TABLE S1. Primers used to quantify total and different methanogen groups from the Everglades soil DNAs

Target methanogen	Primer[a]/probe	Primer sequence (5'-3')	Tm[b]	Reference
Total methanogens	mlas (F)	GGT GGT GTM GGD TTC ACM CAR TA	58.5	Steinberg and Regan (2009)[1]
Total methanogens	mcrA-rev (R)	CGT TCA TBG CGT AGT TVG GRT AGT	58.8	Steinberg and Regan (2009)
Methanomicrobiales	MM-F (F)	AAR TAC AAR GTY GAC TGG MRR RAC	56.2	This study
Methanosaetaceae	MST-F (F)	CAA GTW **Y**[c]GG MGG ATT **C**GC CAA GG	60.4	This study
	msa (Probe)	CAA GTW CGG MGG ATT YGC CAA GG	60.5	Steinberg and Regan (2009)
Methanobacteriaceae	MB_F (F)	A**A**G CAC C**W**A ACA MCA TGG A**H**A C**H**G T	59.3	This study
	mabac (Probe)	ARG CAC CKA ACA MCA TGG ACA CWG T	61.6	Steinberg and Regan (2009)

[a] F, forward primer; R, reverse primer.

[b] Tm, melting temperature of primer, which was determined in the online calculation with OligoAnalyzer 3.1 at Integrated DNA Technologies (https://www.idtdna.com/site).

[c] The different nucleotide between our primer and Steinberg and Regan's probes was underlined.

Bae et al., 2015

TABLE S2. Primer used to quantify total and different methanogen groups from the Everglades soil DNA

Combination	Target microbial groups	Standard curves				Reference for primer sequence
		Slope	y intercept	R^2	Efficiency (%)	
mlas/mcrA-Rev	Total methanogens	-3.464	32.76	0.9996	94.5	Steinberg and Regan (2009)[1]
MM-F/mcrA-Rev	*Methanomicrobiales*	-3.491	32.30	0.9984	93.4	this study
MST-FmcrA-Rev	*Methanosaetaceae*	-3.433	32.69	0.9968	95.5	this study
MB-F/MCRA-Rev	*Methanobacteriaceae*	-3.403	32.20	0.9995	96.7	this study
DSRp2060F/DSR4R	Sulfate reducers	-4.4496	38.07	0.9998	94.9	Foti et al. (2007)[2]

TABLE S3. Diversity measurements of gene *mcrA* based on OTUs generated from deduced amino acid sequences of these gene and transcripts retrieved from the WCA-2A of Everglades.

Gene	Study site	Clone no. screened	Cutoff % on deduced amino acid sequences	No. of OTUs	Chao1 richness (CI)[a]	Shannon diversity (CI)	Coverage (%)
mcrA	F1	86	5%	41	74 (53, 131)	3.4 (3.1, 3.6)	71
			10%	31	39 (33, 60)	3.1 (2.9, 3.3)	85
			15%	24	30 (25, 51)	2.8 (2.6, 2.9)	89
			20%	12	22 (18, 49)	2.5 (2.3, 2.7)	94
	F4	82	5%	38	71 (49, 133)	3.3 (3.1, 3.5)	73
			10%	25	31 (26, 52)	2.9 (2.7, 3.1)	89
			14%	18	19 (18, 26)	2.6 (3.6, 3.9)	96
			20%	12	12 (12, 0)	2.1 (1.9, 2.3)	99
	U3	87	5%	42	58 (47, 89)	3.6 (3.4, 3.7)	78
			10%	33	44 (36, 73)	3.3 (3.1, 3.4)	85
			15%	25	28 (25, 40)	3.0 (2.9, 3.2)	93
			19%	18	19 (18, 25)	2.7 (2.5, 2.8)	97

[a] 95% confidence interval.

Bae et al. 2015

TABLE S4. Composition of *mcrA* and phylogenetic affiliation of OTUs with known sequences

