Project NO. RM-13

ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
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PROJECT TITLE:

RM-13: **THE ROLE OF NITRIFICATION IN RICE SYSTEMS TO SUPPORT NITROGEN USE EFFICIENCY**

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EXPERIMENTS CONDUCTED, BY LOCATION, TO ACCOMPLISH SPECIFIC OBJECTIVES:

Objectives:

The overall objective of this research is to determine the extent that nutrient use efficiency (NUE), yield, nitrogen (N) acquisition and translocation are linked to nitrification of ammonical N fertilizer in popular California rice varieties. Specific objectives to meet this overall objective are:

1. Investigate whether mixed NH_4^+/NO_3^- nutrition increases nitrification potentials in CA paddy soils.
2. Determine the extent of nitrification occurring in California rice cultivars that vary in yield potentials.
3. Identify the importance of nitrification for increased NUE, yield, N acquisition and translocation in rice plants.
4. Characterize the degree of variability between popular California rice cultivars in objectives 2 and 3.
5. Determine which plant traits, such as POR, rooting density, etc. can be used as accurate predictors of the nitrification potential in popular California rice varieties.

Experiments conducted to accomplish the objectives:

Two independent experiments were conducted. Experiment I was a pilot laboratory incubation to determine whether mixed NH_4^+/NO_3^- nutrition increase soil nitrification potential and N transformation (Objective 1), while experiment II was a pot study in controlled environment growth chambers to accomplish objectives 2-5.

Experiment I: Soils were collected from paddy fields at the CA Rice Research Experimental Station. The fields were a part of an established N rate trial, receiving no and 175 k N ha^{-1} ammonical fertilizer. Three soil cores were sampled from each of eight fields (two N rates, four replicates each), transported on ice to the University of California Davis, and stored at 4°C. On the next day, the three soil cores were composited after the removal of vegetation, rice roots, and dead plant residues, homogenized by hands and used for the incubation in dark at room temperature to determine nitrification potential with ^{15}N isotopic pool dilution method. To determine whether co-application of NO_3^- affects nitrification potential and plant-available NH_4^+, KNO_3 was added at rates of 0%, 10%, and 20% of field recommended N rates. The results suggest that application of NO_3^- with NH_4^+tends to suppress soil nitrification potential (**Fig. 1A**), while additions of NH_4^+ with NO_3^- did not affect NO_3^- consumption (**Fig. 1B**).

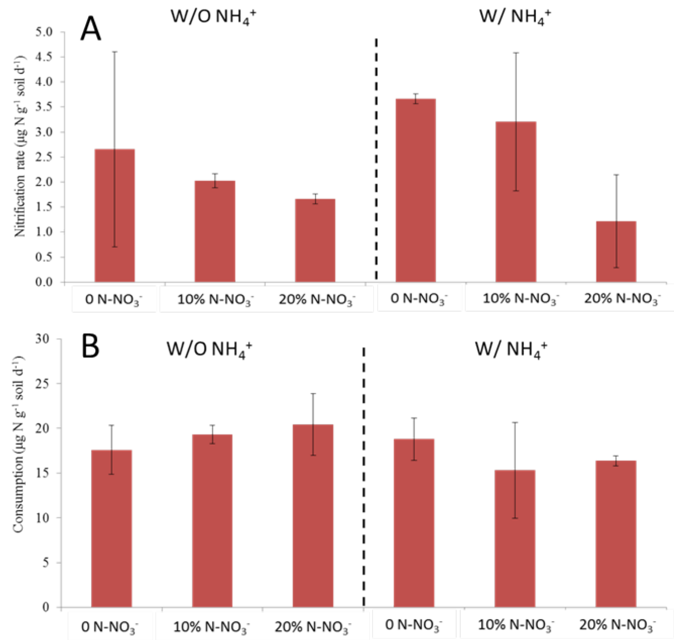


Fig. 1. Nitrification potential (**A**) and NO_3^- consumption potential (**B**) of paddy soils receiving various mixed rates of NH_4^+/NO_3^- nutrition.

