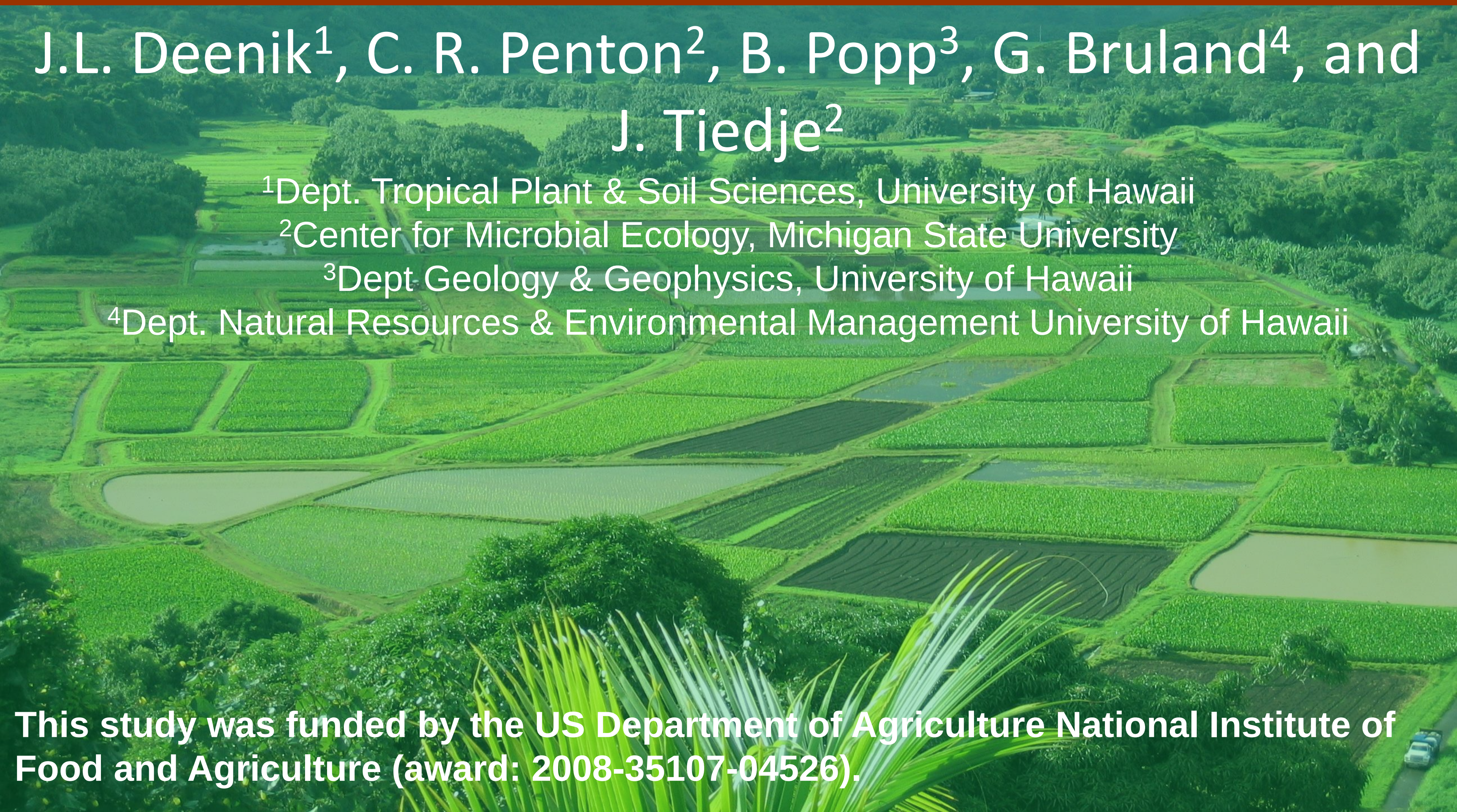
Anammox Activity and Nitrogen Dynamics in Taro Soils of Hawaii



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Research Questions and Hypotheses

1. Is anammox present and active in flooded agroecosystems?

   H$_1$: *Anammox is present and contributes significantly to N loss in taro fields*

2. How important are rhizosphere mediated nitrification-denitrification reactions in the overall N balance of agroecosystems?

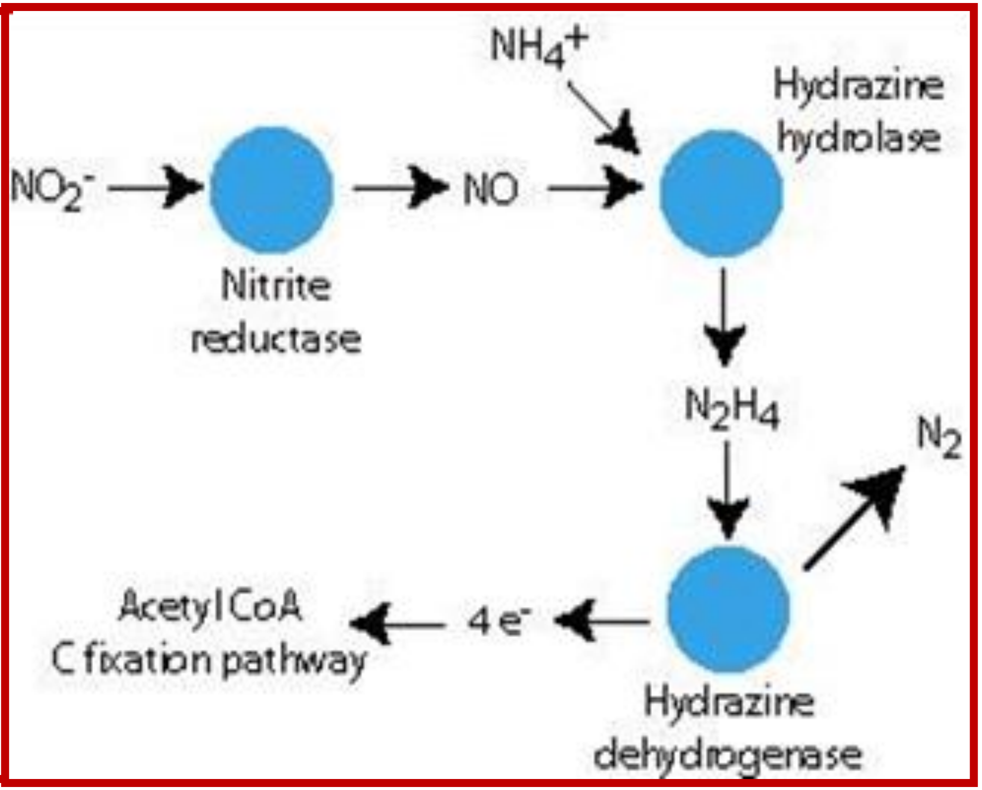
   H$_2$: *The presence of arenchymous tissue and an oxidized rhizosphere in taro will promote coupling of nitrification and denitrification contributing to significant N losses*

