Hydraulic and biotic impacts on neutralisation of high-pH waters

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Supplementary Information

Materials and Methods

SI Section S1. Detailed experimental conditions

Duplicates of the experiments were run for each set of conditions. Sterilized runs were performed without biofilm in both types of systems, for 20 days. In a parallel sterilised cascade experiment, air was excluded for the first 15 days, after which time the experiment was ventilated. Between the runs of the sterilized experiments, the systems were washed with detergent, rinsed with tap water, followed by 10 % HNO_3, ethanol and deionized water (15 MΩ). For the biotic experiments, biofilm was collected from settlement ponds with steel slag leachate at Scunthorpe, UK to reflect alkaliphile communities established under field conditions in wetland systems (53°34'46.3"N 0°35'33.8"W). Samples of biofilm sufficiently thick to enable sections of it to be removed were collected together with water and placed in the systems within one hour of sampling. A macrophyte alga (*Ulva flexuosa*) was also collected from the ponds at Scunthorpe, together with colonized plastic mesh pads that were attached to the vegetation in the ponds for a month. The three sources (direct samples, algae, and pads) were used to colonize the systems with biofilm, following the approach of Rogerson et al. (2008b). The biofilm incubation and colonization period lasted eight weeks.

During colonization and in the biotic experiments, the mesocosm was subjected to a 12:12 day: night light cycle (light: Thorn Lopak 250 W HPS-T Na lamp), in a windowless, air-conditioned laboratory (ambient air temperature 20 ± 1°C). The mesocosms were placed in full light conditions (6.82 klux) and allowed to run without samples being taken to allow the system to equilibrate fully for 8 weeks. During the biofilm incubation, the steel slag leachate was prepared with tap and deionized water, in similar amounts. The pH of the leachate during incubation was 10.5 (Table 1) permitting rapid development of the biofilm, which was found to be impeded under higher pH. After full colonization and between runs of replicates of the biotic experiment, the systems were gradually fed with steel slag leachate at pH 11.5 for five days to prevent an abrupt increase in pH, which might damage the biofilm. The experiment started immediately after, and monitoring and sampling took place over a 20 day period. To characterise cycles in bulk physio-chemical parameters and trace metal concentration, a day and night sampling regime was implemented in the last run of the experiment to fully assess the control exerted by biofilms.