Study site			Sum	Representative OUT	(sub)Cluster I.D.	Strains closely related (accession number)	Inferred phylogeny		
F1	F4	U3					Class	Order	Family
5	1	0	6	F1-F04	MM-I	*Methanomicrobium mobile* BP DSM 1539 (AAL29293)	*Methanomicrobia*	*Methanomicrobiales*	*Methanomicrobiaceae*
2	0	2	4	U3-H06	MM-I	*Methanocorpusculum bavaricum* DSM 4179 (AAL29298)	*Methanomicrobia*	*Methanomicrobiales*	*Methanomicrobiaceae*
0	0	2	2	U3-E08	MM-I	*Methanocorpusculum labreanum* Z (ABN07725)	*Methanomicrobia*	*Methanomicrobiales*	*Methanomicrobiaceae*
1	0	0	1	F1-C11	MM-I	*Methanocorpusculum parvum* DSM 3823 (AAL29294)	*Methanomicrobia*	*Methanomicrobiales*	*Methanomicrobiaceae*
0	1	0	1	F4-A09	MM-I				
0	0	2	2	U3-D09	MM-I				
1	0	0	1	F1-G01	MM-I				
1	0	0	1	F1-C07	MM-I				
0	1	0	1	F4-G06	MM-I				
0	3	0	3	F4-H02	MM-II	*Methanoplanus petrolearius* DSM 11571 (ADN36741)	*Methanomicrobia*	*Methanomicrobiales*	*Methanomicrobiaceae*
0	1	0	1	F4-H09	MM-II	*Methanoculleus marisnigri* JR1 (ABN56546)	*Methanomicrobia*	*Methanomicrobiales*	*Methanomicrobiaceae*
0	2	0	2	F4-B05	MM-II				
0	1	0	1	F4-A12	MM-II				
1	5	0	6	F4-D02	MM-II				
0	1	0	1	F4-H10	MM-II				
0	1	0	1	F4-B10	MM-II				
0	1	1	2	U3-G01	MM-II				
1	0	0	1	F1-B10	MM-II				
0	3	0	3	F4-G10	MM-II				
3	1	0	4	F4-B03	MM-II				
0	1	0	1	F4-F04	MM-II				
3	4	0	7	F4-B08	MM-II				
0	0	1	1	U3-D04	MM-II				
8	9	3	20	F4-F08	MM-II				
0	0	7	7	U3-H08	MM-III	No reference sequences included			
0	0	1	1	U3-F12	MM-III				
0	0	1	1	U3-G09	MM-IV	No reference sequences included			
7	6	2	15	F1-B07	MM-IV				
2	2	0	4	F4-H01	MM-IV				
1	0	0	1	F1-G05	MM-IV				
1	0	0	1	F1-E03	MM-IV				
2	2	0	4	F1-H08	MM-IV				
0	1	0	1	F4-D03	MM-IV				
1	0	0	1	F1-E05	MM-V	*Methanolinea tarda* NOBI-1 (EHF09948)	*Methanomicrobia*	*Methanomicrobiales*	*Methanoregulaceae*
0	1	0	1	F4-F03	MM-V				
1	1	4	6	U3-E04	MM-V				
1	0	2	3	F1-G08	MM-V				
0	1	2	3	U3-F07	MM-V				
0	1	0	1	F4-H04	MM-V				
1	0	3	4	U3-A03	MM-VI	No reference sequences included			
0	0	3	3	U3-H01	MM-VI				
0	0	1	1	U3-B02	MM-VI				
0	2	0	2	F4-C03	MM-VI				
2	0	0	2	F1-D12	MM-VII	*Methanoregula boonei* 6A8 (ABS55100)	*Methanomicrobia*	*Methanomicrobiales*	*Methanoregulaceae*
0	0	4	4	U3-E07	MM-VII				
1	0	0	1	F1-A08	MM-VII				
1	0	0	1	F1-D10	MM-VII				
0	0	1	1	U3-F05	MM-VII				
0	0	1	1	U3-B03	MM-VII				
1	0	0	1	F1-G04	MM-VII				
8	8	2	18	F1-B11	MST-I	*Methanosaeta harundinacea* 6Ac (ADQ42341)	*Methanomicrobia*	*Methanosarcinales*	*Methanosaetaceae*
1	1	0	2	F4-D06	MST-I	*Methanosaeta thermophila* PT (ABK14360)	*Methanomicrobia*	*Methanosarcinales*	*Methanosaetaceae*
0	0	1	1	U3-C07	MST-I				
0	2	0	2	F4-B06	MST-I				
0	0	1	1	U3-C12	MST-I				
0	0	3	3	U3-D01	MST_II	*Methanosaeta concilii* DSM 3671 (AAK16832)	*Methanomicrobia*	*Methanosarcinales*	*Methanosaetaceae*
1	1	0	2	F4-B09	MST_II	*Methanosaeta concilii* VeAc9 (AAK16833)	*Methanomicrobia*	*Methanosarcinales*	*Methanosaetaceae*
0	1	0	1	F4-C02	MST_II				
1	0	0	1	F1-A04	MST_II				
0	1	0	1	F4-G05	MST_II				
0	0	1	1	U3-D11	MST_II				
0	0	2	2	U3-F10	MST_II				
0	0	1	1	U3-F09	MST_II				
3	0	0	3	F1-E08	MST_II				
0	0	4	4	U3-E02	MST_II				
0	1	0	1	F4-F11	MST_II				
3	0	0	3	F1-D05	MST_II				
0	1	0	1	F4-E02	MST_II				
0	3	0	3	F4-H07	MST_II				
0	0	2	2	U3-C09	MST_II				
1	4	0	5	F4-D05	MST_II				
0	3	0	3	F4-B01	MST_II				
0	0	1	1	U3-D10	MSR	*Methanomethylovorans hollandica* ZB (AAP20897)	*Methanomicrobia*	*Methanosarcinales*	*Methanosarcinaceae*
1	0	1	2	U3-D05	MSR	*Methanococcoides burtonii* DSM 6242 (ABE53268)	*Methanomicrobia*	*Methanosarcinales*	*Methanosarcinaceae*
						Methanohalobium evestigatum Z-7303 (ADI73798)	*Methanomicrobia*	*Methanosarcinales*	*Methanosarcinaceae*
						Methanohalophilus mahii DSM 5219 (ADE36137)	*Methanomicrobia*	*Methanosarcinales*	*Methanosarcinaceae*
						Methanosarcina thermophila TM-1 (AAC43426)	*Methanomicrobia*	*Methanosarcinales*	*Methanosarcinaceae*
						Methanosarcina mazei C16 (AAC43413)	*Methanomicrobia*	*Methanosarcinales*	*Methanosarcinaceae*
						Methanosarcina acetivorans C2A (AAM07885)	*Methanomicrobia*	*Methanosarcinales*	*Methanosarcinaceae*
						Methanosarcina barkeri Fusaro (CAA68357)	*Methanomicrobia*	*Methanosarcinales*	*Methanosarcinaceae*
2	0	1	3	U3-A12	MCEL-I	*Methanocella paludicola* SANAE (BAI60588)	*Methanomicrobia*	*Methanocellales*	*Methanocellaceae*
0	0	3	3	U3-C03	MCEL-I	*Methanocella conradii* HZ254 (AEH76000)	*Methanomicrobia*	*Methanocellales*	*Methanocellaceae*
0	0	1	1	U3-E05	MCEL-I				
1	0	0	1	F1-D02	MCEL-II				
1	0	0	1	F1-F08	MB-I	*Methanobacterium palustre* NBRC 105230 (BAI67109)	*Methanobacteria*	*Methanobacteriales*	*Methanobacteriaceae*
						Methanosphaera stadtmanae DSM 3091 (ABC56731)	*Methanobacteria*	*Methanobacteriales*	*Methanobacteriaceae*
						Methanobrevibacter smithii ATCC 35061 (ABQ87107)	*Methanobacteria*	*Methanobacteriales*	*Methanobacteriaceae*
						Methanobacterium ivanovii NBRC 104952 (BAI67108)	*Methanobacteria*	*Methanobacteriales*	*Methanobacteriaceae*
2	0	0	2	F1-H12	MB-II	*Methanobacterium petrolearium* Mic5c12 (BAI67093)	*Methanobacteria*	*Methanobacteriales*	*Methanobacteriaceae*
4	2	0	6	F4-G09	MB-II	*Methanobacterium aarhusense* H2-LR (AAR27839)	*Methanobacteria*	*Methanobacteriales*	*Methanobacteriaceae*
2	1	0	3	F1-C04	MB-II	*Methanobacterium alcaliphilum* NBRC 105226 (BAI67095)	*Methanobacteria*	*Methanobacteriales*	*Methanobacteriaceae*
0	0	1	1	U3-F06	MB-II	*Methanobacterium lacus* AL-21 (ADZ08605)	*Methanobacteria*	*Methanobacteriales*	*Methanobacteriaceae*

