Groundwater Monitoring&Remediation

Illuminating Subsurface Microbial Water Quality Patterns Using Adenosine Triphosphate and Dynamic Time Warping Approaches

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Abstract

Aquifer microbial water quality evaluations are often performed by collecting groundwater samples from monitoring wells. While samples collected from continuously pumped sources are seldom disputed as representative of the aquifer, natural biofilm present in the vicinity of well screens may introduce unwanted microbial artefacts in monitoring wells that are only periodically sampled. The need for well water purging to obtain samples void of these artefacts has been widely recognized. However, purging methods are not standardized; many approaches presume that physico-chemical water quality stability achieved through the removal of 3 to 5 well volumes is indicative of the stability of target analytes. Using a data set collected from a shallow unconfined aquifer in Southern Ontario, Canada, the need for using dedicated approaches that account for the time-dependent nature of microbial water quality changes was demonstrated. Specifically, the utility of adenosine triphosphate (ATP) as a rapid, field-ready biochemical indicator of microbial water quality stability was investigated. This work shows that ATP concentrations reflect time-limited (bio)colloid transport processes that are consistent with other microbial water quality parameters monitored, but different from commonly measured physical and chemical water quality indicators of well purging adequacy. ATP concentrations occasionally fluctuated even after 3 or 4 h of purging, indicating that microbial artefacts attributable to biofilms in the vicinity of the well screen can still persist. The recurrence of characteristic ATP patterns in each well was systematically examined through the novel application of dynamic time warping (DTW), a nonparametric time series analysis approach. These patterns are believed to be linked with seasonal hydrogeological conditions, which warrant consideration in the design and interpretation of subsurface microbial water quality investigations.

Introduction

Representative samples of aquifer microbial water quality—specifically, the composition and concentrations of microorganisms normally suspended in the aquifer pore water—are crucial for aquifer source vulnerability assessments to pathogen/fecal contamination. However, the ubiquity of microorganisms presents a significant challenge to ensuring the integrity and representativeness of samples. For example, Harter et al. (2014) demonstrated the need for extended well purging to control for significant fecal microbial indicator contamination of the well-head, within the well casing, and within the immediate vicinity of the well screen. Although there is general alignment to suggest that extended purging/continuous operation of wells would all but ensure that a sample representative of the microbial composition in the aquifer can be collected (Cullimore 2007; Harter et al. 2014), it is often impractical or infeasible. Therefore, evaluations of subsurface microbial water quality parameters can greatly benefit from an inexpensive, sensitive field-ready tool that can support the contextualization of microbial results and aid in determining the minimal level of purging.

Owing to the contrast in hydrogeochemical conditions between a well and the surrounding aquifer, naturally occurring conglomerates of sessile microorganisms and their metabolites—collectively known as biofilm—form distinctive patterns on solid surfaces in the vicinity of a well (i.e., well casing, well screen, and sediment surfaces). These subsurface biofilms are known to underpin microbial water quality when water is initially pumped (Cullimore 2007). The dynamic hydraulic conditions invoked by groundwater pumping can cause biofilm mobilization and entrainment (Puls and Barcelona 1996; Cullimore 2007; Smith and Comeskey 2009), therefore potentially introducing unwanted microbial artefacts into a water sample. Intuitively, aquifer water quality during extended purging is less impacted by biofilm and increasingly representative of suspended microorganisms (Cullimore 2007; Smith and Comeskey 2009; Korbel et al. 2017; van Driezum et al. 2017). The biofilm can be characterized throughout well purging activities by examining "zones of interrogation projections" in wells—increasingly sparse time-based intervals—as proposed by Cullimore (2007). These zones feature irregularly spaced sampling intervals that focus on capturing and describing microbial water quality shifts at key points in time during purging and in absence of continuous monitoring.

Accordingly, it is both desirable and pragmatic to establish the minimum length of time—using relevant microbial

doi: 10.1111/gwmr.12397

indicator(s)—required for well purging activities to limit artefacts attributable to the subsurface biofilms. Amongst a multitude of potential microbiologically relevant indicators, adenosine triphosphate (ATP) is a biochemical measurement of microbial activity suggested to be useful in the delineation of biofilm expanse and microbial densities (Webster et al. 1985; Jensen 1989; Metge et al. 1993; Hammes et al. 2010). ATP is ubiquitous in living cells and lost rapidly from dead cells; it is present in fairly constant concentrations in microorganisms and can be measured rapidly with high sensitivity using bioluminescence when assayed with the firefly enzyme luciferase (McElroy 1947). In highly oxic conditions, elevated ATP concentrations reflect elevated microbial densities (McCarthy 1991; Abelho 2005; van der Kooij et al. 2017) or enhanced metabolism of fast-growing microorganisms without substantial nutrient limitations (Howsam 1988; Knezev and van der Kooij 2004). The relative microbial activity as manifested by ATP fluctuations monitored throughout well purging activities has been regarded as an indicator for characterizing subsurface microbial biofilms (Cullimore 2007). These measurements hold particular promise as they are field-ready and have been recently adapted in laboratory instrumentation for near real-time monitoring applications (e.g., Hach® EZ7300 microbial load ATP analyzer, Loveland, Colorado, USA).

