Microbes and the Nitrogen Cycle

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March 28, 2014

Significance

Malthus: *An Essay on the Principle of Population* (1798)

- Human population increases exponentially
- Agriculture production increases geometrically
- Thus, food is a huge limit on population growth

Haber-Bosch process: 1910

- Atmospheric nitrogen can be converted to bioavailable nitrogen (NH_3)
- Industrial production started 1913
- Half of anthropogenic nitrogen fixation comes from this process (Fowler 2012)

Haber-Bosch

$N_2 + 3H_2 \rightarrow 2NH_3$

- Break triple-bonded N_2 via iron-based catalyst (rate-limiting step)
- With hydrogen in excess, allow formation of NH_3

Led to a huge increase in food security. However…

N is not fully utilized when applied, so excess leaks into environment (see Fowler et al. 2012)

Has definitely contributed to crop yields, but…what else?

N and Soil Microbes

Lots of work done on nitrogen application and plant physiology, community, etc.

Not so much known on microbial end of things…

N and Soil Microbes

Open

The ISME Journal (2012) 6, 1007–1017

www.nature.com/ismej

ORIGINAL ARTICLE

Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients

Noah Fierer[1,2], Christian L Lauber[1], Kelly S Ramirez[2], Jesse Zaneveld[3], Mark A Bradford[4] and Rob Knight[5,6]

Summary

The authors analyzed soil bacteria across three levels of nitrogen (low, medium, high) at two different sites.

- Phylogenetics: who is there?
- Metagenomics: what are they doing?
- Catabolic profiling: what are they eating?

Summary

The authors analyzed soil bacteria across three levels of nitrogen (low, medium, high) at two different sites.

- Phylogenetics: who is there?
- Metagenomics: what are they doing?
- Catabolic profiling: what are they eating?

Why the focus on bacteria?

How does this relate to Prosser and Nichol 2012?

Recall from Prosser & Nicol 2012

"Growth constants of cultivated strains predict that AOA will outcompete AOB if ammonia limitation is the major factor determining specific growth rate. This potentially explains the frequently observed higher abundance of AOA in soil. If cultivated strains are representative of natural communities, the challenge is to explain the presence of AOB in soil."

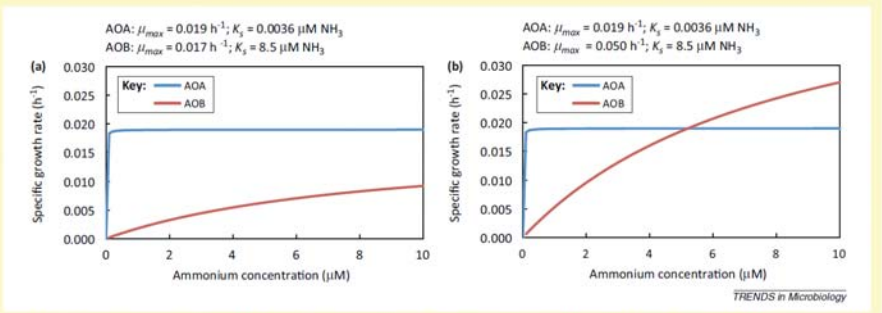
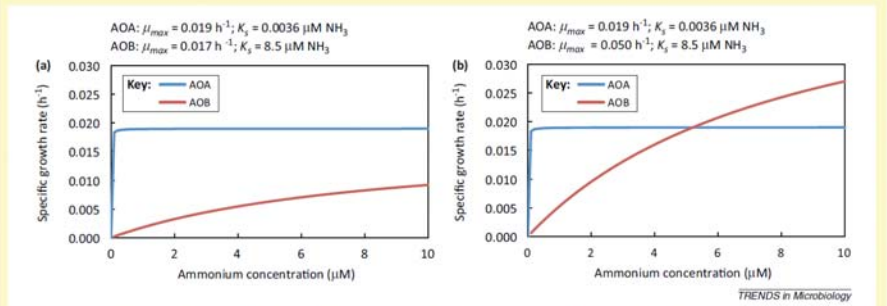


Figure I. The influence of ammonia concentration on specific growth rates of AOA and AOB described by Monod kinetics. Both simulations assumed AOA μ_{max} = 0.019 h^{-1} and K_s values of 0.0036 and 8.5 µM NH_3 for AOA and AOB, respectively. AOB μ_{max} values are 0.017 and 0.050 h^{-1} in (a) and (b), respectively.