Experiment II: The aim of this experiment was to explore the importance of nitrification and its connection with the mobility of ammonical N, nitrogen use efficiency (NUE), and yield among several California rice varieties. Three popular rice cultivars (CM101, M206, and M401) were tested. To determine the extent that root development affects nitrification potential, we used nylon mesh bags with different pore sizes (0.01 and 0.4 inches) to isolate the influence of rice roots on soil processes (**Fig. 2**).

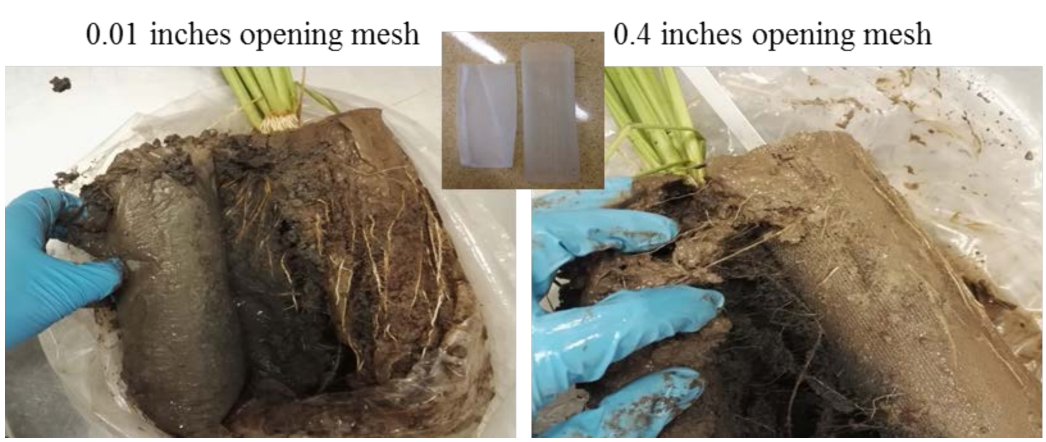


Fig. 2. Nylon bags to exclude (left) or allow (right) rice root development and growth into soils within the bag.

Dried soils were collected from paddy fields at the California Rice Research Experimental Station, sieved (2mm), and used in growth chambers for this study. Approximately 6 kg of soils were well mixed with field recommended K and P rates in a plastic bag and placed in tree pots (15.2 cm in diameter, 30.5 cm in depth). Pots were randomly selected for nylon bag installation (0.01 inch or 0.4 inches opening mesh) and ammonical fertilizer

($(NH_4^+)_2SO_4$) was injected evenly into soils inside each bag at field recommended rates. Controls without bags were prepared in parallel, except that $(NH_4^+)_2SO_4$ was added to the bulk soil along with K and P fertilizers. Rice plants were incubated in a growth chamber with the following environmental settings: 14 h light/10 h dark, 30 °C in day light/28°C in dark night, and 70% moisture content. Each cultivar was allowed to grow until the late tillering stage (8-10 tillers). At the end of the growth period, soil samples inside (soil with minimal root influences – **non-rhizosphere soil**) and outside (**rhizosphere soil**) each nylon bag were collected for analysis of plant-available NH_4^+, NO_3^-, and nitrification potentials. To determine the effect of plant cultivars on microbial community composition, total DNA was extracted from each soil sample for high-throughput sequencing of the 16S rRNA marker gene and quantitative-PCR (qPCR) to quantify populations of ammonia oxidizing archaea (AOA) and bacteria (AOB). Harvested plants were sacrificed to determine biomass production, root density, tissue N content, and leaf nitrate reductase enzyme activity (NRA).

