Assessing Pathways to Potential N_2O Emissions in Different Land Use Systems Using Targeted Pyrotag, Whole Metagenome and Soil Assay Approaches

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Introduction

- Assessing how microbial communities change under different land management practices is central to understanding how land use affects biogeochemical cycles.
- The field of soil molecular and metagenomic analysis continues to advance rapidly. This has opened doors for more detailed study, but also a need for robust methods.
- We compared a suite of methods across different study sites to address fundamental questions about the ecology of soil denitrification.

Types of denitrification

Heterotrophic denitrification

nirK

$NO_3^- \longrightarrow NO_2^- \longrightarrow N_2O \dashrightarrow N_2$

Autotrophic denitrification by ammonia oxidizing bacteria (AOBs)

$NH_4^+ \xrightarrow{O_2,\ H_2O} NH_2OH \longrightarrow NO_2^- \xrightarrow{nirK} N_2O$

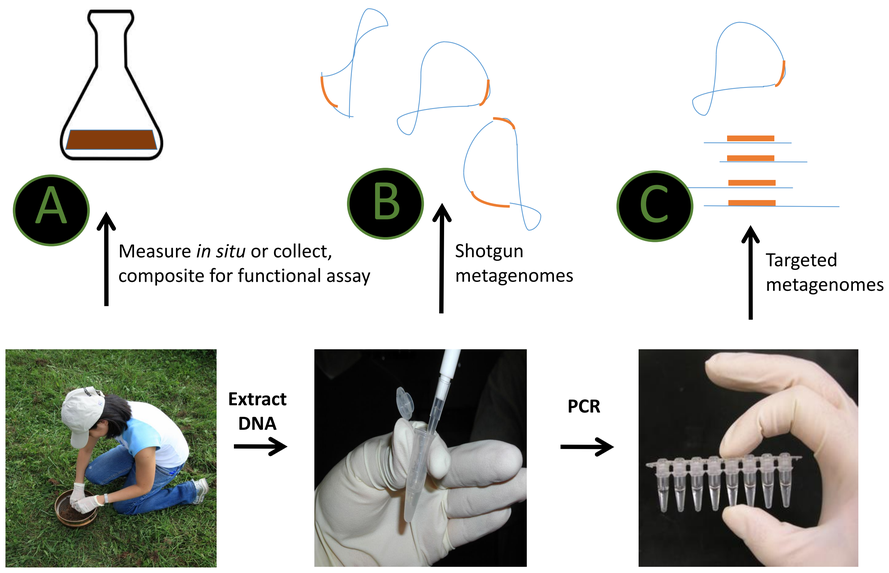
Conditions	Function
Anoxic	Energetics
Oxic	Nitrite detox.

In denitrification, oxidized forms of nitrogen (N) are reduced to gaseous forms like N_2O (a potent greenhouse gas) and N_2. Two groups of bacteria carry out denitrification, under distinct conditions and for different reasons. Both groups share similar pathways such as a nitrite reductase gene (*nirK*) which can be distinguished using molecular approaches.

Summary

- Assays for soil denitrification along with shotgun and targeted approaches demonstrate how land use reshapes the functional potential of the denitrifier community.
- Agriculture enriches for genes in the denitrification pathway which remain into early succession. Autotrophic denitrification by AOBs are associated with highest N_2O fluxes.

Methods



Methodological approaches: A) assess N_2O gas flux in the field or with targeted assays for nitrogen mineralization or denitrification potential. B) Extract soil DNA and shotgun sequence entire genome for analysis. C) Selectively amplify genes of interest (nirK) for targeted analysis

Study Sites: Related methodological approaches taken on two experimental gradients at the Kellogg Biological Station - LTER, Hickory Corners, MI.

