

SUPPORTING INFORMATION

Improving the nutrient removal performance of surface
flow constructed wetlands in winter using hardy
submerged plant-benthic fauna systems

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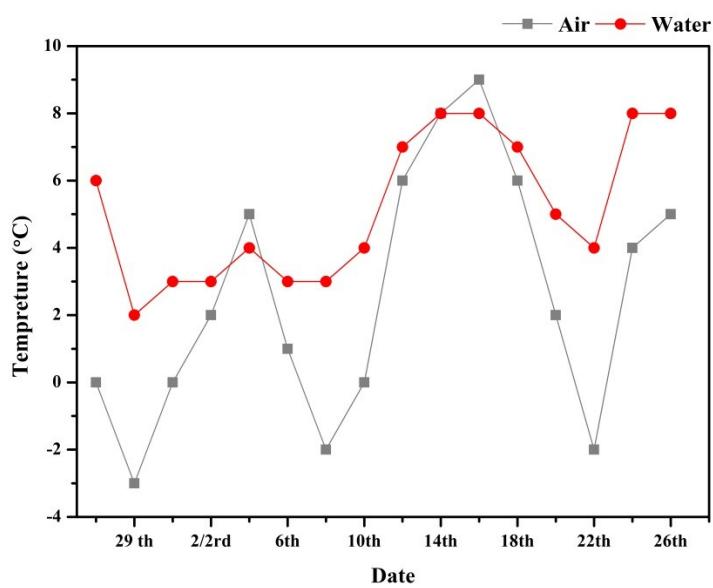


Fig.S1. Air, water temperature during the experiment period

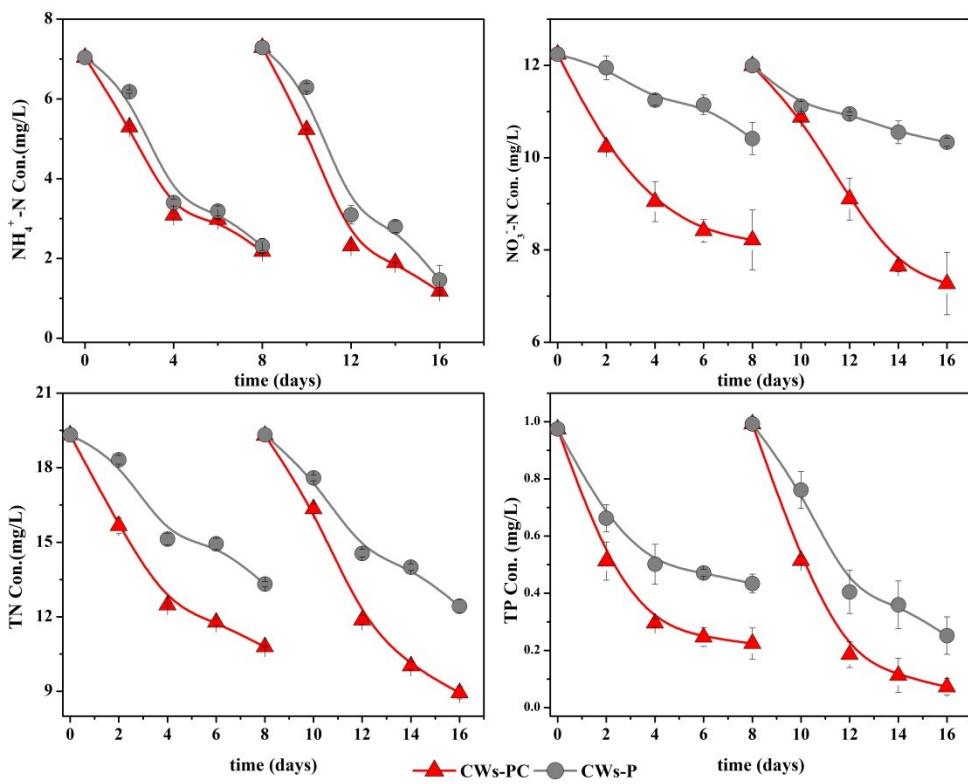


Fig.S2. Treatment performance on NH_4^+ -N, NO_3^- -N, TN and TP for each group during two unstable operating cycles