Results demonstrated that:

1. Rhizosphere soils had higher nitrification potentials than non-rhizosphere soils for cultivars CM101 and M206, but not for M401 (**Fig. 3A**), suggesting that root development and growth may affect the mobility of ammonical N and its allocation, while the magnitude of root influences likely vary by cultivar variety. No significant differences in rhizosphere nitrification potentials were detected between cultivars (**Figure 3A**).
2. Non-rhizosphere soils had higher concentrations of plant-available NH_4^+ than rhizosphere soils, with the degree of difference for each cultivar largely in the order of CM101 > M206 > M401 (**Fig. 3B**). This suggests that differences in nitrification potentials may have contributed to higher N mobility and N uptake by rice cultivars.
3. No differences in plant-available NO_3^- were observed between rhizosphere and non-rhizosphere soils or among rice cultivars (**Fig. 3C**).
4. Bacterial communities in the rhizosphere did not differ by cultivar variety (**Fig. 4**). Furthermore, across all samples, no significant differences in bacterial alpha diversity (species richness and Shannon index) were observed between cultivar varieties, rhizosphere to non-rhizosphere soils, or bag size. Together, these results suggest that nitrification potentials for each cultivar are not linked to changes in bacterial diversity or specific compositional states.
5. Compared to the other cultivar varieties, soils planted with cultivar CM101 had the highest abundance of both AOA and AOB, with higher overall abundances of AOB than AOA across all samples (**Fig. 5**).
6. Cultivar CM101 produced the highest biomass (fresh weight) and total biomass N compared to cultivars M206 and M401 (**Fig. 6**). AOA and AOB abundance exhibited a significant positive correlation with plant biomass across all cultivars (**Fig. 7**), suggesting that ammonia oxidizer activities are associated with production of rice biomass.
7. Cultivar M401 had the highest nitrate reductase activity in leaf tissue compared to the other varieties (**Fig. 8**), indicating that this cultivar has potential to have the highest nitrogen use efficiency (NUE). Interestingly, the low biomass and total biomass N content of cultivar M401 (**Fig. 7**) suggests that further research on N cycling, especially on N loss during nitrate assimilation by rice plants is needed.

