Buried traits in the rhizosphere: crop control of nitrification

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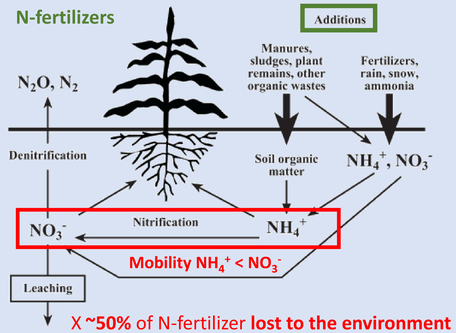
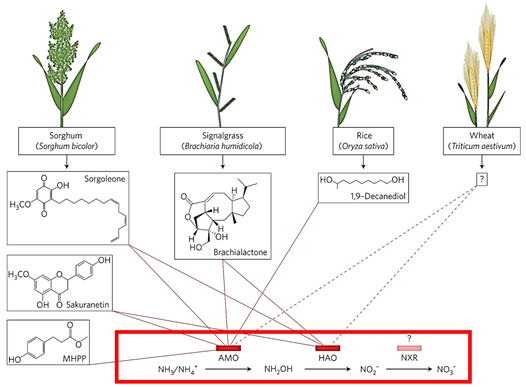
Overview: Biological Nitrification Inhibitors

✓1/2 half the food produced in the world supported by N-fertilizers



X ~50% of N-fertilizer **lost to the environment** by leaching/gaseous emissions

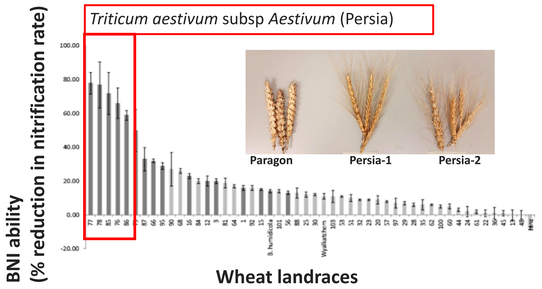


Biological nitrification inhibitors (BNIs) in root exudates reduce nitrification rates [1]**:**

- increasing N retention time in the root zone – mobility NH_4^+<<NO_3^-;
- decreasing fertilizer need and the associated environmental impact;
- providing an alternative to synthetic nitrification inhibitors.

Potential for breeding this trait into modern, elite wheat cultivars.

Historical wheat landraces with higher BNI capacity than equivalent modern wheat cultivars [2]:



Objectives:

- To develop a set of robust, complementary methodologies for rhizosphere phenotyping
- To quantify the variation across wheat landraces in the impact on rhizosphere nitrification rates;
- To link rhizosphere nitrification rates with trait loci in wheat landraces.

References:
1. Coskun *et al*. 2017 Nat. Plants 3: 17074
2. O'Sullivan *et al*. 2016 Plant Soil 404:61-74
3. Winfield *et al*. 2018 Plant Biotechnol. J. 16:165–175
https://www.seedstor.ac.uk/search-browsecollections.php
5. Cai *et al*. 2016 Anal. Chem. 89: 1178-1184
6. Kautsar *et al*. 2017 Nucleic Acids Res. 3;45:W55-W63

Phase I: Optimization of methodologies to measure nitrification rate

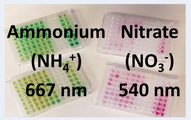
Watkins Historic Collection of Landrace Wheats
Wheat genetic diversity before the onset of modern breeding practices [3].
Core collection: 120 Watkins accessions [4].

Rhizosphere soil

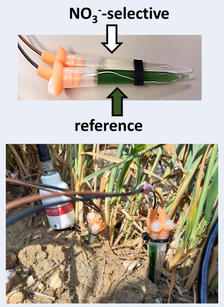
Chemical methods

1. Spectrophotometric assay:

- **KCl soil extraction**
- **soil pore water**
- **Bioavailable N-NO_3^-:** diffusive gels with (NO_3^-) – selective resins



2. Nitrate (NO_3^-) – selective sensors

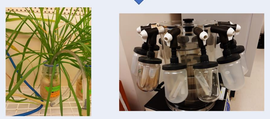


Biological methods

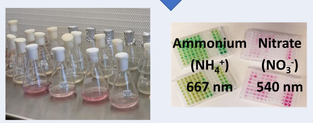
3. Bioassay:

Measurement of inhibitory effect of root exudates and root tissue in nitrification: rate of nitrifying bacteria [2]:

Selected wheat landraces: hydroponics/field



Collection of root exudates and root tissue
1 h 1mM NH_4^+ – 24 h water – freeze-dried



Nitrate production rate of *Nitrosomonas europaea* and *Nitrosospira multiforme* *cultures in the presence of root exudate/tissue*

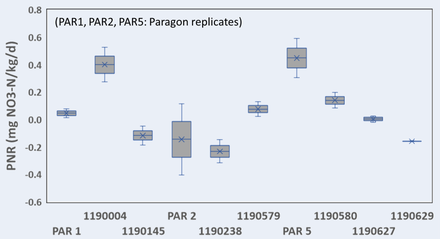


Figure 1. Potential nitrification rate (PNR) for rhizosphere soil collected from 7 historical wheat landraces [4] and a modern cultivar (Paragon) following addition of 100 mg N-NH_4 / kg soil.

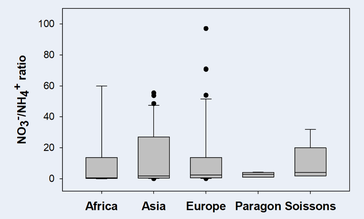


Figure 2. Ratio of NO_3^-/NH_4^+ for rhizosphere soil collected from 90 historical wheat landraces [4] and two modern cultivars (Paragon and Soissons) after harvest.

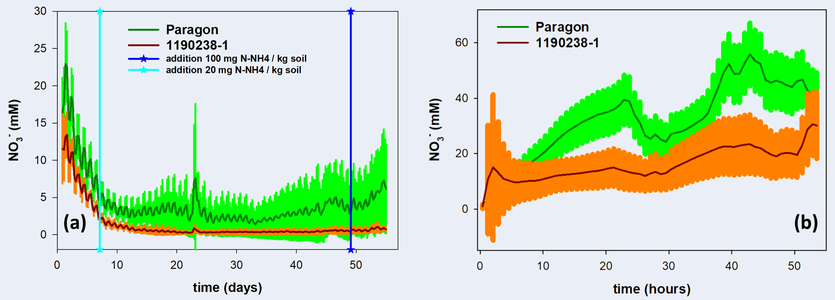


Figure 3. Data collected from nitrate (NO_3^-) selective sensors for rhizospheres of a modern wheat cultivar (Paragon) and an historical landrace (1190238) grown in greenhouse (a) or in the field (b). Data variability is partly associated to the distance of the sensor to the rhizosphere area.

Preliminary results suggest that growing some of the historical wheat landraces can result in a decrease in nitrification rates in the soil rhizosphere. High variability is reported for geographic origin and replicates of modern cultivars.

Phase II

i) Genome mining using the plantiSMASH platform [6] to identify genes clusters involved in BNIs biosynthesis pathways;
ii) Linkage analysis to identify trait loci (QTL) associated with BNI activity and ultimately identify potential breeding targets for BNI's [3]

in progress …

Phenotyping population of 88 individuals of the wheat landrace candidate in a field experiment. The target trait, decrease of nitrification rate in rhizosphere soil, is being phenotyped for inhibition of nitrification by *N. europea* and pool of bioavailable NO_3 using selective resins.

Acknowledgements:
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