Supporting Information for:

Nitrate Removal in Shallow, Open-Water Treatment Wetlands

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14 pages
10 figures
2 tables

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Table SI 1. Wetland Temperatures[a]

Month	Average Temperature (˚C)
January	8.6±0.4
February	9.8±0.3
March	11.9±0.3
April	14.4±1.0
May	16.8±0.7
June	20.0±0.3
July	22.0±0.2
August	22.3±0.4
September	21.7±0.5
October	17.5±0.3
November	12.4±0.5
December	9.3±0.3

[a] Average monthly temperatures reported in Livermore, CA from 2007-2012 ± standard error of the mean.[5]

Table SI 2. QPCR Primers and Thermal Profiles

Primer Name	Primer Sequence 5'-3'	[Primer] (nM)	Amplicon Size	Target Gene	Thermal Profile	Reference
cd3aF	GTSAACGTSA AGGARACSGG	500	425	nirS	95°C 10 min, 6 touchdown cycles [95°C 15 s, 63–58°C (−1°C per cycle) 30 s, 72°C 30 s,] then 35 cycles (95°C 15 s, 58°C 30 s, 72°C 30 s)	Throbäck et al., 2004[1]
R3cd	GASTTCGGRT GSGTCTTGA	500				
nirK876	ATYGGCGGVA YGGCGA	500	164	nirK	95°C 10 min, 6 touchdown cycles [95°C 15 s, 63–58°C (−1°C per cycle) 30 s, 72°C 30 s,] then 35 cycles (95°C 15 s, 58°C 30 s, 72°C 30 s)	Henry et al. 2004[2]
nirK1040	GCCTCGATCA GRTTRTGGTT	500				
hzsA1597F	WTYGGKTATC ARTATGTAG	400	260	hzs	95°C 3min, 40 cycles (95°C 30 s, 55°C 30 s , 72°C 30 s)	Harhangi et al., 2011[3]
hzsA1857R	AAABGGYGAA TCATARTGGC	400				
EUB338	ACTCCTACGG GAGGCAGCAG	1000	180	16S	95°C 3min, 40 cycles (95°C 60 s, 53°C 30 s , 72°C 60 s)	Fierer et al., 2005[4]
EUB518	ATTACCGCGG CTGCTGG	1000				

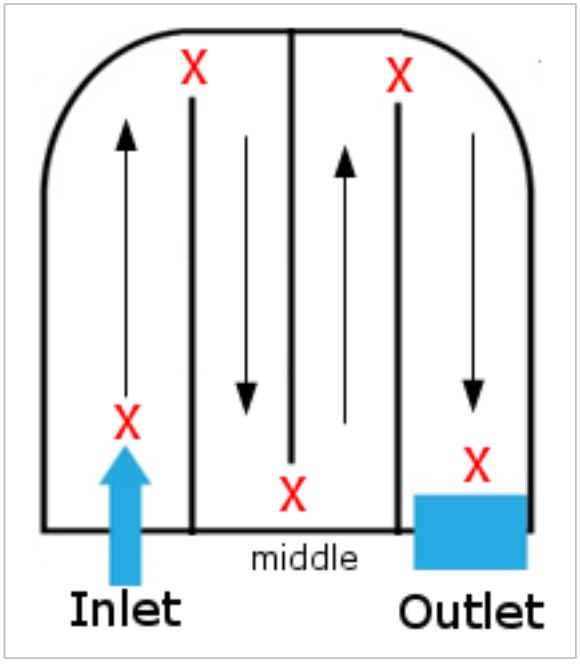


Figure SI 1. Schematic of open-water wetland cell. X symbols represent sampling points. Black arrows show flow direction.