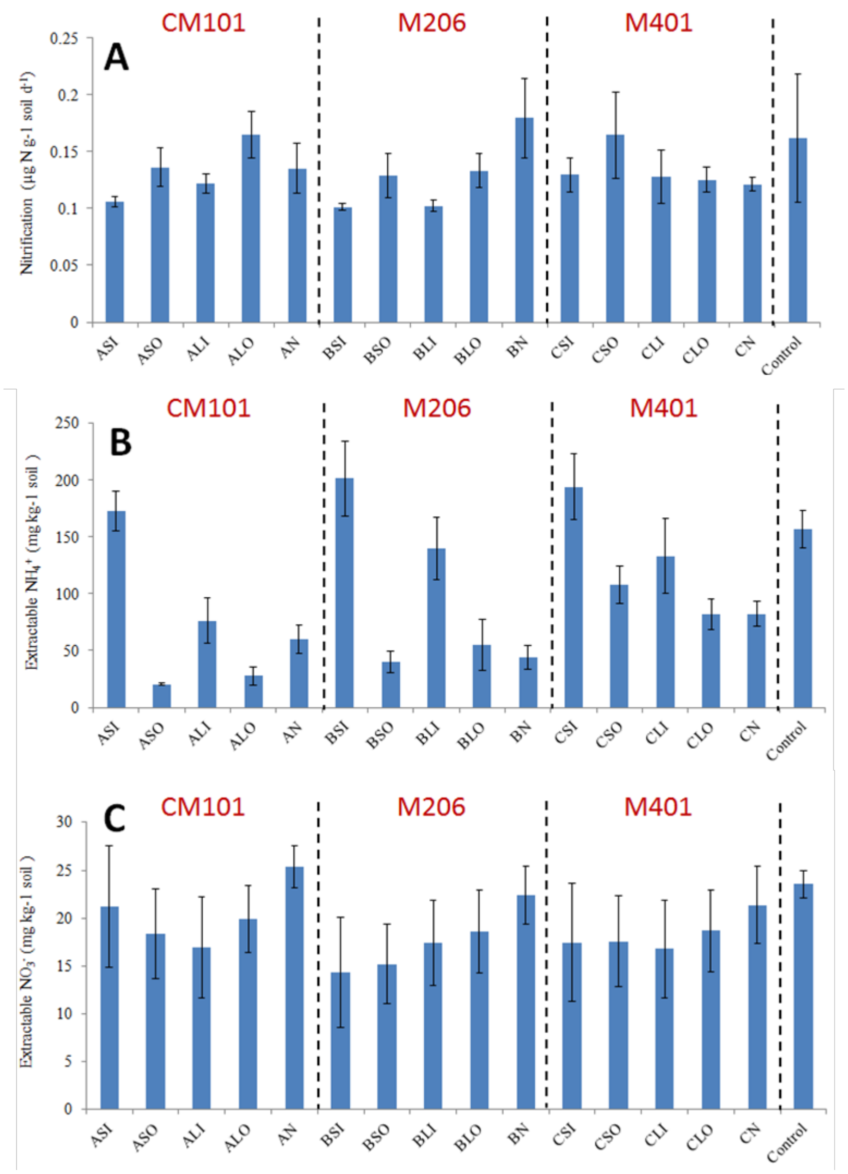


Fig. 3. Nitrification potentials (**A**), plant-available NH_4^+ (**B**), and plant-available NO_3^- (**C**) in soils planted with rice cultivar varieties CM101, M206, and M401. Experiments were performed in growth chambers and capital letters indicate treatment effects, with the first letter showing cultivar variety (A, CM101; B, M206; C, M401), the second letter showing whether nylon bags were added to isolate soils from root influences (S, bag with 0.01 inch pore opening; L, bag with 0.4 inch pore opening; N, no bag control), and the third letter identifying rhizosphere soils (O) from non-rhizosphere soils (I). Bulk soils without rice plants were used as controls.

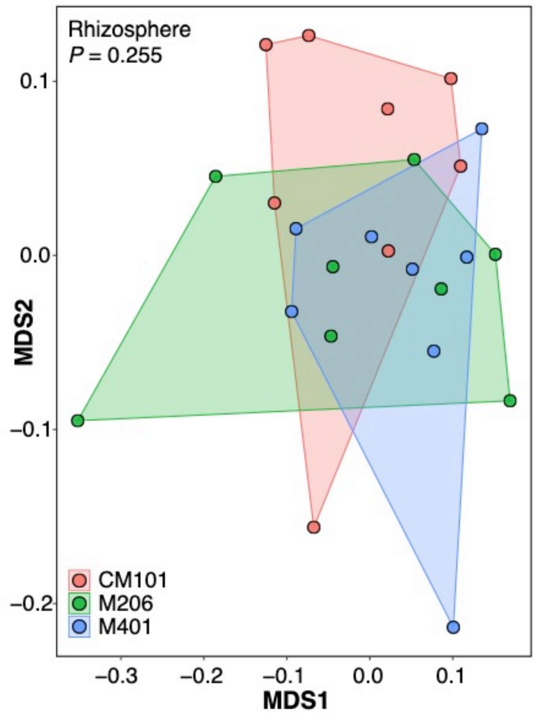


Fig. 4. Non-metric multidimensional scaling plot based on Bray-Curtis dissimilarities of rhizosphere bacterial communities of different rice cultivars. No significant differences in bacterial community structure between rice cultivars were detected.