The Anammox Reaction

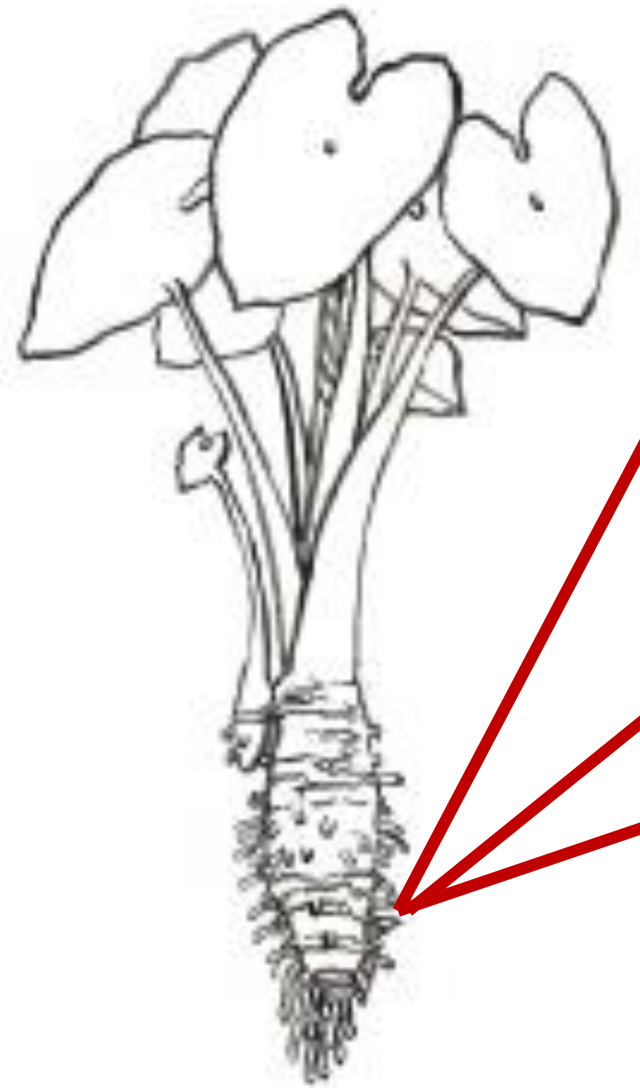
anaerobic ammonium oxidation

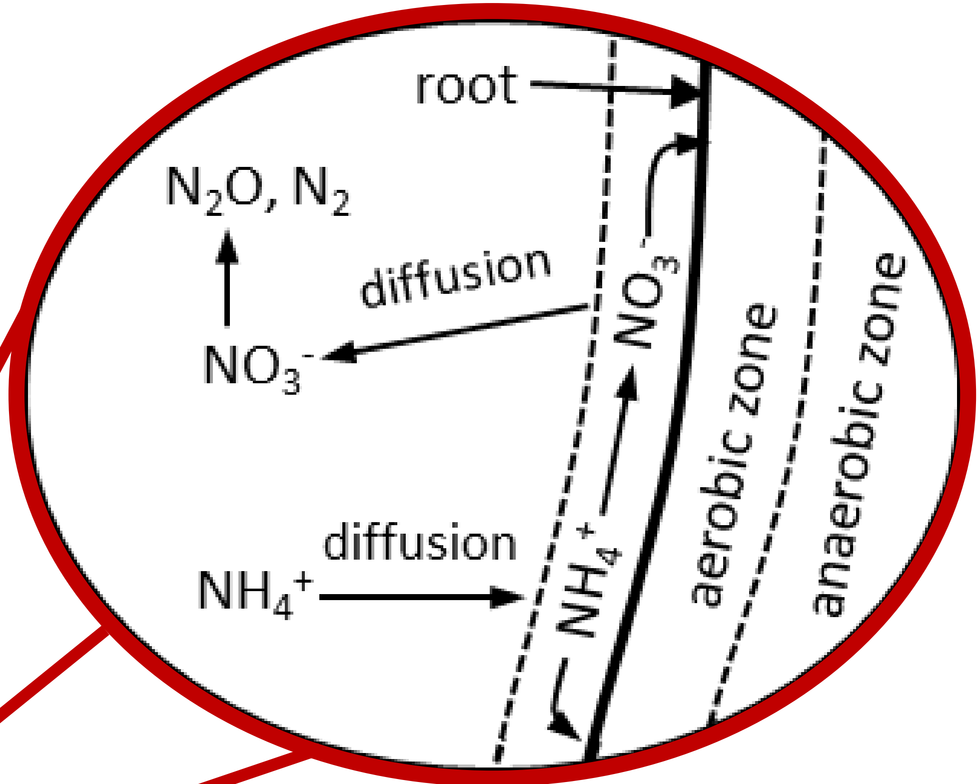
$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$

- Anammox bacteria discovered in waste water treatment
- Share unique organelle – *anammoxosome* where NH_4^+ and NO_2^- combine via hydrazine to yield N_2
- Present and active in diverse range of environments where oxic/anoxic interfaces occur