ATP behavior during well purging activities has not been reported; the evolution of its behavior over spatial and seasonal time scales has also not been documented. Therefore, the main goal of this work was to examine the potential of ATP measurements as an indicator of purging sufficiency for collecting aquifer-representative microbial water quality samples. We examine ATP concentrations in groundwater samples collected sequentially throughout concurrent well purging activities at two locations in the same shallow unconfined aquifer, exhibiting practically equivalent physical and chemical water quality characteristics. The following questions were therefore addressed: (1) how do ATP concentrations fluctuate throughout well purging and (2) how do they compare with other physical and chemical water quality parameters measured concurrently? (3) Will ATP concentrations measured simultaneously in these two wells converge to suggest microbial water quality conditions that are increasingly representative of the aquifer? Lastly, we explore (4) spatio-temporal scales over which ATP patterns are observed to gain insights related to the dynamics of subsurface biofilms. For this purpose, the novel application of a parameter-free approach that leverages information within sequential ATP measurements was demonstrated. While ATP concentrations may be analyzed solely based on their magnitude and summary statistics, additional insights arising from the relative microbial activity throughout purging may be overlooked unless underlying features captured by the sequentially collected data are considered. However, given the irregularly spaced sample collection intervals applied throughout purging that preclude parametric time series analysis approaches, a nonparametric time series analysis approach—dynamic time warping (DTW)—was applied. This approach facilitates the comparison of time series of differing lengths and irregular sampling intervals by generating a metric to describe the (dis)similarity of any features exhibited. This metric, the DTW distance, was calculated for all pairs of time series and subsequently ordinated using non-metric multidimensional scaling (NMDS) to portray the collective relationships between ATP patterns and explore potential spatio-temporal influences.

Materials and Methods

Study Site

The study site (Figure 1) is situated near drinking water production wells 6 km south of Woodstock, Ontario, Canada. The topography of the site comprises rolling hills and

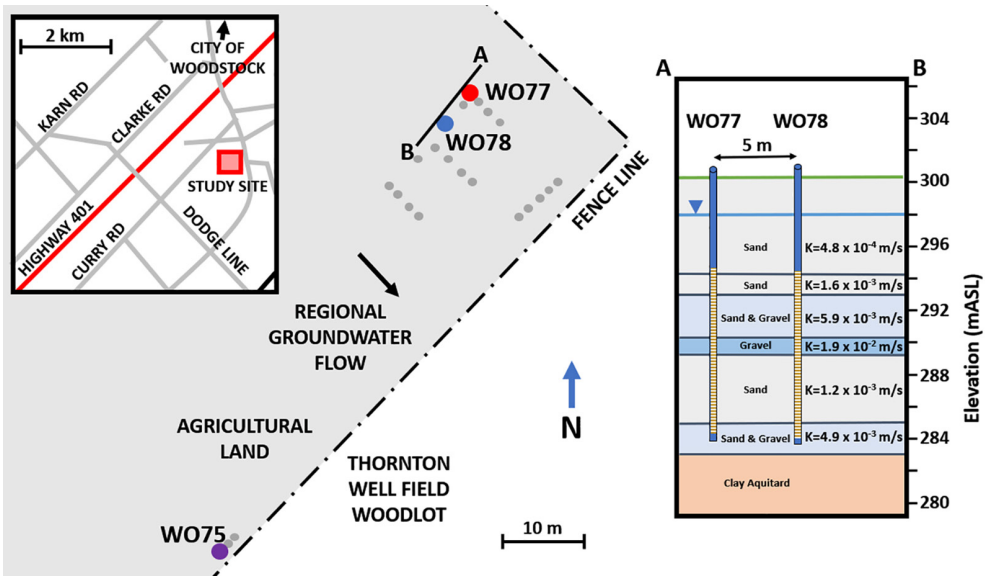

Figure 1. Study site and monitoring well locations. Monitoring wells used only for regional hydraulic gradient estimation in this study are represented by gray circles. Cross-section of transect A-B shows the hydrogeological conceptual model previously developed at the site (adapted from Critchley 2010).

drumlin features. This site was selected because this glacial outwash aquifer system features extremely high groundwater velocities and is highly aerobic (mean dissolved oxygen [DO] during study period = 7.9 mg/L) (Devlin et al. 2012; Critchley et al. 2014). Therefore, it provides ideal conditions that strengthen the aforementioned relationships between ATP concentrations and microbial densities (McCarthy 1991; Abelho 2005; Hammes et al. 2010; van der Kooij et al. 2017). The geochemistry within this aquifer system is known to be rather uniform (Critchley et al. 2014). Regional groundwater flow is generally in a southeasterly direction (Critchley 2010).

The monitoring wells (WO77, WO78) of interest are developed in the shallowest unconfined aquifer of the regional aquifer system, comprising primarily of sand and gravels interspersed with discontinuous silty till aquitard units (Haslauer 2005; Critchley 2010; Critchley et al. 2014) (inset transect cross-section, Figure 1). These wells are situated 5 m apart in a direction perpendicular to regional groundwater flow. Both monitoring wells (50.8 mm [mm] diameter, polyvinyl chloride [PVC] construction) were screened at similar depths, between 5.91 to 16.57 m below ground surface (mBGS) and vented to the atmosphere. The average water table elevation occurred at approximately 298 m above sea level [mASL] (≈3 mBGS), which was consistently above the top of the screened interval. The aquifer hydraulic conductivity in the screened intervals was estimated to range from 4.8×10^{-4} to 1.9×10^{-2} m/s (based on collective results from grain size analyses, flowmeter tests, point velocity probes [PVP] measurements [Devlin et al. 2012], tracer tests, and three dimensional finite difference model calibration). Horizontal velocities were estimated to be between 1 m/d and about 13 m/d using a solute tracer study. Hydraulic conductivity profiles generated between these two wells exhibited remarkable similarities (Critchley 2010). Additional well installation details are provided in Critchley (2010).