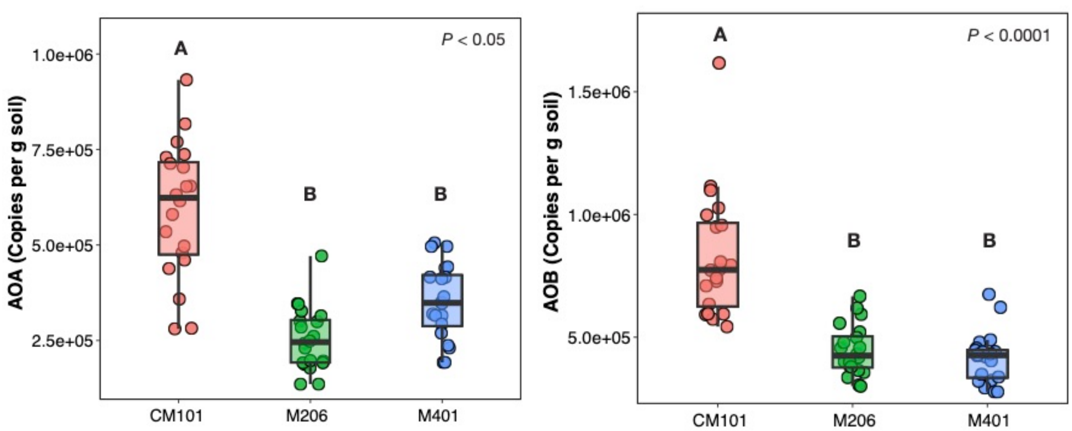


Fig. 5. Abundance of ammonia-oxidizing archaea (AOA) and bacteria (AOB) in soils planted with different rice cultivar varieties. Different letters indicate statistical significance.

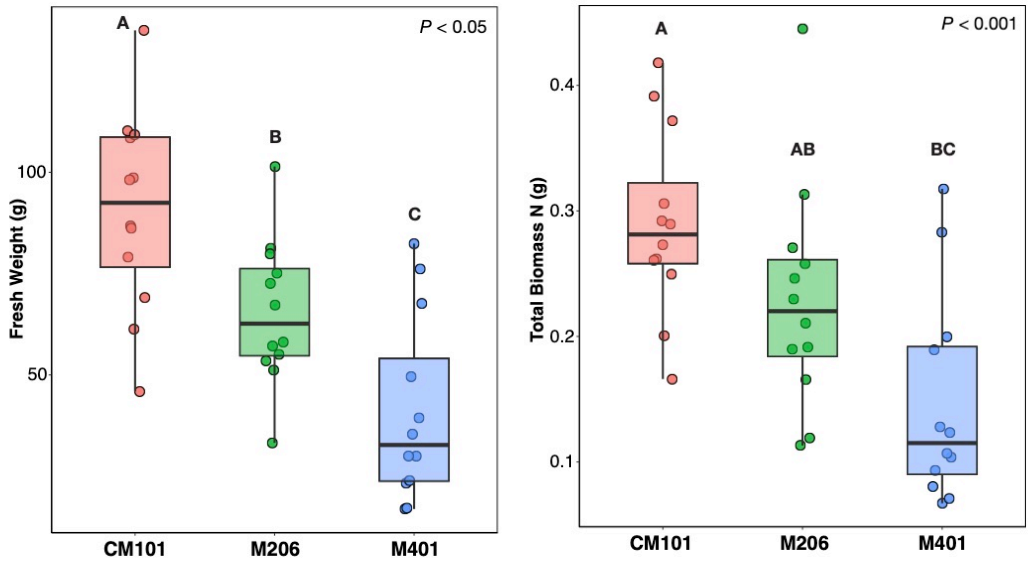


Fig. 6. Total biomass and biomass N content of different rice cultivar varieties. Measurements were determined in each rice plant at the end of the growth experiment. Different letters indicate statistical significance.

