

Supplemental Material

Molybdenum-based diazotrophy in a *Sphagnum* peatland in northern Minnesota

Melissa J. Warren^{1*}, Xueju Lin^{2**}, John C. Gaby^{3***}, Cecilia B. Kretz^{1****}, Max Kolton², Peter L. Morton³, Jennifer Pett-Ridge⁴, David J. Weston⁵, Christopher W. Schadt⁵, Joel E. Kostka^{1,2}, Jennifer B. Glass^{1,2#}

¹School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA, USA

²School of Biology, Georgia Institute of Technology, Atlanta, GA, USA

³Department of Earth, Ocean and Atmospheric Science, Florida State University, Tallahassee, Florida, USA

⁴Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA, USA

⁵Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA

Now at: *CH2M, Atlanta, GA, USA; **InstantLabs, Baltimore, MD, USA; ***Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Oslo, Norway; ****Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

#Corresponding author: Jennifer B. Glass, 404-894-3942; jennifer.glass@eas.gatech.edu

Running Title: Nitrogen fixation in a *Sphagnum* peat bog

Table S1. Dissolved trace metal concentrations in S1 transect 3 (far, mid and near sites).

BD indicates “below detection” and N/A indicates that the element of interest was not analyzed.

Site	Type	Depth (cm)	V	Mn	Fe	Co	Ni	Cu	Zn	Mo
June 2014										
Far	Hummock	0-10	10	487	4,081	1	N/A	30	344	1
		0-10	14	443	1,797	5	N/A	11	208	4
	Hollow	30-50	14	186	1,920	7	N/A	13	275	2
100-150		8	115	1,149	3	N/A	12	228	1	
Mid	Hummock	0-10	13	2,222	3,527	3	N/A	29	534	1
		0-10	11	555	21,112	5	N/A	16	284	1
	Hollow	30-50	21	158	6,800	4	N/A	20	186	4
100-150		5	60	7,907	2	N/A	12	154	7	
Near	Hummock	0-10	8	445	2,483	1	N/A	25	227	1
		0-10	9	1,148	24,030	5	N/A	8	474	4
	Hollow	30-50	12	284	14,139	6	N/A	12	143	2
100-150		12	69	7,071	4	N/A	9	86	5	
September 2014										
Far	Hollow	0-10	BD	289	22,576	9	23	8	211	BD
		30-50	BD	368	11,818	15	83	7	251	5
		100-150	BD	231	9,975	7	17	8	48	BD
Mid	Hollow	0-10	BD	309	34,980	15	34	38	123	BD
		30-50	BD	89	30,816	3	9	20	202	BD
		100-150	BD	213	23,035	10	19	9	223	BD

Table S2. Statistics for metagenome sequences and *nifH* gene cluster I-IV analysis.

Sample	Sample	raw # seqs	# sequences	# functional genes	#nifH genes	I	II	III	IV/V	non assigned
T3M 0cm	T3M 0 R1	23149446	20636767	5419176	1203	23	4	27	42	1
	T3M 0 R2	23149446	20636767	4834626						
T3M 30cm	T3M 30 R1	23396969	21420027	5864331	1304	18	1	53	26	1
	T3M 30 R2	23396969	21420027	5626796						
T3M 75cm	T3M 75 R1	22408842	20698248	4244995	511	34	0	31	34	0
	T3M 75 R2	22408842	20698248	4548589						
T3F 0cm	T3F 0 R1	17800326	16292777	3761428	28944	49	4	21	25	1
	T3F 0 R2	17800326	16292777	3585934						
T3F 30cm	T3F 30 R1	30686580	27965337	7598974	58075	49	5	23	22	1
	T3F 30 R2	30686580	27965337	7374546						
T3F 75cm	T3F 75 R1	909520	824421	195481						
	T3F 75 R2	909520	824421	205646						

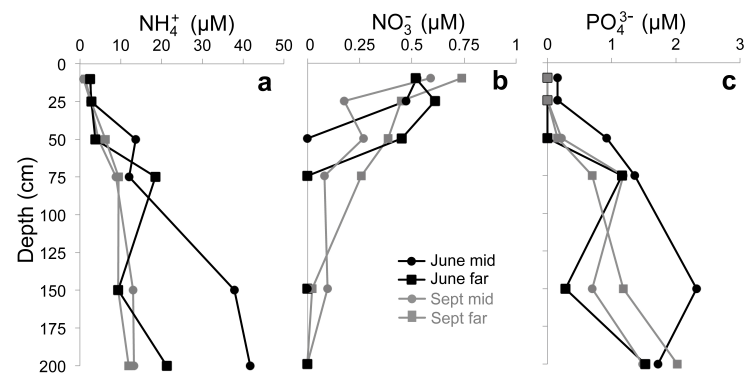


Figure S1. Depth profiles of dissolved (a) ammonium, (b) nitrate, and (c) phosphate from S1 T3 peat bog middle (circles) and far (squares) sites. Black symbols are from June 2014; gray symbols are from September 2014.

