Abstract - summary

- Eutrophication - effects of ammonia and nitrate
- Salicornia depressa
- Roots first, then flower tissue

Introduction - what is it and why do we care?

- Urban areas have fertilizer and sewage runoff
- How does this affect plants in marsh areas?
    - Look at differences in gene expression
        - Between control
        - Between each source
    - See functional differences?
- Most studies focus on the effect of ammonia because that's what's in fertilizers. However, the most common nitrogen containing compound in coastal waters is nitrate. (Johnson et al.)
    - Where is nitrate coming from?
        - Coastal flooding
- Greater effect in low marsh because greater contact with water; weakest effect in high marsh because high marsh receives lowest amount of tidal water (Johnson et al.)
    - Salicornia is a high marsh plant, so it may be less affected by nitrate because it is high marsh. May be more affected by ammonia because it is more exposed to rainwater runoff
    - Potentially increases above-ground biomass (shoots) and decreases below-ground biomass (roots)
- Salicornia store Na+ in above ground shoots
- NaCl stimulates growth (Octavio R. Salazar et al.)
    - Look at genes SOS1, HKT1, and NHX2

Hypotheses - based on previous work, what do we think will happen?

- Roots are longer and thinner when grown with ammonia only
    - Ammonia is a "immobile" form of nitrogen (Bloom et al, 2003)
    - NH4 accelerates cell division
- Roots grow more laterally when grown with nitrate
    - "More nourished"
    -

Methods

- Experimental

- Coding

Citations

- Avinash Sreedasyam, Et al. JGI Plant Gene Atlas: an updateable transcriptome resource to improve functional gene descriptions across the plant kingdom, *Nucleic Acids Research*, Volume 51, Issue 16, 8 September 2023, Pages 8383–8401, https://doi.org/10.1093/nar/gkad616
  Saltmarsh plant responses to eutrophication - Johnson - 2016 - Ecological Applications - Wiley Online Library

- Johnson, D. S., Warren, R. S., Deegan, L. A., & Mozdzer, T. J. (2016). Saltmarsh plant responses to eutrophication. *Ecological Applications*, *26*(8), 2649–2661. https://doi.org/10.1002/eap.1402
- Salazar, O.R., Chen, K., Melino, V.J. *et al.* SOS1 tonoplast neo-localization and the RGG protein SALTY are important in the extreme salinity tolerance of *Salicornia bigelovii*. *Nat Commun* 15, 4279 (2024). https://doi.org/10.1038/s41467-024-48595-5

Key skill set we are learning
Analysis of the pipeline using unix command line tools

Checked the quality to see whether the reads are good or bad

Trimmed off the beginning and end of the read the quality, a lot was not trimmed. Anything not certain were trimmed. The sequencing itself was done in ilumina to do that rna adaptors are sticking up on it, salicornia rna can stick to it and it actually starts sequencing, the adaptor sequence that connects to the plate gets sequenced by mistake because they are not part of the plants
If we find the sequence in the data then we take it off

Aligned the samples to genome so it is Easier for computer to read.

Converted to binary files (sam to bam files) so they are smaller
We sort them so the files get condensed even more
Then we indexed them

Count the expression of each gene, r to make the pretty graph and do statistics

Jeny's notes:

Summary of "Root growth as a function of ammonium and nitrate in the root zone" by Bloom, Jackson, & Smart:

Objective

The study examines how different concentrations of ammonium (NH_4^+) and nitrate (NO_3^-), two major forms of inorganic nitrogen, affect root growth and development in tomatoes. It considers both field-grown tomatoes and those cultivated in controlled solution cultures.

Key Findings

1. **Field Experiments**:
    - **Root Growth and Soil Nitrogen Levels**:
        - Root biomass increased in soil zones with moderate nitrogen levels (2 μg NH_4^+-N g^{-1} soil and 6 μg NO_3^--N g^{-1} soil).
        - Root growth decreased in zones with either lower or higher nitrogen levels.
    - **Root Branching**:
        - Root branching was not significantly influenced by soil nitrogen levels, suggesting that root architecture is relatively insensitive to available mineral nitrogen.
2. **Solution Culture Experiments**:
    - Root systems were larger under NH_4^+ nutrition compared to NO_3^- nutrition, despite shoot biomass being similar in both cases.
    - High concentrations of NH_4^+ (100 mmol m^{-3}) promoted greater root biomass, length, and area compared to equivalent or higher levels of NO_3^-.
3. **Energy Efficiency**:
    - NH_4^+ acquisition required less energy than NO_3^-, allowing for more extensive root development under NH_4^+ nutrition.
    - NO_3^- assimilation consumed a larger portion of photosynthetic energy, possibly limiting carbohydrate availability for root growth.
4. **Soil Nitrogen and pH Effects**:
    - NH_4^+ absorption acidifies the rhizosphere, potentially enhancing root growth by increasing cell wall extensibility.
    - NO_3^- absorption alkalinizes the rhizosphere, which may limit root growth.
5. **Functional Equilibrium Hypothesis**:
    - Root growth is influenced by a balance between nitrogen supply and carbohydrate availability, aligning with the "functional equilibrium" concept where root and shoot growth are interdependent.