0	0	2	2	U3-G12	MB-II	*Methanobacterium bryantii* DSM 863 (AAK16836)	*Methanobacteria*	*Methanobacteriales*	*Methanobacteriaceae*
1	0	0	1	F1-E07	MB-II	Methanothermobacter thermautotrophicus delta H (AAA7344	*Methanobacteria*	*Methanobacteriales*	*Methanobacteriaceae*
0	0	3	3	U3-D03	MB-II				
0	0	1	1	U3-H09	MB-II				
2	0	0	2	F1-F01	UnEury-I	No reference sequences included			
2	0	0	2	F1-H09	UnEury-II	Candidatus *Methanomethylophilus alvus* Mx1201 (AGI85144)	*Methanobacteria*	the seventh order	
0	0	2	2	U3-H05	UnEury-II	*Methanomassiliicoccus luminyensis* B10 (AEO13319)			
1	0	0	1	U3-B06	UnEury-II				
1	0	3	4	U3-A08	UnEury-II				
0	0	1	1	U3-B07	UnEury-II				
0	0	1	1	U3-F08	UnEury-II				
0	0	2	2	U3-G08	UnEury-II				
0	0	4	4	U3-E11	UnEury-II				
86	82	87	255						

Bae et al., 2015

TABLE S5. Gene copies measured using qPCR from soil samples collected in WCAs of the Everglades.

