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Evidence for the Functional Significance of Microbial Diversity among Free-living Diazotrophs in Soils of a Long Term Agricultural Site

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Introduction

Biological N-Fixation by free-living diazotrophs is a globally important process with implications for agriculture. Kennedy & Islam (2001) found that N_2 Fixation by free-living diazotrophs can theoretically account for 50-150 kg N/ha. In addition, Global N_2 fixation by free-living diazotrophs is estimated at 100-290 Tg N/yr in terrestrial systems (Clevland et al. 1999).

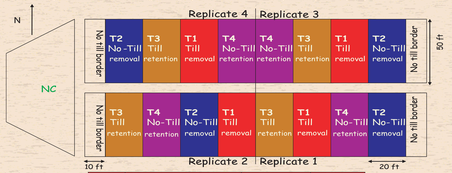
Surveys of *nifH* diversity in soil commonly reveal sequence types that correspond to diverse unidentified diazotrophs. Evidence indicates that these non-cultivated diazotrophs, rather than their cultivated cousins, are the dominant N-fixing organisms in many soil systems.

Agricultural experiments provide an excellent opportunity to study the functional significance of microbial community composition in soil. Long term agricultural experiments are particularly useful for studying microbial community composition and activity with respect to changes in management practice. Both tillage and biomass managements are known to impact the composition and function of the diazotrophic community in soil.

To evaluate whether diazotrophic community composition has functional significance in an agronomic context, we examined the effects of tillage and biomass management on diazotroph community structure, N-fixation, and soil characteristics in a long-term (> 30 years) experimental site in Chazy, Clinton county, NY (44°55.13'N, 73°28.40')

Chazy site

Study site: The William H. Miner Institute in Chazy, Clinton County, New York
The research plots have been maintained as a long term tillage experiment since 1973. Maize (N dependent crop) is the major crop.



T1	Biomass Removal	Till	Tnb
T2	Biomass Removal	NoTill	NTnb
T3	Biomass Retention	Till	Tb
T4	Biomass Retention	NoTill	NTb
NC	Non cultivated		

Results from previous research (Hsu and Buckley, 2009) indicates that:

- Diazotoph diversity was reduced by the long term impacts of agricultural management.
- Within long term agricultural treatments, tillage effects were found to dominate most soil characteristics, but biomass management practices were found to have the largest impact on diazotrophic community composition and N-fixation rates: biomass retention depresses both diazotroph diversity and N-fixation rates.
- Results suggest an association between the diversity of diazotrophic community and N-fixation.

Goal:

Determine whether functional redundancy within the diazotrophic community serves as the mechanism linking changes in diazotroph diversity to changes in N-fixation rate that have been observed in our site.

Hypothesis:

Greater diversity in the diazotrophic community will lead to an increase in functional diversity which will buffer N-fixation rates against variation in environmental conditions.

Experimental Designs:

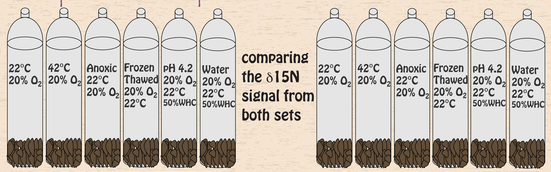
- A cultivation experiment was performed using a range of different N-free media to evaluate whether community diversity corresponds to the diversity of conditions that support the growth of diazotrophs.
- In addition, soil microcosms were exposed to a variety of conditions to determine whether increased diversity in the diazotrophic community is associated with resilience of soil N-fixation to changes in the environment.

Materials &Methods:

Experiment 1

15 soil cores (top 0-5 cm) were taken from each of four field replicates and pooled to represent treatments T2, T4 and NC. N_2 fixation was determined by assessing $^{15}N_2$ incorporation into soil in relation to controls incubated with unlabeled N_2.

Example: NCr1 soil samples



Experiment 2

a) N-free media were used to enumerate different functional classes of diazotrophs by MPN. Growth conditions included differences in carbon sources (C1 malate, C2 cellobiose, C3 sucrose, C4 mannitol, C5 vanillin, and C6 acetate), pH (7.5, 5.5 and 3.5), oxygen levels (20%, 5% and 0%), trace metals (Mo, V and Fe), and temperatures (4, 22, 30, and 42 degree C). All inoculation tests were incubated in 96-wells plates and growth was detected by absorbance at 590 nm after 40 days of incubation.

b) Bacteria were isolated and purified from different growth conditions and characterized by analysis of their 16S rRNA gene sequences.