Conclusions

- Optimal root growth occurs at intermediate soil nitrogen levels, with NH_4^+ generally supporting more robust root systems than NO_3^-.
- Local soil conditions, particularly nitrogen form and concentration, are critical in determining root system development.
- The balance of energy and pH effects between NH_4^+ and NO_3^- plays a key role in mediating root growth responses.

This study reconciles differences between agricultural observations (where high nitrogen inhibits root growth) and ecological studies (where nitrogen stimulates root proliferation). It highlights the importance of understanding localized soil nitrogen dynamics to optimize plant growth and root development.

SOS1 tonoplast neo-localization and the RGG protein SALTY are important in the extreme salinity tolerance of Salicornia bigelovii

The study titled "SOS1 tonoplast neo-localization and the RGG protein SALTY are essential for extreme Na^+ accumulation in Salicornia" investigates the mechanisms enabling Salicornia plants to thrive in highly saline environments. Salicornia species can accumulate high concentrations of sodium ions (Na^+) in their succulent shoots without suffering from ion toxicity. The research identifies two critical components facilitating this salt tolerance:

1. **SOS1 Tonoplast Neo-localization**: SOS1, traditionally known as a plasma membrane Na^+/H^+ antiporter, is found to relocate to the tonoplast (vacuolar membrane) in Salicornia. This neo-localization suggests a unique adaptation allowing efficient sequestration of Na^+ into vacuoles, thereby reducing cytosolic Na^+ toxicity.
2. **RGG Protein SALTY**: The study identifies a novel RGG domain-containing protein named SALTY, which is essential for the extreme Na^+ accumulation observed in Salicornia. SALTY appears to interact with the SOS1 transporter, facilitating its function and stability at the tonoplast.

These findings provide valuable insights into the molecular adaptations of halophytes like Salicornia, offering potential strategies for engineering salt tolerance in crop plants

Plant Gene Atlas

The JGI Plant Gene Atlas is an updatable transcriptome resource developed through a multi-laboratory collaboration, adhering to standardized growth, sequencing, and data analysis protocols for several evolutionarily diverse JGI flagship plant species. This resource provides insights into transcriptional changes across various plant genomes by maintaining consistent tissues and conditions. It also significantly enhances JGI's genome annotation efforts by accurately cataloging gene expression profiles.

Plant Gene Atlas

Currently, the Atlas comprises over 2,000 RNA-seq samples from seventeen JGI plant flagship genomes, including:

- *Chlamydomonas reinhardtii* (algal model)
- *Physcomitrella patens* (moss model)
- *Arabidopsis thaliana* (model for plant genetics and biology)
- *Sphagnum angustifolium* (flat-topped bogmoss)
- *Glycine max* (legume model and crop plant)
- *Medicago truncatula* (forage legume model)
- *Populus trichocarpa* (woody perennial biomass crop)
- *Eucalyptus grandis* (biomass energy crop)
- *Brachypodium distachyon* (a C3 grass model)
- *Panicum virgatum* (herbaceous perennial crop)
- *Panicum hallii var. filipes* (grass model)
- *Panicum hallii var. hallii* (grass model)
- *Setaria italica* (grain and forage crop)
- *Setaria viridis* (a C4 grass model)
- *Sorghum bicolor* (a C4 grass bioenergy crop)
- *Sorghum bicolor Rio* (sweet sorghum, Rio)
- *Kalanchoe fedtschenkoi* (model for Crassulacean acid metabolism photosynthesis)

For each species, samples were collected from coordinated studies encompassing developmental stages, abiotic stress conditions, and a common comparative condition focused on nitrogen metabolism. These comprehensive RNA-seq datasets facilitate comparisons of gene expression within a plant and among orthologous gene sets across diverse plant genomes.

