Supplemental Materials

Methanogens are major contributors to nitrogen fixation in soils of the Florida Everglades

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Table S1. Alpha diversity of N_2-fixing populations estimated from *nifH* and *nifH* mRNA sequence data obained from field soils and incubation soils of WCA-2A

Soils from:		*nifH* from:	Sampling time and site		No. of clones assayed	No. of OTUs	Chao1 richness (CI)[a]	Shannon diversity (CI)	Good's coverage (%)
Field		DNA	October 2009	F1	71	48	211 (105, 502)	3.6 (3.3, 3.8)	41
				F4	69	52	130 (85, 234)	3.8 (3.7, 4.0)	42
				U3	64	53	311 (151, 738)	3.9 (3.7, 4.1)	28
		mRNA	August 2011	F1	45	17	108 (50, 268)	1.9 (1.5, 2.4)	69
				F4	34	13	16 (13, 30)	2.3 (1.9, 2.6)	85
			August 2012	F1	61	36	171 (80, 443)	3.2 (2.9, 3.5)	52
				F4	77	35	54 (41, 94)	3.3 (3.1, 3.5)	77
				U3	68	22	31 (24, 60)	2.6 (2.4, 2.9)	85
Incubation	H_2+CO_2 (80:20, vol:vol)	mRNA	August 2011	F1	30	11	25 (13, 78)	1.9 (1.6, 2.2)	80
				F4	43	11	13 (11, 27)	1.9 (1.6, 2.2)	91
				U3	43	14	32 (18, 95)	2.1 (1.8, 2.4)	79
	cellulose	mRNA	January 2012	F1	67	28	79 (43, 202)	2.9 (2.7, 3.2)	73
				F4	78	16	25 (17, 61)	1.9 (1.6, 2.2)	90
				U3	75	18	23 (19, 41)	2.2 (2.0, 2.5)	89

[a]95% confidence interval.

Table S2. Primers used for qPCR analysis, and PCR efficiency from standard curves made from each primer during qPCR analysis in this study

Target gene	Primer			Standard curve: Slope and y axis intercept (R^2 value)	PCR efficiency (%)
	I. D.	Seqs (5' to 3')	Reference		
16S rRNA	1055f	ATGGCTGTCGTCAGCT	1	y = -3.42x + 39.55 (0.9999)	96.25
	1392r	ACGGGCGGTGTGTAC			
AOA *amoA*	Crenamo A23f	ATGGTCTGGCTWAGACG	2	y = -3.47x + 33.03 (0.9998)	94.13
	Crenamo A616r	GCCATCCATCTGTATGTCCA			
AOB *amoA*	amoA 1f	GGGGTTTCTACTGGTGGT	3	y = -3.43x + 34.72 (0.9947)	95.80
	amoA 2r	CCCCTCKGSAAAGCCTTCTTC			
nirK	nirK 876	ATYGGCGGVCAYGGCGA	4	y = -3.46x + 34.75 (0.9996)	94.62
	nirK 1040	GCCTCGATCAGRTTRTGGTT			
nirS	nirS Cd3aF	AACGYSAAGGARACSGG	5	y = -3.40x + 33.06 (0.9999)	96.84
	nirS R3cd	GASTTCGGRTGSGTCTTSAYGAA			
nrfA	nrfAF2aw	CARTGYCAYGTBGARTA	6	y = -3.46x + 33.62 (0.9994)	94.67
	nrfAR1	TWNGGCATRTGRCARTC	7		
nifH	nifH-F	GGHAARGGHGGHATHGGNAARTC	8	y = -3.45x + 32.60 (0.9997)	94.84
	nifH-R	GGCATNGCRAANCCVCCRCANAC			