Groundwater levels were regularly monitored in 28 additional monitoring wells within a 500-m radius of the two wells of interest; the mean regional groundwater gradient over the study period was estimated to be 0.0069 using HydrogeoEstimatorXL (Devlin and Schillig 2017). Groundwater levels were highest in the spring months (April/May) and lowest during late fall/early winter (December) (Supporting Information Figure S1). Annual recharge at this location is estimated to be 396 mm/year (Koch 2009). Local infiltration travel time through the unsaturated zone to the water table is estimated to be on the order of 2.8 to 5.6 years (Sousa 2013). These observations are generally consistent with the seasonal hydrogeological fluctuations observed historically at this site (Haslauer 2005; Christie et al. 2009; Koch 2009; Critchley 2010; Brook 2012). Water level measurements do not support vertical flow at this site to be a significant factor (Critchley 2010).

Field and Laboratory Analyses

Twelve groundwater sampling events occurred between May 2017 and May 2018. A minimum of 2 weeks between sampling events was used in this study to limit interferences from irreversible subsurface biofilm perturbations associated with the preceding sampling event(s) (Lundkvist et al. 2007; Tolhurst et al. 2008; Worley-Parsons 2015). During each event, a series of samples was collected from one or both monitoring wells throughout continuous well purging from quiescence to over 3 h time. All samples were collected using dedicated pumps with PVC tubing that were pre-sterilized in the laboratory by soaking and pumping a dilute bleach solution (0.6% sodium hypochlorite) through the tubing for a minimum of 1 h followed by a sterile deionized water flush. Sterile deionized water was also used on site to prime the pumps (Flojet model No. 4105 Series diaphragm pump, Irvine, California) as necessary. Water levels were measured prior to pumping and the rate of water extraction from the wells was tracked periodically (average pumping rate = 5.8 liters/minute [L/min]). The pumping rates applied are consistent with the goal of minimizing drawdown within the well as in low-flow purging to minimize artificial mobilization and entrainment of particulates (Puls and Barcelona 1996; Barcelona et al. 2005); however, low levels of shear stress under laminar flow regimes are still expected to result in some degree of biofilm detachment, mobilization and entrainment processes (Rittman 1982). Temperature, dissolved oxygen (DO), pH and electrical conductivity (EC) were measured on-site throughout the well purging process using dedicated portable multiparameter meters (YSI Quattro Professional Plus, YSI Inc./Xylem Inc., Milford, Ohio). Turbidity was also monitored using a portable turbidimeter (Hach®, Loveland, Colorado). All probes and meters were calibrated using standards as per the manufacturers' instructions.

Additional parameters relevant for microbial water quality were measured for selected samples. Flow cytometry was deployed for the evaluation of microbial cell densities using the FACSCalibur™ flow cytometer (BD Biosciences, San Jose, California). Culture-based methods, in the form of Biological Activity Reaction Tests (BART™, Droycon Bioconcepts Inc., Regina, Canada) for iron-related bacteria (IRB BART™), sulfate reducing bacteria (SRB BART™), slime-forming bacteria (SLYM BART™), and algae (ALGE BART™ including grass-green algae, blue-green algae, desmids, diatoms and euglenoids) were also deployed in select samples to corroborate the relative abundance of specific groups of microorganisms. A molecular method for microbial community analysis (16S rRNA gene amplicon sequencing) was performed concurrently with some samples. As these analyses were the focus of another study (Chik et al. 2020), only key findings relevant for the interpretation of the ATP data are presented in Supporting Information Table S4 and discussed. The parameters were first monitored in WO78 for four additional sampling campaigns prior to initiating sample collection from WO77 on the fifth sampling event.

Determination of ATP Concentrations

All ATP measurements were determined using the Lumitester C-110 luminometer (Kikkoman Food Products Company, Tokyo, Japan) with the Quench-Gone Aqueous (QGA™) test kit (LuminUltra, Fredericton, New Brunswick, Canada) in accordance to the manufacturer's procedures (compliant with ASTM Standard D4012) in the laboratory. Briefly, 120 mL of sample was collected and passed through a 25-mm diameter glass microfiber syringe

filter with a 0.7 μm nominal pore size (Whatman™ GD/X, Florham Park, New Jersey). Internal tests conducted by the manufacturer have ascertained the ability of these depth filters to capture microorganisms down to 0.2 μm. The larger analytical volume was used compared to that recommended by the manufacturer (25 to 50 mL for this type of source water matrix) to improve method sensitivity, which would therefore allow for the quantification of microbial activity levels anticipated to be near the nominal method detection limit. The syringe filter was first removed from the syringe barrel to avoid the application of excessive negative pressure to the filter membrane upon removal of the syringe plunger, and reattached to the barrel. One (1) mL of UltraLyse 7 was added to the barrel and passed through the filter and collected in a new 9-mL UltraLute (dilution) tube. The sample was capped and inverted three times to mix the contents. Dedicated pipette tips were used to transfer 100 μL of the contents of the dilution tube and 100 μL of luminase enzyme to a new assay tube. The tube was gently swirled five times and immediately inserted into the luminometer for measurement. The relative light units (RLU) associated with the cellular ATP is measured using the luminometer, recorded and converted to cellular ATP concentration in units of picograms/mL (pg-ATP/mL). The luminometer was calibrated for each set of samples analyzed as recommended by the manufacturer's instructions. All ATP measurements were conducted on the same day of sample collection. Duplicate aliquots were collected every 15 samples to characterize intra-sample variation; negative controls were also measured to evaluate potential cross-contamination throughout sample collection and analysis. A total of 20 ATP time series between two wells were collected over a year at near-monthly intervals; the collection of additional time series would be necessary to facilitate more rigorous statistical tests and was beyond the scope of this initial proof-of-concept demonstration.