Study site	Sampling time	T-MCRA	MM	MST	MB	DsrB
F1	Oct. (2009)	1.2E+9(5.1E+8)	6.4E+8(3.8E+8)	3.4E+8(2.4E+8)	2.3E+7(1.2E+7)	ND
	Apr. (2010)	1.2E+9(9.4E+8)	5.8E+8(4.4E+8)	3.0E+8(1.7E+8)	6.7E+7(5.1E+7)	4.4E+8 (1.6E+8)
	Aug. (2011)	6.5E+8(1.3E+8)	1.0E+9(2.6E+8)	6.2E+8(1.6E+8)	3.9E+7(1.6E+7)	1.2E+9 (2.7E+8)
	Jan. (2012)	2.4E+9(9.8E+8)	1.3E+9(4.7E+8)	9.8E+8(4.7E+8)	1.2E+8(5.2E+7)	3.5E+9 (1.3E+9)
	Aug (2012)	6.0E+8(1.1E+8)	7.1E+8(3.3E+7)	4.5E+8(5.4E+7)	5.5E+7(2.8E+7)	1.1E+9 (2.2E+8)
	Dec. (2012)	6.5E+8(8.9E+7)	1.5E+9(1.4E+8)	7.2E+8(8.0E+7)	6.7E+7(1.4E+7)	3.2E+9 (4.0E+8)
	average	**1.1E+9(8.3E+8)**	**9.6E+8(4.5E+8)**	**5.7E+8(3.1E+8)**	**6.2E+7(4.2E+7)**	**1.9E+9 (1.4E+9)**
F4	Oct. (2009)	4.4E+8(2.7E+8)	5.2E+8(5.5E+8)	2.2E+8(8.5E+8)	5.1E+6(3.2E+6)	ND
	Apr. (2010)	8.5E+8(4.2E+8)	3.8E+8(1.7E+8)	1.6E+8(7.4E+7)	1.0E+7(8.8E+5)	5.1E+8 (1.2E+8)
	Aug. (2011)	6.7E+8(8.5E+7)	1.1E+9(3.9E+8)	5.5E+8(8.1E+7)	2.6E+7(7.0E+6)	1.1E+9 (6.8E+7)
	Jan. (2012)	2.1E+9(4.8E+8)	9.3E+8(1.5E+8)	9.6E+8(2.6E+8)	1.9E+8(8.4E+7)	3.5E+9 (1.0E+9)
	Aug (2012)	3.8E+8(8.0E+7)	2.3E+8(8.1E+7)	3.3E+8(1.0E+8)	1.1E+7(3.8E+6)	7.6E+8 (1.6E+8)
	Dec. (2012)	6.4E+8(2.2E+8)	8.7E+8(2.9E+7)	3.6E+8(9.7E+7)	2.0E+7(9.2E+6)	1.8E+9 (6.2E+8)
	average	**8.4E+8(6.4E+8)**	**6.8E+8(4.2E+8)**	**4.3E+8(3.0E+8)**	**4.4E+7(7.3E+6)**	**1.5E+9 (1.2E+9)**
U3	Oct. (2009)	1.5E+9(2.2E+9)	8.9E+7(4.5E+7)	1.0E+8(1.3E+8)	1.6E+7(2.5E+7)	ND
	Apr. (2010)	2.4E+8(1.6E+8)	1.3E+8(1.0E+8)	7.8E+7(4.1E+7)	7.2E+6(2.3E+6)	2.4E+8 (9.7E+7)
	Aug. (2011)	2.4E+8(7.5E+7)	2.1E+8(1.2E+8)	1.8E+8(4.7E+7)	9.1E+6(5.4E+6)	4.4E+8 (6.4E+7)
	Jan. (2012)	8.7E+8(2.2E+8)	5.0E+8(1.3E+8)	3.5E+8(9.5E+7)	1.9E+7(3.3E+6)	1.4E+9 (3.1E+8)
	Aug (2012)	2.9E+8(9.0E+7)	2.2E+8(5.3E+7)	1.5E+8(5.7E+7)	8.6E+6(6.2E+6)	3.8E+8 (5.2E+7)
	Dec. (2012)	1.0E+8(6.1E+7)	1.5E+8(7.2E+7)	1.5E+8(7.0E+7)	8.2E+6(3.6E+6)	8.5E+8 (4.1E+8)
	average	**5.3E+8(9.1E+8)**	**2.2E+8(1.6E+7)**	**1.7E+8(1.1E+8)**	**1.1E+7(1.0E+7)**	**6.7E+8 (4.9E+8)**
W3	Feb. (2012)	2.9E+7 (1.9E+7)	2.5E+7 (3.6E+6)	3.4E+7 (2.3E+7)	6.3E+6 (3.2E+6)	9.5E+7 (6.6E+7)
	Mar. (2013)	6.4E+7 (6.6E+7)	4.0E+7 (2.6E+7)	5.1E+7 (2.9E+7)	1.8E+7 (1.5E+7)	7.6E+7 (4.2E+7)
	Apr. (2013)	1.2E+8 (4.1E+7)	3.0E+7 (5.3E+6)	8.6E+7 (1.4E+7)	1.9E+7 (8.3E+6)	8.6E+7 (1.5E+7)
	average	**9.8E+7 (6.0E+7)**	**1.9E+7 (1.7E+7)**	**7.6E+7 (2.3E+7)**	**1.4E+7 (1.0E+7)**	**8.6E+7 (4.1E+7)**