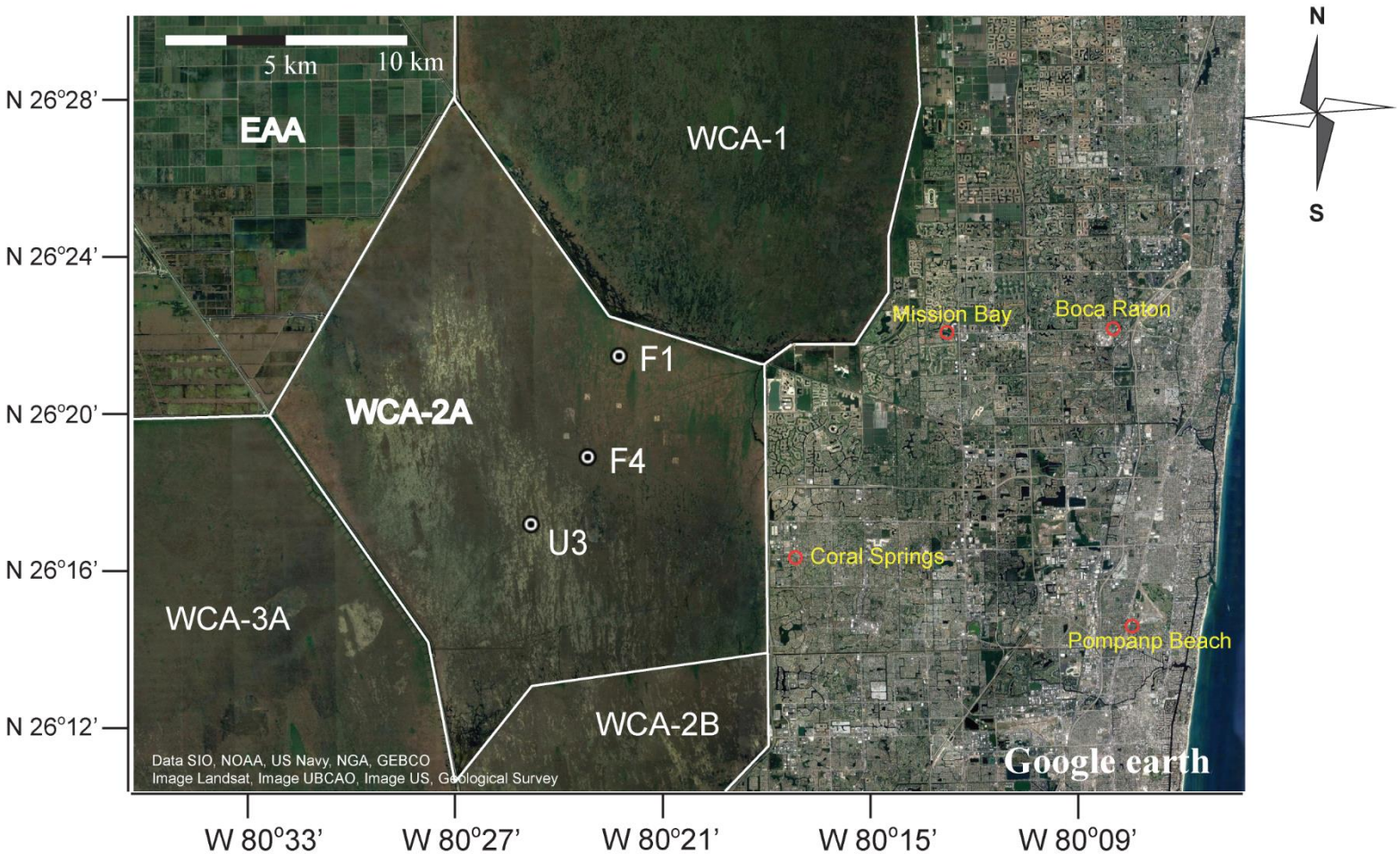

Fig. S1. Sites F1, F4, and U3 representing nutrient impacted (P loaded), intermediate and unimpacted zone, respectively, within Water Conservation Area 2A (WCA-2A) of the northern Everglades. EAA: Everglades Agricultural Area.