Table 1 Number of conditions that supported growth of N-fixing bacteria compared across all MPN experiments (20,000 cells/g of soil as cut-off of growth)

	# of growth at 20000 cut off	Sum	Avg	Std	U-test
T4r1	6				
T4r2	8				
T4r3	7				
T4r4	8	29	7.25	0.9574	ns*
T2r1	7				
T2r2	12				
T2r3	9				
T2r4	8	36	9	2.1602	ns
NCr1	12				
NCr2	14				
NCr3	13				
NCr4	11	50	12.5	1.2910	NC>T4

*ns: No siginificant

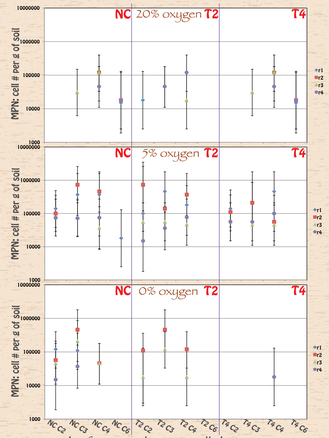


Fig 1 MPN results for growth in C2 cellobiose, C3 sucrose, C4 mannitol and C6 Acetate under 3 levels of oxygen.

Table 2 Numbers of conditions that support growth of N-fixers compared across different levels of oxygen. (20,000 cells/g of soil as cut-off of growth)

	aerobic	microaerobic	anoxic	sum
T4	1	12	0	13
T2	3	12	5	20
NC	5	12	10	27
Fisher exact test	ns*	ns	NC,T2>T4	NC>T4 (p=0.015)

*ns: No siginificant

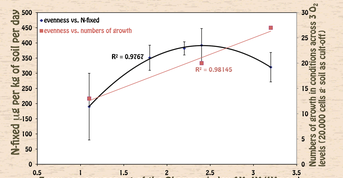


Fig 2 Diazotroph diversity plotted in relation to N-fixation rate and functional diversity.

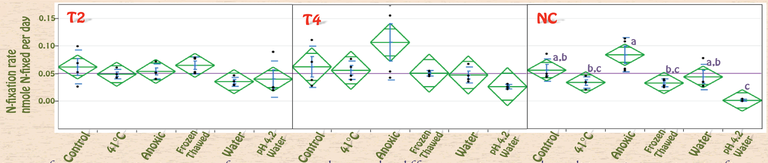


Fig 3 Nitrogen fixation rates in microcosms from T2, T4, and NC under different environmental conditions. (comparing significant effect of conditions within each treatment, p<0.05)

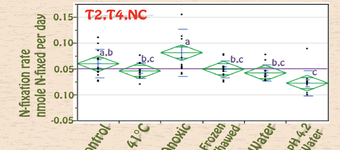


Fig 4 Nitrogen fixation rates in microcosms under 6 different environmental conditions across 3 treatments. (significant p<0.05)

Table 4 ANOVA table for N-fixation rates under conditions within each treatment.

	DF	Sum of Sq	Mean Sq	F-Value	P-Value
NC*6 conditions	5	0.0151	0.0030	8.8243	0.0002
T2*6 conditions	5	0.0028	0.0006	1.2897	0.3116
T4*6 conditions	5	0.0142	0.0028	2.6090	0.0607

Table 3 ANOVA table for N-fixation rate under 3 treatments or 6 inoculation conditions.

	DF	Sum of Sq	Mean Sq	F-Value	P-Value
T2, T4, NC	2	0.0021	0.0011	1.1418	0.3254
6 conditions	5	0.2300	0.0046	6.6677	<0.0001

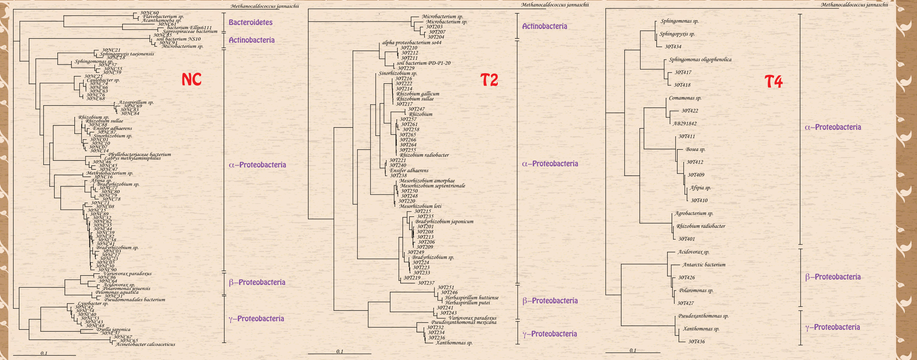


Fig 6 Phylogenetic analyses of bacteria isolated from MPN experiments in different C sources and N-Free media under 30 degree C growth condition.

Conclusions:

- The cultivation experiment showed that more diverse diazotrophic communities could respond to a greater range of growth conditions
- In soil microcosms no relationship was observed between diazotroph diversity and resiliance to environmental perturbation.

Further directions:

- Use *nifH* primers to evalute the isolates and compare to *nifH* clone libraries recovered directly from soil.
- Evaluate the portion of the diazotrophic community that was recovered through cultivation.

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