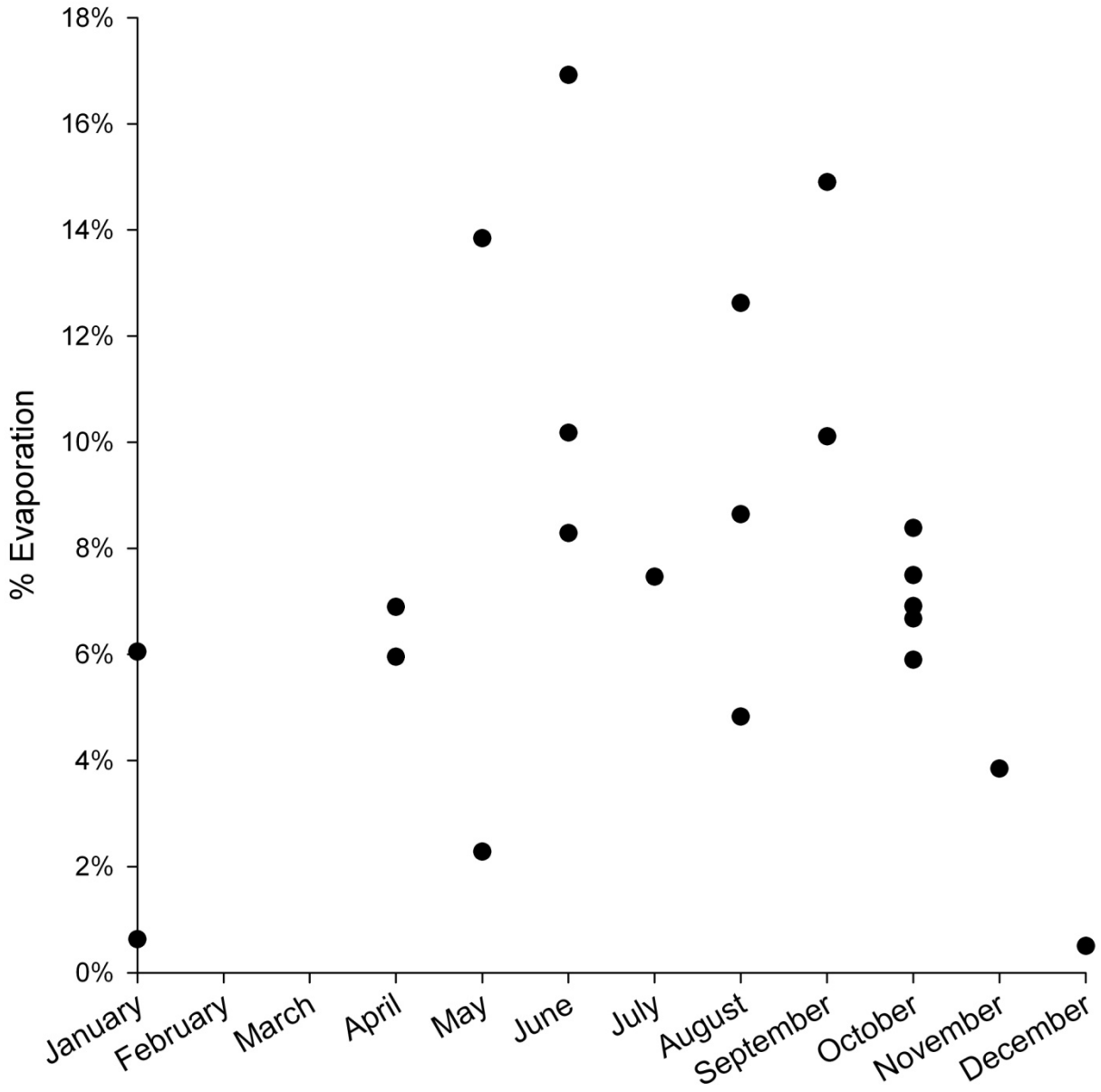


Figure SI 2. Percent evaporation between inlet and outlet of pilot-scale wetland cell throughout the year, calculated based on Cl^- concentrations.