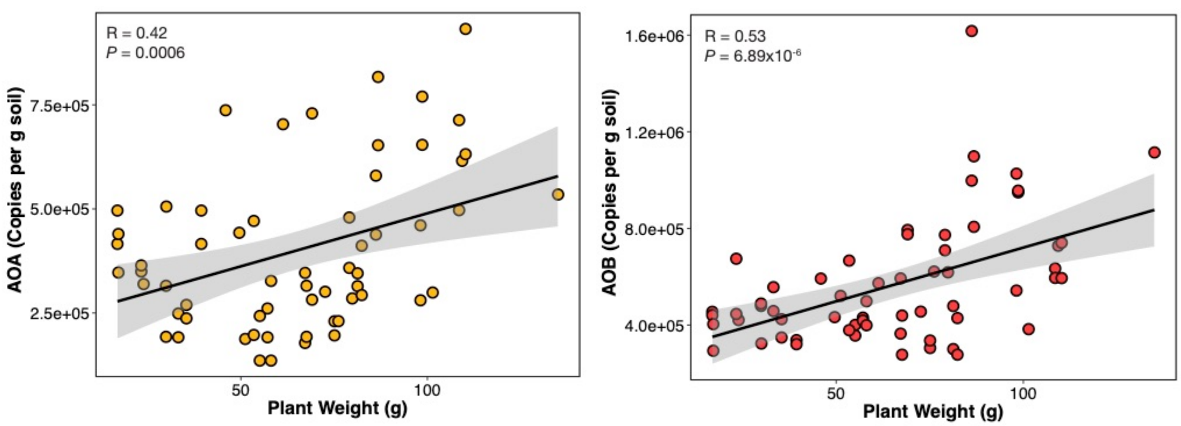


Fig. 7. Pearson correlations of ammonia-oxidizing archaea (AOA) and bacteria (AOB) abundances with plant biomass among all rice cultivar varieties.

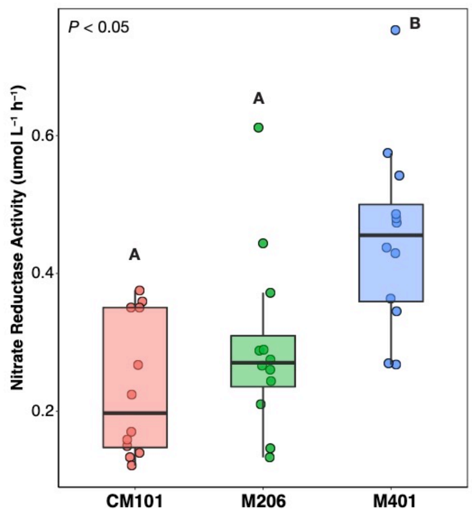
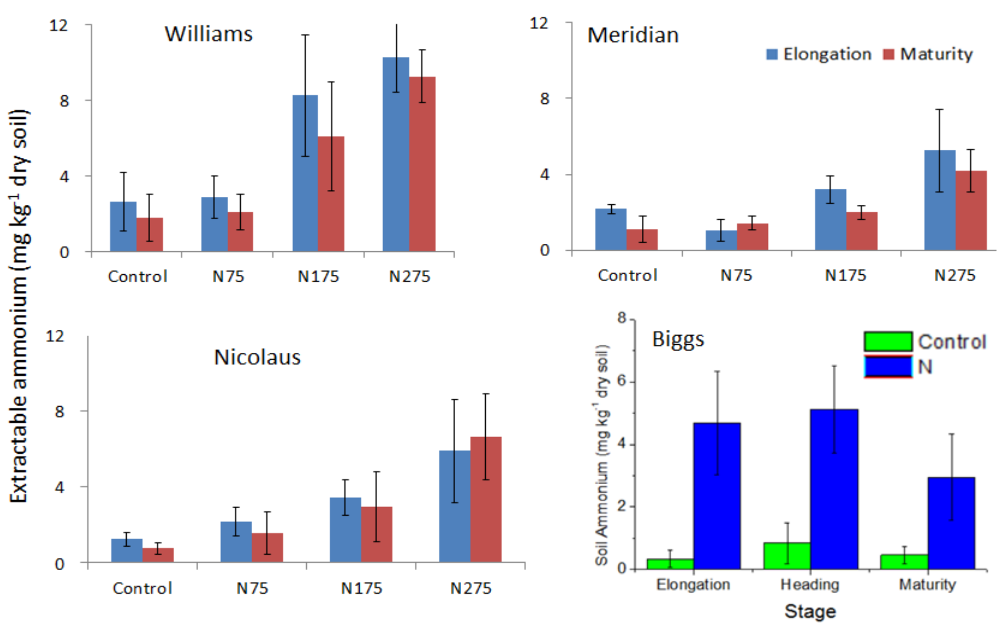