S2. Results

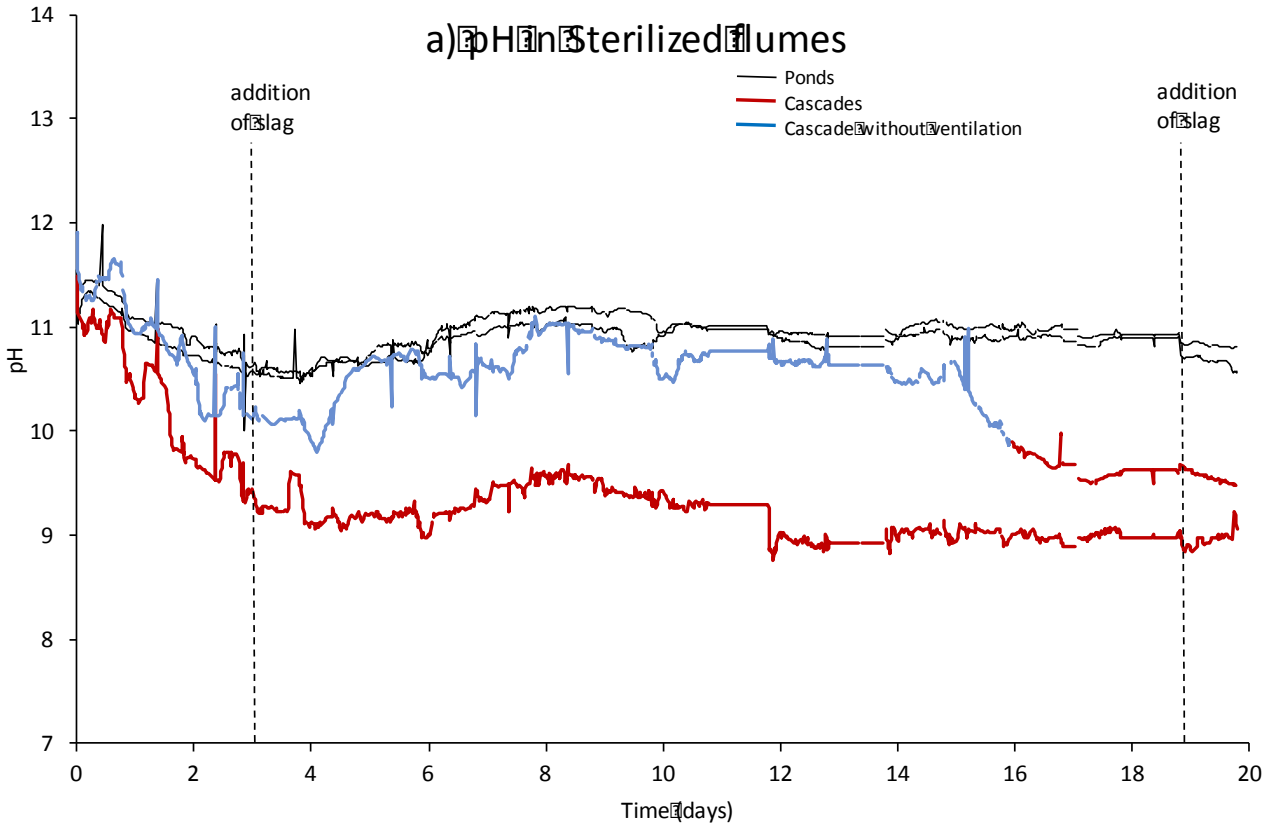

Figure S1. pH values in the first run of the experiments including results of the unventilated cascade (blue line). The data from this test assists understanding the role of gas exchange with air in these systems, but it has been removed from all subsequent statistical analyses.

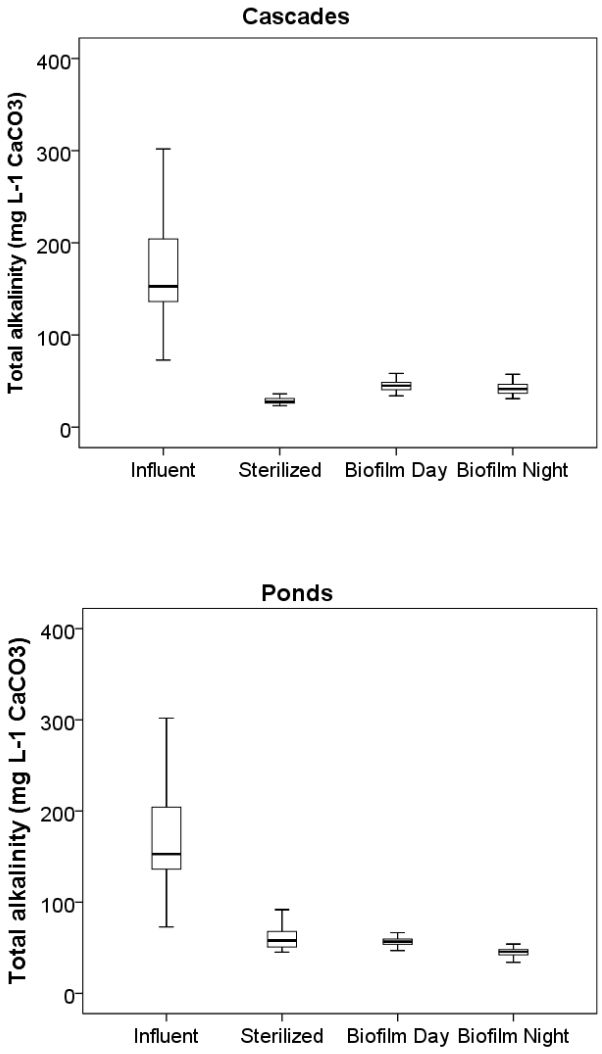

Figure S2. Boxplots of total alkalinity measured in the influent and the effluent of the sterilized and colonized ponds and cascades, during day and night in the biofilm experiments colonized mesocosms.