Recall from Prosser & Nicol 2012

Thoughts on this?



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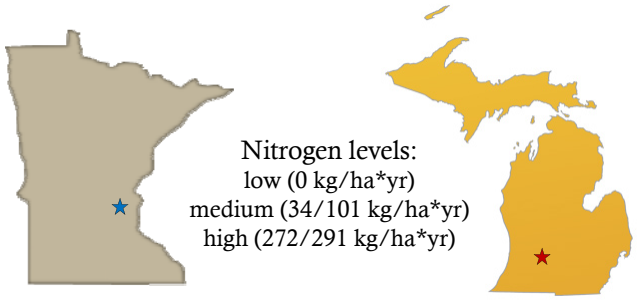
Hypothesis

The amount of nitrogen added to soil will change the structure and function of the microbial community.

Methods

Two field sites, three plots per site:

- Cedar Creek (CC) in Minnesota
- Kellogg Biological Station (KBS) in Michigan



Methods

Phylogenetic analysis

- barcoded pyrosequencing of 16S gene

Metagenomic analysis

- shotgun sequencing via 454
- 518Mbp total data: 1.35M reads, average 384bp/read

Catabolic profiling

- measure CO_2 emissions when soil is incubated with one of 22 various substrates

Methods

Metagenomic analysis: MG-RAST

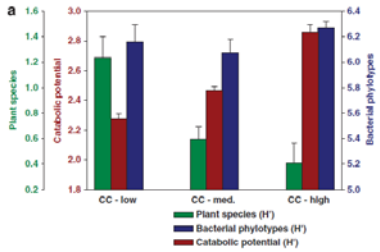
Methods

Any questions about methods?

Figures 1 and 2

Figure 1

CC



KBS

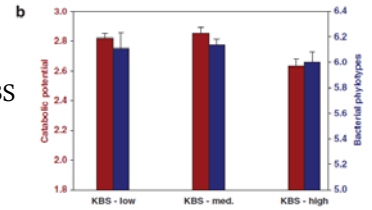
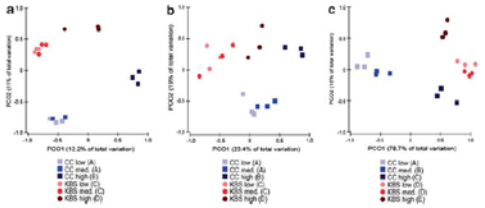


Figure 2

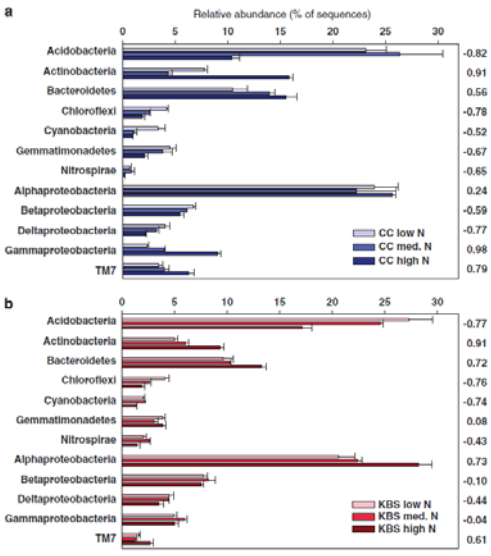


Very <u>general</u> summary of these figures?

Figure 3

CC

KBS



Figures 1-3

What are these 3 figures telling us?