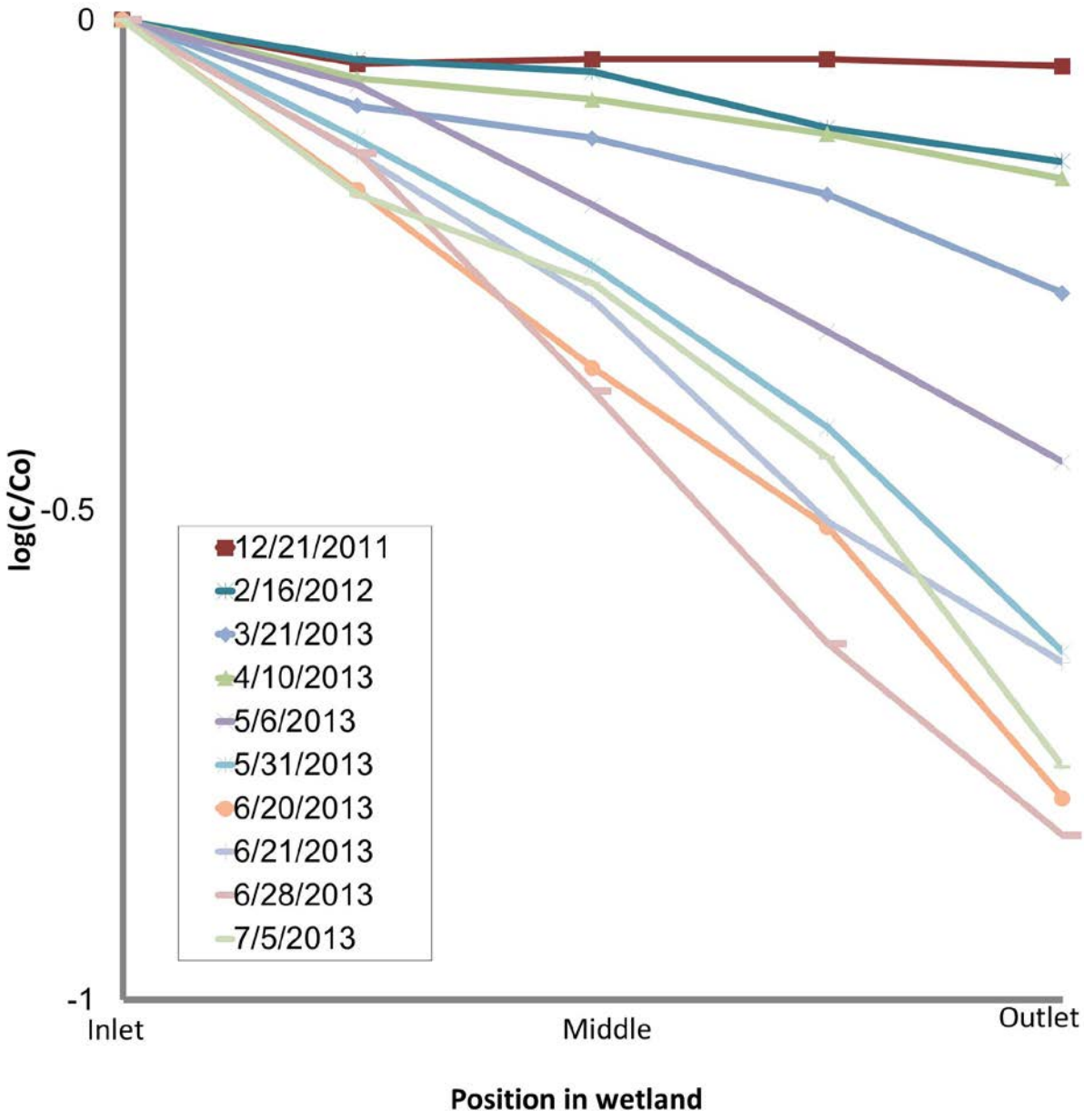


Figure SI 3. Semi-log plot of NO_3^- removal along flow-path in Discovery Bay pilot-scale open-water wetland cell. For clarity, a limited data set is plotted. Other data exhibited similar kinetics.

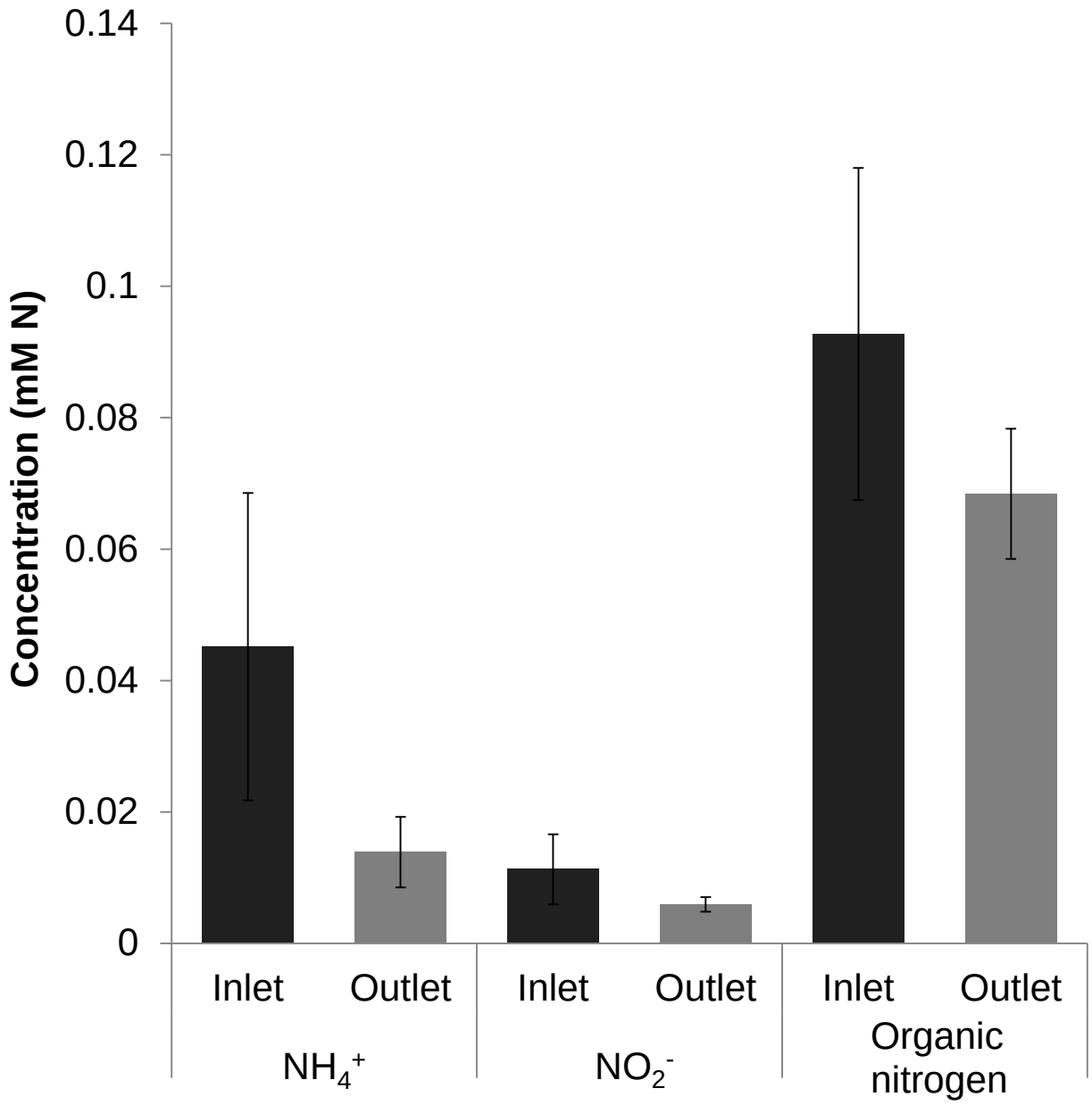


Figure SI 4. Average inlet and outlet concentrations of nitrogen species ± standard error of the mean (n=3-5).