Fig. 8. Nitrate reductase enzyme activity in leaf tissues of different rice cultivar varieties. Different letters indicate statistical significance.

Experiment III: The objective of this experiment was to characterize dynamic changes in mineral nitrogen concentrations at different stages of rice growth and to explore its potential correlation with nitrification rates and rice yield. Rice cultivar M206 was grown in field plots across four sites (Biggs – Rice Experiment Station, Williams, Meridian, and Nicolaus) and treated with varying rates of nitrogen (Biggs, 0 and 171 kg N ha^{-1}; other three sites, 0, 75, 175, and 275 kg N ha-[1]). Soils were sampled and extractable NH_4^+ and NO_3^- levels were quantified at three different stages of rice plant growth (Elongation, Heading, and Maturity). Field plots without rice plants were used as controls.

Results demonstrated that:

Field trials suggest that soil ammonium content is low after rice elongation and nitrate is the major N source after rice elongation (**Figs. 9 & 10**).



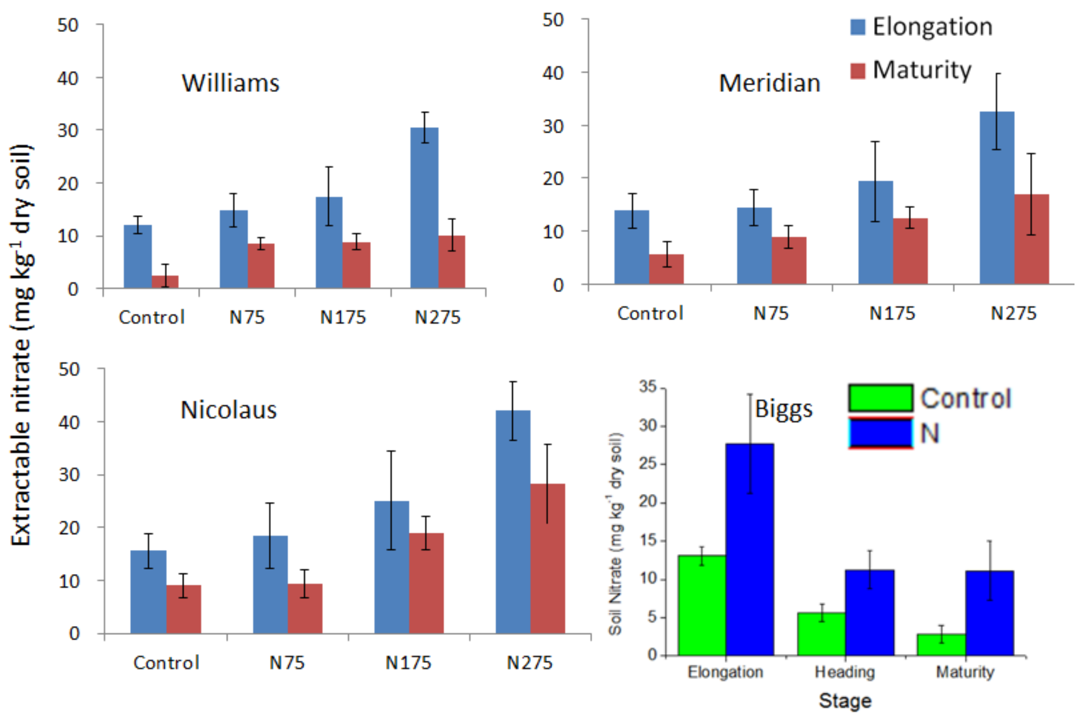


Fig. 10. Extractable soil NO_3^- levels at different stages of rice plant (cultivar M206) growth determined across four field sites.