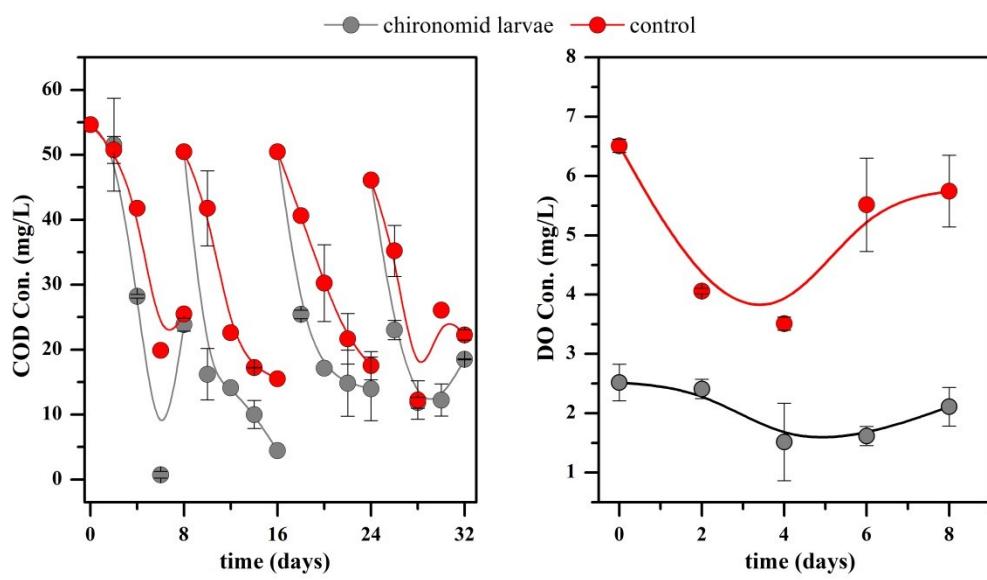


Fig.S3. Treatment performance on COD for each group during four typical operating cycles (a)
Typical DO profile in each group (b)