NMDS of ATP Time Series Using DTW Distances

DTW was originally developed for speech recognition to facilitate pairwise comparisons between sequences of different lengths with irregularly spaced observations (Sakoe and Chiba 1978; Mueen and Keogh 2016). DTW has been recently used to elucidate spatial, temporal and/or seasonal dynamics for phosphorus transport in watersheds (Dupas et al. 2015), geophysical seismic images to delineate geological strata (Hale 2013), and sewer flow monitoring (Dürrenmatt et al. 2013). Here, the novel application of time series analysis using DTW to investigate ATP patterns is demonstrated. This technique realigns the most similar features of each time series to those of another based on imposed constraints, before a distance metric representing the (dis)similarity between the time series is computed. This temporal realignment allows for time series of differing lengths and irregular sampling intervals to be compared, based on morphological features of the biofilm as manifested by the ATP time series. During a given purging sequence, peaks in microbial parameters (such as ATP) have been interpreted as the location(s) of elevated microbial biomass attributable to enhanced biofilm growth in the vicinity of the well from which water is abstracted (Cullimore 2007). As the *relative* microbial activity is of interest, each ATP time series was normalized by re-scaling each ATP concentration measurement between the minimum and maximum values for each well and sampling event. Thus, the maximum ATP value within a time series would be indicative of the foci of biomass from subsurface microbial biofilms while lower values can be expected with extended purging (Cullimore 2007). Details of the calculation of DTW distances are provided in the Supporting Information S2. In this work, DTW was implemented using the *dtw* package (v1.20-1) in *R* (Giorgino 2009) with local Euclidean distances. These distances were computed for all pairs of time series and compiled in a distance matrix.

NMDS is a robust, indirect ordination approach that can be applied to any (dis)similarity or distance matrix involving any quantitative, semi-quantitative, or qualitative variables (Kenkel and Orloci 1986). A low-dimensional portrayal of ATP time series relationships was generated using NMDS, which iteratively places each time series in a position that reflects the order of the pairwise distances calculated. Accordingly, the scale of the axes and the ordination of the plot are arbitrary and do not reflect the magnitude of the pairwise distances. The emergence of patterns in the ordination allows for the corroboration of existing knowledge, generation of hypotheses, or design of further sampling campaigns to target any observed variation(s) (Kenkel and Orloci 1986). It also provides an exploratory tool to contextualize the strength of the (dis)similarities between the objects ordinated. NMDS solutions with stress values above 0.20 should be interpreted with caution and those with stress above 0.30 are highly suspect (Buttigieg and Ramette 2014). The NMDS ordination approach has been discussed extensively elsewhere (Kenkel and Orloci 1986; Legendre and Legendre 2012; Buttigieg and Ramette 2014).

Results

Physical and Chemical Water Quality Characteristics

Physical and chemical water quality parameters (Supporting Information Table S3) were generally consistent with those observed historically at this site (Critchley et al. 2014). In accordance with common practice for groundwater chemistry evaluations, purging was considered adequate when pH stabilized within 0.1 Standard Units (SU), EC fluctuated by less than approximately 5%, DO stabilized within 0.2 mg/L or 10% saturation (whichever is greater), and/or turbidity either stabilized or fell below 10 nephelometric turbidity units (NTUs) (Striggow 2017). Temperature is subject to rapid changes when collected for parameter measurement and therefore was not typically used for determining well purging adequacy (Striggow 2017). In all sampling campaigns, these criteria were all achieved within the time taken to purge three to five well volumes, which corresponds to approximately 10 to 15 minutes depending on exact purging flow rate.

To facilitate comparisons between the various parameters measured, the coefficient of variation was evaluated for a running window of three consecutive measurements for each parameter throughout purging (Figure 2). The apparent fluctuations exhibited by turbidity are attributable to a