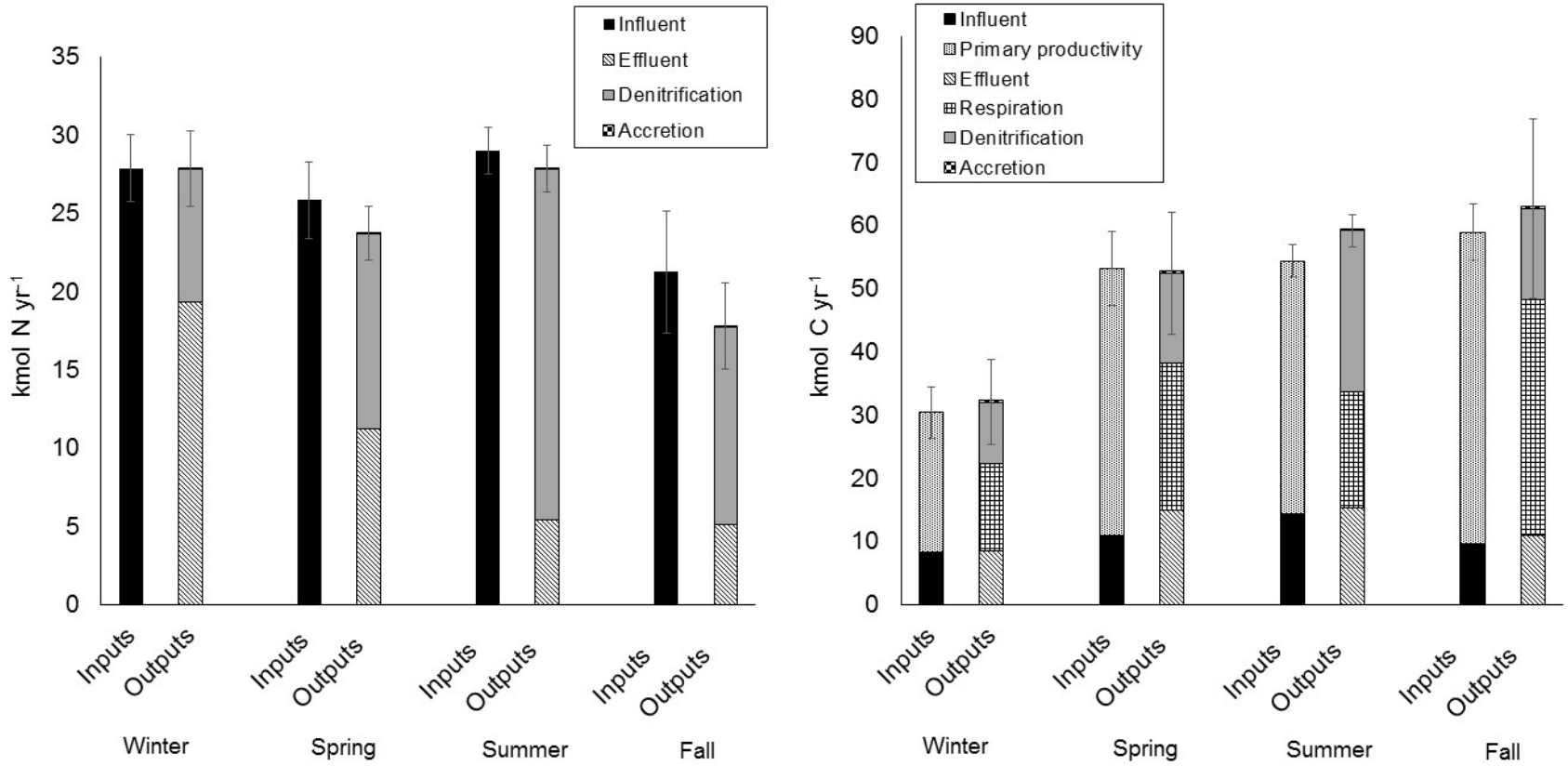


Figure SI 5. Nitrogen (left) and carbon (right) seasonal mass balances (average ± standard error of the mean) for Discovery Bay open-water wetland during 2012-2013. Accretion fluxes are based on measured biomat elemental composition (32±1% organics) and measured accumulation rate. Primary productivity and aerobic respiration fluxes are based on diurnal oxygen profiles. Denitrification fluxes calculated using rates measured in acetylene-block microcosms, corrected and averaged by monthly average temperatures.