Rhizosphere Coupling of Nitrification-Denitrification



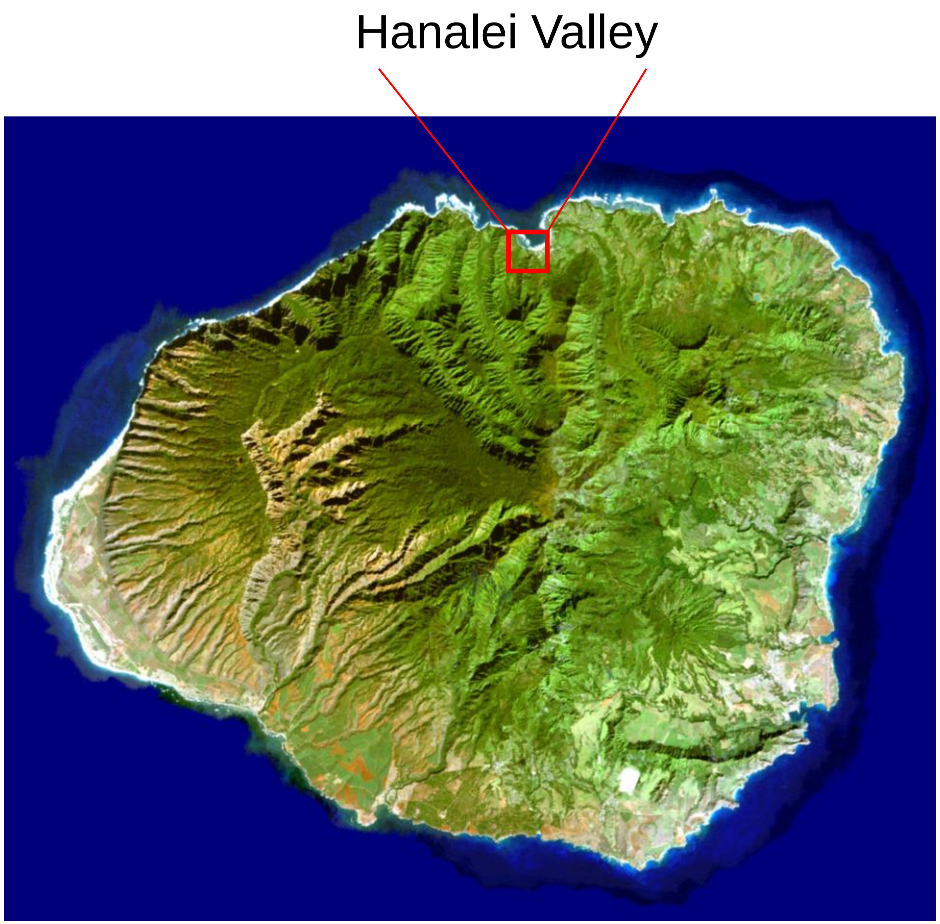


1. NH_4^+ nitrified in the aerobic rhizosphere
2. NO_3^- diffuses back out into the anaerobi bulk soil, and denitrified

Objectives

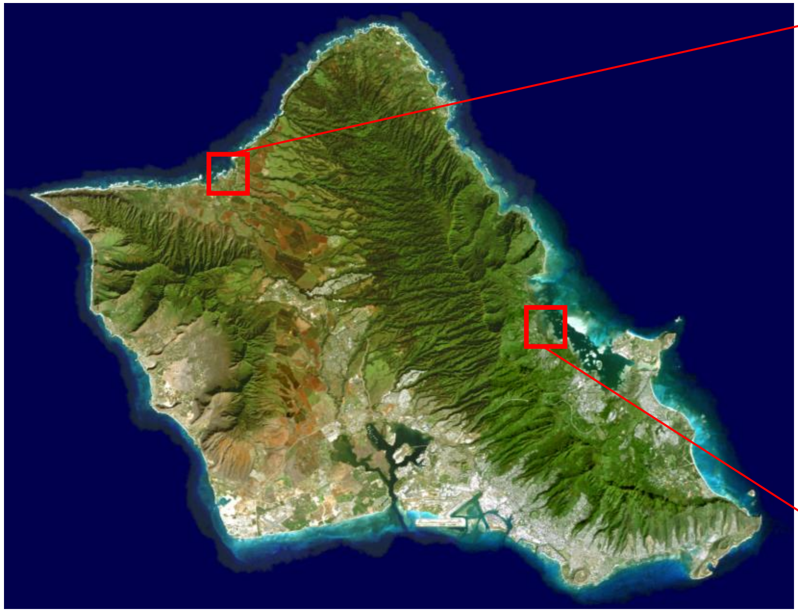
1. To determine the N balance of flooded taro agricultural fields using conventional and isotope-based measurements.
    a. Evaluate the contribution of anammox and coupled nitrification-denitrification to gaseous N looses
    b. Investigate N functional gene in the subsurface
    c. Assess impact of different N fertilization practices
2. To characterize N transformations in intensively managed flooded agroecosystems
    a. Evaluate O2 flux from the root rhizosphere as a mediator of sub-surface nitrification
    b. Assess the relative importance of sub-surface N cycling processes to overall NH3 losses
    c. Investigate N functional gene abundance in the sub-surface in relation to N cycling activities
    d. Assess overall N balance in intact taro cores

