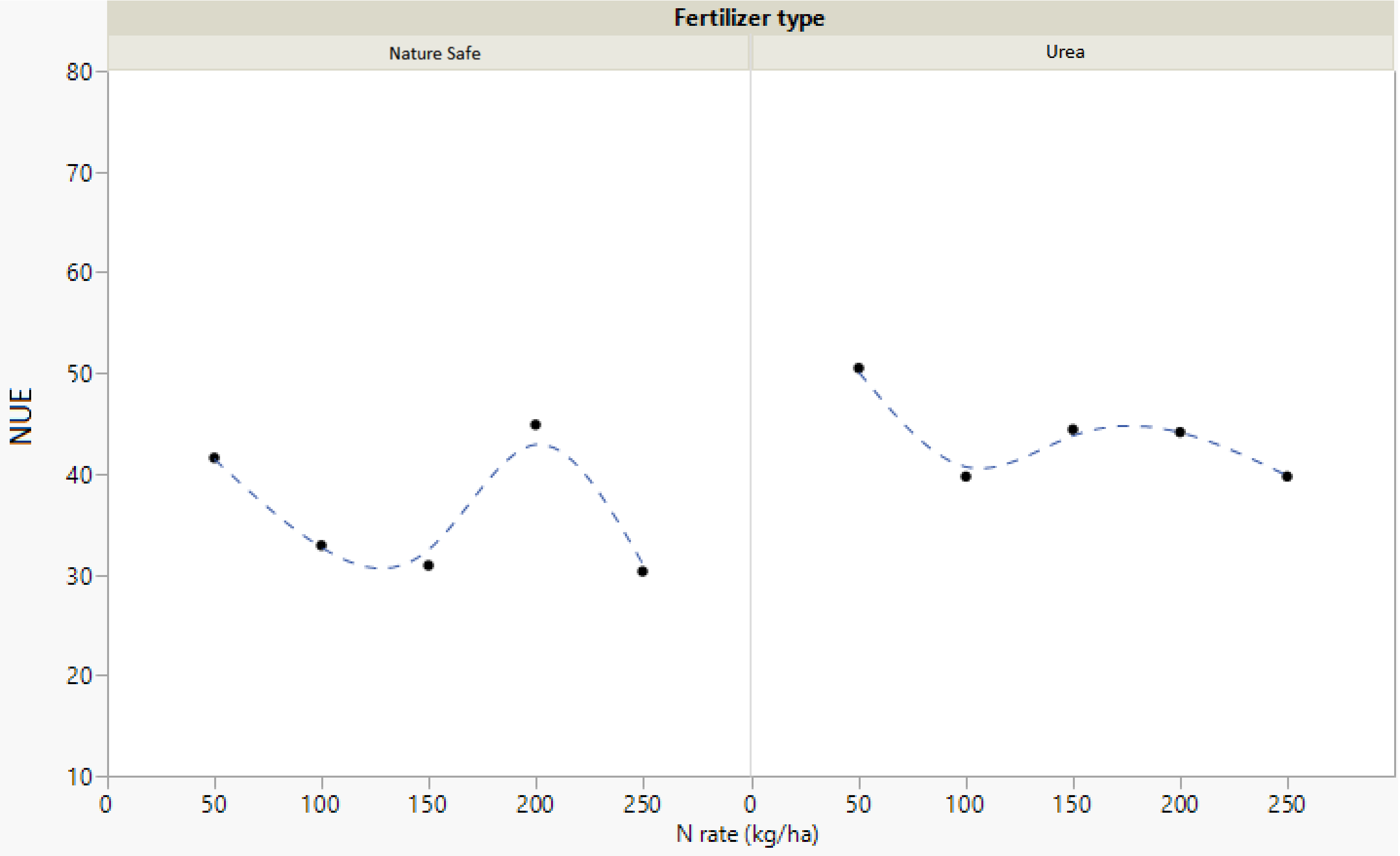


Figure 2.9. Nitrogen use efficiency (NUE) vs N rate.