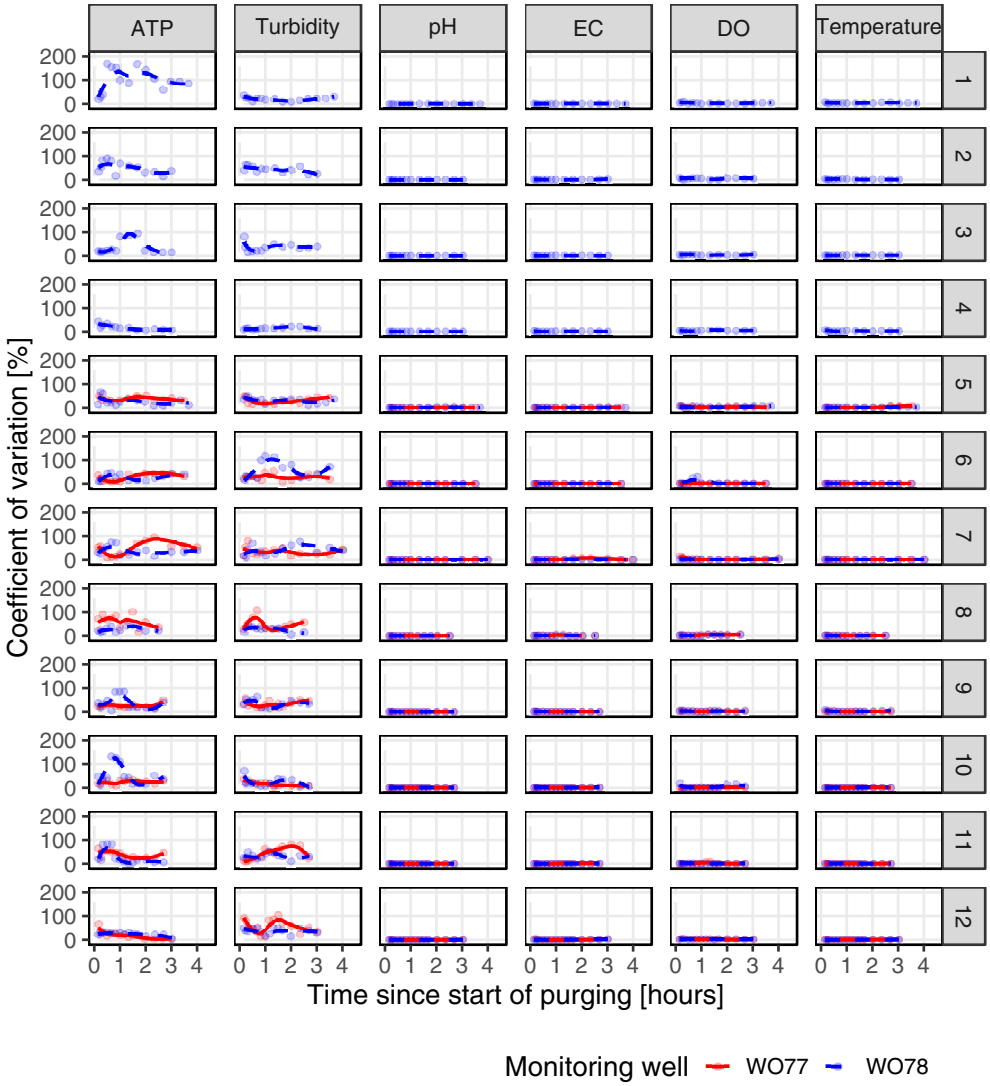

Figure 2. Stabilization history of groundwater quality parameters monitored, expressed as the coefficient of variation evaluated for running windows of three consecutive measurements throughout purging. Red and blue markers/lines are used to denote monitoring wells WO77 and WO78, respectively. Each row of the plot denotes a sampling event (corresponding dates provided in Figure 5). The panel columns correspond to the following parameters: adenosine triphosphate (ATP), turbidity, pH, electrical conductivity (EC), dissolved oxygen (DO), and temperature.

large standard deviation relative to the low turbidity values measured (i.e., consistently less than 10 NTUs). Meanwhile, the maximum coefficients of variation observed for pH, EC, DO, and temperature were 3%, 6%, 30%, and 8%, respectively. The largest fluctuations in DO concentrations were traceable to measurements taken when the flow-through cell was disturbed during purge water sampling.

ATP Measurements

Groundwater microbial ATP concentrations ranged between 0.046 and 58.6 pg-ATP/mL. Median ATP concentrations across all sampling events were 0.27 pg-ATP/mL ($n = 126$) and 0.38 pg-ATP/mL ($n = 190$) for wells WO77 and WO78, respectively. Across sampling events, ATP concentrations exhibited a slight decreasing trend from the end of summer (sampling event 5) to late spring (sampling event 12) (Figure 3). For sampling events during which ATP concentrations were measured concurrently in both wells, ATP concentrations were generally higher in WO78 than in WO77, presumably due to local subsurface biofilm heterogeneities. A notable exception was the ATP concentrations observed in WO77 during sampling event 8 (Figure 3), which was likely attributable to a higher average flow rate (12 L/min) sustained by the alternate pump (Simer 2825SS, Delavan, WI) used. Within a purge sequence, the coefficient of variation evaluated for three consecutive ATP measurements fluctuated as high as 168% but was usually less than 50% toward the termination of most sampling events (Figure 2). Ninety-five percent confidence intervals on the paired differences of ATP concentrations between wells all contained zero, regardless of the extent of purging achieved (Table 1). However, the margins of error calculated for these confidence intervals progressively reduced with extended purging, decreasing by nearly an order of magnitude (0.70 to 0.08 pg-ATP/mL) amongst samples collected in the first 30 min of purging ($n = 35$) compared to samples collected after 1.5 h

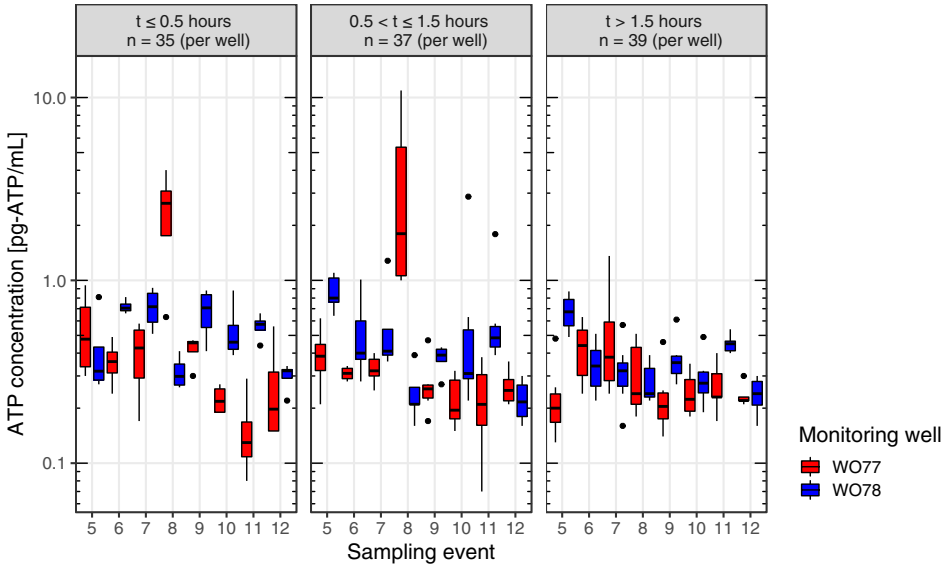

Figure 3. Stabilization of ATP concentrations throughout purging in both wells. Red and blue fill are used to denote monitoring wells WO77 and WO78, respectively. Each column panel denotes the stage of purging attained. The dates corresponding to each sampling event are provided in Figure 5.