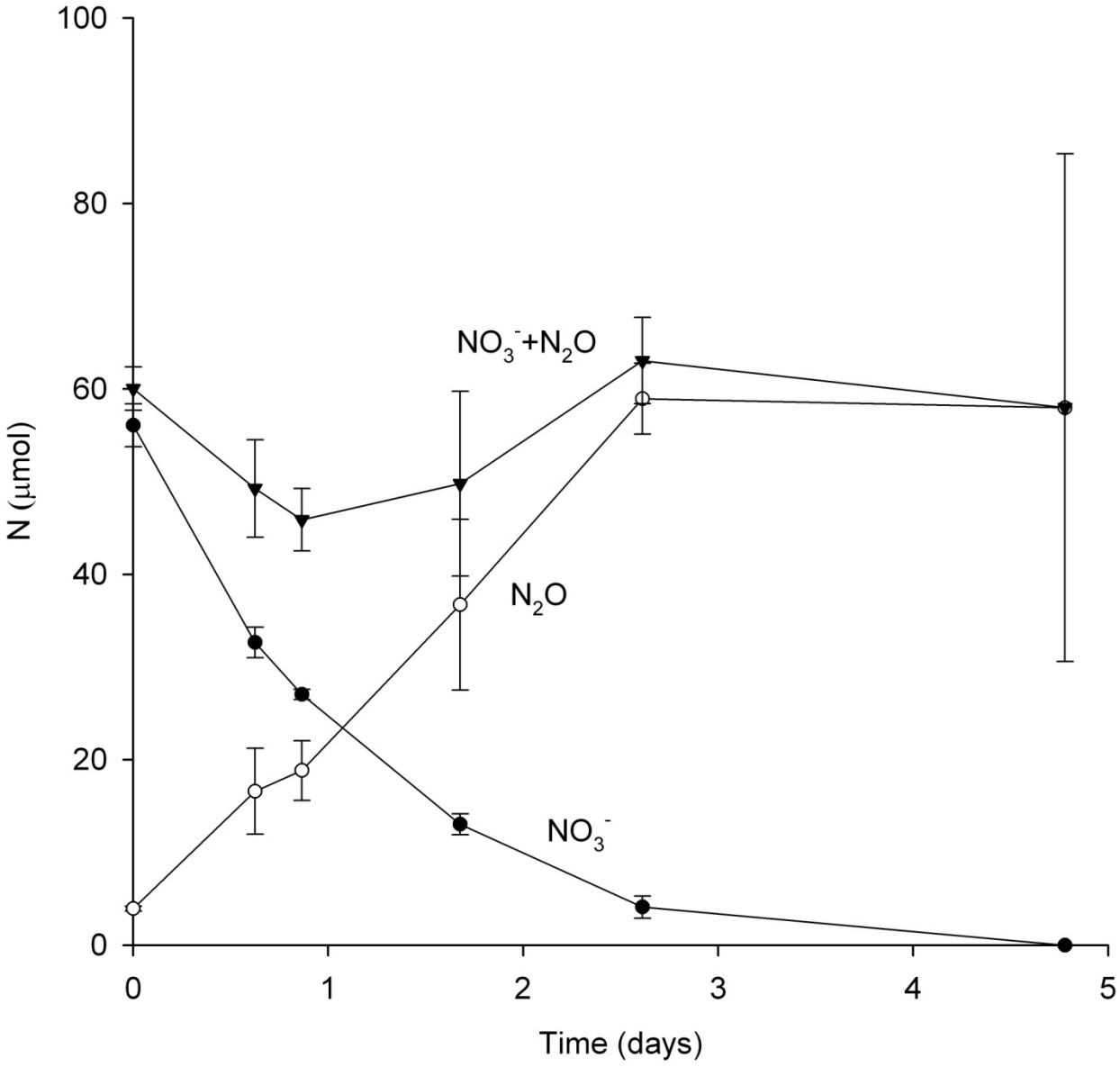
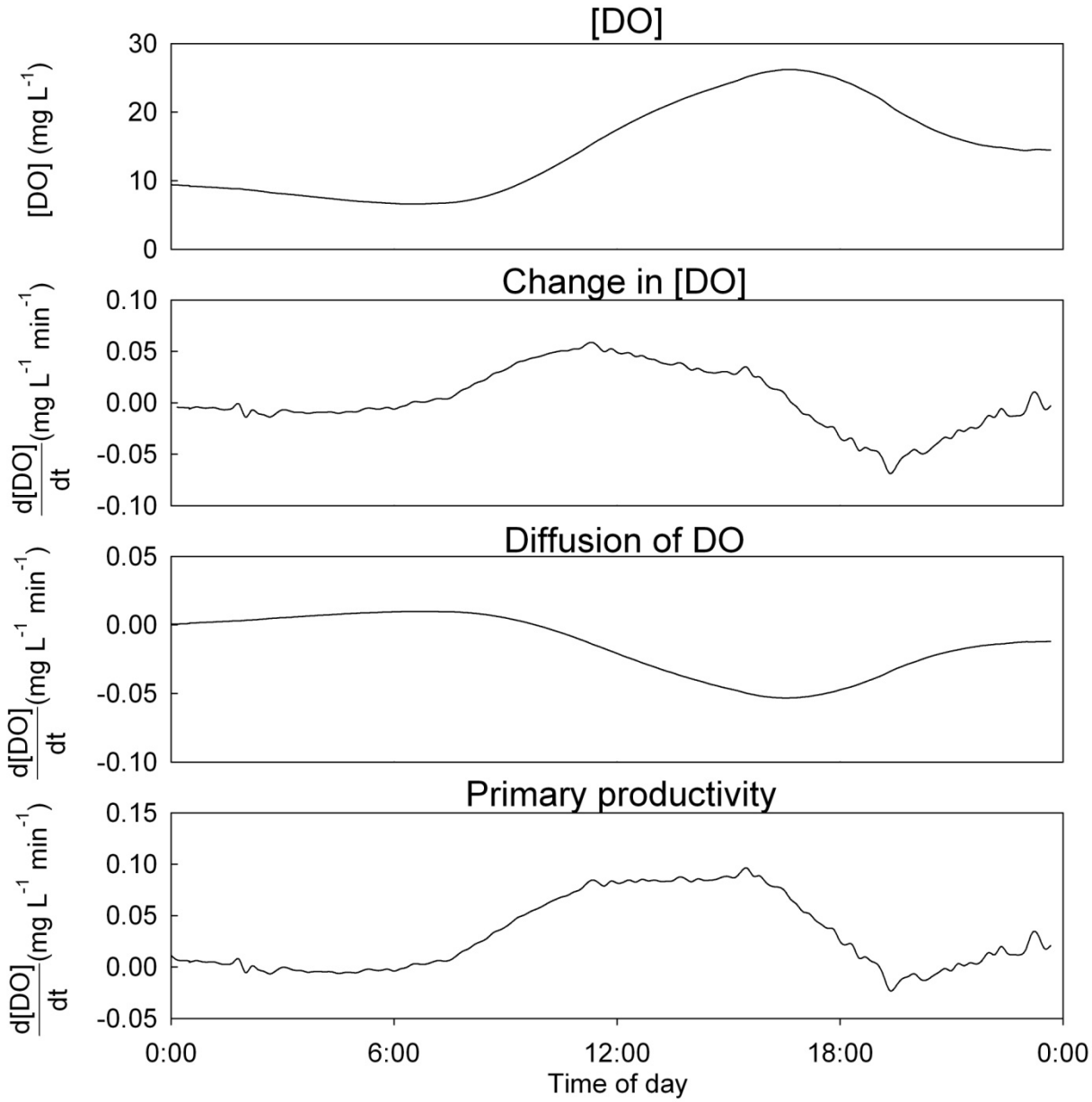