Kauai Sites



Oahu Sites

Hybrid

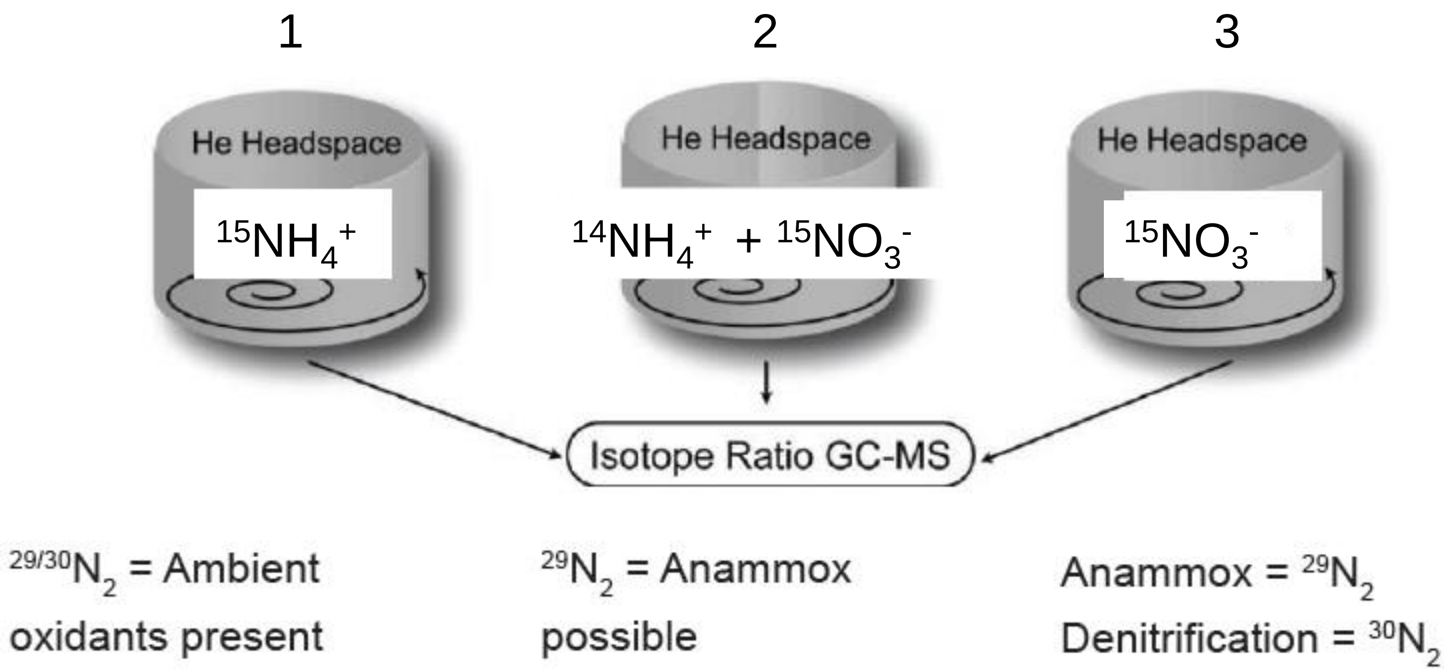


Organic

Methods Objective 1

- In Situ O_2 profile measurements.
- Porewater NH_4^+, NO_2^- and NO_3^- and sediment porosity determined at 0.5 cm depth increments
- Estimation of potential denitrification and nitrification rates (incubations).
- Anammox and denitrification activity measured using isotope pairing incubations

Methods



1. $^{15}NH_4^+$: Validation for anaerobic conditions and consumption of all available oxidants. No $^{30/29}N_2$ indicates valid incubation conditions.
2. $^{14}NH_4^+ + ^{15}NO_3^-$: Measures the overall potential for anammox activity considering possible ammonium limitation.
3. $^{15}NO_3^-$: Production of $^{29}N_2$ indicates anammox activity while $^{30}N_2$ indicates denitrification.

Results - Anammox Activity

- Anammox present in taro fields
- Anammox activity was low to undetectable in all samples
- Either anammox bacteria were inactive at the time of sampling or alternative metabolic pathways were being used to maintain a significant population over time.

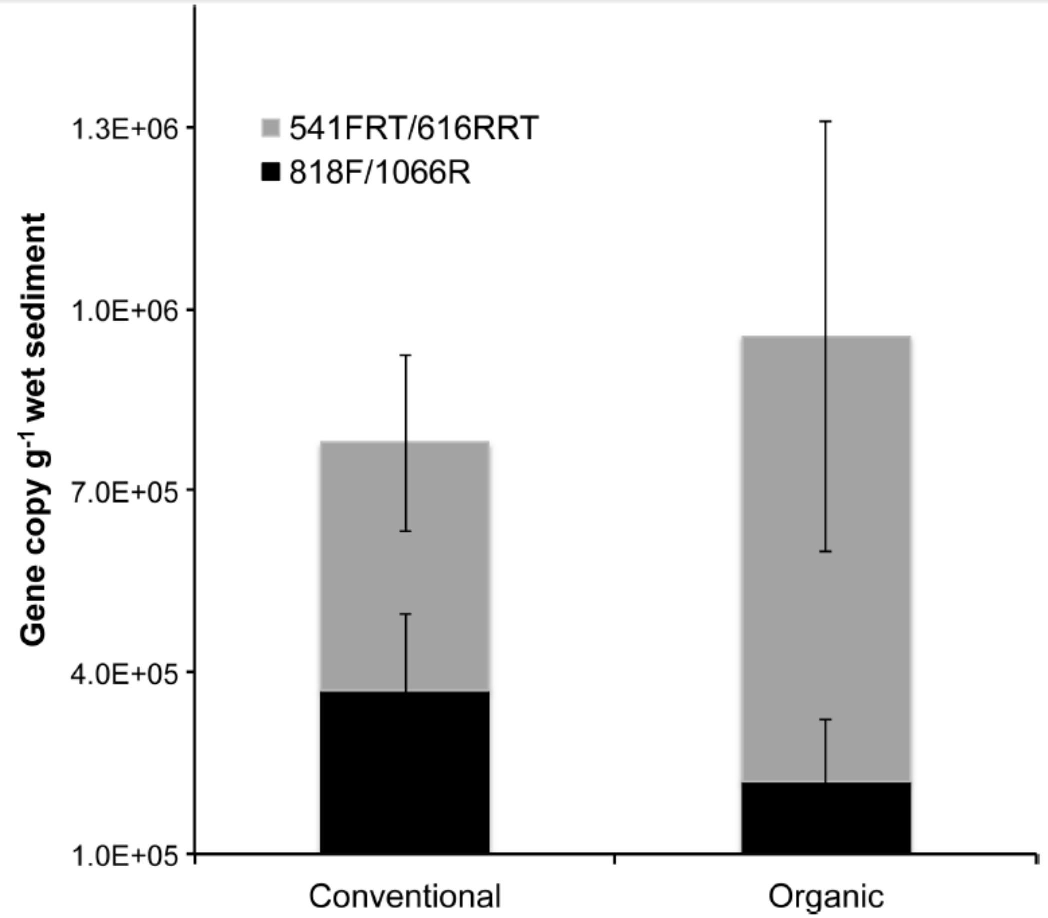


FIG. 2. Anammox 16S rRNA gene copies ($\times 10^6$) per gram wet sediment for the *Candidatus* "Scalindua"-type anammox (541FRT-616RRT; dark gray) and the "Brocadia," "Kuenenia," and "Jettenia" anammox (818F-1066R; black) from the conventional and organic management sites.