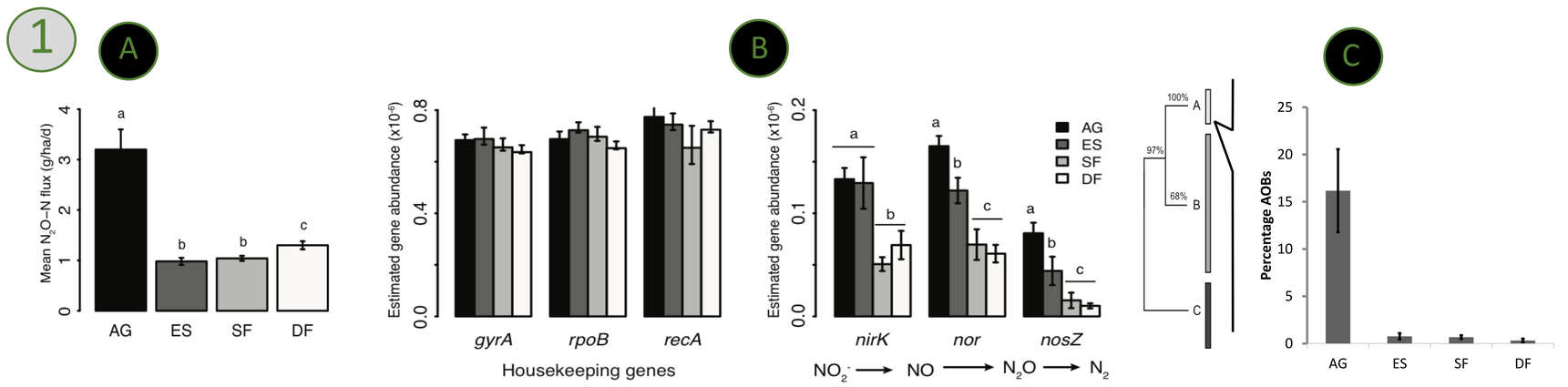
1 Agricultural and successional gradient

- AG – **Conventional row crop agriculture**
- ES – **Early successional field**
- SF – **Successional forest**
- DF – **Deciduous forest**

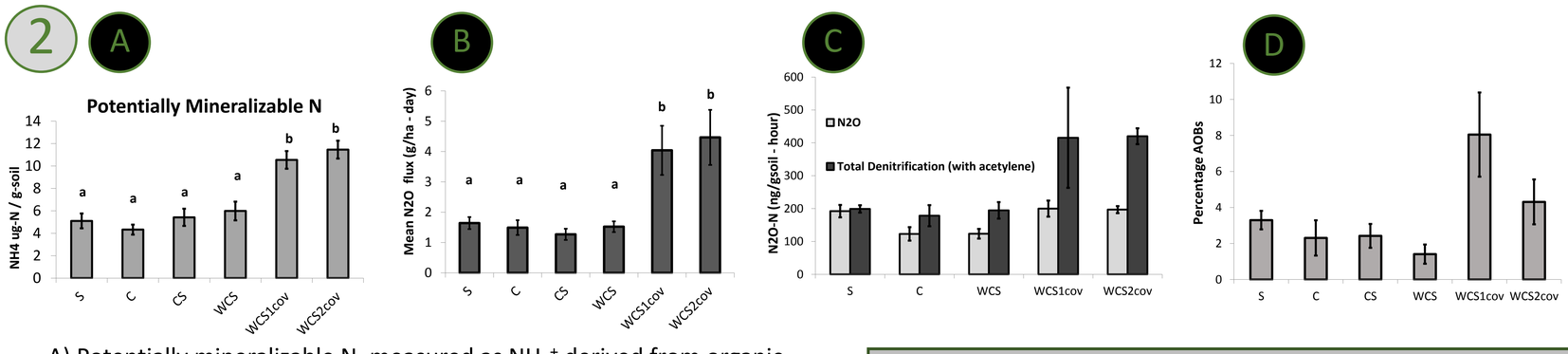
2 Cropping diversity – no external inputs over 15 years

- **C – continuous corn**
- **S – continuous soy**
- **CS – corn and soy rotation**
- **WCS - wheat corn soy rotation (w-c-s)**
- **WCS1cov – w-c-s with clover cover crop**
- **WCS2cov – w-c-s with rye and clover covers**

Results



A) Field N_2O gas fluxes from different land use types. B) Annotated genes from shotgun metagenome. Housekeeping genes normalized to genome size show similar total numbers of bacterial cells, whereas denitrification genes are over-represented in agricultural and early successional fields. C) Targeted *nirK* genes a larger proportion of *nirK* are from AOBs- not heterotrophic denitrifiers - in the agricultural site.



A) Potentially mineralizable N, measured as NH_4^+ derived from organic matter breaking down in soil. B) Field N_2O gas flux from cropping diversity gradient C) In the denitrification potential enzyme assay acetylene is used to block the last step in heterotrophic denitrification ($N_2O \rightarrow N_2$), the result indicates a larger proportion of nitrogen gas release as di-nitrogen under cover cropped treatments. D) Targeted nirK genes: a larger proportion of nirk are from ammonia oxidizing species, not heterotrophic denitrifiers, under cover crops.

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