Project NO. RM-13

ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2017 -December 31, 2017

PROJECT TITLE: THE ROLE OF NITRIFICATION IN RICE SYSTEMS TO SUPPORT NITROGEN USE EFFICIENCY

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OBJECTIVES AND EXPERIMENTS CONDUCTED, BY LOCATION, TO ACCOMPLISH OBJECTIVES:

Objectives:

The overall objective of this research is to determine if 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 increase nitrification potential in CA paddy soils.
2. Determine the extent of nitrification occurring in California rice varieties with a range of yield potentials.
3. Identify the importance of nitrification for increased NUE, yield, N acquisition and translocation in rice plants.
4. Determine if there is significant variability between popular California rice cultivars for objective 2 and 3.
5. Determine which plant traits, such as, rooting density, etc. can be used as accurate predictors of the nitrification potential in popular California rice varieties.

OBJECTIVES AND EXPERIMENTS CONDUCTED, BY LOCATION, TO ACCOMPLISH 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 a established N rate trial at CA Rice Research Experiment Station. In this trial, two N fertilizer treatments (0 or175 k N ha^{-1} ammonical fertilizer) and four replicates for each treatment (total eight plots) were applied. Three soil cores were taken by auger borings to a depth of 20 cm from each plot, transported in ice chest to the University of California Davis. The three soil cores were composited after the removal of vegetation, rice roots, and dead plant residues, homogenized by hands and stored at 4°C. For the lab incubation, the composited soils were sieved to 2 mm and incubated in dark at room temperature to determine nitrification potential with ^{15}N isotopic pool dilution method. To determine the effect of NO_3^- applied with NH_4^+ on nitrification potential and plant-available NH_4^+, KNO_3 was added at rates of 0, 10, and 20% of the field recommended N rates.

The results suggest that the application of NO_3^- with NH_4^+ tends to suppress soil nitrification potential (Fig. 1A) but has no effect on NO_3^- consumption (Fig. 1B).

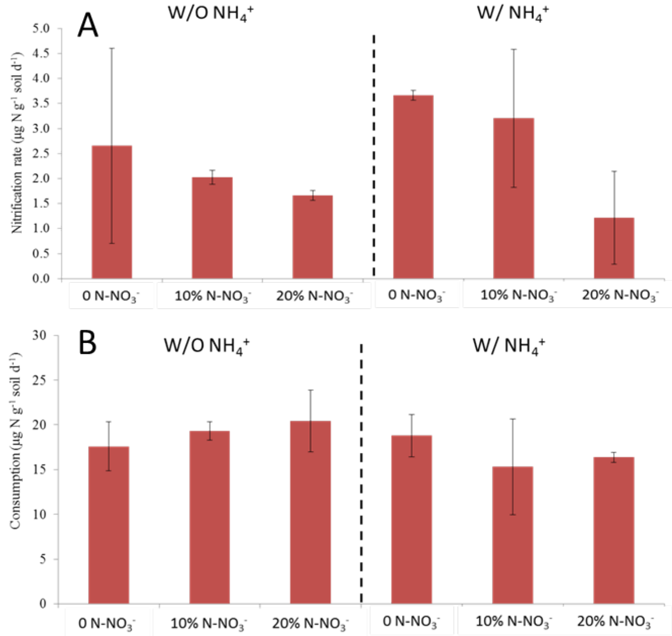

Fig. 1. Nitrification potential (A) and NO_3^- consumption potential (B) of paddy soils receiving various mixed rates of NH_4^+/NO_3^- nutrition.

Experiment II: This experiment was designed to explore the importance of nitrification in CA rice and its influence on making fertilizer ammonical N more mobile/available and therefore affects on NUE and rice yield. Three rice cultivars (CM101, M-206, and M-401) were tested. To determine the effect of root development on nitrification potential, nylon bags with different opening size (0.01 inch or 0.4 inches) were used to restrain the influences of rice roots in soil processes (Fig. 2).