Source: Penton, et al., 2014

Results – Management Effects on O_2, Nitrification, and Denitrification

TABLE 2. Summary of Management Effects on Selected Soil Properties

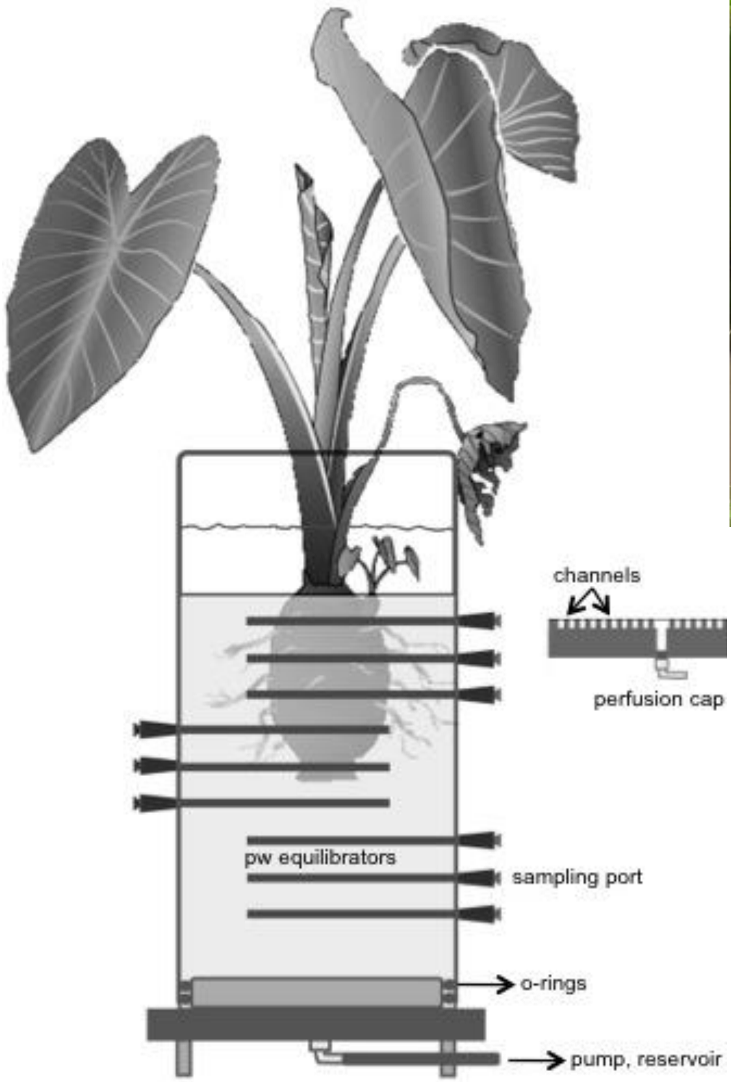
Management	TN g kg^{-1}	TOC g kg^{-1}	O_2 penetration depth, mm	Slurry nmol N_2 g^{-1} dry sediment h^{-1}	Slurry mmol N_2 m^{-2} day^{-1}	Nitrification nmol N-NO_3^- g^{-1} dry sediment h^{-1}	NO_3^- flux μmol N m^2 day^{-1}	NH_4^+ flux umol N m^2 d^{-1}	Denit. flux μmol m^{-2} d^{-1}
Conventional	0.98 ± 0.14^{A}	12.02 ± 1.37^{A}	0.3 ± 0.2^{A}	40.1 ± 4.1^{A}	12.3 ± 0.9^{A}	13.6 ± 12.0^{A}	-33.3 ± 19.3^{A}	1503 ± 454^{A}	16.7 ± 9.6^{A}
Hybrid	1.09 ± 0.08^{A}	12.23 ± 0.88^{A}	6.0 ± 2.3^{B}	38.9 ± 6.1^{A}	9.1 ± 1.4^{A}	10.3 ± 1.8^{A}	-9.1 ± 1.3^{AB}	294 ± 205^{B}	4.6 ± 0.7^{AB}
Organic	0.98 ± 0.06^{A}	11.44 ± 0.97^{A}	2.8 ± 0.6^{AB}	18.4 ± 6.7^{B}	4.3 ± 1.7^{B}	6.6 ± 1.6^{A}	0.1 ± 1.8^{B}	102 ± 26.2^{B}	0.6 ± 0.6^{B}

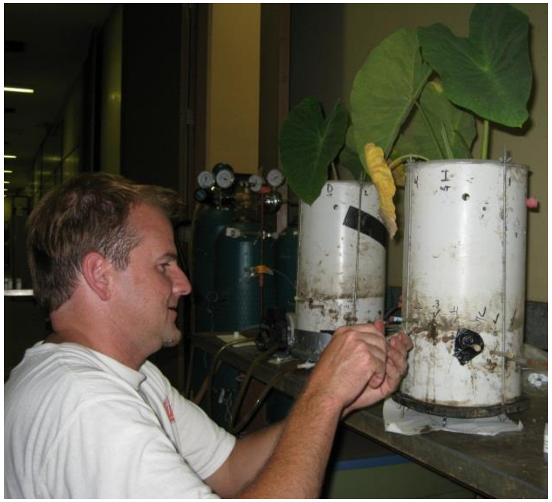
Total N (TN) and TOC expressed as grams per kilogram. Potential slurry denitrification rates estimated from the linear accumulation of $^{30}N_2$ in the $^{15}NO_3^-$ amended incubation. Slurry rates extrapolated to m^{-2} day^{-1} were corrected for soil porosity and depth of NO_3^- penetration. Potential nitrification rates estimated from the accumulation of NO_3^- after aerobic incubation of added NH_4^+. O_2 fluxes are modeled from oxygen microelectrode porewater profiles ($n = 3$). Surface fluxes of NO_3^- and NH_4^+ (μmol N m^2 day^{-1}) estimated from porewater profiles (surface water to −2 cm) and denitrification rates from fluxes were estimated from porewater modeling. Superscript letters indicate grouping based on Fisher least square differences (LSD).