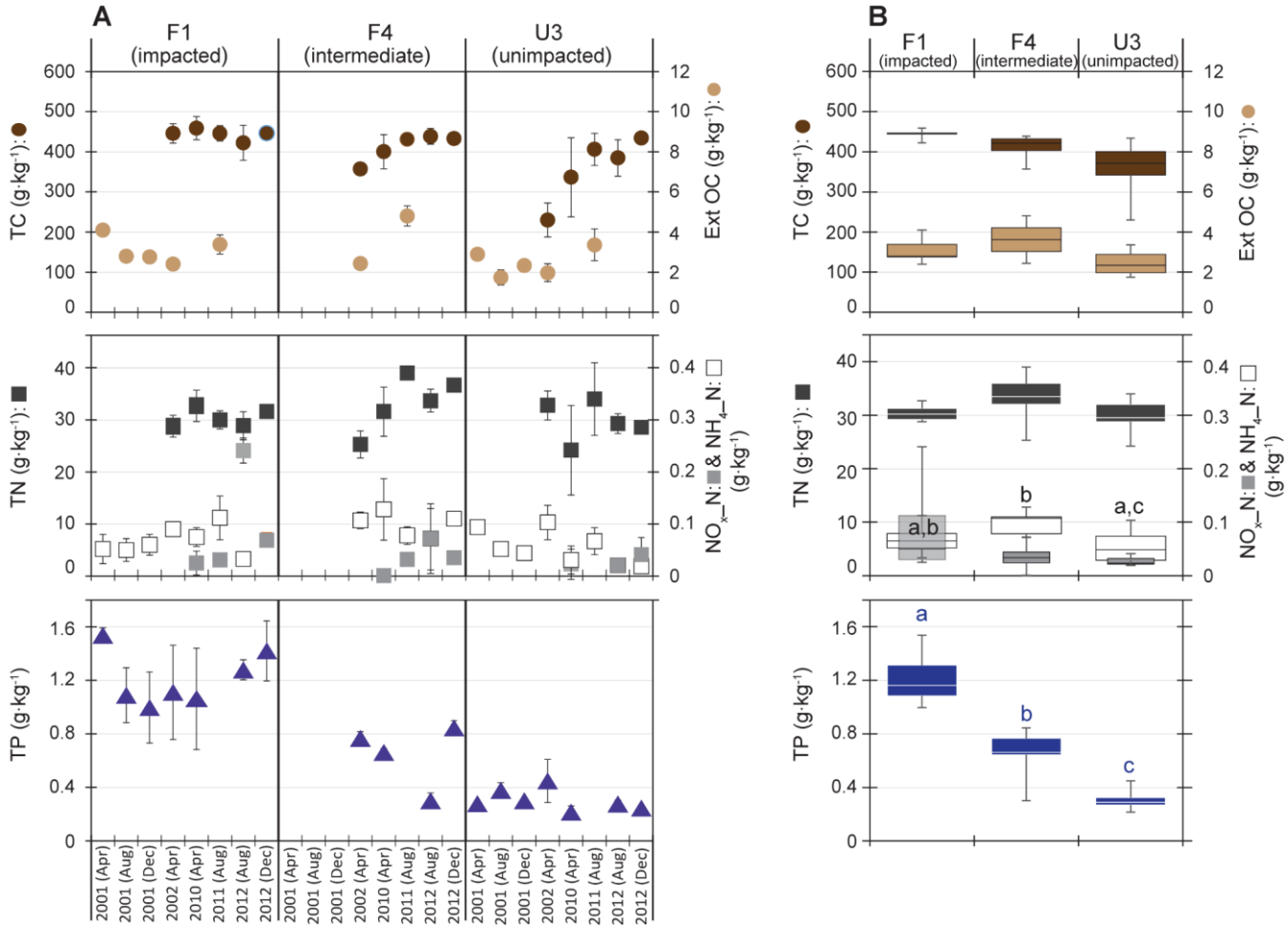

Fig. S2. Geochemical parameters determined over 11 years (2001-2012) in the sites of WCA-2A of Everglades wetland across nutrient gradient; F1 (impacted), F4 (intermediate), U3 (unimpacted). While NO_x-N and NH_4^+-N concentrations from soil samples collected in April 2010, August 2012, December 2012 were determined in this study, other data were adopted from the previous studies measured in the same study sites (9-12). A: temporal profiles of each geochemical parameter, with bars representing Mean±S.D. (n=3). B: Box-and-Whisker plot created from the pooled data from the temporal profile. Boxes depict the medians (horizontal lines in the boxes) and the lower and upper quartiles (bottoms and tops of the boxes, respectively). The vertical bars (whiskers) show the highest and the lowest values, excluding outliers. The different letters denoted on the box of TP and NO_x_N indicate a significant difference among sites ($P \leq 0.05$ in the Tukey-Kramer HSD test).