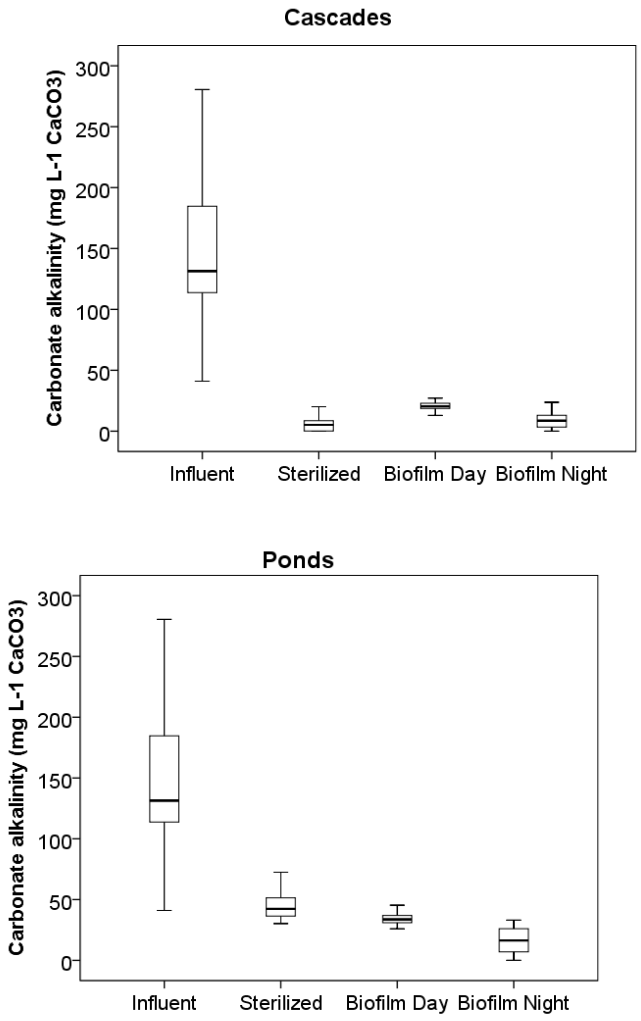

Figure S3. Boxplots of carbonate alkalinity measured in the influent and the effluent of the sterilized and colonized ponds and cascades, during day and night in the biofilm colonized mesocosms.