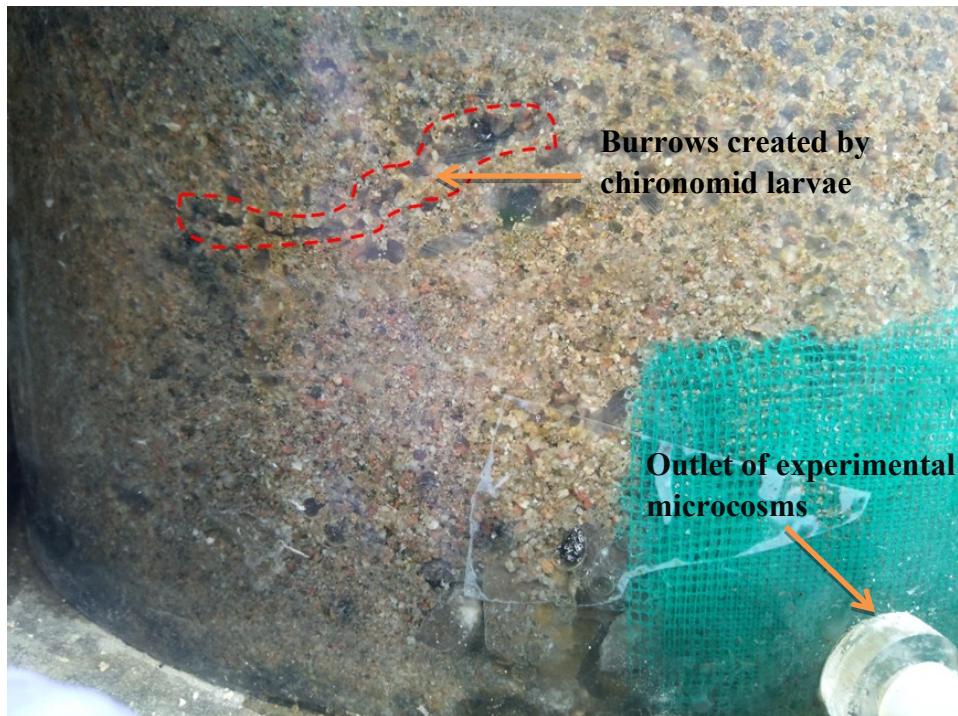


Fig. S4 Picture showing the burrows produced by chironomid larvae at the US layer

Table S1. Primers of target genes used in qPCR analysis.

Target gene	Primer	Primer sequence (5'-3')	Amplification size (bp)	Reference
Bacterial 16S rRNA	338F	ACTCCTACGGGAGGCAGCAG	180	(Muyzer et al., 1993)
	518R	ATTACCGCGGCTGCTGG		
<i>amoA</i>	amo598f	GAATATGTTCGCCTGATTG	120	(Dionisi et al., 2002)
	amo718r	CAAAGTACCAACCATAACGCAG		
<i>nirK</i>	nirK583F	TCA TGGTGCTGCCGKGACGG	326	(Liu et al., 2003)
	nirK909R	GAA CTTGCCGGTKGCCAGAC		
<i>nirS</i>	nirScd3aF	GT(C/G)AACGT(C/G)AAGGA(A/G)AC(C/G)GG	425	(Kandeler et al., 2006)
	nirSR3cd	GA(C/G)TTCGG(A/G)TG(C/G)GTCTTGA		

Table S2. Total biomass over the course of the experiment

	Total dry weight of plant (g)		Total dry weight of larvae (g)	
	initial	final	initial	final
CWs-PC	0.23 ± 0.02	4.10 ± 0.83	18.26 ± 0.44	19.07 ± 0.12
CWs-P	0.20 ± 0.03	1.30 ± 0.14	-	-

--: means no data

Table S3. Comparison of phylotype coverage, diversity and richness estimators at a phylogenetic distance of 3%

CWs	Sample	OTUs	Shannon	Simpson(10 ⁻¹)	ACE	Chao	Good's coverage(%)
CWs-P	Superincumbent substrate layer	2899	9.11	9.90	3488.34	3468.78	98.2
	Underlying substrate layer	1589	6.16	9.12	2076.12	2023.16	98.8
CWs-PC	Superincumbent substrate layer	2935	9.48	9.96	3476.69	3493.08	98.2
	Underlying substrate layer	1727	6.44	9.30	2241.50	2248.49	98.7
	Larvae body	2001	6.11	9.27	3296.32	3658.37	97.7

References for supplementary materials

- Dionisi, H.M., Layton, A.C., Harms, G., Gregory, I.R., Robinson, K.G., Sayler, G.S., 2002. Quantification of Nitrosomonas oligotropha-like ammonia-oxidizing bacteria and Nitrospira spp. from full-scale wastewater treatment plants by competitive PCR. *Appl. Environ. Microb.* **68**(1), 245-253.
- Kandeler, E., Deiglmayr, K., Tscherko, D., Bru, D., Philippot, L., 2006. Abundance of narG, nirS, nirK, and nosZ genes of denitrifying bacteria during primary successions of a glacier foreland. *Appl. Environ. Microb.* **72**(9), 5957-5962.
- Liu, X., Tiquia, S.M., Holguin, G., Wu, L., Nold, S.C., Devol, A.H., Luo, K., Palumbo, A.V., Tiedje, J.M., Zhou, J., 2003. Molecular diversity of denitrifying genes in continental margin sediments within the oxygen-deficient zone off the Pacific coast of Mexico. *Appl. Environ. Microb.* **69**(6), 3549-3560.
- Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microb.* **59**(3), 695-700.