Source: Penton, et al., 2014

- O_2 penetration limited, but deepest in hybrid managed system
- High NH_4^+ porewater concentration in conventional and hybrid systems reflecting urea fertilization practices
- High denitrification potential in conventional fields
- Significantly lower denitrification rates in organic fields

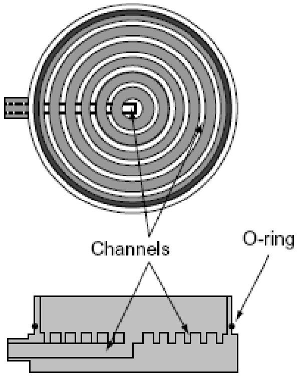
2. Whole core experiment to determine taro N balance including coupling of nitrification-denitrification





Whole Core Methods

1. Withdraw porewater after bubbling with He to remove O_2
2. Cores labeled via bottom perfusion cap with $^{15}NH_4^+$ to >90% enrichment
3. Incubate in light vs. dark
4. Extract equilibrated porewater and determine $^{15}NO_3^-$, $^{30}N_2$, $^{15}NH_4^+$
5. Sacrifice plant tissue for $^{15}NH_4^+$



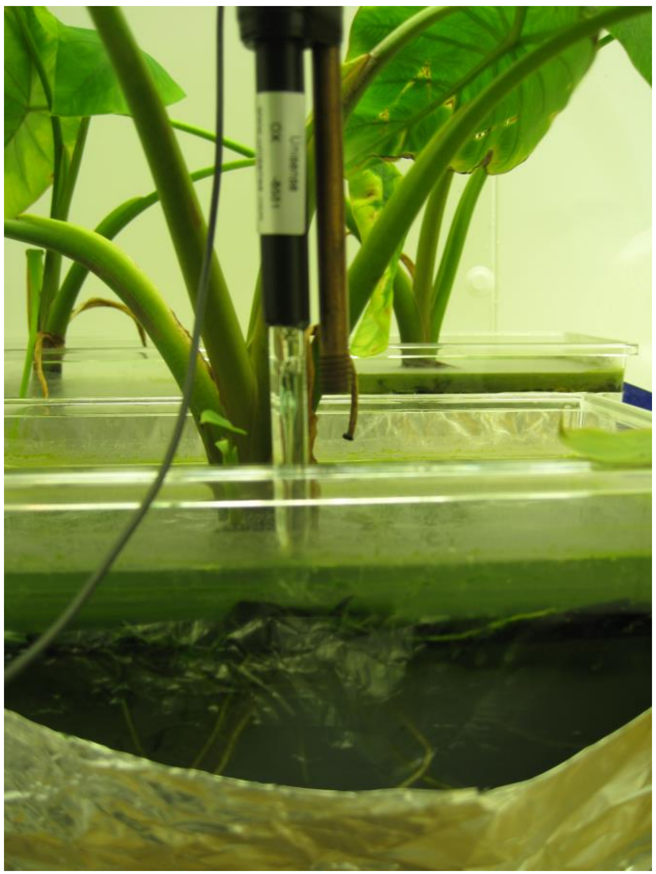
Evidence for Rhizosphere O_2

- Field cores show extensive root oxygenation with oxidized Fe

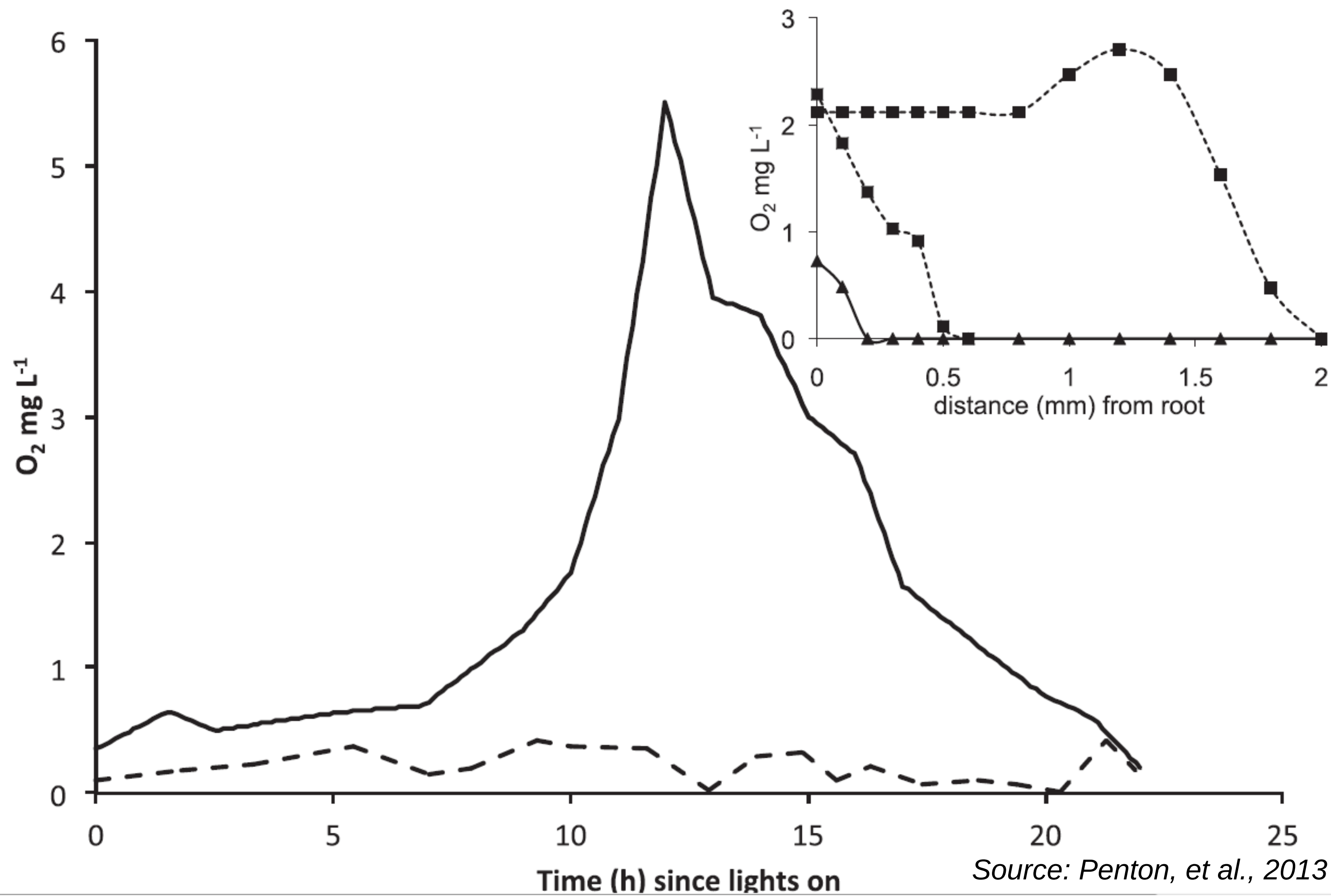
- Our initial set of cores showed some oxidized Fe in root channels