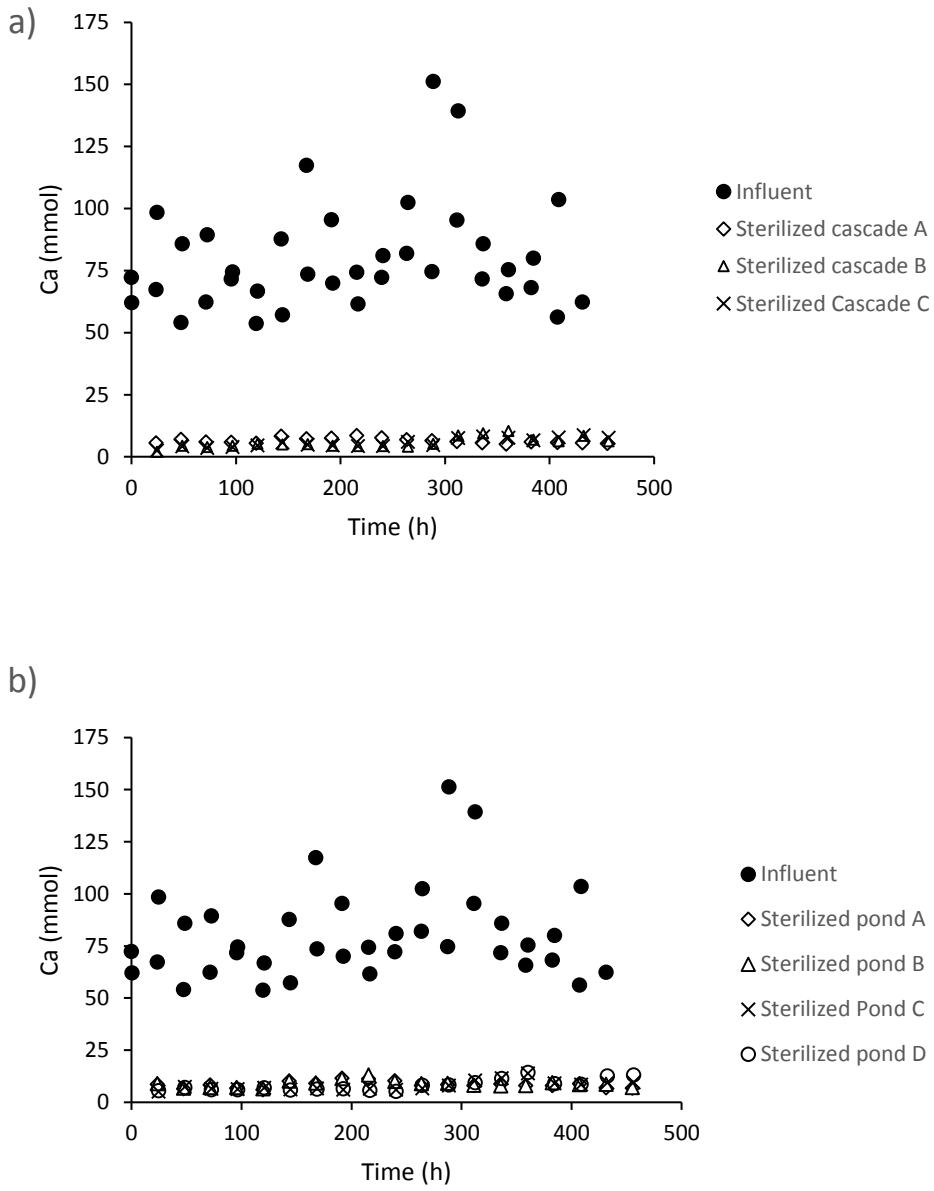

Figure S4. Calcium input and output (mmol) in the sterilized systems a) cascades and b) ponds during the experiments.