Figure SI 6. Conversion of NO_3^- to N_2O via partial denitrification in anoxic microcosms amended with acetylene gas (5 mL; ~10% v/v). Experiment conducted at 22°C. Error bars represent ± one standard deviation (n=3).



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16 **Figure SI 7.** Example of dissolved oxygen (DO) profiles used to calculate annual primary
17 productivity and respiration rates. Data shown from April 8th, 2013 at outlet of pilot-scale
18 wetland (Manta MultiProbe, Eureka Environmental, Austin, TX). Diffusion was based on [DO]
19 before sunrise and after sunset; respiration was based on consumption of DO during night;
20 Primary productivity=Change in [DO]+Respiration-Diffusion of DO.[6]

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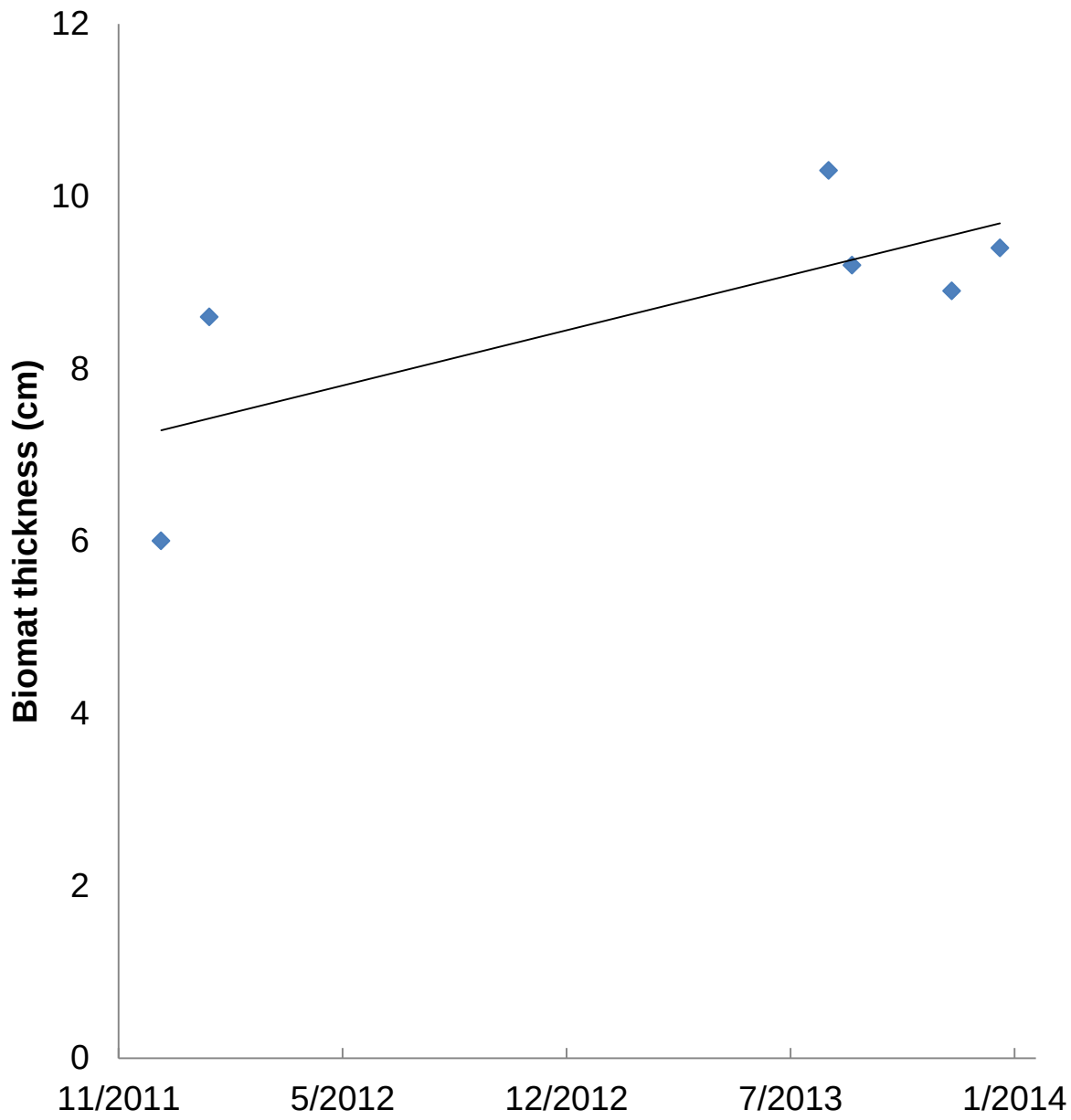