ND, Not determined

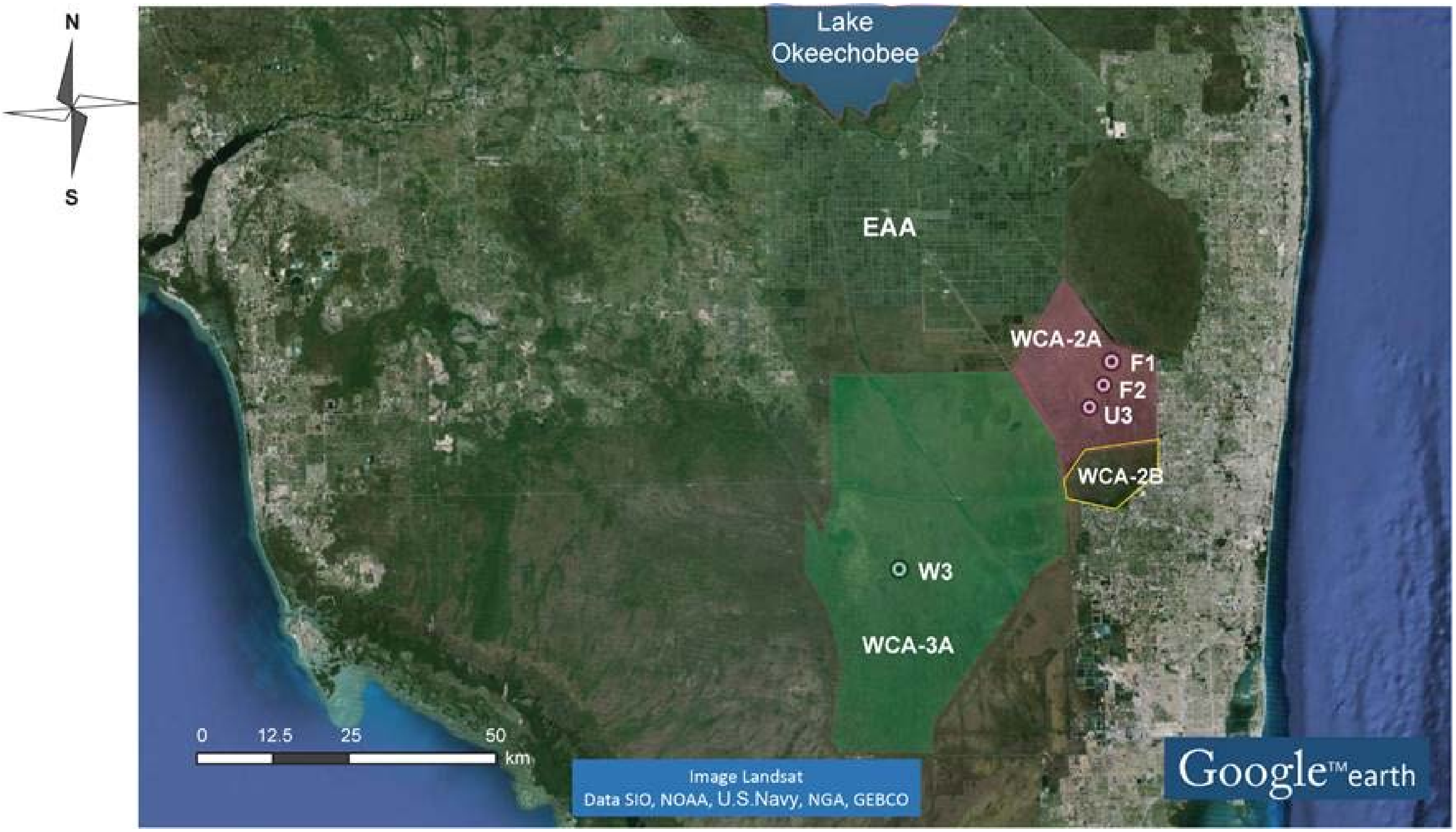

Image Landsat
Data SIO, NOAA, U.S.Navy, NGA, GEBCO

FIG. S1. Study sites F1, F4, U3 and W3 within the Everglades Conservation Areas.

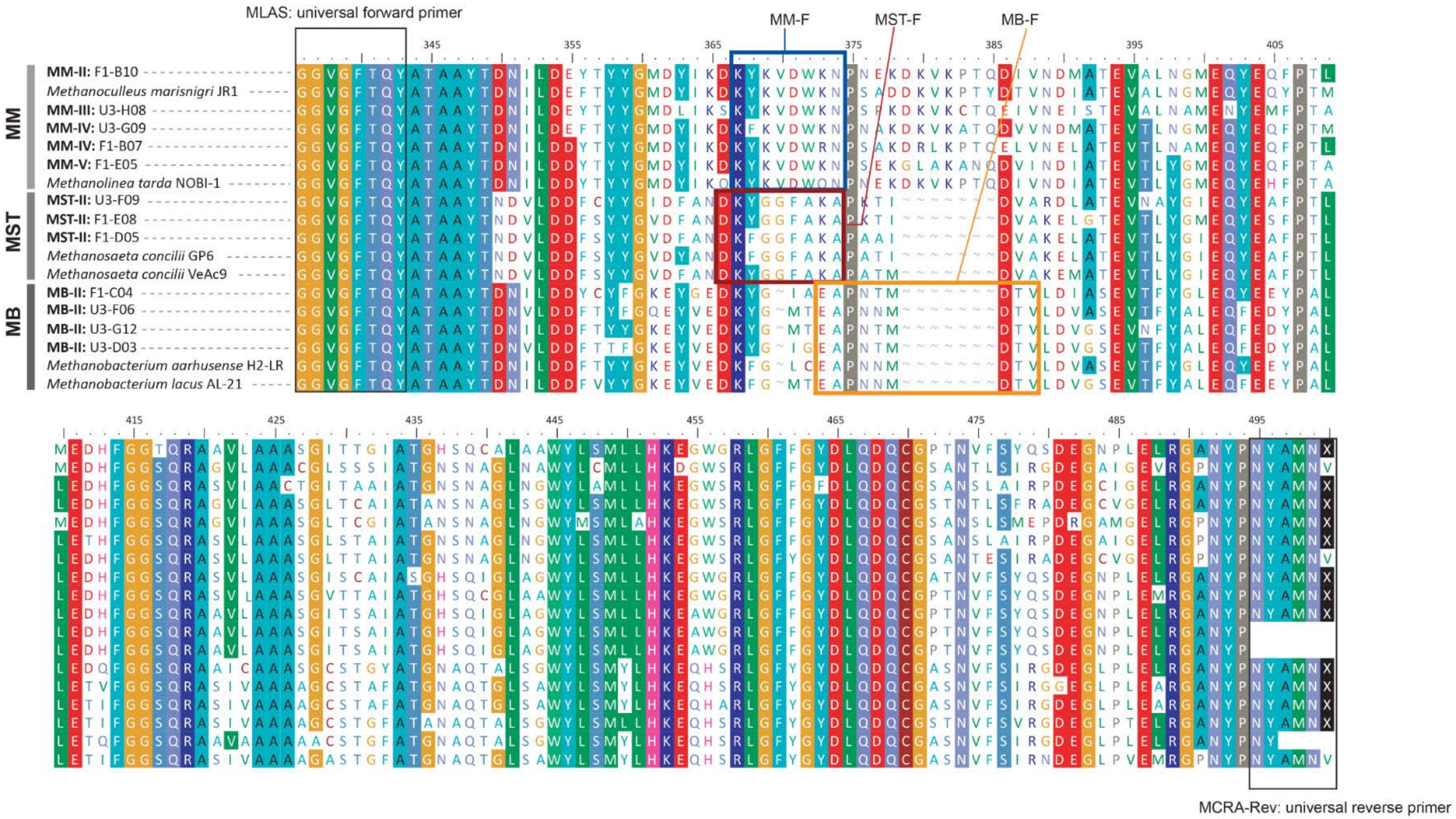

FIG. S2. Binding region of *mcrA* primers MLAS, MCR-Rev, MM-F, MST-F and MB-F used in qPCR in this study.