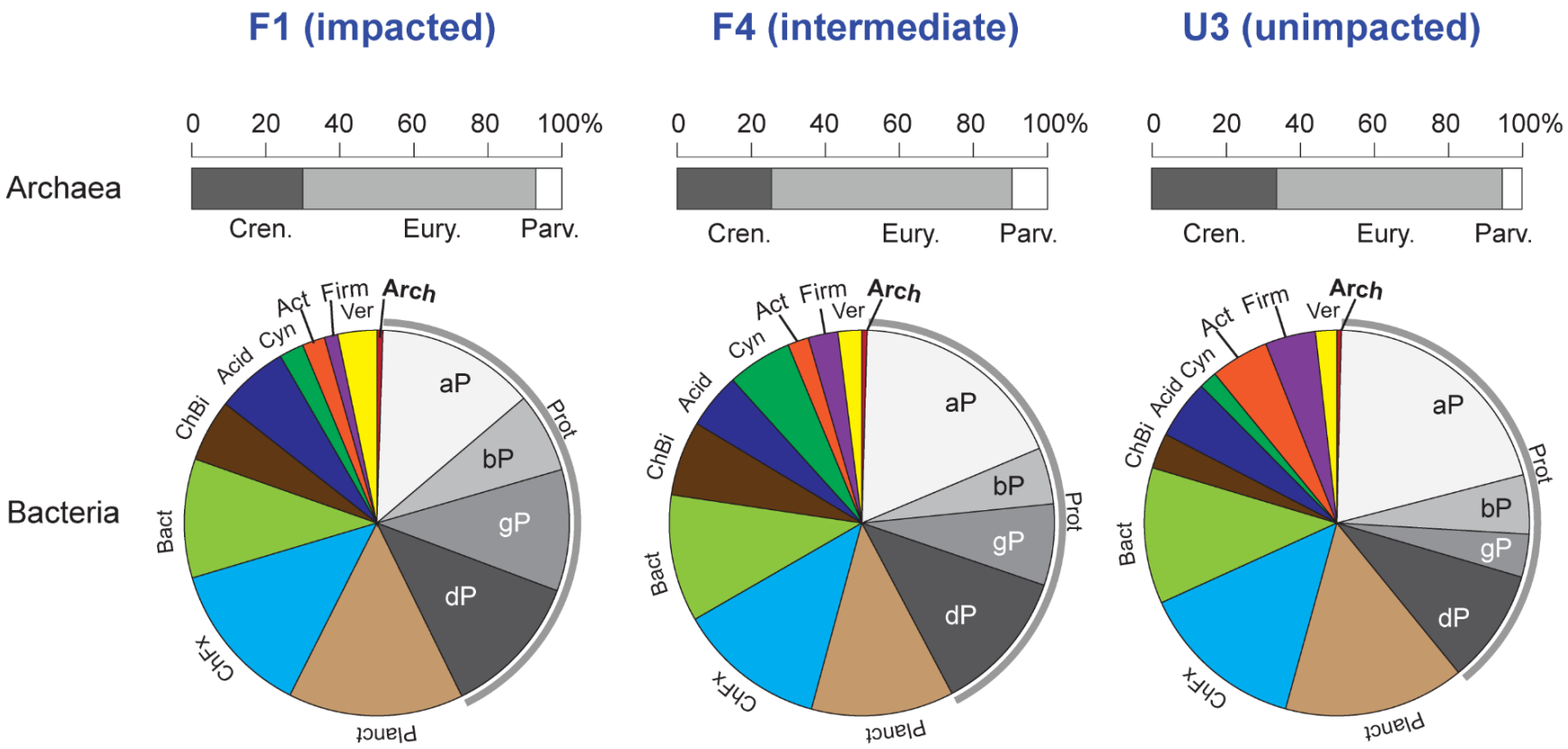

Fig. S3. Composition of bacteria and archaea determined in soil samples taken from sites F1, F4 and U3 of WCA-2A taken in October 2009. Majority of phyla (>1%) within bacteria or archaea were included. Proportions of each taxa presented in above pie graphs are the mean value obtained from triplicate soil samples. Abbreviation: Prot, *Proteobacteria* (a, b, g, dP: *Alpha-*, *Beta-*, *Gamma-*, *Delta-proteobacteria)*; Planct, *Planctomycetes*; Bact, *Bacteroidetes*; ChBi, *Chlorobi*; Acid, *Acidobacteria*; Cyn, *Cyanobacteria*; Act, *Actinobacteria*; Firm, *Firmicutes*; Ver, *Verrucomicrobia*; Cren, *Crenarchaeota*; Eury, *Euryarchaeota*; Parv, *Parvarchaeota*.