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

Soil used for this pot study was collected from the paddy field at CA Rice Research Experiment Station and sieved (2mm). Approximately, 6 kg of soil was mixed with K and P fertilizers at the field recommended rate and placed in a pot (15.2 cm in diameter, 30.5 cm in depth). Nylon bags (0.01 inch or 0.4 inches opening mesh) were installed in randomly selected pots. Ammonical fertilizer ($(NH_4^+)_2SO_4$) was added evenly into each nylon bag at the field recommended rate. A control without nylon bag but with $(NH_4^+)_2SO_4$, K, and P fertilizers was prepared in parallel. Each treatment was replicated three times. Rice was grown in a growth chamber with the following environmental settings: 14 hours of light/10 hours of dark, 30 °C in day light/28°C in dark night, and 70% huminity. Rice was grown to late tillering stage (8-10 tillers). Soil samples inside (I, soil with limited or no root influence) and outside (O, rhizosphere soil) of the nylon bag were collected for the analysis of plant-available NH_4^+, NO_3^-, nitrification potential, and microbial community composition, while plant samples were collected to determine tissue N content, nitrate reductase activity (NRA), biomass production, and root density.

Our results showed the following:

1. Rhizosphere soils (containing roots) tend to have higher nitrification potential than those without root influence in CM101 and M-206, but not in M-401 (Fig. 3A); this suggests that root development and growth can largely affect the mobility of ammonical N through nitrification into mobile NO_3^-, increasing it's uptake and allocation into the plant. Results suggest the magnitude of influences likely vary across CA rice varieties.
2. Soils without root influence have higher NH_4^+ than rhizosphere soils, with the magnitudes of difference in the order of CM101> M-206 > M-401 (Fig. 3B), which suggests that higher nitrification potentials caused higher N mobility and hence lead to higher N uptake by plants, although the NUE may differ across rice varieties.
3. No difference in plant-available NO_3^- was observed between rhizosphere soils and soils without root influence or among rice varieties(Fig. 3C).
4. The presence of rice increases the abundance of ammonia oxidizing *Archaea* (AOA) and *Bacteria* (AOB) in soil (Fig. 4). Compared to other rice varieties, CM101 has higher abundance of AOA and plant biomass (Figs. 5&6). This indicates that rice biomass might be associated with ammonia oxidizer (nitrification) activities. However, this connection was only tested for one variety and not confirmed with others.
5. M401 has higher nitrate reductase activity in rice leaves than other varieties (Fig. 7), suggesting that this variety has potential to use nitrate more efficiently. The low biomass in M401 suggests that further research focus on N cycling especially on N loss during the process of nitrate assimilation in rice plant is needed.
6. Across all samples, no significant differences in alpha diversity (species richness and Shannon index) or composition of the soil microbial communities were observed among the treatments with different rice variety, bag position, and bag size. (Fig. 8, 9 and 10).