Figure SI 8. Average biomat thickness measured throughout the pilot-scale open-water wetland throughout the study period. Slope=1.2±0.5 cm yr^{-1}. r^2=0.55.

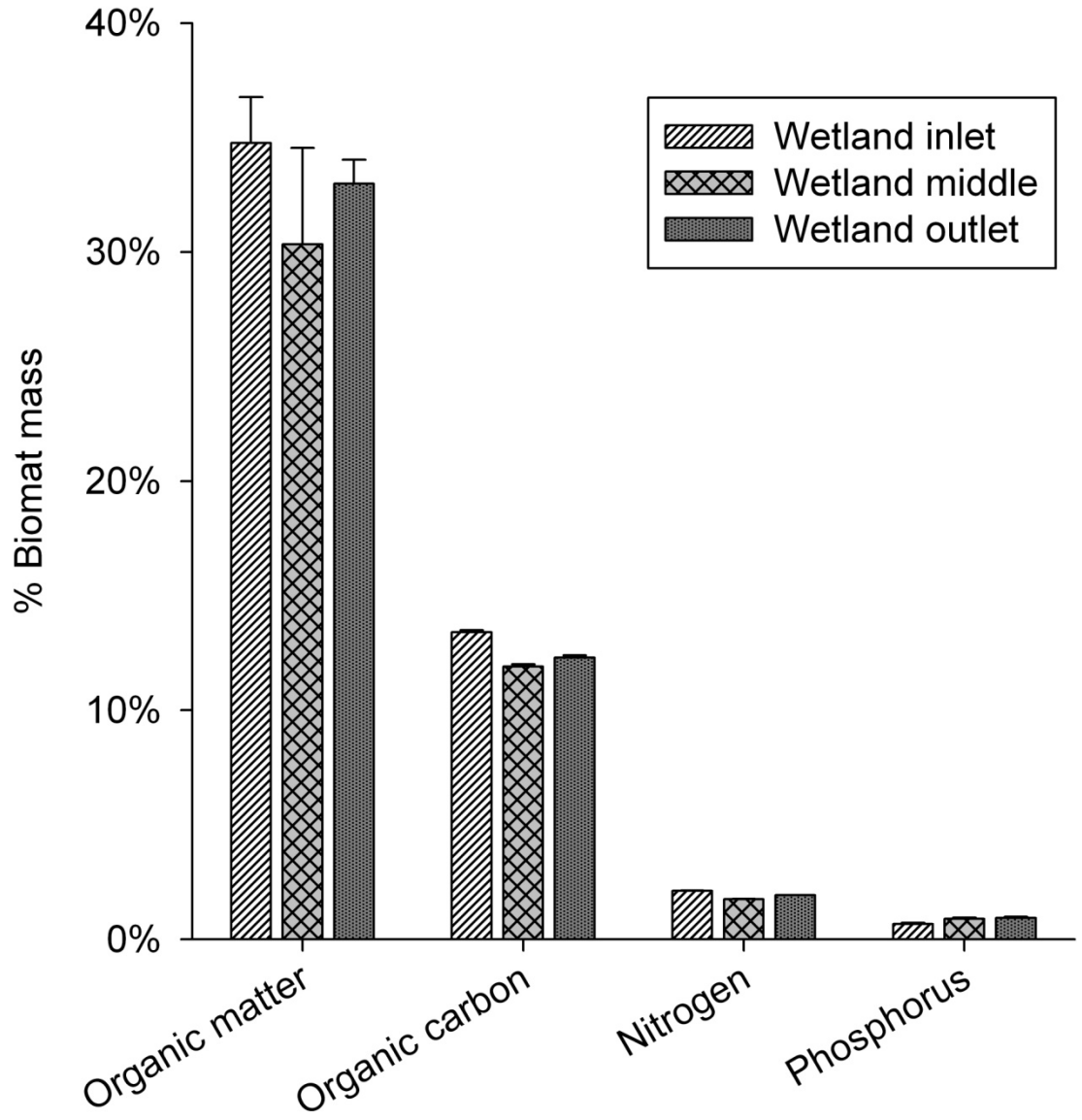

Figure SI 9. Percent phosphorous, nitrogen, organic carbon, and organic matter (i.e., volatile solids) by mass in wetland biomat at the inlet, middle, and outlet of open-water wetland on a dry weight basis. Remainder consisted of non-volatile organic matter (i.e., minerals).