Table 1

Paired *t*-Test Results Comparing ATP Concentrations Between WO77 and WO78

Time since Start of Purging [Hours]	*n*	Mean Difference [pg-ATP/mL]	Standard Error [pg-ATP/mL]	95% Confidence Interval [Lower, Upper Bound]	t-Value	*p*-Value
$t \leq 0.5$	35	0.29	0.36	[−0.40, 0.99]	0.86	0.40
$0.5 < t \leq 0.5$	37	0.04	0.18	[−0.31,0.39]	0.22	0.83
$t > 1.5$	39	0.01	0.04	[−0.06,0.09]	0.36	0.72

Note: Tests were performed against the alternative hypothesis that the true difference in means is not equal to zero.

of purging ($n = 39$). This observation is consistent with the decreasing median of relative differences between ATP concentrations in both wells with extended purging (Figure 4).

The relative microbial activity (i.e., normalized ATP concentrations) in each well was used to characterize the foci of biomass from subsurface microbial biofilms during each sampling event (Figure 5). This normalization effectively addresses potential biases attributable to the individual sampling campaign and well-specific factors (e.g., steady-state flow rate attained, generally higher ATP concentrations in WO78). Comparisons of these patterns were made visually. A generally monotonic, decreasing trend was sometimes observed; however, elevated ATP concentrations can also be observed at later stages of purging. This indicates that the highest levels of microbial activity (and by association, densities) are not always highest in the immediate vicinity of the well casing. Recurring ATP patterns were noted in each well over consecutive sampling campaigns in September and November (Figure 5, sampling events 6 and 7) and again in April 2018 (sampling events 10 and 11). During these periods, WO77 ATP patterns typically exhibited two peaks during the latter stages of purging, while a single prominent peak was exhibited within the first hour of purging in WO78. These peaks reflect order-of-magnitude changes in microbial activity. The ATP patterns observed on August 30, December 20, and May 30 (sampling events 5, 8, and 12, respectively) within each well also bear some resemblance to each other, albeit with some visually erratic fluctuations. Similar ATP patterns were also noted during the sampling event on January 30, 2018 (sampling event 9) *between* the wells. This event coincided with the first major increase in groundwater elevation (+20 cm) captured during the study period. To confirm that the variation in these data is not inherent to the method of analysis, additional microbial water quality analyses were conducted—these are described below and detailed in Supporting Information Table S4.

Additional Microbial Water Quality Parameters

Microbial cell densities estimated using flow cytometry generally yielded sample concentration estimates (mean≈500 cells/mL, median≈60 cells/mL) below the limit of reliable quantification using this technique (i.e., ≈1000 cells/mL) (Hammes and Egli 2010) (Supporting Information Table S4). These estimated densities are consistent with those that can be estimated using ATP concentrations as microbial equivalents (by assuming an average of 0.001 pg-ATP/microorganism as per Deininger and Lee 2001), which confirmed that cell densities were largely below this threshold. Despite the majority of flow cytometry cell densities falling below the limit of reliable quantification, a weak positive correlation between ATP concentrations and sample microbial cell concentration estimates

was noted (Spearman's $\rho = 0.147$, $p = 0.03$). 16S rRNA gene sequences attributable to bacterial taxa (e.g., *Paenibacillus* and *Rhizobia*) that are known to produce extracellular polymeric substances (EPS) and are prevalent in the biofilm of reactors mimicking aerobic groundwater conditions (Ross et al. 2001) were generally higher in abundance during the early and intermediate stages of purging (<60 min, by which time at least 10 well volumes have been purged, Chik et al. 2020). Sequences linked with *Sphingomonadales*, which are known to co-aggregate with other bacteria and play a quantitatively important role in freshwater biofilm communities (Rickard et al. 2002), were also in higher abundance during the early and intermediate stages of purging. The vast majority of microbial taxa did not significantly increase after an hour of purging (Chik et al. 2020). This is consistent with more prominent expression of BART™ reactions observed for iron-related and slime-forming bacteria conducted for samples collected during the early stages of purging. Low levels of sulfate reducing bacteria and algae were consistently observed (Supporting Information S4).

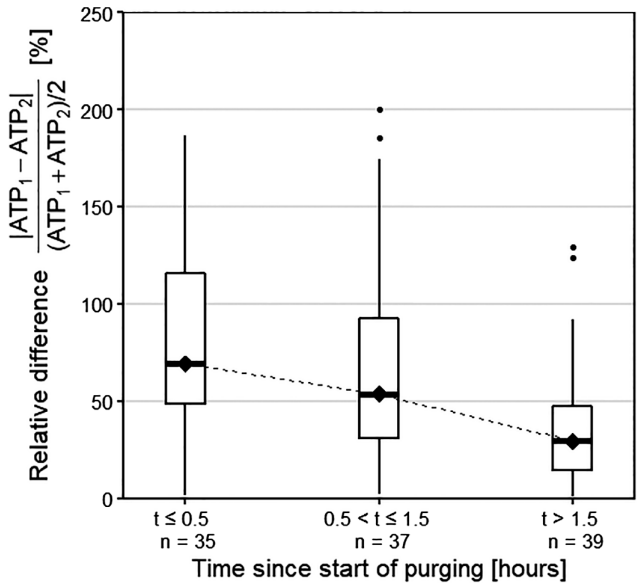

Figure 4. Box-and-whisker plots of relative differences between WO77 (ATP_1) and WO78 (ATP_2) paired ATP measurements throughout concurrent purging activities, where *t* represents the time elapsed since start of purging in hours and *n* reflects the number of paired samples falling within the specified period. The medians are marked by diamonds, the boundaries of the box indicate the 25th- and 75th-percentile, and the whiskers indicate the highest and lowest values of the results excluding extreme values.