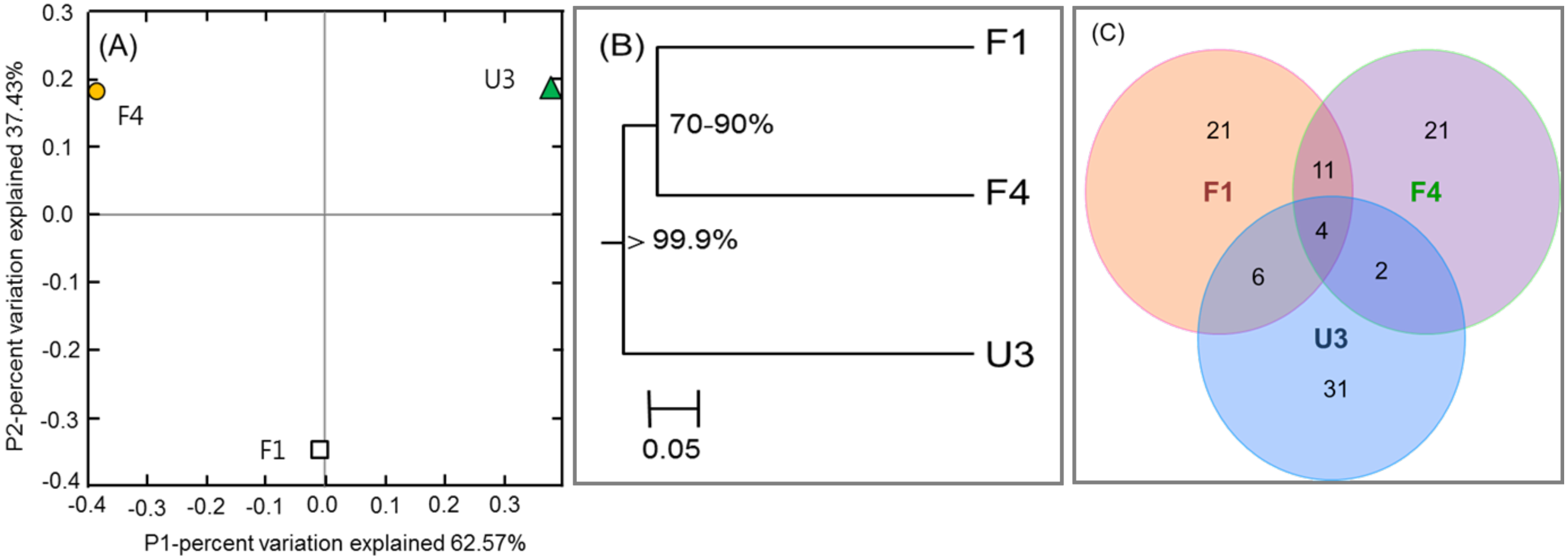

FIG. S3. Distribution of methanogen communities determined by McrA in F1, F4, U3 of the Everglades Water Conservation Areas. (A) Unweighted PCoA plot and (B) Dendrogram with Jackknif values cluster, and (C) Venn diagram showing the shared number of OTUs between sites.

