Supplemental Information

Understanding the Impact of Different Source Water Types on the Biofilm Characteristics and Microbial Communities of Manganese Removing Biofilters

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Additional Materials and Methods

Water Quality Analysis

TOC was measured with a TOC analyzer (TOC-VCSH TOC Analyzer, Shimadzu) following Method 5310 B.[1] True colour was measured using the Platinum-Cobalt Standard Method on a HACH DR5000 following Method 8025.[1] Alkalinity was measured using a T50 auto titrator (Mettler Toledo) following Method 2320.[1] Phosphate and ammonia were measured using a HACH DR5000 following Method 8048 and the salicylate method (#8155) respectively.[1] To quantify dissolved metals, samples were filtered through a 0.45 μm cellulose nitrate membrane. Filtered and unfiltered samples were acidified with trace metals nitric acid to a pH of < 2 and analysed by inductively coupled plasma mass spectrometry (ICP-MS, Thermofisher X Series II) following Standard Method 3125 to determine the concentration of total and dissolved metals respectively.[2-4]

Cellular ATP was quantified in duplicate for 50 mL aliquots of raw water and filter effluent water using commercial test kits for aqueous samples (Quench Gone Aqueous, LuminUltra Technologies Ltd.). Raw water and filter effluent water were also filtered through a sterile 0.22 um nitrocellulose membrane and genomic DNA was extracted from the filters using a commercial test kit (QIAGEN DNeasy Power Water Kit, QIAGEN). All analyses were initiated within 24 hrs of sample collection.

Filter Media Analysis

Mn and Fe on the surface of filter media was analysed following EPA Method 3050B.[5] Filter media was dried at 105°C for approximately 72 hrs before being digested. Particles were removed from solution by decanting, centrifugation, and filtration (0. 45 μm). Mn and Fe in the filtered digestate were quantified by ICP-MS following Standard Method 3125.[2] Total ATP was quantified in duplicate using a commercial test kit for biofilm samples (Deposit and Surface Analysis, LuminUltra Technologies Ltd.) and EPS was extracted in triplicate following the method of.[6] The protein fraction of EPS was quantified in duplicate using a commercial test kit (Pierce BCA Protein Assay Kit, Thermo Scientific) and the carbohydrate fraction was quantified in duplicate following the method of DuBois et al., (1956) as modified by Keithley and Kirisits (2018).[6,7] HPC enumeration was performed following Method 9215.[1] Genomic DNA was extracted using a commercial kit for biofilm samples (DNeasy Power Biofilm Kit, QIAGEN). Wet filter media was weighed in triplicate then dried at 105°C for 72 hrs and weighed again to determine a dry weight correction factor. The dry weight correction factor was applied to the measurement of metals, tATP, protein, and carbohydrate on filter media.

Additional Data

Table S1. Duration, in seconds, for each stage of the backwash cycle at the M-GW/SW, Shediac, and W-GW biofiltration plants.

Backwash Cycle	M-GW/SW	S-GW	W-GW
Level Down (s)	120	400	600
Air Scour (s)	120	120	120
Air + Water (s)	200	180	240
Rinse (s)	1100	180	300
Pre-Filtration (s)	780	500	720

Table S2. Average (± S.D.) accumulation of Mn and Fe on filter media taken from the M-GW/SW, S-GW, and W-GW biofiltration plants before and after backwashing. Filter media mass has been corrected to dry weight.

	Sample Location	M-GW/SW	S-GW	W-GW
Mn (mg/g dw)	Pre-Backwash	55 ± 13	55 ± 7	76 ± 26
	Post-Backwash	44 ± 7	52 ± 7	112 ± 38
Fe (mg/g dw)	Pre-Backwash	18 ± 14	52 ± 11	0.4 ± 0.2
	Post-Backwash	12 ± 6	50 ± 17	0.8 ± 1.0