NMDS of ATP Time Series Based on DTW Distances

Pairwise (dis)similarities between ATP time series calculated as DTW distances were compiled in a matrix and ordinated using NMDS to generate a two-dimensional representation of these relationships (Figure 6). ATP time series that have similar features will typically exhibit a lower DTW distance metric than those that do not. Accordingly, NMDS uses the order of the distance metrics between ATP time series (each represented by a single marker) to position similar ATP time series closer together. ATP time series associated with each well and sampling event is represented by a colored marker (red circles for WO77 and blue triangles for WO78) denoted with the corresponding sampling event number. To aid visualization and interpretation, the regions represented by each of the wells were also shaded consistently with the marker colors.

Notably, the markers denoting ATP time series collected from the two wells during the same sampling campaign generally occupied different quadrants of this ordination, with the exception of the ATP time series collected from both wells during the sampling event on January 30 (Figure 6, sampling event 9). ATP time series collected during the

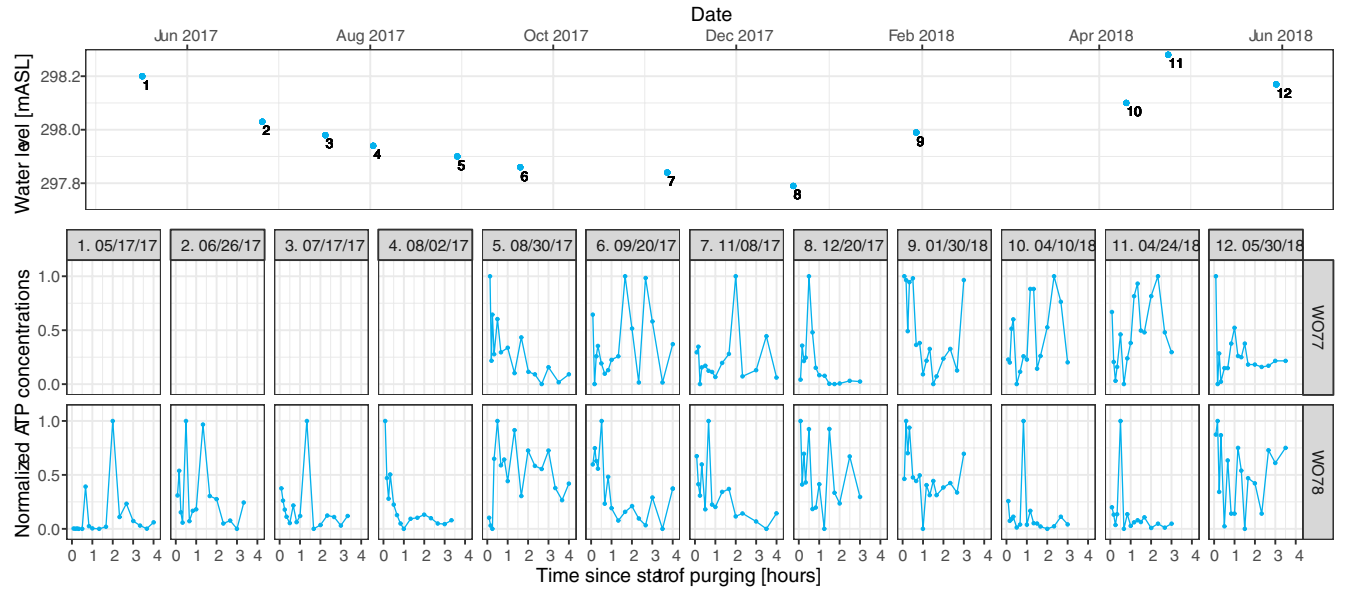

Figure 5. Normalized ATP concentrations observed throughout well purging activities. The positions of groundwater levels observed in WO78 during each sampling event are indicated above the plots of normalized ATP concentrations. Continuous groundwater levels monitored in a nearby well WO75 are summarized in the Supporting Information S1.