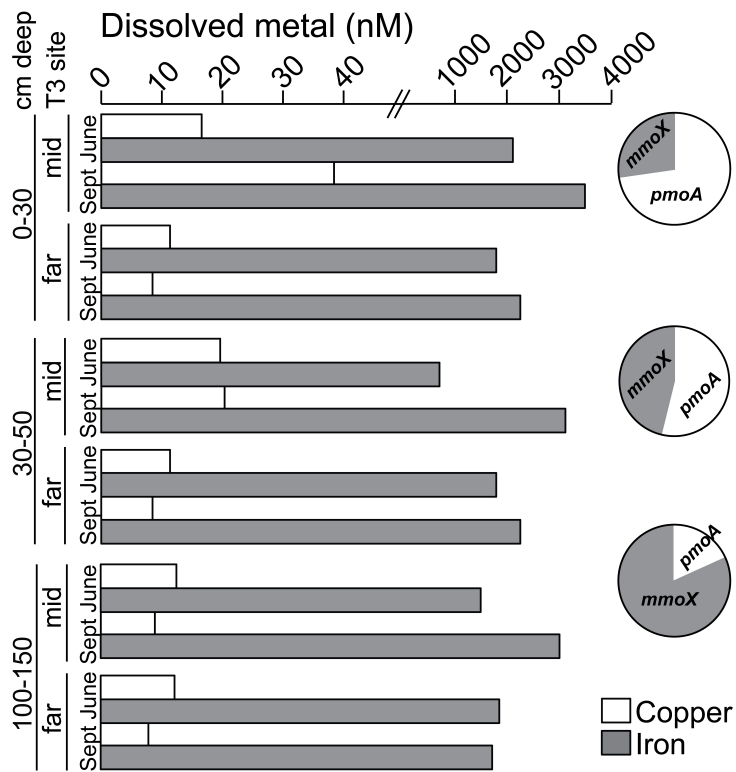


Figure S2. Dissolved copper (white) and iron (shaded) concentrations in pore water from three depths in S1 peat hollows (mid and far sites along T3 transect) in June 2015. Pie charts show the relative abundance of *pmoA* (Cu-containing particulate methane monooxygenase) and *mmoX* (Fe-containing soluble methane monooxygenase) sequences recovered from metagenomes from T3 mid sites (0-10, 25-50, and 75-100 cm).

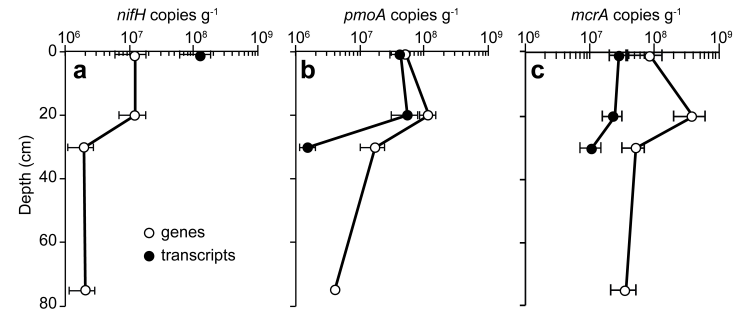


Figure S3. Depth profiles of copy numbers for DNA (white) and cDNA (black) for (a) *nifH*, (b) *pmoA* and (c) *mcrA* in units of copies per gram of dry weight for the S1 bog T3 mid site. Panel (b) is modified from Esson, et al. (1).