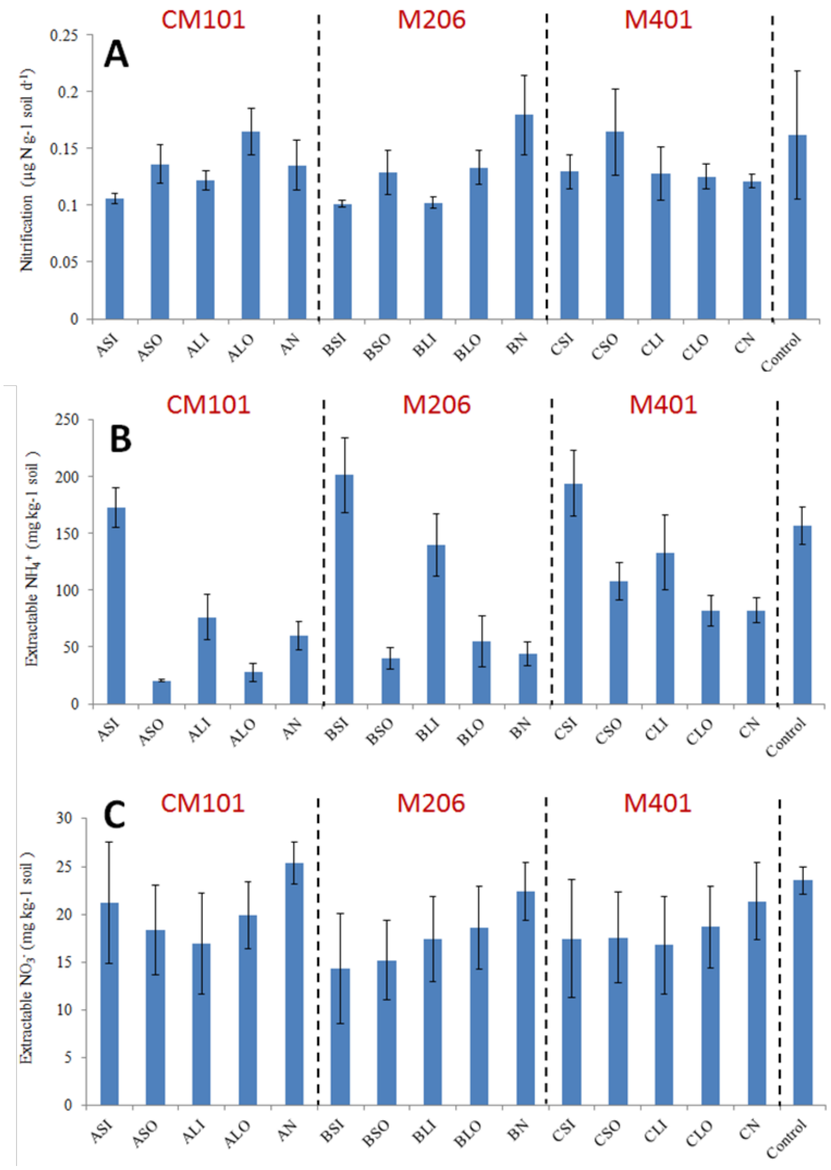

Fig. 3. Nitrification potentials (A), plant available ammonium (B), and plant available nitrate (C) in paddy soils with rice cultivar CM101, M206, and M401. The experiment was a pot study in growth chambers. Capital letters in the X-axis indicate treatment effects, in which the first letter shows rice cultivar (A, CM101; B, M206; C, M401), the second letter indicates whether a nylon bag was placed to create soils without root influences (S, a bag with an opening size of 0.01 inch; L, a bag with an opening size of 0.4 inches; N, no bag was placed), while a third letter indicates rhizosphere soils (O) and soils without root influences (I); Control indicate bulk soils without rice planted.

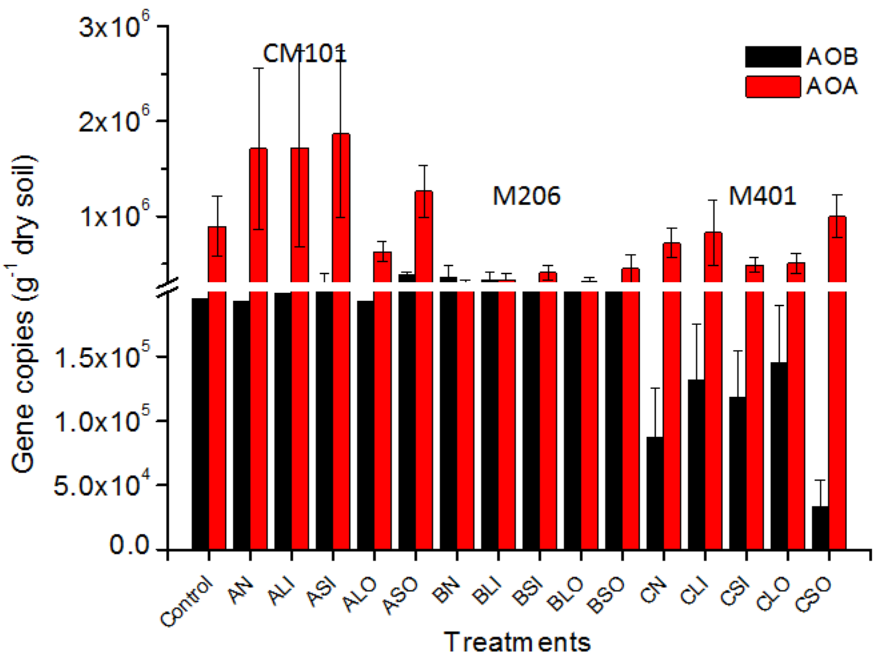

Fig. 4. Abundance of ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) in paddy soil with rice cultivar CM101, M-206, and M-401. For treatment names: the first letter shows rice cultivar (A, CM101; B, M-206; C, M-401), the second letter indicates whether a nylon bag was placed to exclude the influence of root on soil ammonia oxidizers (S, a bag with an opening size of 0.01 inch; L, a bag with an opening size of 0.4 inches; N, no bag was placed), while the third letter represents rhizosphere soil (O) or soil without root influences (I); control represents bulk soil without rice planted.