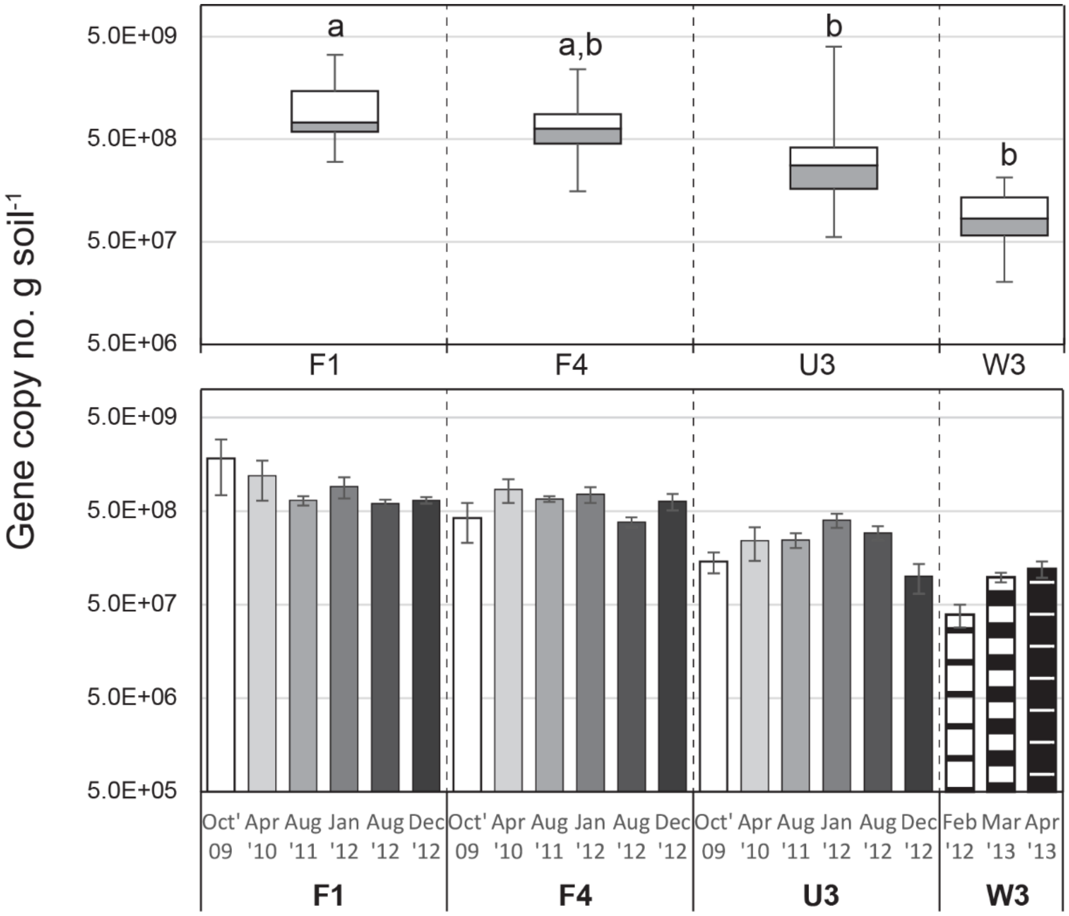

FIG. S4. Box and Whisker plot (upper) and temporal profiles (lower) of the *mcrA* copies for total methanogens measured in the soil samples collected from sties F1, F4, U3 and W3 from October 2009 to April 2013. Error bars in temporal profiles within each site represent +/- 1 SE from 3 soil cores. Box and Whisker plot was constructed based on pooled data from the temporal measurements within each site. Boxes depict the medians (horizontal lines) and the lower and upper quartiles (bottoms and tops of boxes). The vertical bars (whiskers) show the highest and the lowest values, excluding outliers. Lower case letters on each box represent the difference obtained from comparisons for all pairs of sites: similar letters indicate no significant difference; different letters indicate a significant difference ($P < 0.05$ in Tukey-Kramer HSD test).

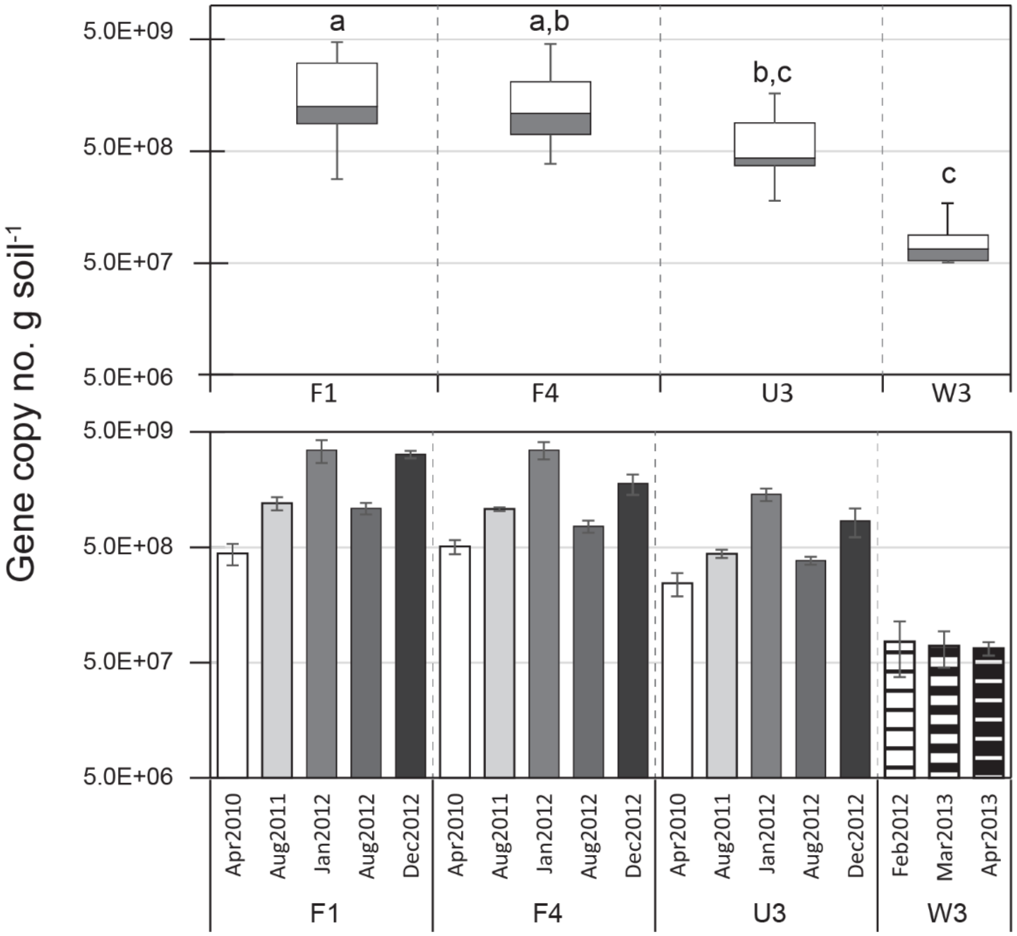

FIG. S5. Box and Whisker plot (upper) and temporal profiles (lower) of the *dsrB* copies measured in the soil samples collected from sties F1, F4, U3 and W3 from April 2010 to April 2013. Error bars in temporal profiles represent +/- 1 SE from 3 soil cores. Box and Whisker plot was constructed from the pooled data of temporal measurements within each site. Boxes depict the medians (horizontal lines) and the lower and upper quartiles (bottoms and tops of boxes). The vertical bars (whiskers) show the highest and the lowest values, excluding outliers. Lower case letters on each box represent the difference obtained from comparisons for all pairs of sites: similar letters indicate no significant difference; different letters indicate a significant difference (P <0.05 in Tukey-Kramer HSD test).