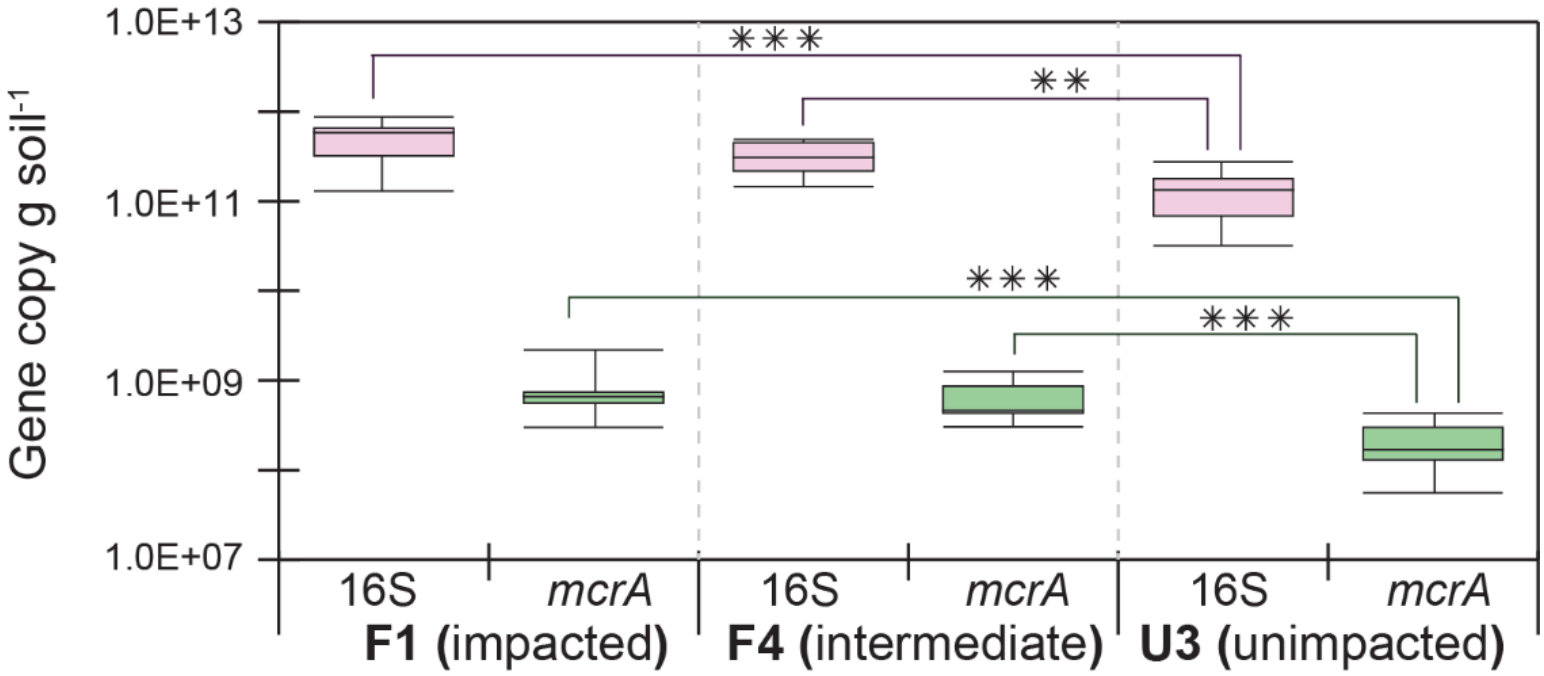

Fig. S4. Box-and-Whisker plots representing the copy numbers of genes 16S rRNA (bacterial) and *mcrA* measured in WCA-2A soils sampled in April 2010, August and December 2012. Data on *mcrA* copies were adopted from our previous study (11). Boxes depict the medians (horizontal lines in the boxes) and the lower and upper quartiles (bottoms and tops of the boxes, respectively). The vertical bars (whiskers) show the highest and the lowest values, excluding outliers. Significant difference between samples was indicated with ***P < 0.001, **P < 0.01, *P < 0.05.