Plant Gene Atlas

The Atlas is accessible online, providing a hub for data access, including expression viewers, genome browsers, and data downloads. Users are encouraged to adhere to the data usage policy and cite the resource appropriately in publications

JGI Plant Gene Atlas: an updateable transcriptome resource to improve functional gene descriptions across the plant kingdom

The JGI Plant Gene Atlas is a dynamic transcriptome resource aiming to enhance functional gene descriptions across the plant kingdom. It spans 18 diverse plant species, offering comprehensive RNA sequencing data to address knowledge gaps in plant functional genomics. Key points include:

1. Data Collection and Coverage: The resource contains over 2,090 RNA-seq samples and 15.4 trillion RNA bases. It includes flagship genomes and model organisms like Arabidopsis thaliana, Eucalyptus grandis, and Sorghum bicolor.
2. Standardized Protocols: Experimental conditions (e.g., light, temperature, and nitrogen regimes) are standardized to ensure consistent data quality and comparability.
3. Functional Annotation Improvements: Through co-expression analysis, differential gene expression studies, and ortholog-based comparisons, over 64,000 genes without prior functional descriptions now have additional biological information.
4. Conserved and Tissue-Specific Patterns: Expression profiles reveal conserved transcriptional patterns and tissue-specific expression, facilitating insights into plant regulatory mechanisms and evolution.
5. Tools and Accessibility: The Gene Atlas is accessible via its dedicated portal and Phytozome, supporting data visualization, differential expression analysis, and co-expression clustering.

This resource aims to integrate phylogenetic, environmental, and developmental insights, enabling advancements in plant functional genomics and improving tools for crop improvement and ecological studies.

Root Development and Absorption of Ammonium and Nitrate from the Rhizosphere

The study titled "Root Development and Absorption of Ammonium and Nitrate from the Rhizosphere" by Bloom et al. (2003) examines how plant roots respond to the availability of ammonium (NH_4^+) and nitrate (NO_3^-) in the soil. Key findings include:

- Root Growth Response: Roots tend to proliferate in soil zones rich in nitrogen. Notably, root density and extension are greater in nutrient solutions containing ammonium compared to those with nitrate as the sole nitrogen source.

- Rhizosphere pH Alteration: The absorption of NH_4^+ and NO_3^- by roots alters the rhizosphere's pH and redox potential. These changes, in turn, regulate root cell proliferation and mechanical properties, influencing overall root development.

Nitrogen Acquisition and Root Development: Nitrogen required for cell division and expansion primarily comes from the root apex absorbing NH_4^+ and NO_3^- from the rhizosphere. This localized nitrogen uptake is crucial for sustaining root growth and development.

The study highlights the complex interactions between nitrogen forms in the soil and root developmental processes, emphasizing the importance of localized nitrogen availability in shaping root architecture.

Summary of the Article: "Root growth as a function of ammonium and nitrate in the root zone"

Objective

The study examines the effects of soil ammonium (NH_4^+) and nitrate (NO_3^-) levels on root growth in field-grown tomatoes and in controlled solution culture experiments. It seeks to understand the interplay between nitrogen availability and root development.

Key Findings

Field Experiment Results:

Root Distribution:

Roots were sparse in soil zones with low nitrogen levels.

Root biomass peaked at intermediate levels of soil nitrogen: 2 μg NH_4^+-N g^{-1} soil and 6 μg NO_3^--N g^{-1} soil.

Excessive levels of NH_4^+ or NO_3^- reduced root growth.

Root Branching:

Root branching was relatively insensitive to soil nitrogen concentrations, remaining uniform across zones.

Solution Culture Results:

NH_4^+ vs. NO_3^- Nutrition:

Roots exhibited greater biomass, length, and area under NH_4^+ nutrition compared to NO_3^-, despite similar shoot biomass in both conditions.

Growth Response:

Root parameters, including length and branching, were enhanced at moderate nitrogen concentrations but inhibited at higher concentrations, especially for NO_3^-.

Energy and pH Effects:

NH_4^+ absorption was less energy-intensive than NO_3^-, allowing more energy for root growth.

NH_4^+ absorption acidified the rhizosphere, enhancing root cell wall extensibility, while NO_3^- absorption alkalinized it, reducing growth.

1. Functional Equilibrium Hypothesis:
    - Root growth depends on a balance between nitrogen supply and carbohydrate availability from shoots.
    - High nitrogen levels can lead to local carbohydrate shortages, limiting root development in those zones.

Conclusions

- Optimal root growth in tomatoes occurs at moderate levels of NH_4^+ and NO_3^-, with NH_4^+ being more beneficial than NO_3^- for root system development.
- Localized nitrogen availability and root-zone pH play critical roles in regulating root architecture.
- The findings reconcile prior discrepancies in observations from agricultural and ecological studies on nitrogen's role in root growth.

This study highlights the importance of managing soil nitrogen levels to optimize root growth, which has implications for crop management and sustainable agriculture.