No differences in diversity across nitrogen gradients, though low/medium and high nitrogen groups are distinct.

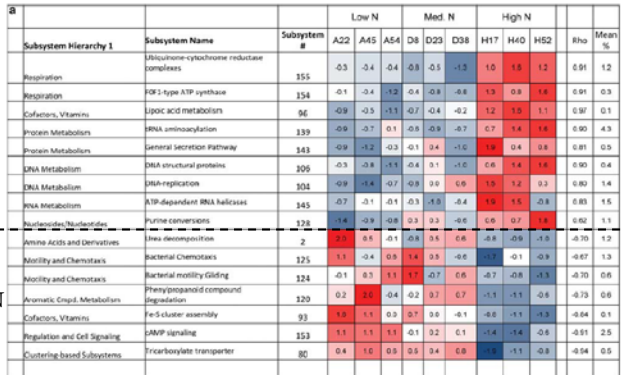
Differences in levels of Proteobacteria, Bacteroidetes, Actinobacteria and Acidobacteria, especially

Figure 4

CC site:

Better in high N conditions

Better in low N conditions



a				Low N			Med. N			High N				
	Subsystem Hierarchy 1	Subsystem Name	Subsystem #	A22	A45	A54	D8	D23	D38	H17	H40	H52	Rho	Mean %
	Respiration	Ubiquinone-cytochrome reductase complexes	155	-0.3	-0.4	-0.4	-0.8	-0.5	-1.2	1.0	1.5	1.2	0.91	1.2
	Respiration	F0F1-type ATP synthase	154	-0.1	-0.4	-1.2	-0.4	-0.8	-0.6	1.3	0.8	1.6	0.91	0.3
	Cofactors, Vitamins	Lipoic acid metabolism	96	-0.9	-0.5	-1.1	-0.7	-0.4	-0.2	1.2	1.9	1.1	0.97	0.1
	Protein Metabolism	tRNA aminoacylation	139	-0.9	-0.7	0.1	-0.6	-0.9	-0.7	0.7	1.4	1.6	0.90	4.3
	Protein Metabolism	General Secretion Pathway	143	-0.9	-1.2	-0.3	-0.1	0.4	-1.0	1.9	0.4	0.8	0.81	0.5
	DNA Metabolism	DNA structural proteins	106	-0.3	-0.8	-1.1	-0.4	0.1	-1.0	0.6	1.4	1.6	0.90	0.4
	DNA Metabolism	DNA-replication	104	-0.9	-1.4	-0.7	-0.9	0.0	0.6	1.9	1.2	0.3	0.80	1.4
	RNA Metabolism	ATP-dependent RNA helicases	145	-0.7	-0.1	-0.1	-0.3	-1.0	-0.4	1.9	1.5	-0.8	0.83	1.5
	Nucleosides/Nucleotides	Purine conversions	128	-1.4	-0.9	-0.8	0.1	0.3	-0.6	0.6	0.7	1.8	0.82	1.1
	Amino Acids and Derivatives	Urea decomposition	2	2.0	0.6	-0.1	-0.8	0.6	0.6	-0.8	-0.9	-1.0	-0.70	1.2
	Motility and Chemotaxis	Bacterial Chemotaxis	125	1.1	-0.4	0.9	1.4	0.5	-0.6	-1.7	-0.1	-0.9	-0.67	1.3
	Motility and Chemotaxis	Bacterial motility Gliding	124	-0.1	0.3	1.1	1.7	-0.7	0.6	-0.7	-0.8	-1.3	-0.70	0.6
	Aromatic Cmpd. Metabolism	Phenylpropanoid compound degradation	120	0.2	2.0	-0.4	-0.2	0.7	0.7	-1.1	-1.1	-0.6	-0.73	0.6
	Cofactors, Vitamins	Fe-S cluster assembly	93	1.0	1.1	0.3	0.7	0.0	-0.1	-0.6	-1.1	-1.3	-0.64	0.1
	Regulation and Cell Signaling	cAMP signaling	153	1.1	1.1	1.1	-0.1	0.2	0.1	-1.4	-1.4	-0.6	-0.91	2.5
	Clustering-based Subsystems	Tricarboxylate transporter	80	0.4	1.0	0.9	0.5	0.4	0.8	-1.9	-1.1	-0.8	-0.94	0.5