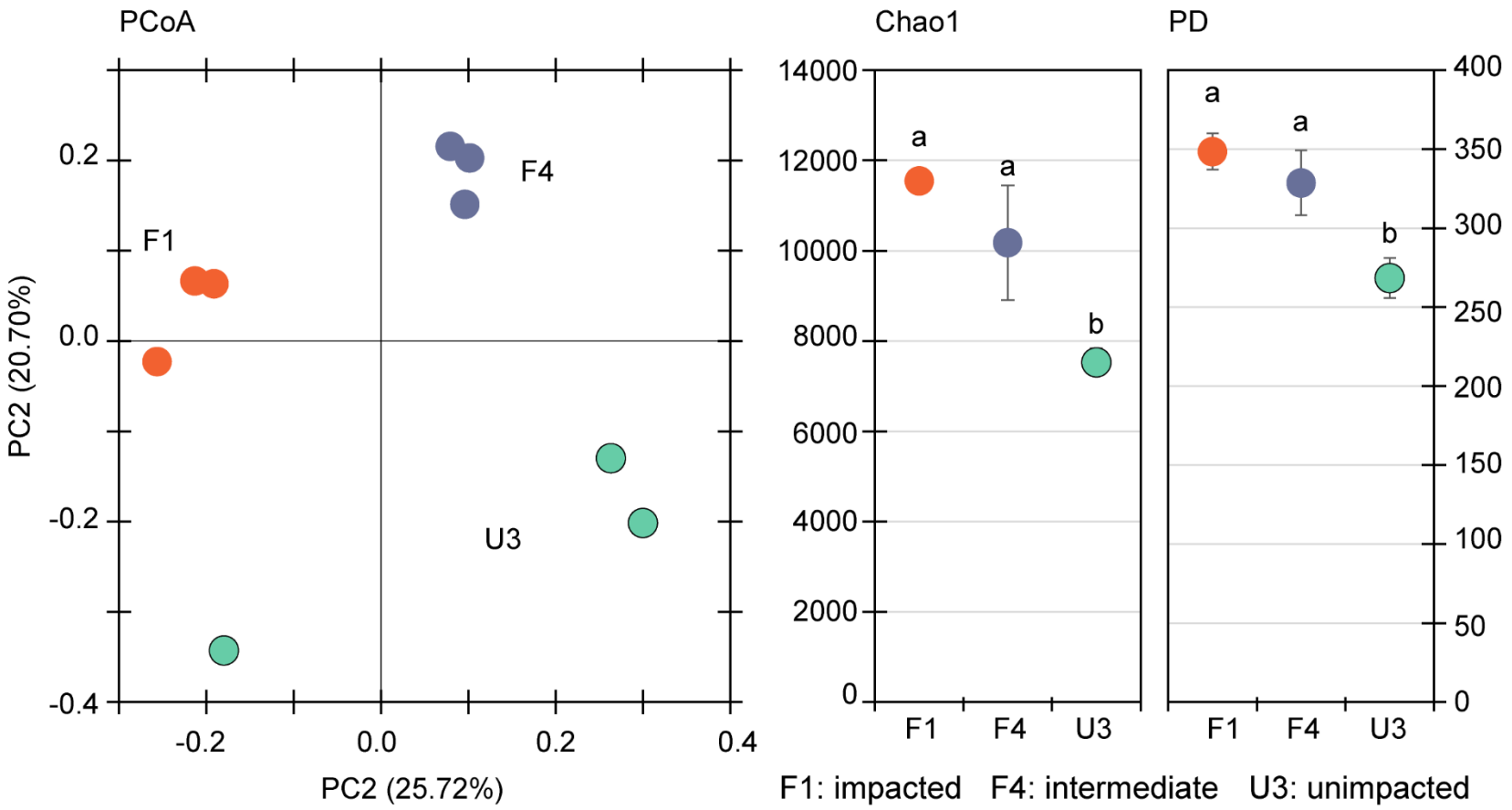

Fig. S5. Diversity of prokaryotes inhabiting WCA-2A soils sampled in October 2009 across the nutrient gradient. PCoA biplot (Left) represents distribution of microbial communities from sites F1, F4 and U3. Alpha-diversity of prokaryotes was presented by Chao1 richness and Faith's phylogenetic diversity (PD) (Right side). The different letters on the symbols of sites show a significant difference in beta-diversity among sites ($P \leq 0.05$ in the Tukey-Kramer HSD test). Error bars in Chao1 and PD graph represent Mean±S.D. (n=3).

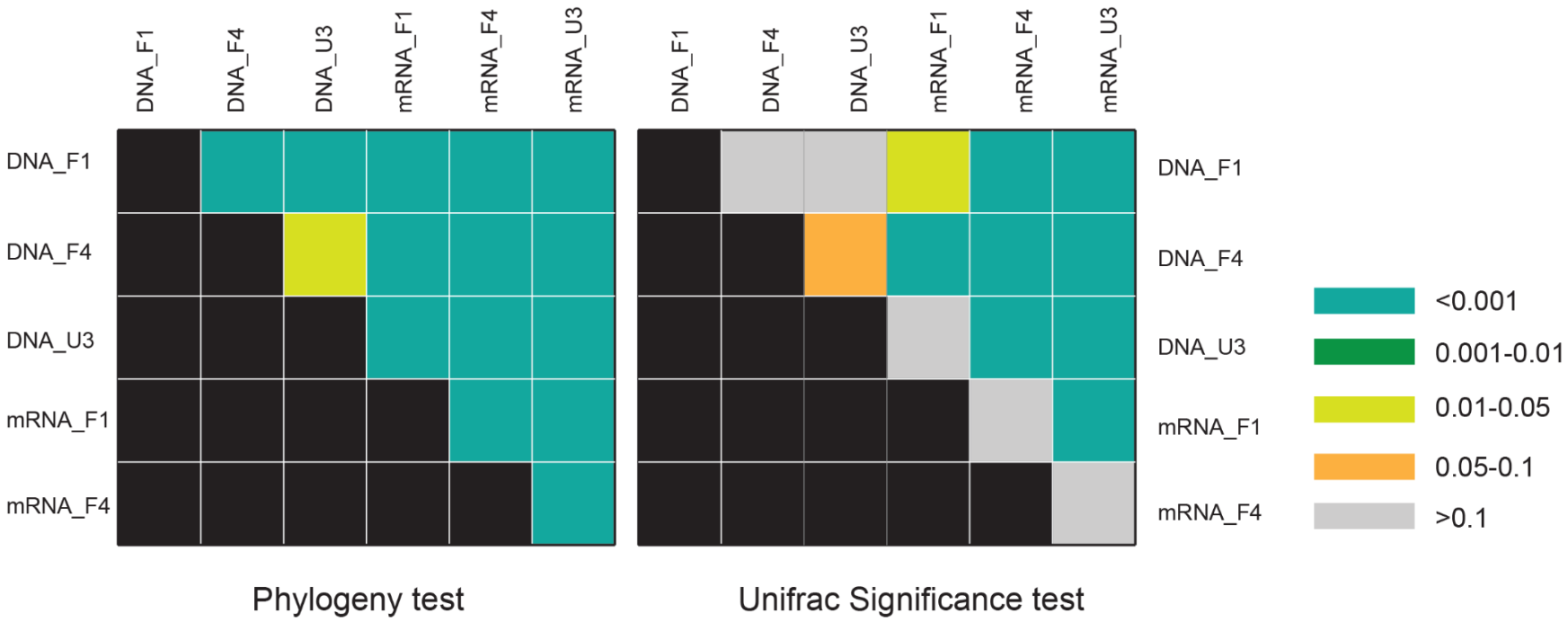

Fig. S6. P values from phylogeny tests and Unifrac Significance tests showing significant differences between N_2-fixing assemblages from sites F1 (impacted), F4 (intermediate) and U3 (unimpacted). N_2-fixing assemblages determined by *nifH* and *nifH* mRNA sequences were denoted by DNA and mRNA on the figure tailed by site.