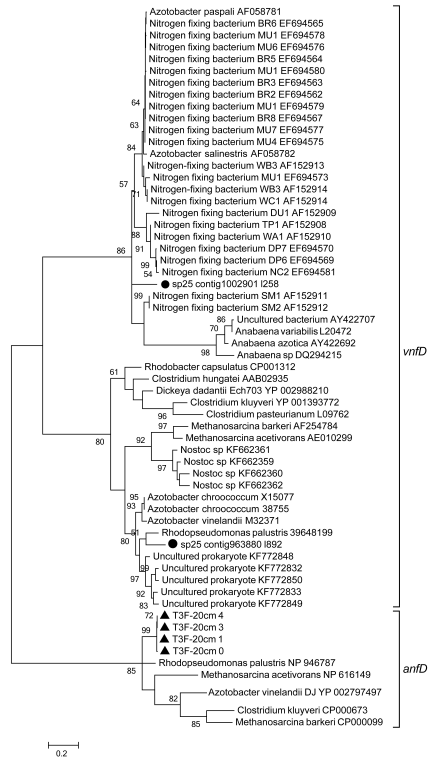


Figure S4. Evolutionary history of retrieved *vnf* and *anf* genes inferred using the Maximum Likelihood method. Black circles represent two assembled metagenomic *vnfD* reads from the S1 bog T3F site (25 cm depth). Black triangles represent *anfD* amplicons from S1 bog T3F site (20 cm depth). The percentage of trees in which the associated taxa clustered together is shown next to the branches.

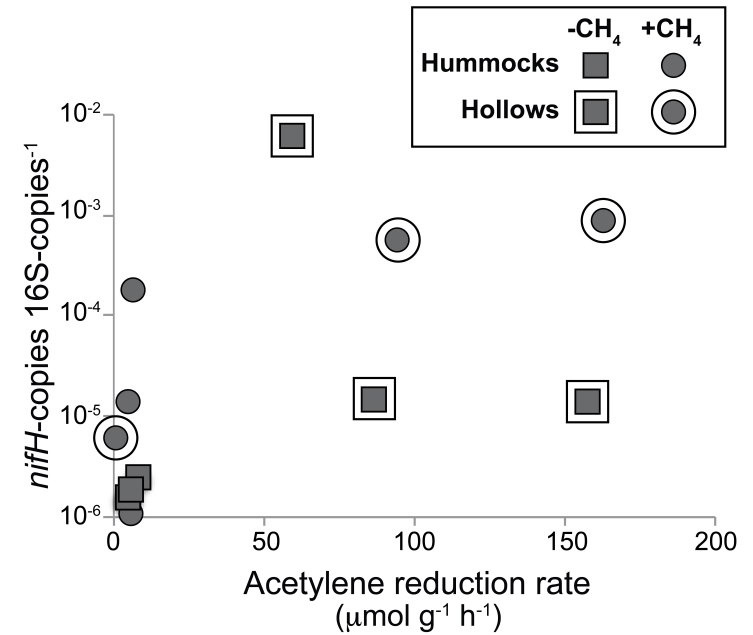


Figure S5. Correlation between *nifH*:16S rRNA cDNA transcript ratios and acetylene reduction rates for hummock and hollow (line around symbols) peat bog samples from June 2014 incubated under 80%N₂+20% CO₂ headspace with (circles) and without (squares) 1% CH₄. When normalized to dry peat, these data range from 10³ to 10⁷ *nifH* copies g⁻¹.

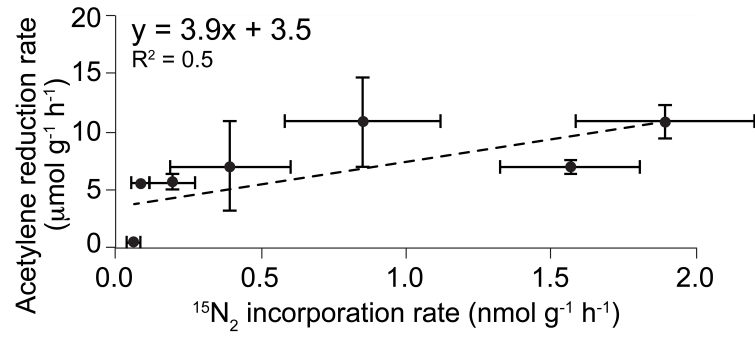


Figure S6. Correlation between ARA and ¹⁵N₂ incorporation. The slope of the graph is the conversion factor (3.9).