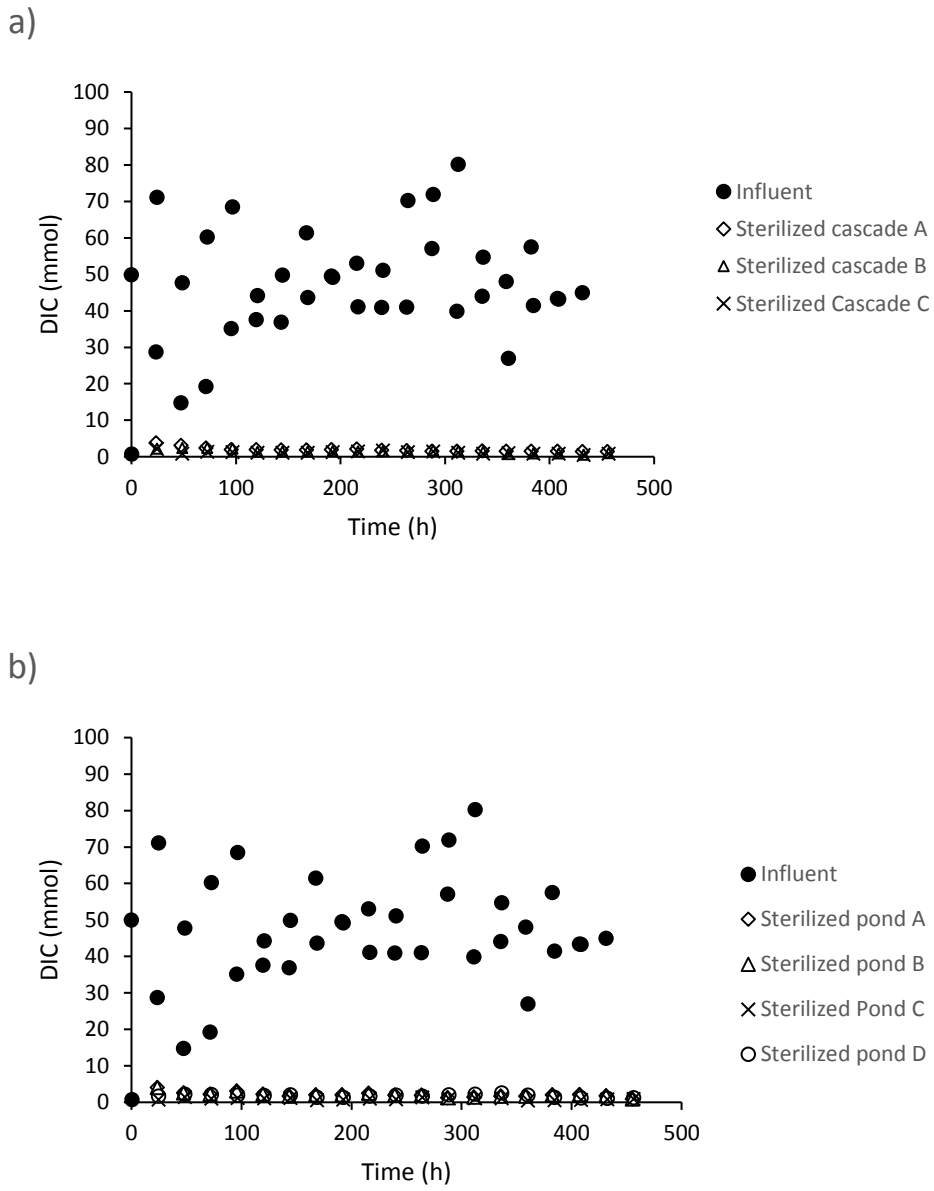

Figure S5. Dissolved Inorganic Carbon (DIC) input and output (mmol) in the sterilized systems a) cascades and b) ponds during the experiments.

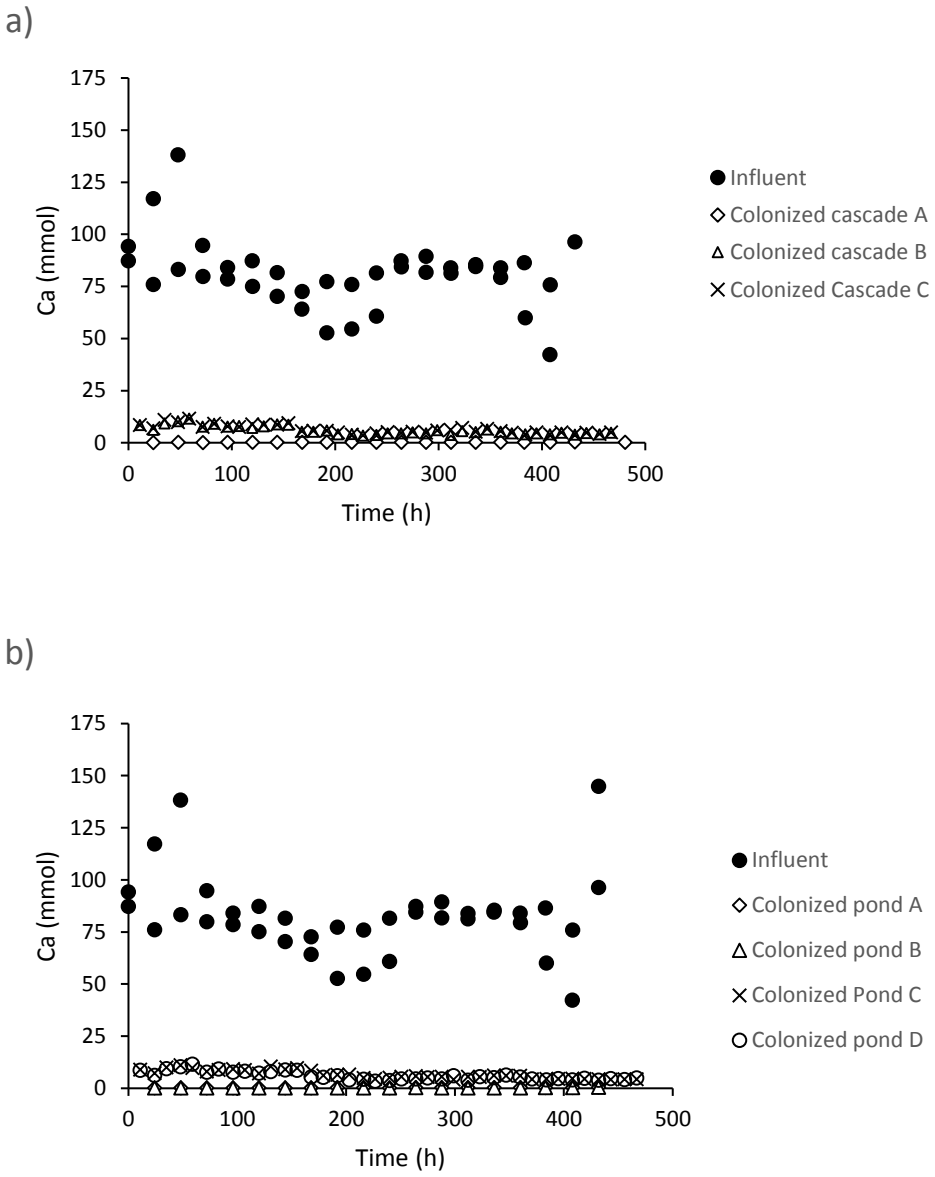

Figure S6. Calcium input and output (mmol) in the colonized systems a) cascades and b) ponds during the experiments.