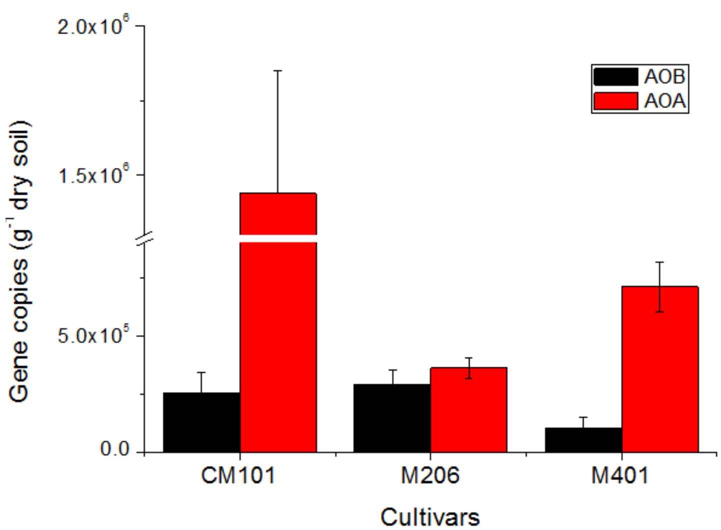

Fig. 5. Abundance of ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) in paddy soils planted with three rice cultivars.

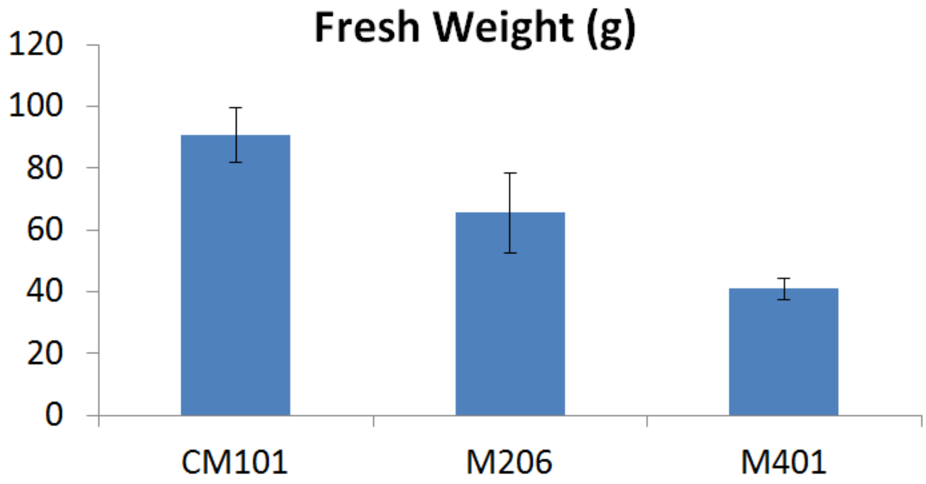

Fig. 6. Fresh weight of rice plants for three rice varieties.

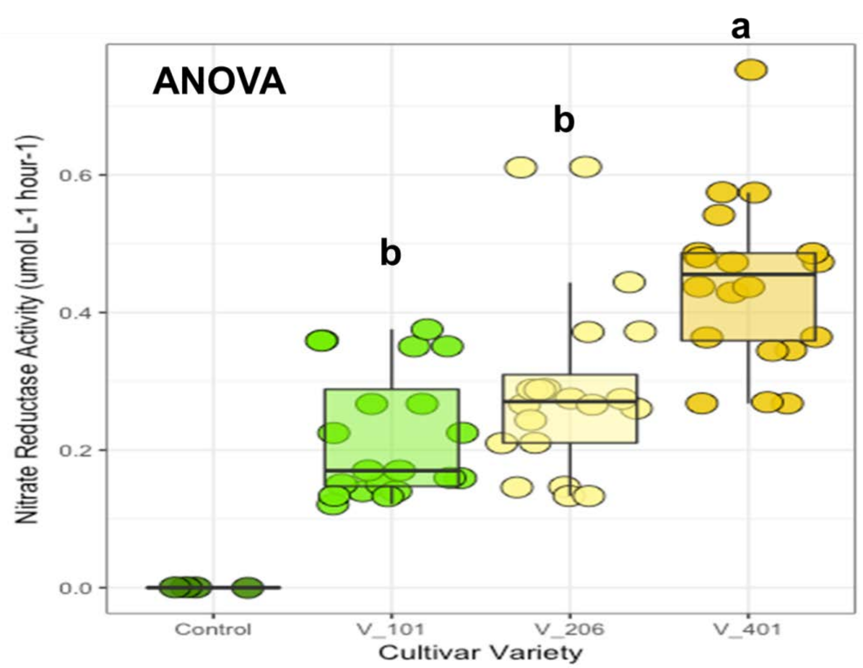

Fig.7. Nitrate reductase activity in rice leaves. V-101 represents CM101 cultivar, V-206 represents M206 cultivar, and V-401 represents M401 cultivar.

