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Examination of Biological Nitrification Inhibition by a Fine Fescue Root Exudate

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AOB synthetic inhibitors

Dicyandiamide (DCD)

3,4-Dimethylpyrazole phosphate (DMPP)

Nitrapyrin

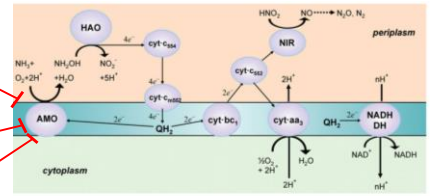


FIGURE 2. Metabolic mechanism and electron transfer pathway of ammonia oxidation by AOB (based on Arp and Stein, 2003). AMO = ammonia monoxygenase; HAO = hydroxylamine oxidoreductase; cyt-bc1; cyt-c_{552} = cytochrome c_{552}; cyt-c_{554} = cytochrome c_{554}; cyt-aa_3 = cytochrome aa_3; cyt-c_{m552} = membrane cytochrome c552; NIR = nitrite reductase. (Color figure available online).

Guo et al. (2013)

Nitrogen losses in perennial grass systems

Occur when fertilization and irrigation results in potential nitrate (NO_3^-) loss and nitrogen gas (N_2O and N_2) production.

Urbanization will continuously increase (79% by 2025) replacing natural or agricultural ecosystems by perennial grass systems.

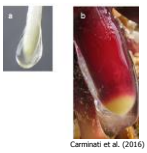
=>Need for innovative solutions to mitigate future nitrogen losses in perennial grass systems

Bremer D. (2006)

Rhizodeposition

Ability by living plants roots to release carbon compounds
→ facilitating plant-microbe communication

Mucilage



Carminati et al. (2016)

Polysaccharide, protein, phospholipid
Protection from toxic metal and drought

Root exudates

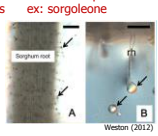
Major source of soil organic C
Amino acids, organic acids, sugars, phenolics and secondary metabolites

Symbiosis interaction

Manage pathogens

Reduce competition ex: sorgoleone

Inhibition of nitrication (**BNI**) ex: sorgoleone

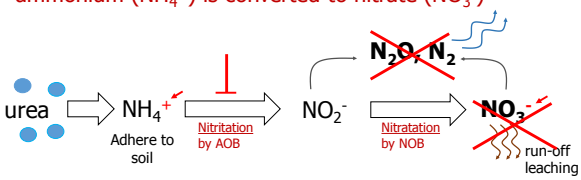


Weston (2012)

Haichar et al. (2014)

Nitrogen losses occur through nitrification

Nitrification is a two-step process during which ammonium (NH_4^+) is converted to nitrate (NO_3^-)



AOB abundance regulates nitrification
Inhibiting AOB → ↓ nitrogen losses

Biological Nitrification Inhibition

Biological Nitrification Inhibition (BNI) is the natural ability of plants to release chemical substances from their roots that have a suppressing effect on nitrifier activity and soil nitrification.

Subbarao et al., (2006)

Benefit of BNI versus synthetic inhibitors

Synthetic nitrification inhibitors have been extensively used but present several drawbacks:

application (labor)
cost
degradation

1. BNI are produced and secreted into the soil (↓ cost)
2. BNI have long lasting effect (↓ cost)
3. BNI are locally produced (↑ efficiency)
4. BNI are already found in different monocotyledons

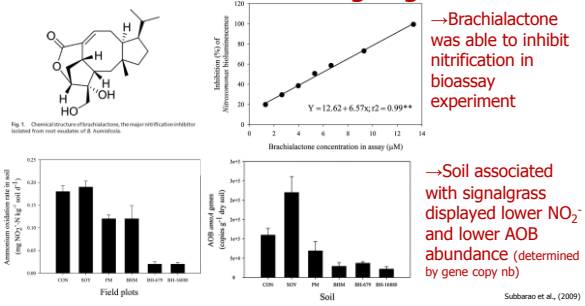
What about fine fescue species ?

5 different species : hard fescue, strong creeping red fescue, slender creeping red fescue, Chewings fescue and sheep fescue

Low input: ↘ mowing
↘ irrigation
↘ fertilization

Objective: Determine if low input fine fescue species have the ability to reduce nitrification through BNI.

BNI first found in signalgrass



→Brachialactone was able to inhibit nitrification in bioassay experiment

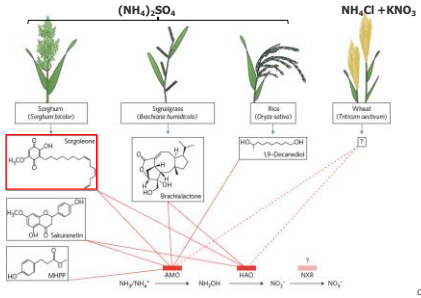
→Soil associated with signalgrass displayed lower NO_2^- and lower AOB abundance (determined by gene copy nb)

Subbarao et al., (2009)

Research questions

1. Can fine fescue species suppress nitrification in their rhizosphere?
2. What is the compound produced and secreted by fine fescue species leading to putative nitrification inhibition?