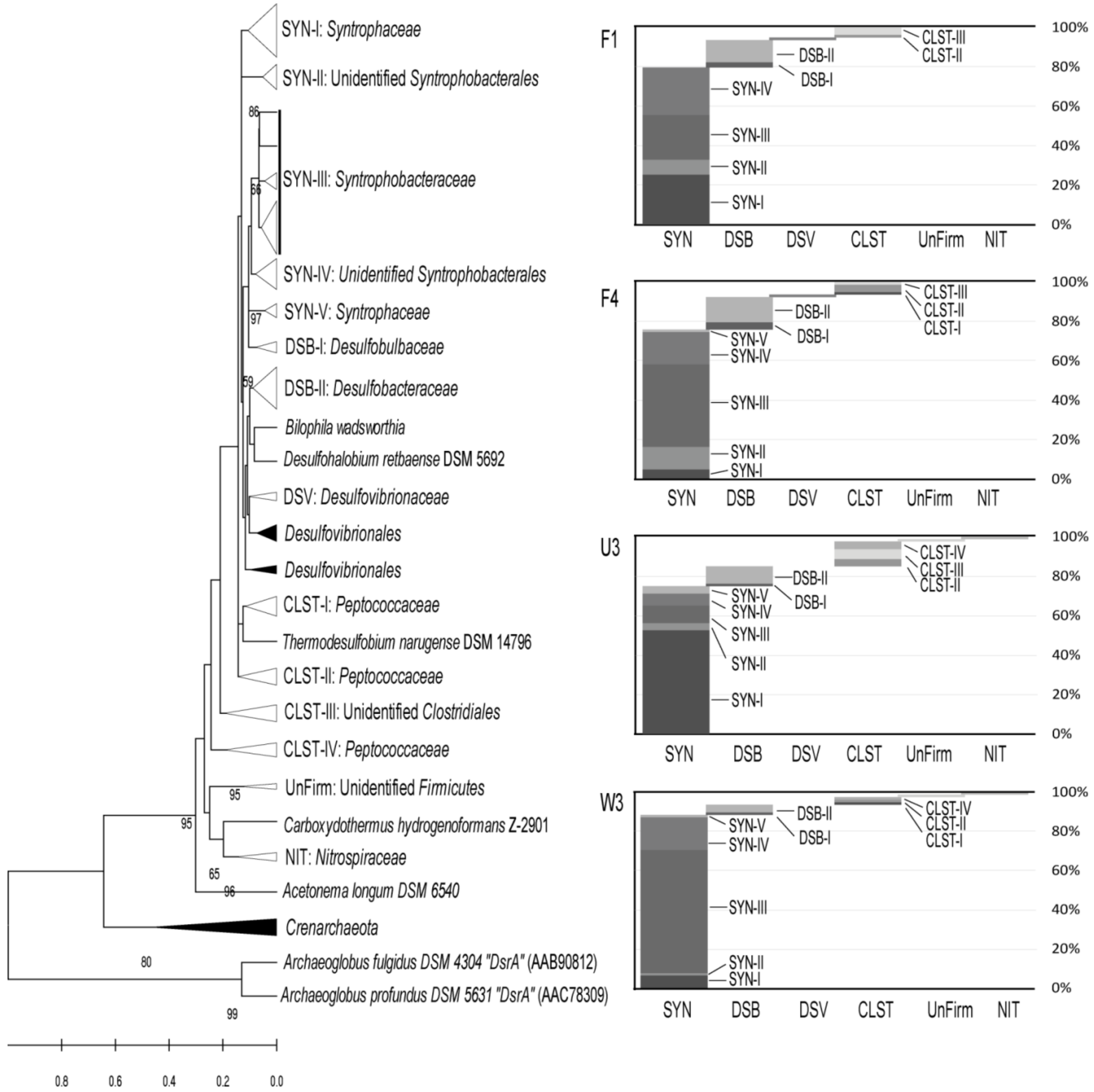

FIG. S6. Maximum Likelihood tree representing the phylogeny of *dsrB* transcripts from study sites F1, F4, U3 and W3. Deduced amino acid sequences from *dsrB* transcripts were used for constructing this tree. Bootstrap values of ≥50% from 1000 re-assemblages were placed at branch points. The relative percentage of clades generated from the ML tree and were presented in the stacked column graphs.

References

1. Steinberg LM, Regan JM. 2009. *mcrA*-Targeted Real-Time Quantitative PCR Method To Examine Methanogen Communities. Appl. Environ. Microbiol. 75:4435-4442.

2 Foti M, Sorokin DY, Lomans B, Mussman M, Zacharova EE, Pimenov NV, Kuenen JG, Muyzer G. 2007. Diversity, Activity, and Abundance of Sulfate-Reducing Bacteria in Saline and Hypersaline Soda Lakes. Appl. Environ. Microbiol. 73: 2093–2100.