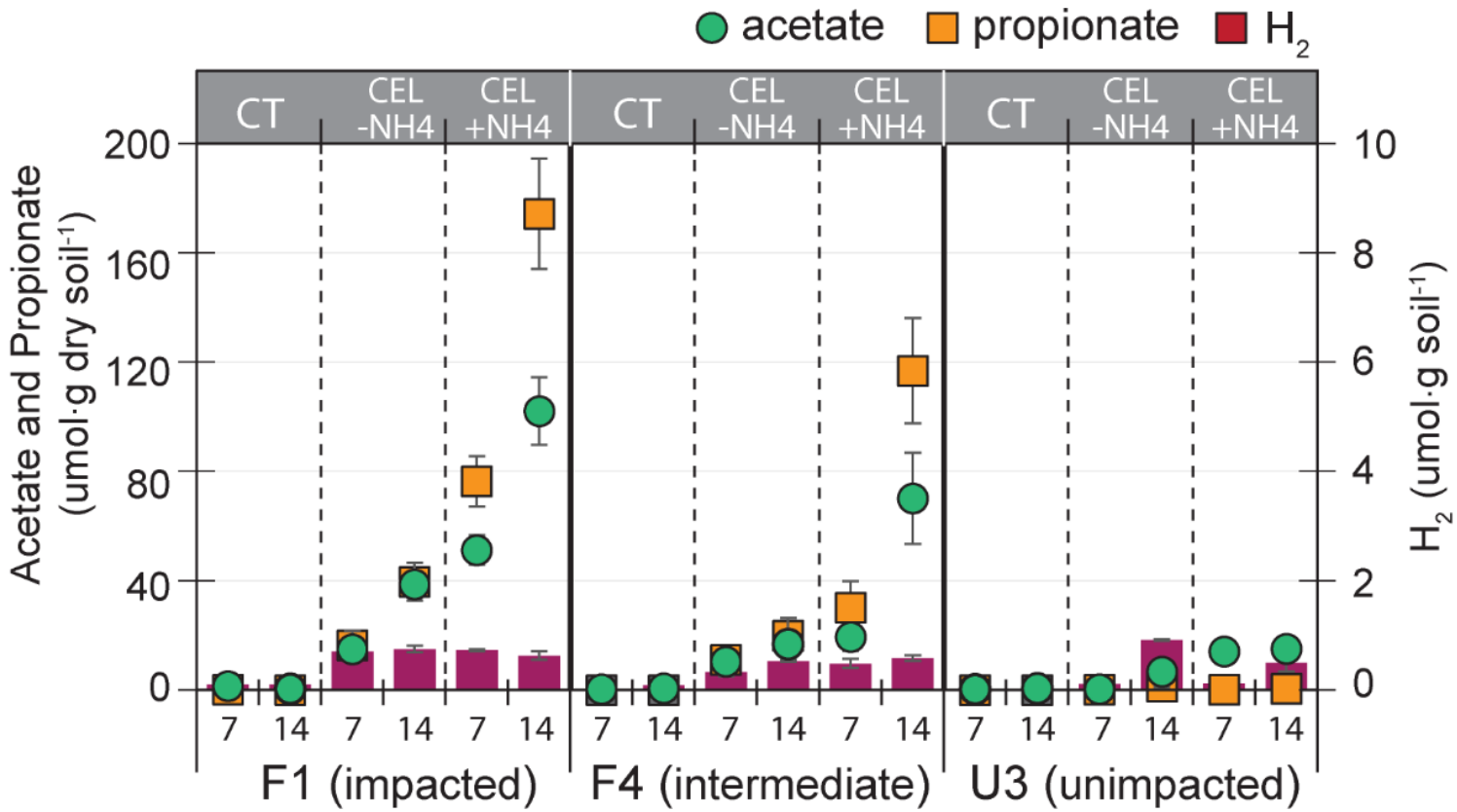

Fig. S7. Acetate, propionate and H_2 produced during soil incubation with cellulose without 100 mM NH_4^+ (CEL-NH4), with 100 mM NH_4^+ (CEL+NH4). Incubation with no cellulose was used as control (CT). Soil sampled in January 2012 was used for this incubation study. Error bars represent Mean±S.D. (n=3).

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