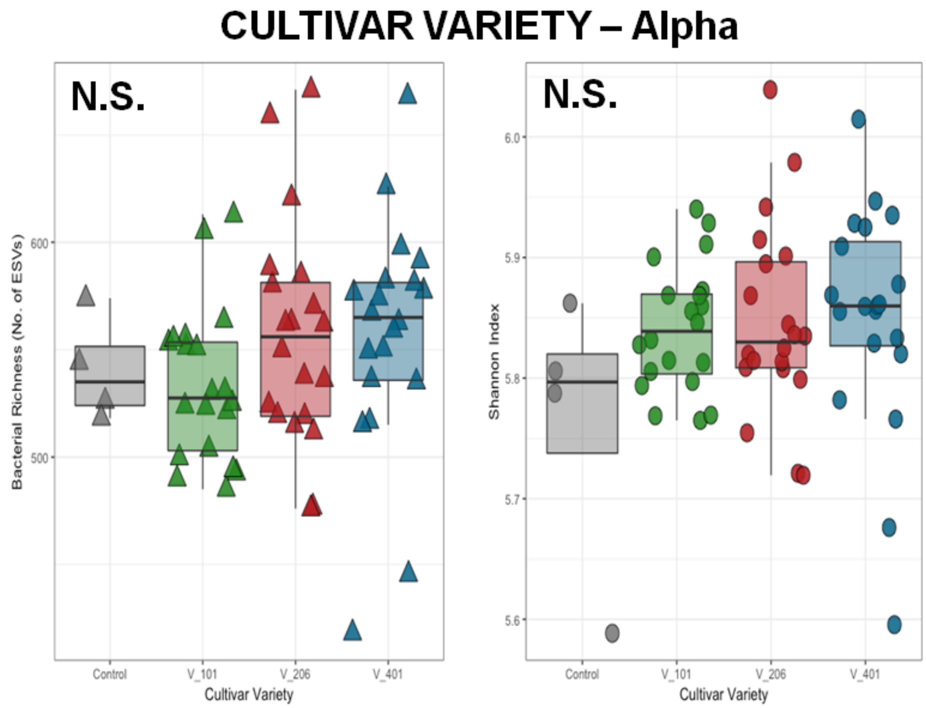

Fig. 8. Soil bacterial diversity indices across rice cultivars: (A) Richness and (B) Shannon index.