Microsensor Analysis of O_2 in the Rhizosphere

- Root oxygenation experiment with nutrient agar and bed of FeS for O_2 scavenging



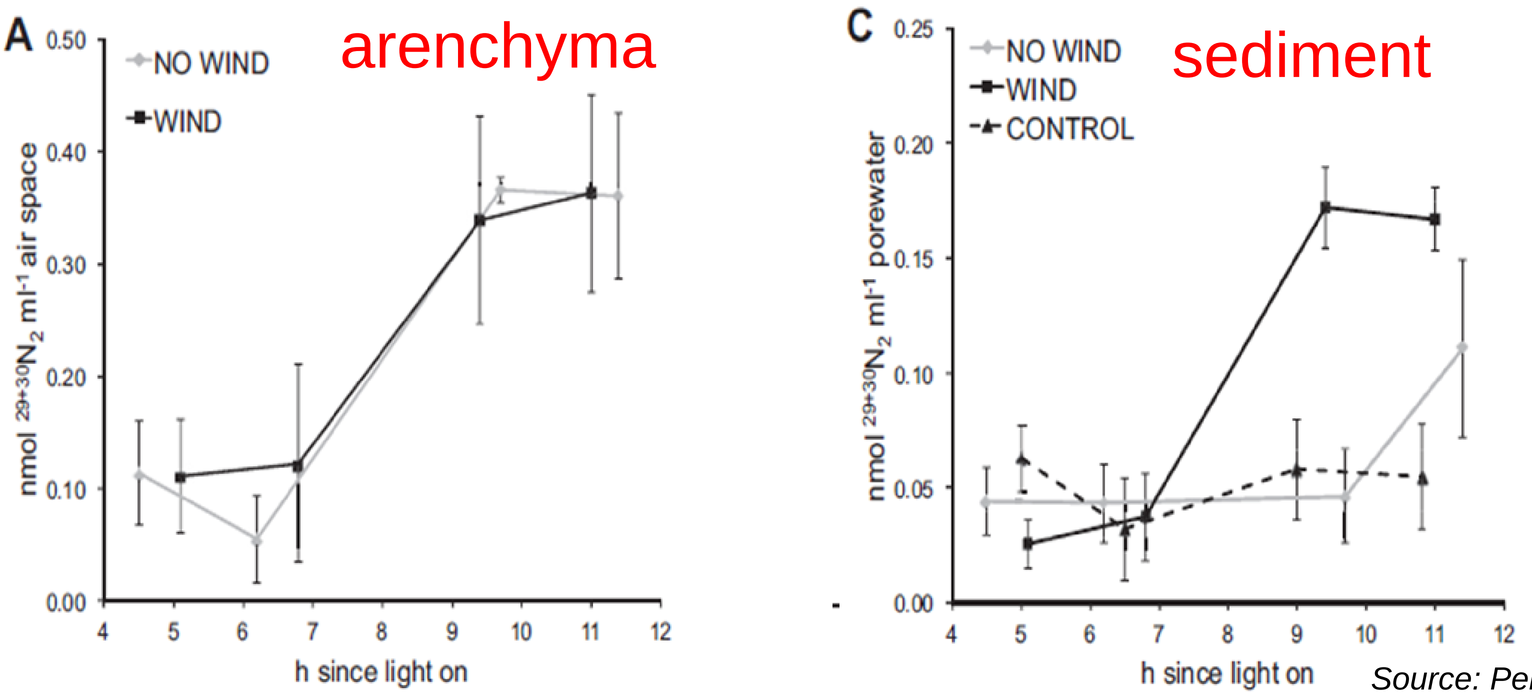
Oxic Rhizosphere



Source: Penton, et al., 2013

- Root study demonstrated the presence of an oxic rhizosphere
- O_2 flux to roots responds to wind, photosynthesis and root age (inset).

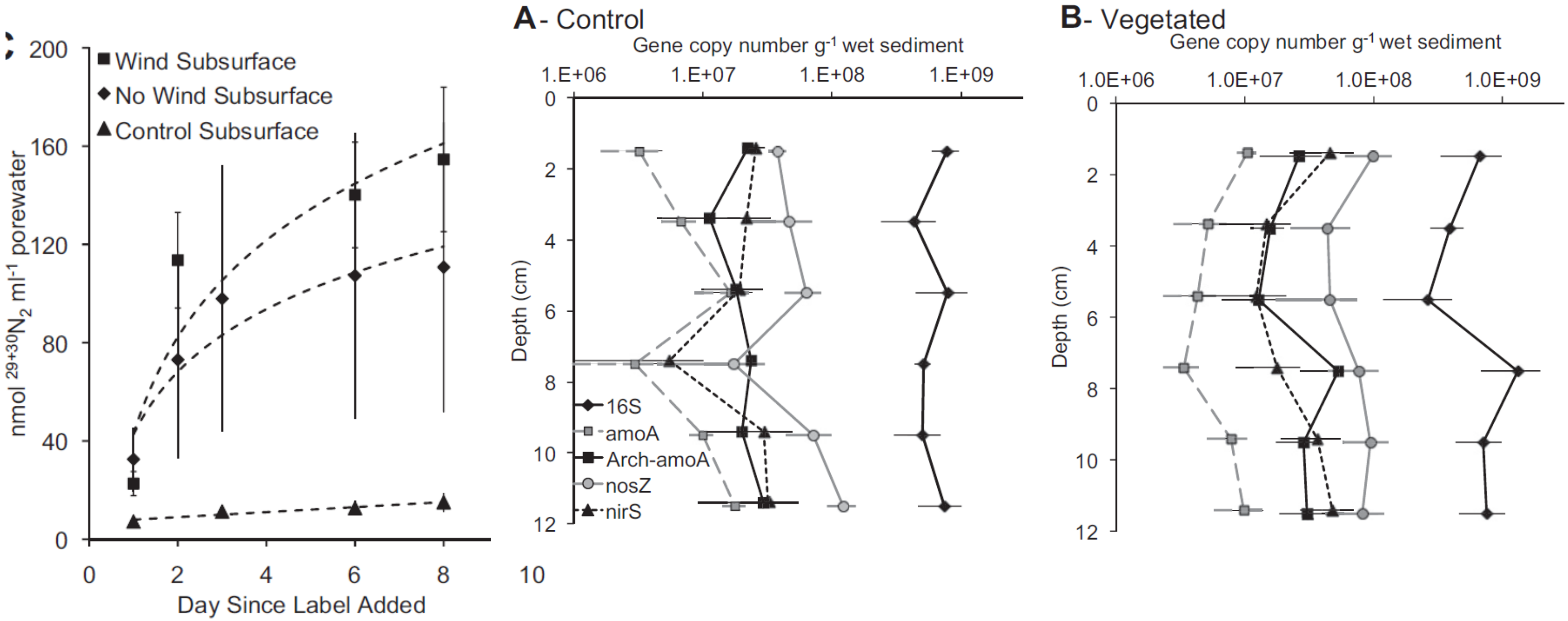
Rhizosphere Coupling of Nitrification/Denitrification