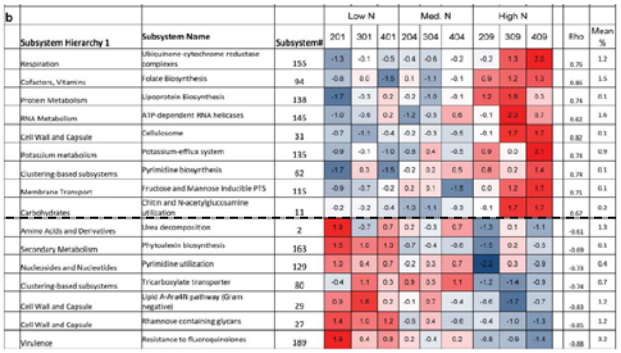
Also note: statistics! Nice for metagenomic data, where looking for patterns can lead to false positives, etc.

Figure 4

KBS site:

Better in high N conditions

Better in low N conditions



b				Low N			Med. N			High N				
	Subsystem Hierarchy 1	Subsystem Name	Subsystem#	201	301	401	204	304	404	209	309	409	Rho	Mean %
	Respiration	Ubiquinone cytochrome reductase complexes	155	-1.3	-0.1	-0.5	-0.4	-0.6	-0.2	-0.2	1.3	2.0	0.75	1.2
	Cofactors, Vitamins	Folate Biosynthesis	94	-0.8	0.0	-1.5	0.1	-1.1	-0.1	0.9	1.2	1.3	0.86	1.5
	Protein Metabolism	Lipoprotein Biosynthesis	138	-1.7	-0.3	0.2	-0.2	-1.0	-0.1	1.2	1.6	0.3	0.74	0.1
	RNA Metabolism	ATP-dependent RNA helicases	145	-1.0	-0.6	0.2	-1.2	-0.5	0.6	-0.1	2.0	0.7	0.62	1.6
	Cell Wall and Capsule	Cellulosome	31	-0.7	-1.1	-0.4	-0.2	-0.3	-0.5	-0.1	1.7	1.7	0.82	0.1
	Potassium metabolism	Potassium-efflux system	135	-0.9	-0.1	-1.0	-0.8	2.4	-0.5	0.9	0.0	2.1	0.76	0.9
	Clustering-based subsystems	Pyrimidine biosynthesis	62	-1.7	0.3	-1.5	-0.2	3.2	0.5	0.8	0.2	1.4	0.74	0.1
	Membrane Transport	Fructose and Mannose inducible PTS	115	-0.9	-0.7	-0.2	0.2	3.1	-1.8	0.0	1.2	1.7	0.71	0.1
	Carbohydrates	Chitin and N-acetylglucosamine utilization	11	-0.2	-0.2	-0.4	-1.3	-1.1	-0.3	-0.1	1.7	1.7	0.67	0.0
	Amino Acids and Derivatives	Urea decomposition	2	1.9	-0.7	0.7	0.2	-0.3	0.7	-1.3	0.1	-1.1	-0.61	1.3
	Secondary Metabolism	Phytoalexin biosynthesis	163	1.3	1.0	1.3	-0.7	-0.4	-0.0	-1.5	0.2	-0.5	-0.69	0.1
	Nucleosides and Nucleotides	Pyrimidine utilization	129	1.2	0.4	0.7	-0.2	0.3	0.7	-2.2	0.3	-0.9	-0.73	0.4
	Clustering-based subsystems	Tricarboxylate transporter	80	-0.4	1.1	0.3	0.9	0.5	1.1	-1.2	-1.4	-0.9	-0.74	0.7
	Cell Wall and Capsule	Lipid A-Ara4N pathway (Gram negative)	29	0.9	1.8	0.2	-0.1	0.7	-0.4	-0.6	-1.7	-0.7	-0.83	1.2
	Cell Wall and Capsule	Rhamnose containing glycans	27	1.4	1.0	1.2	-0.5	2.4	-0.6	-0.4	-1.0	-1.3	-0.85	1.2
	Virulence	Resistance to fluoroquinolones	189	1.9	0.4	0.8	0.2	-0.4	0.2	-0.6	-0.9	-1.4	-0.88	3.3