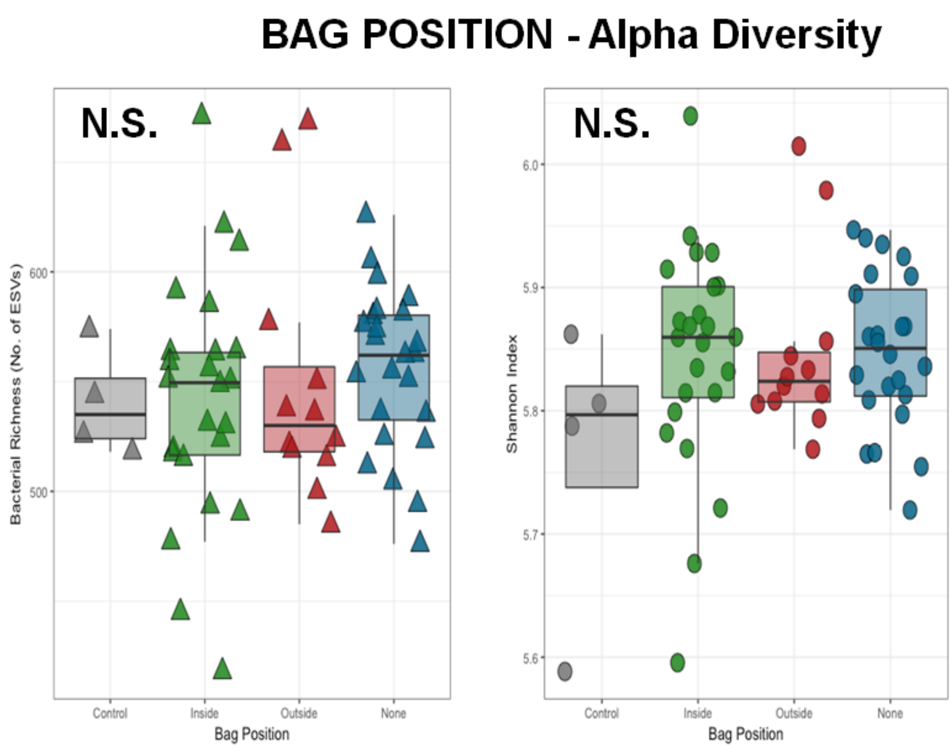

Fig. 9. Soil bacterial diversity indices for different bag positions: (A) richness and (B) Shannon index

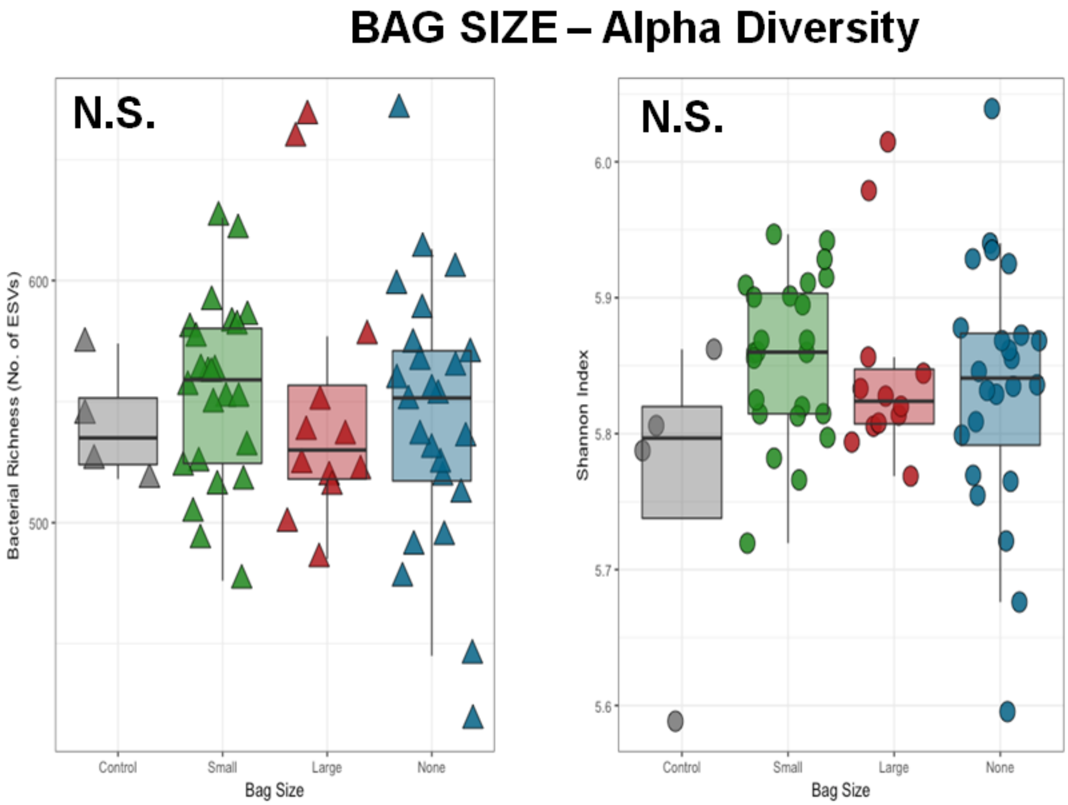

Fig. 10. Soil bacterial diversity indices for different bag sizes: (A) Richness and (B) Shannon index.

Experiment III: This experiment was conducted to determine the importance of nitrification in CA field rice and its connection with the mobility of ammonical N, NUE, and rice yield. Rice cultivar M206 was in four sites: Biggs (Rice Experiment Station), Williams, Meridian, and Nicolaus. On Biggs, two nitrogen rates were applied (0 and 171 kg N ha^{-1}). On other three sites, four nitrogen rates were applied (0, 75, 175 and 275 kg N ha^{-1}). The dynamics of soil NH_4^+ and NO_3^- contents at different rice stages and the relationships among nitrification potential, abundance of ammonia oxidizers, and rice yield were tested.