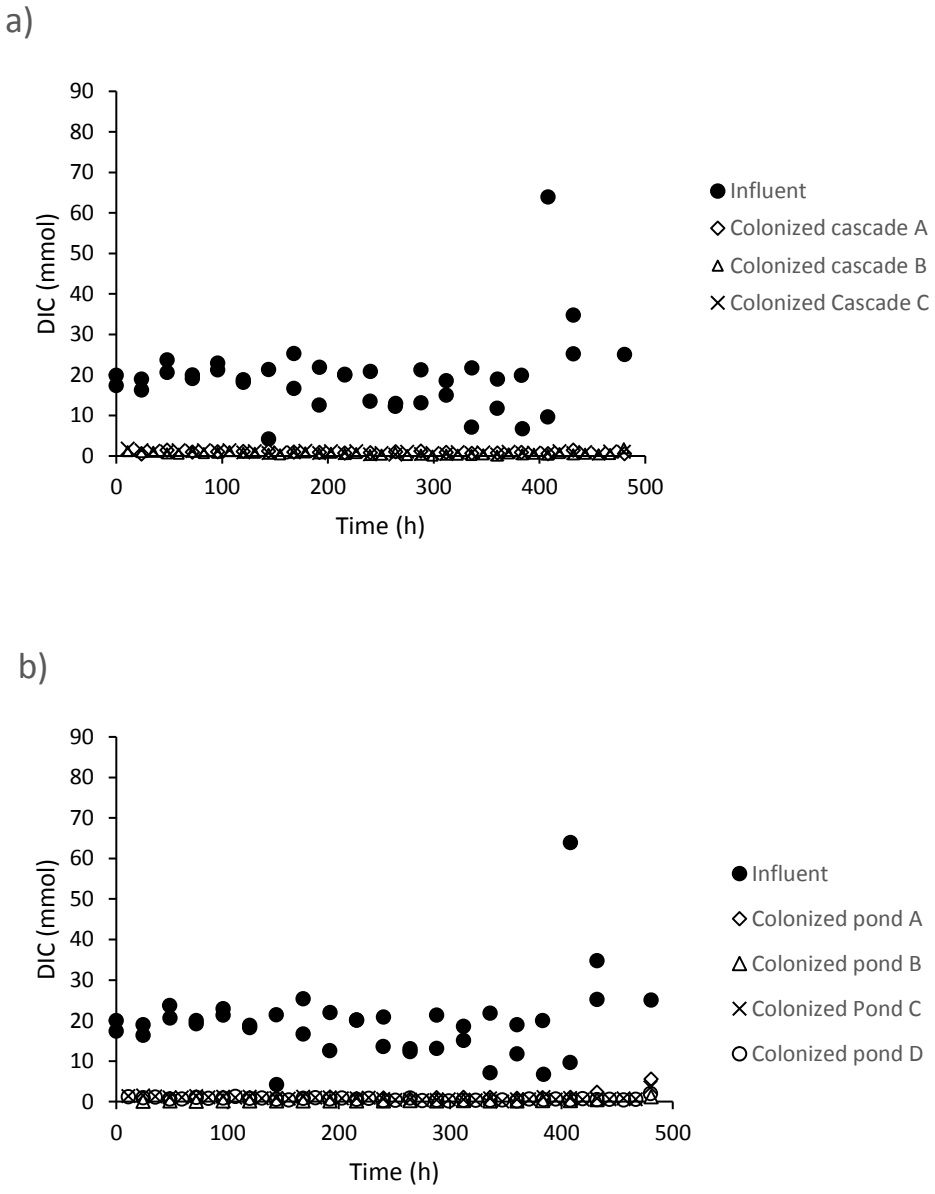

Figure S7. DIC input and output (mmol) in the colonized systems a) cascades and b) ponds during the experiments.