Figure S7. Universal *nifH* primer sequences aligned with corresponding template from methanogen (cluster III) *nifH* genes.

primer name (position*)	PolF (115-134)	PolR (457-476)	F1 (25-50)	R6 (457-473)
primer sequence (5' to 3')	TGGGATGCGAARIBBAGACTC	ATBGGCATCATYTRCCGGGA	TAYGGIARBRGGIGIAYGGIARITC	GCCATCATYTCIGGGA
Mbo ^b	---C-G-A-G---T---	-G---G---C---	-C-A-A-C-T-C-A-A-	---G-T-C-G---
Mac	---C-A-A-A---	-T---T-T-ACT---	-T-A-G-C-C-T-A-A-	---G-T-T-ACT---
Mmg	-T-C-C-C-A-A---	-T---T-C-C-ACT---	-C-A-G-C-C-T-A-A-	---G-T-C-ACT---
Mpa	---C-C-A-C---	-C---C-C---	-C-A-A-C-A-C-A-G-	---C-C-C-G---
Mba	-T-C-G-G-A-T---	-A---T-T-ACT---	-C-A-A-T-A-T-A-A-	---T-T-ACT---
Mpe	---C-G-G-C-T---	-T---T-T---	-C-A-G-T-F-C-A-G-	---T-T-T---
Mcp	---C-C-A-A---	-A---C-T---	-C-C-A-C-A-C-C-G-	---C-T-G---
Mli	-T-G-G-G-C---	-C---C-G-C---	-C-C-A-C-C-C-C-G-	---C-G-C---
Mnt	-T-C-C-A-G---	-T---T-C-ACT---	-C-A-G-C-C-T-A-A-	---T-C-ACT---
primer name (position*)	nifH2 (115-131)	nifH (460-476)	IGK3 (19-47)	DVV (388-413)
primer sequence (5' to 3')	TGAGATGAAARINIGIA	ADNGGCATCATYTRCC	GIWVHTAYGGIARBRGGIGIARIGGGLAA	AVTGRRAALICIGRUALIACITC
Mbo	---C-C-G-A-G---	-GG---G-T-C---	--AA-T-C-A-A-C-T-C-A-	--G-A-A-A-C-G-G-C-A-
Mac	-C-C-A-A-A---	-TT---T-T---	--AA-T-A-A-G-C-C-T-A-	-G-A-A-A-T-G-C-G-G-
Mmg	-T-C-C-A-A---	-TT---T-C---	--AA-C-C-A-G-C-T-A-A-	-G-A-A-T-G-C-G-G-
Mpa	-C-C-C-A-C---	-GC---C-C---	--AA-C-C-A-A-C-A-C-A-	-C-G-C-C-G-G-G-A-
Mba	-T-C-G-G-A---	-TA---T-T---	--AA-C-C-A-A-T-A-T-A-	-A-A-T-A-A-C-A-A-
Mpe	---C-G-G-C---	-GT---T-T---	--AA-T-C-A-G-T-F-C-A-	-T-G-T-F-G-T-G-A-
Mcp	-C-C-A-A---	-GA---C-T---	-CA-T-C-C-A-C-A-C-C-	-A-G-G-G-G-G-G-G-
Mli	-C-T-G-G-C---	-GC---C-G---	--AA-T-C-A-A-C-C-C-C-	-G-G-G-G-G-G-G-G-
Mnt	-T-C-C-A-G---	-TT---T-C---	--AA-C-C-A-A-G-C-T-A-	--G-A-A-T-G-T-G-T-G-

* Relative to the *nifH* sequence of *Acetobacter vinlandii* (Genbank ACCN9 M20568)

^b The following abbreviations represent species names: Mbo, Candidatus *Methanoregula boomeri* GA8; Mac, *Methanosarcina acetivorans* C2A; Mmg, *Methanosarcina mazei* Go1; Mpa, *Methanosphorobacter palustris* EU-9; Mba, *Methanosarcina barkeri* str. Fusaro; Mpe, *Methanoplanus porphyreus* DSM 11571; Mep, *Methanosarcina concili* GP9; Mli, *Methanofollis liminatans* DSM 4140; Mnt, *Methanosarcina muzzeyi* Tuzo1. The - symbol represents conserved positions whose nucleotides match those of the primer sequence above, bold letters indicates nucleotides in degenerate positions of the primer, and red letters indicate primer-template mismatches.

References

1. **Esson, K. C., X. Lin, D. Kumaresan, J. P. Chanton, J. C. Murrell, J. E. Kostka.** 2016. Alpha-and gammaproteobacterial methanotrophs codominate the active methane-oxidizing communities in an acidic boreal peat bog. *Appl Environ Microbiol* **82**:2363-2371.
-