Our results generally support our assumption that nitrification is important in CA rice varieties and that the mobility of fertilizer N increased in soils with root influences; however, the degree of these influences vary greatly across the cultivars. Our on-going analyses of plant tissues (e.g. total N, shoot and root nitrate reductase activity, and root density), more rice cultivars (S102, M105, M209) and soil microbial community (e.g., composition of AOA and AOB) will further demonstrate connections with nitrification potential, N assimilation, and rice yield. Based on these preliminary results, it is highly expected that rice yield/nitrogen use efficiency (NUE) is associated with the activity of ammonia oxidizers in rhizosphere and nitrate reductase in rice leaf.

SUMMARY OF 2018 RESEARCH (major accomplishments), BY OBJECTIVE:

Objective 1: We demonstrated in laboratory incubation that mixed NH_4^+/NO_3^- nutrition did not increase nitrification potential in one common CA paddy soil without rice plants. In contrast, additions of NO_3^- tended to suppress soil nitrification potentials. More work is needed to determine whether co-application of NO_3^- promotes nitrification in paddy soils (with rice planted), restrain the mobility of ammonical N, and hence decrease NUE and rice yield.

Objectives 2-5: We demonstrated that nitrification occurred at significant rates in all tested rice varieties, i.e., CM101, M206, and M401 (Objective 2), which seemingly increased the mobility of ammonical fertilizer $((NH_4^+)_2SO_4)$ and plant NH_4^+/NO_3^-uptakes. However, the rates of nitrification and its effects on N allocation and uptakes varied among the three cultivars (Objective 3). Our ongoing plant and soil testing will further demonstrate the connections between nitrification and N acquisition, NUE, and rice yield (Objectives 2 and 4). It is highly possible that there is a strong link between NO_3^- assimilation and increased rice yields by increasing the mobility of ammonical N through nitrification in the rice rhizosphere. Cultivar CM101 recruited the highest abundances of ammonia-oxidizer populations. Higher abundances of ammonia oxidizer populations were found in soils planted with cultivar CM101, which also produced the highest biomass and total biomass N content. Given that the abundance of ammonia oxidizers was significantly positively correlated with plant biomass, the recruitment of rhizosphere ammonia oxidizers by rice plants may be a useful trait for rice breeders to select for increased yields in the development of new varieties. Significant differences in nitrate reductase activity were also found across rice varieties. It is highly expected that rice yield/nitrogen use efficiency (NUE) is associated with the activity of ammonia oxidizers in rhizosphere and nitrate reductase in rice leaf. (Objectives 2-5)

PUBLICATIONS OR REPORTS: *In preparation*

CONCISE GENERAL SUMMARY OF CURRENT YEAR'SRESULTS:

We explored the importance of nitrification in three CA rice (CM101, M206, M401) in the context of N acquisition, NUE and rice yield. Nitrification occurs at significant rates in CA rice, which, however, varied largely across the tested cultivars resulting in different concentrations of plant-available NH_4^+ in soils, but not NO_3^-. It is highly possible that the difference among the tested rice would lead to different uptake rates of $NH_4^+$and NO_3^-, and hence the NUE and rice yields.

We also discovered that the presence of the rice rhizosphere owns more ammonia oxidizers and increases nitrate reductase activity in rice leaves among tested cultivars. It is highly expected that rice yield/nitrogen use efficiency (NUE) is associated with the activity of ammonia oxidizers in rhizosphere and nitrate reductase in rice leaf.