Our results showed that:

1. Soil NH_4^+ content was low after rice elongation and NO_3^- was the major N source for plant growth after rice elongation (prior to panicle initiation) (Figs. 11&12). These results generally support our assumption that nitrification is important in CA rice and the mobility of fertilizer N increased under root influences; however the magnitude of influences vary greatly across the cultivars.
2. Our on-going analyses of plant tissues (e.g. total N, shoot and root nitrate reductase activity, and root density), testing more rice cultivars (S102, M105, M209 including lower yielding Koshi variety, possibly analyzing low yielding rejects from breeder trails), and exploring the changes of soil microbial communities (e.g., abundance of AOA and AOB) can further be used to understand the connections among nitrification potential, NUE, and rice yield. Based on the preliminary results, it is

highly expected that rice yield/ NUE is associated with the activity of ammonia oxidizers in rhizosphere and nitrate reductase in rice leaf.

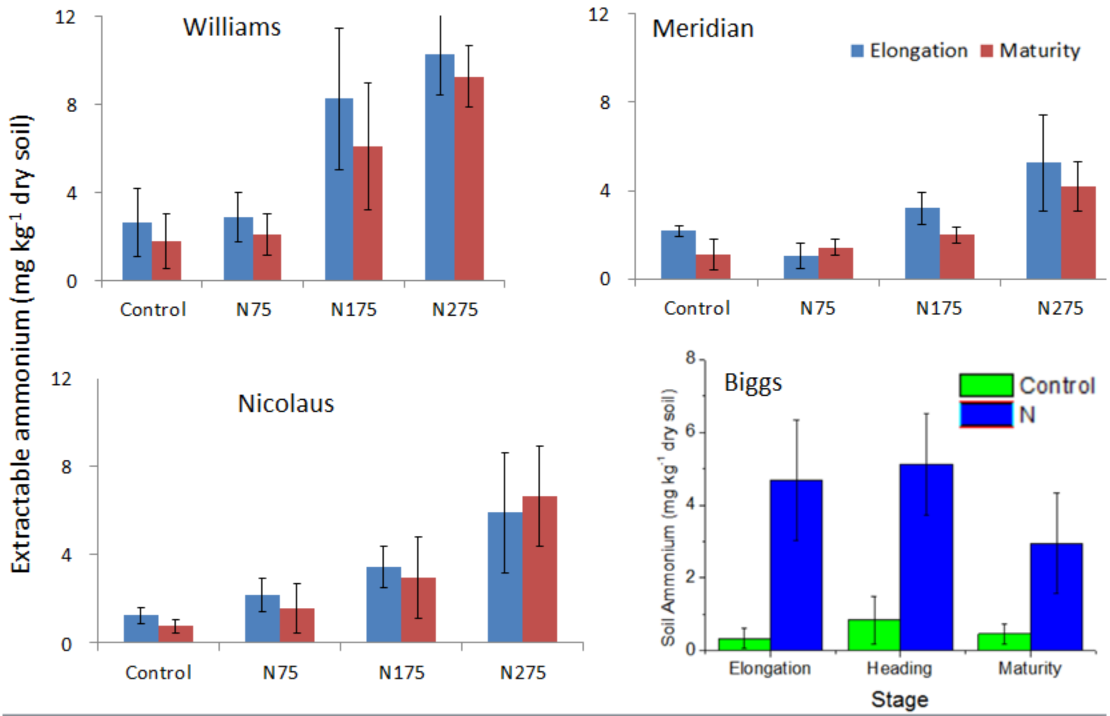

Fig. 11. Dynamic soil ammonium contents at different stages and sites.