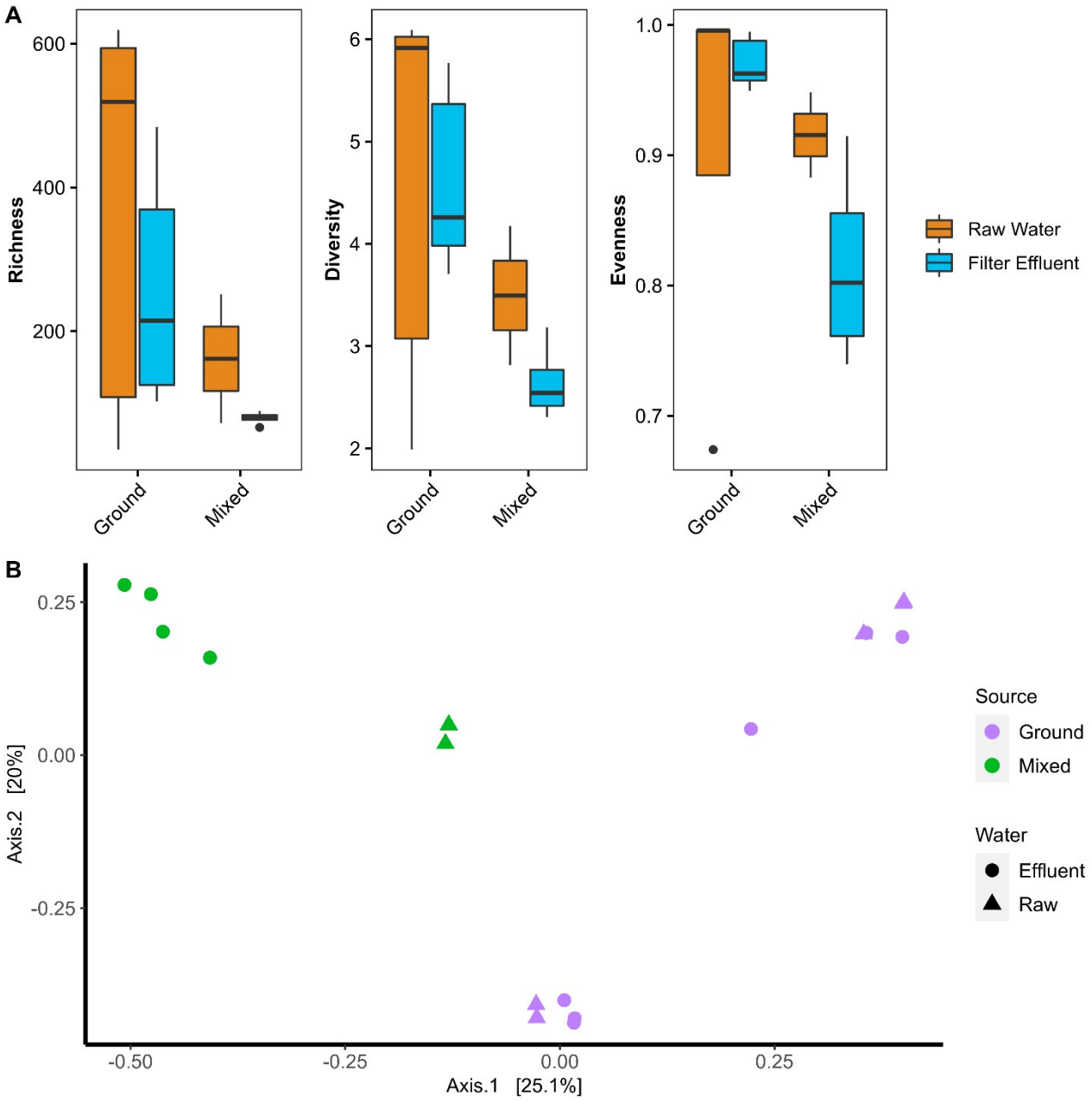

Figure S1. (A) Richness (observed ASVs), diversity (Shannon's Index), and evenness (Simpson's Index) of communities within groundwater and mixed-water biofilters. Richness ($p < 0.05$), diversity ($p < 0.05$), and evenness ($p < 0.05$) were all significantly greater the mixed-water community than in the groundwater communities (Wilcoxon rank sum test with a Benjamini-Hochberg correction for FDR). The horizontal line within the box indicates the median, the upper and lower bounds of the box indicate the third and first quartiles, respectively, and the whiskers extend to the largest value no greater than 1.5 times the interquartile range. Values beyond the whiskers are outliers. (B) PCoA ordination of Bray Curtis dissimilarity matrix of microbial

communities in raw and effluent water samples from groundwater and mixed-water biofilters. The groundwater community was significantly different ($p < 0.001$) from the mixed-water community (Adonis test, 999 permutations).

References

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