BNI are found in other monocotyledons



Coskun et al. (2017)

Research question

1. Can fine fescue species suppress nitrification in their rhizosphere?

Rhizosphere experiment

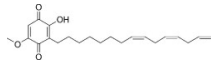
Experimental design:

Plants were seeded in cones with top soil

- Negative control: bulk soil (no inhibition of nitrification)
- Positive control: sorghum (presence of nitrification inhibition)
- Fine fescue sp. tested: "Chantilly"(STCR) and "Radar" (CHF)

Possible BNI compound in fine fescue species

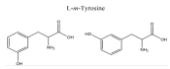
Sorgoleone



→ Produced by sorghum
→ Phyto-allelopathic
→ Hydrophobic
→ BNI activity

Dayan et al. (2010)
Subbarao et al. (2013)

L-*m*-tyrosine

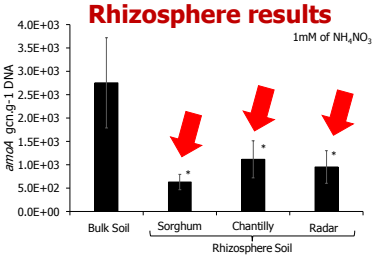


→ Produced by fine fescue sp
→ Phyto-allelopathic
→ Non-polar (hydrophobic)
→ Toxic to bacteria

➡ Potential BNI candidate for fine fescue species

Credit Dr. Dominic Petrella/ Bertin et al. (2009)

Rhizosphere results



→ Sorghum, Chantilly and Radar possess similar AOB abundance suggesting that STRC and CHF could inhibit nitrification in their rhizosphere

L-m-tyrosine is toxic to *Bacillus* species

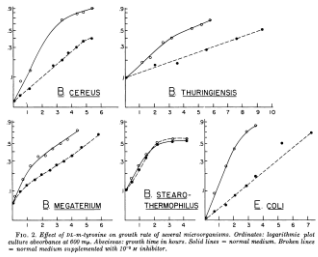


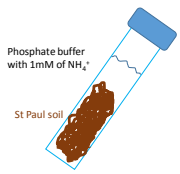
FIG. 2. *Effect of DL-m-tyrosine on growth rate of several microorganisms. Ordinates: logarithmic plot cultures absorbance at 650 mμ. Abscissae: growth time in hours. Solid lines = normal medium. Broken lines = normal medium supplemented with 10^{-4} M inhibitor.*

Aronson and Wermus. (1965)

Research question

2. What is the compound produced and secreted by fine fescue species leading to putative nitrification inhibition?

Soil-slurry experiment with L-*m*-tyrosine



Soil slurry experiments are designed to test the soil potential nitrification

Modified protocol from Hart et al. (1994)

Experimental design:

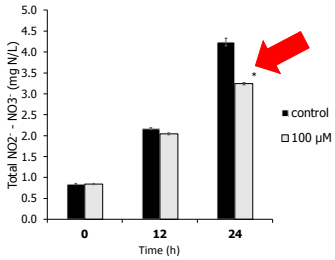
Soil only (3 replicates)
Soil containing 100 µM of L-*m*-tyrosine (3 replicates)

Soil solution filtered 12 hours and 24 hours after addition of the buffer solution

Solution analyzed for total NO_2^- - NO_3^- using a flow-trough injection Lachat

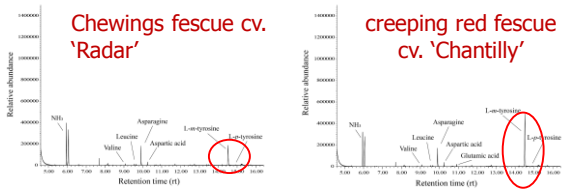
Hypothesis: soil containing L-*m*-tyrosine will show lower amount of total NO_2^- - NO_3^-

L-*m*-tyrosine reduced nitrification



Is this reduction in nitrification due to L-*m*-tyrosine or due to a toxicity effect of tyrosine?

L-*m*-tyrosine is present in the roots of the tested cultivars



Credit Dr. Dominic Petrella

Soil-slurry experiment with L-*m*-tyrosine and tyrosine

Experimental design:
Soil only (3 replicates)
Soil containing 1µM, 10µM, 100µM and 1mM of L-*m*-tyrosine and tyrosine (3 replicates)

Soil solution filtered 24 hours after addition of the buffer solution

Solution analyzed for total NO_2^- - NO_3^- using a flow-trough injection Lachat

Hypothesis: only soil containing L-*m*-tyrosine will show lower amount of total NO_2^- - NO_3^-

Conclusion and moving forward

Chewings fescue and strong creeping red fescue are able to present lower AOB abundance in their rhizosphere

- Confirm nitrification is reduced in rhizosphere →Soil slurry experiment with rhizosphere soil

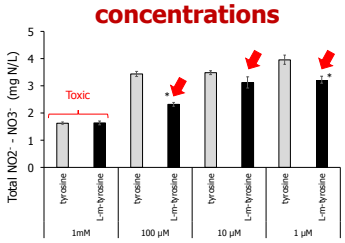
L-*m*-tyrosine may inhibit nitrification

- Confirm L-*m*-tyrosine inhibits AOB →bioassay experiment
- Is L-*m* tyrosine the (unique) rhizosphere inhibitor? →bioassay experiment

Is L-*m*-tyrosine production either ammonium only or ammonium and nitrate dependent?

- Confirm and quantify L-*m*-tyrosine secretion by cv. Radar and Chantilly grown with various source of N

Tyrosine affects nitrification only at very high concentrations



→L-*m*-tyrosine is an inhibitor of nitrification

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Thank you for your attention