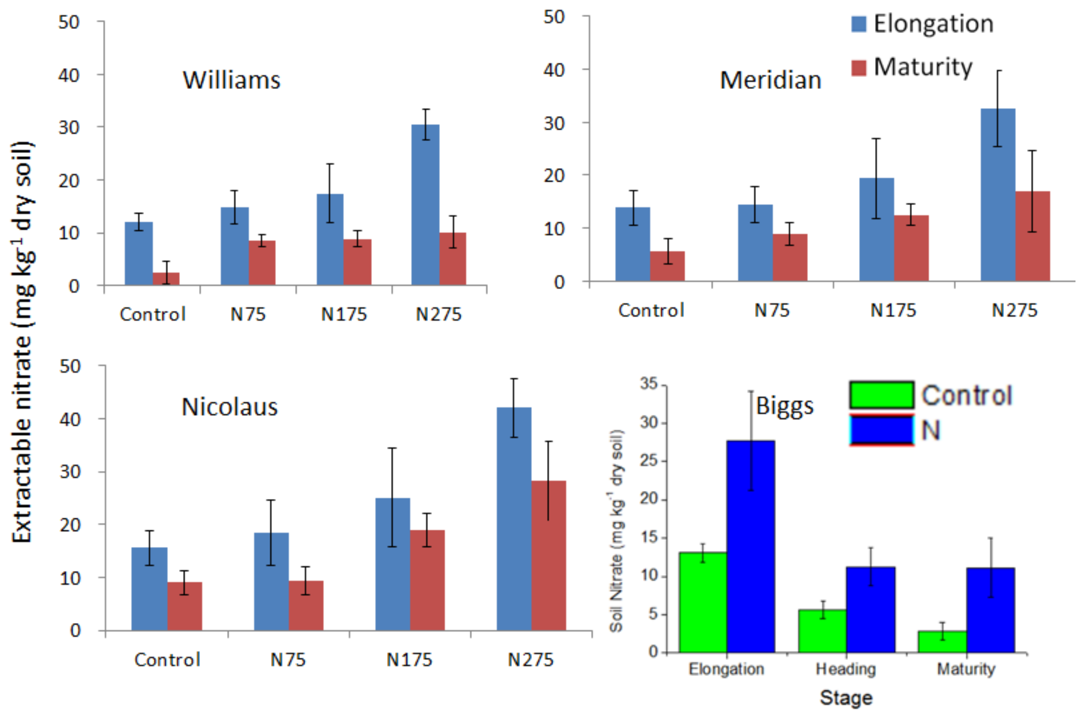

Fig 12. Dynamic soil nitrate contents at different stages and sites.

SUMMARY OF 2017 RESEARCH (major accomplishments), BY OBJECTICVE

Objective 1: In laboratory incubation, we demonstrated that mixed NH_4^+/NO_3^- nutrition did not increase nitrification potential in one CA paddy soil without the presence of rice plant. In contrast, the addition of NO_3^- fertilizer tends to suppress soil nitrification potential. More work is needed to determine whether the application of NO_3^- mixed with NH_4^+ promotes the soil nitrification process in paddy soils (with the presence of rice plant), restrains the mobility of ammonical N, and hence decreases NUE and rice yield.

Objective 2-5: We demonstrated that nitrification occurred at a high rate in all the 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^-uptake. However, the rates of nitrification and its effects on N allocation and uptakes differed among the three tested cultivars (Objective 3). Our ongoing plant and soil testing is expected to further demonstrate the connection between nitrification and rice N acquisition, NUE, and rice yield (Objectives 2 and 4). Our results suggest a strong link between AOA abundance and the increased rice biomass, as a result of the increased mobility of ammonical N through nitrification in rice rhizosphere. A higher population of ammonia oxidizers was found in rhizosphere affected by rice roots. Significant difference of ammonia oxidizers' abundance and nitrate reductase activity was found across rice varieties. It is highly expected that rice yield/ NUE is associated with the activity of ammonia oxidizers in rhizosphere and nitrate reductase in rice leaf. (Objectives 2-5)

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PUBLICATIONS OR REPORTS: Not available

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

We explored the importance of nitrification in three CA rice (CM101, M206, M401) by examining N uptake, NUE and rice yield. Nitrification occurs at significant rate in CA rice, which, however, varied largely across the tested cultivars, resulting in different concentrations of plant-available fertilizer NH_4^+ in soils. It suggests that the difference among the tested rice varieties can lead to different crop uptake rates of $NH_4^+$and NO_3^-, and therefore NUE and rice yields.

We also discovered that the presence of the rice rhizosphere increases the abundance of ammonia oxidizers and increases nitrate reductase activity in rice leaves among tested cultivars. It is assumed that rice yield/ NUE is associated with the activity of ammonia oxidizers in rhizosphere and nitrate reductase in rice leaf.

These activities in the soil and root likely benefit high yielding rice and should be included in evaluating the performance of new rice varieties.