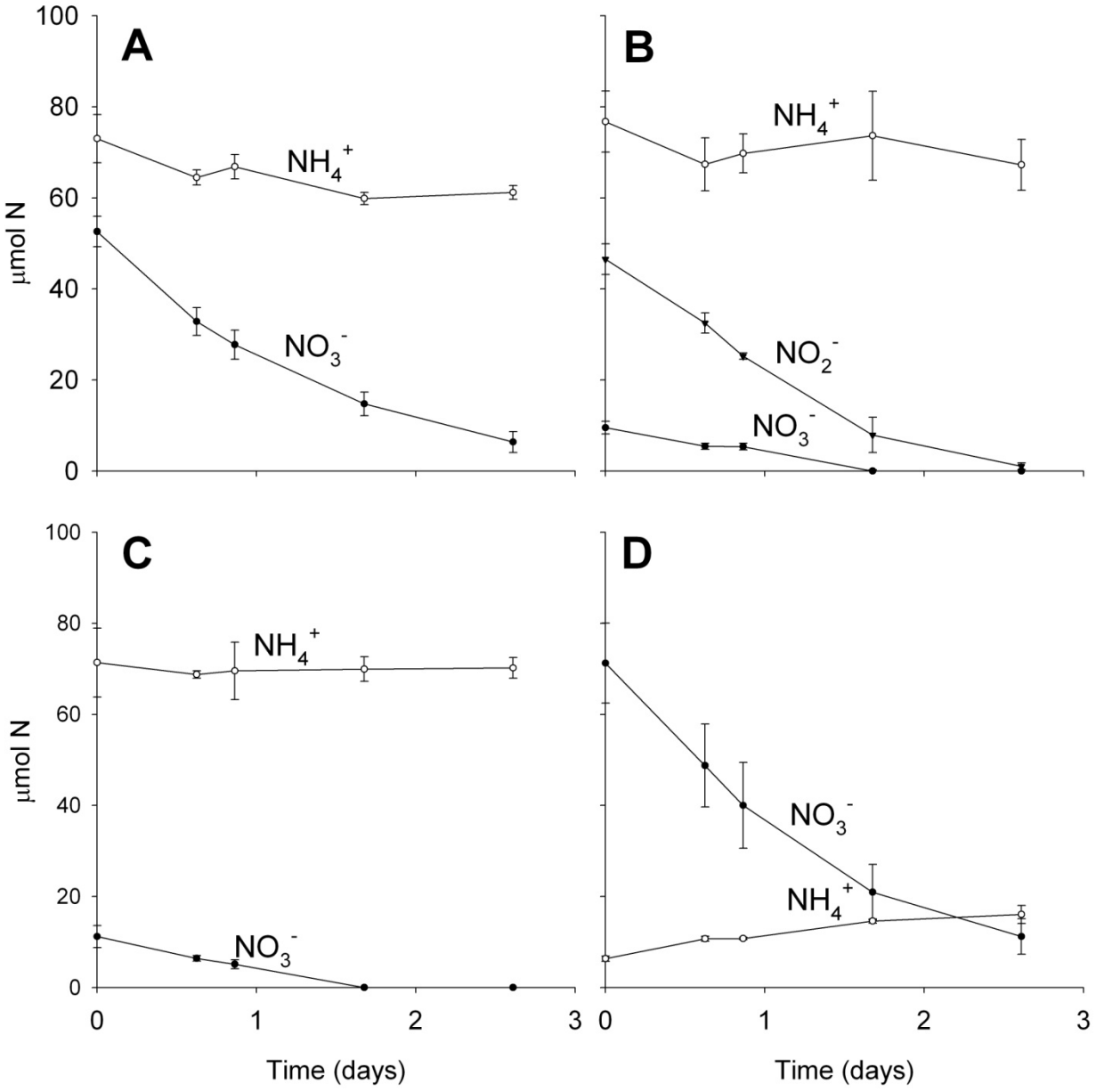

Figure SI 10. Concentrations of nitrogen species in anoxic microcosms amended with **A**: NH_4^+ and NO_3^-; **B**: NH_4^+ and NO_2^-; **C**: NH_4^+; **D**: NO_3^-. Experiment conducted at 22±2°C. Error bars represent ± one standard deviation (n=3).

References

(1) Throbäck, IN; Enwall, K; Jarvis, Å; Hallin, S. Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. *FEMS Microbiol. Ecol.* **2004**, *49* (3), 401–417.

(2) Henry, S; Baudoin, E; López-Gutiérrez, JC; Martin-Laurent, F; Brauman, A; Philippot, L. Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. *J. Microbiol. Methods*. **2004**, *59* (3), 327–335.

(3) Harhangi, HR; Roy, ML; Alen, T van; Hu, B; Groen, J; Kartal, B; Tringe, SG; Quan, Z-X; Jetten, MSM; Camp, HJMO den. Hydrazine synthase, a unique phylomarker with which to study the presence and biodiversity of anammox bacteria. *Appl. Environ. Microbiol.* **2012**, *78* (3), 752–758.

(4) Fierer, N; Jackson, JA; Vilgalys, R; Jackson, RB. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR Assays. *Appl. Environ. Microbiol.* **2005**, *71* (7), 4117–4120.

(5) Metar. Weather history for Livermore, CA. **2013**. Available at: http://www.wunderground.com/history/airport/KLVK. Accessed December 17, 2013.

(6) Odum, HT. Primary production in flowing waters. *Limnol. Oceanogr.* **1956**, *1* (2), 102–117.