Source: Penton, et al., 2014

- Increase in $^{29+30}N_2$ mirrors light effect on O_2 in arenchyma and sediment.
- In the presence of light and wind significantly enhances rhizosphere coupling of nitrification and denitrification in sediment of vegetated cores
- Arenchyma not only transports O_2 to the rhizosphere, but also acts as a conduit for N_2 from denitrification

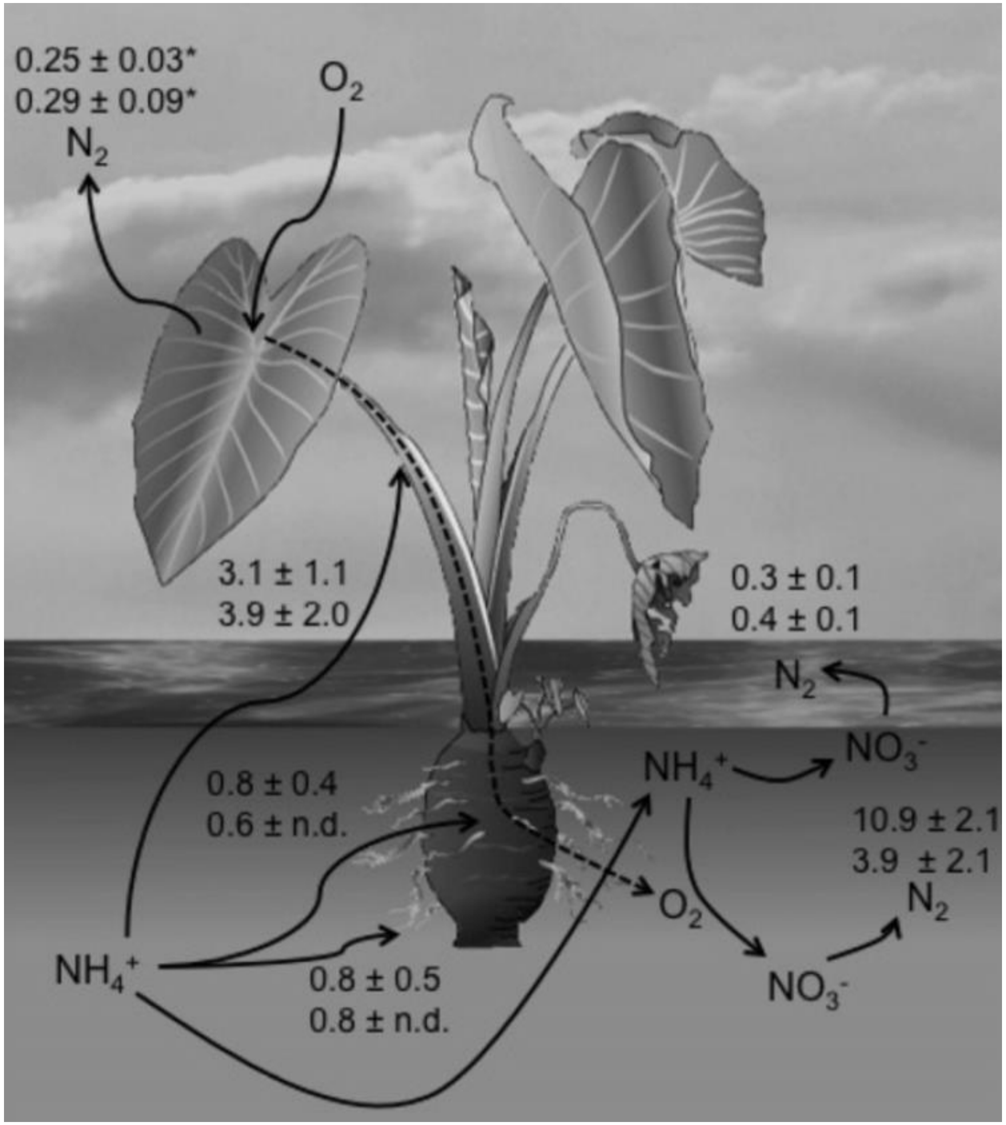
Rhizosphere Coupling of Nitrification/Denitrification



Logarithmic increase in $^{29+30}N_2$ in the vegetated cores illustrates that added $^{15}NH_4^+$ is lost immediately following fertilization.

- Non-vegetated and vegetated sediments showed high populations of nitrifying and denitrifying bacteria
- Taro sediments poised for denitrification provided oxic/anoxic interface present

N Balance



Wind Treatment

Assimilation: 24.1%

Subsurface loss: 67.5%

Surface H_2O: 1.86%

Arenchyma: 1.55%

No Wind Treatment

Assimilation: 47.5%

Subsurface loss: 39.4%

Surface H_2O: 4.04%

Arenchyma: 2.90%

Significance

- Novel use of a $^{15}NH_4^+$ intact whole core method to trace N fate in a large emergent macrophyte under flooded conditions.
- Study demonstrated the overwhelming importance of rhizosphere coupling of nitrification/denitrification in subsurface suggesting that subsurface application of NH_4^+ may not improve fertilizer N efficiency
- Large pulses of NH_4^+ should be avoided

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