Also note: statistics! Nice for metagenomic data, where looking for patterns can lead to false positives, etc.

Figure 4

High nitrogen:

- ↑ DNA/RNA metabolism, protein metabolism, respiration
- ↓ motility and transport systems, secondary metabolite production

Low nitrogen:

- ↑ motility and transport systems, urea decomposition, secondary metabolite production, virulence factors
- ↓ DNA/RNA metabolism, protein metabolism, respiration

Figure 4

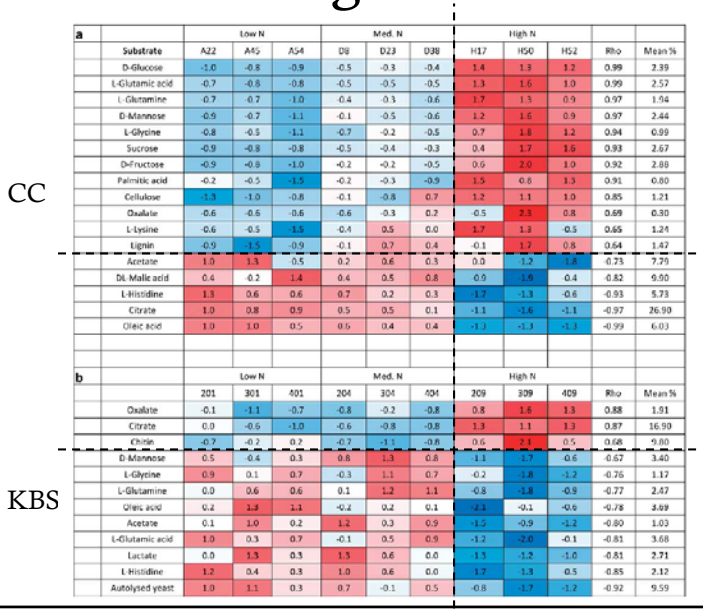
High nitrogen:

- ↑ growth and replication
- ↓ scrounging

Low nitrogen:

- ↑ scrounging
- ↓ growth and replication

Figure 5



CC

a		Low N			Med. N			High N				
	Substrate	A22	A45	A54	D8	D23	D38	H17	H50	H52	Rho	Mean %
	D-Glucose	-1.0	-0.8	-0.9	-0.5	-0.3	-0.4	1.4	1.3	1.2	0.99	2.39
	L-Glutamic acid	-0.7	-0.8	-0.8	-0.5	-0.5	-0.5	1.3	1.6	1.0	0.99	2.57
	L-Glutamine	-0.7	-0.7	-1.0	-0.4	-0.3	-0.6	1.7	1.3	0.9	0.97	1.94
	D-Mannose	-0.9	-0.7	-1.1	-0.1	-0.5	-0.6	1.2	1.6	0.9	0.97	2.44
	L-Glycine	-0.8	-0.5	-1.1	-0.7	-0.2	-0.5	0.7	1.8	1.2	0.94	0.99
	Sucrose	-0.9	-0.8	-0.8	-0.5	-0.4	-0.3	0.4	1.7	1.6	0.93	2.67
	D-Fructose	-0.9	-0.8	-1.0	-0.2	-0.2	-0.5	0.6	2.0	1.0	0.92	2.88
	Palmitic acid	-0.2	-0.5	-1.5	-0.2	-0.3	-0.9	1.5	0.8	1.3	0.91	0.80
	Cellulose	-1.3	-1.0	-0.8	-0.1	-0.8	0.7	1.2	1.1	1.0	0.85	1.21
	Oxalate	-0.6	-0.6	-0.6	-0.6	-0.3	0.2	-0.5	2.3	0.8	0.69	0.30
	L-Lysine	-0.6	-0.5	-1.5	-0.4	0.5	0.0	1.7	1.3	-0.5	0.65	1.24
	Lignin	-0.9	-1.5	-0.9	-0.1	0.7	0.4	-0.1	1.7	0.8	0.64	1.47
	Acetate	1.0	1.3	-0.5	0.2	0.6	0.3	0.0	1.2	1.8	0.73	7.79
	DL-Malic acid	0.4	-0.2	1.4	0.4	0.5	0.8	0.9	1.9	0.4	0.82	9.00
	L-Histidine	1.3	0.6	0.6	0.7	0.2	0.3	-1.7	-1.3	-0.6	-0.93	5.73
	Citrate	1.0	0.8	0.9	0.5	0.5	0.1	-1.1	-1.6	-1.1	-0.97	26.90
	Oleic acid	1.0	1.0	0.5	0.6	0.4	0.4	-1.3	-1.3	-1.3	-0.99	6.03

KBS

b		Low N			Med. N			High N				
		201	301	401	204	304	404	209	309	409	Rho	Mean %
	Oxalate	-0.1	-1.1	-0.7	-0.8	-0.2	-0.8	0.8	1.6	1.3	0.88	1.91
	Citrate	0.0	-0.6	-1.0	-0.6	-0.8	-0.8	1.3	1.1	1.3	0.87	16.90
	Chitin	-0.7	-0.2	0.2	-0.7	-1.1	-0.8	0.6	2.1	0.5	0.68	9.80
	D-Mannose	0.5	0.4	0.3	0.8	1.3	0.8	-1.1	-1.7	-0.6	-0.67	3.40
	L-Glycine	0.9	0.1	0.7	-0.3	1.1	0.7	-0.2	-1.8	-1.2	-0.76	1.12
	L-Glutamine	0.0	0.6	0.6	0.1	1.2	1.1	-0.8	-1.8	-0.9	-0.77	2.47
	Oleic acid	0.2	1.3	1.1	-0.2	0.2	0.1	-2.1	-0.1	-0.9	-0.78	3.69
	Acetate	0.1	1.0	0.2	1.2	0.3	0.9	-1.5	-0.9	-1.2	-0.80	1.03
	L-Glutamic acid	1.0	0.3	0.7	-0.1	0.5	0.9	-1.2	-2.0	-0.1	-0.81	3.68
	Lactate	0.0	1.3	0.3	1.3	0.6	0.0	-1.3	-1.2	-1.0	-0.81	2.71
	L-Histidine	1.2	0.4	0.3	1.0	0.6	0.0	-1.7	-1.3	-0.5	-0.85	2.12
	Autolysed yeast	1.0	1.1	0.3	0.7	-0.1	0.5	-0.8	-1.7	-1.2	-0.92	9.59

Figure 5

Confusing: helpful/not helpful?

Why might there be disagreement between sites?

How does catabolic profiling contribute to our understanding of these microbes?

Conclusions

Overall conclusions about effects of nitrogen on diversity and lifestyle:

- Diversity was not affected by nitrogen level
- Differences in lifestyle were observable only at the highest nitrogen levels
- Lifestyle switched from oligotrophy at low/intermediate nitrogen levels to copiotrophy at high nitrogen levels

Conclusions

How does this relate to big picture things like…

- Agricultural practices?
- Nitrogen cycle?
- Global change?

Conclusions

Your thoughts:

Satisfactory? Unsatisfactory?

What would you like to see in the future?