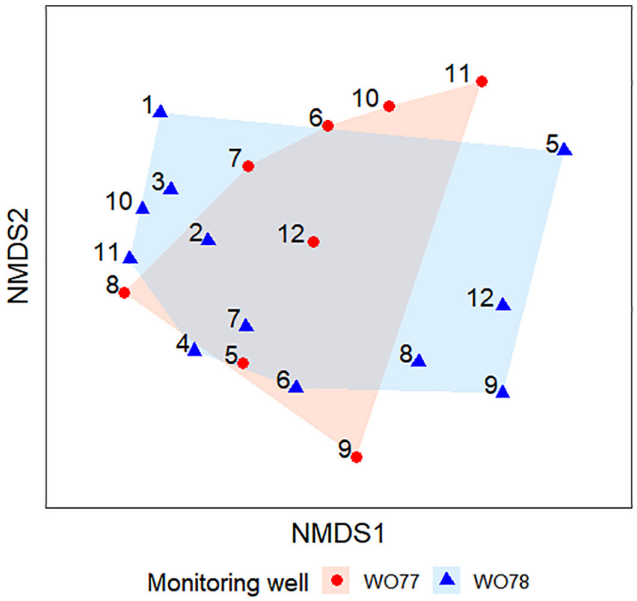

Figure 6. (Dis)similarities between all ATP time series portrayed through nonmetric multidimensional scaling (NMDS). Each red circle or blue triangle represents an ATP time series corresponding to wells WO77 and WO78, respectively, and is labeled sequentially in order of sampling events. Markers appearing closer together reflect greater similarities between the time series. The shaded regions are consistent with the marker colors used for each well. This NMDS solution resulted in a stress value of 0.139, indicating that this solution captures the order of (dis)similarities reasonably well.

same pairs of consecutive events (i.e., sampling events 6/7, 10/11) in each well can also be noted. These observations of (dis)similarities are consistent with those identified through a visual inspection of normalized ATP patterns (Figure 5). Notably, not all (dis)similarities can be perfectly portrayed by NMDS and DTW distances. For example, events 5, 8, and 12 were not clustered in this particular ordination. The inability of this ordination to perfectly represent all time series (dis)similarities is reflected by the stress value of this NMDS solution (0.139), which indicates their good, but not excellent portrayal.

Discussion

Physical and Chemical Indicators Do Not Reflect Purging Adequacy for Microbial Water Quality Evaluations

As would be expected, ATP does not follow the same types of trends as other physical and chemical water quality parameters as it is directly and exclusively linked to the presence of microorganisms. Fluctuations in this biochemical must be interpreted with consideration of the method used to measure it. Hammes et al. (2010) demonstrated a strong, significant correlation between microbial ATP and estimated cellular densities using flow cytometry across a range of different source waters (including groundwater). While the same extent of correlation was not observed for this study (due to microbial densities falling below the threshold deemed reliably quantifiable by flow cytometry), a statistically significant correlation was nonetheless observed between these parameters. Microbial ATP is measured only after its extraction from a discrete number of living microorganisms—that are in essence, particles—in the sample. Strategies for informing microbial water quality that rely on ATP must therefore reflect particle transport behavior rather than that of dissolved solutes. Particle and (bio)colloid transport through the subsurface are subject to advection, dispersion, physico-chemical filtration (attachment/detachment), size-exclusion, and straining processes (Ginn et al. 2005). Subsurface biofilms are conglomerates of microorganisms and their metabolites (Palmer and White 1997). Thus, they are also subject to these processes once detached and entrained in pore water during purging (Liu et al. 2018). Accordingly, the breakthrough of these conglomerates, and ATP by association, is likely to exhibit extended tailing resulting from attachment/detachment processes. Moreover, the transport of these conglomerates can lead to increased variability in microbial observations (i.e., overdispersion) relative to that which would be expected if microorganisms were not clustered. Patterns of ATP concentrations were consistent with the occurrence of these phenomena, thereby indicating its utility for signifying fluctuations in microbial water quality.

The use of continuous physical and chemical water quality measurements for the determination of purging adequacy has rarely been disputed for evaluations of chemical water quality sampling. However, this work underscores an important caveat: the use of these conventional indicators of purging adequacy is not likely appropriate for *bio*chemical water quality parameters related to microbiological water quality. This disparity between the transport of dissolved-phase substances in contrast with the mobilization and transport of (bio)particles has been a widely observed phenomenon (e.g., Schijven and Hassanizadeh 2000; Ginn et al. 2005; Bradford and Torkzaban 2008; Emelko and Tufenkji 2010; Molnar et al. 2015) and critically underscores the need to consider dedicated purge volume-based and purging time-based approaches for chemical and microbial water quality evaluation, respectively. Purge volume-based approaches presume that the substance measured in a sample occupies negligible volume within the voids of subsurface sediments in order to evaluate the position from which the sample originated relative to the well. Conversely, all particles occupy volume; biofilm biomass and other inorganic particles can reduce effective porosity through obstructing connected flow paths through the subsurface. When the degree of obstruction becomes significant, the estimation of the sample's position using purge volume based-approaches also becomes increasingly inaccurate. Therefore, extrapolations beyond the time of sample collection using the same purging protocol (e.g., similar flow rates, type of pump, etc.) has not been recommended for comparisons of field scale microbial water quality data acquired from purge water sampling (Cullimore 2007).

ATP Measurements Can Be Useful for Indicating Aquifer-Representative Microbial Water Quality

The utility of ATP concentrations as indicators of minimal purging requirements for informing microbial water quality sampling was evaluated. ATP evaluation is use-

ful in describing microbial activity and density (Webster et al. 1985; Jensen 1989; Metge et al. 1993; Hammes et al. 2010). Over the past decades, advances of the ATP method to address possible matrix interference effects have resulted in greater sensitivity (limit of detection [LOD] ≤ 0.1 pg-ATP/mL) than the first ATP assays developed (LOD≥10 pg-ATP/mL, e.g., McElroy 1947). Thus, it should be useful in differentiating between microorganisms suspended in aquifer pore water and the often higher densities of sessile microorganisms on well screens and unconsolidated sedimentary aquifer materials in the vicinity of wells (Harvey et al. 1984; Hazen et al. 1991; Griebler and Lueders 2009; Sorensen et al. 2013), especially at highly aerobic conditions that should strengthen the correlation between ATP concentrations and microbial densities (McCarthy 1991; Abelho 2005; van der Kooij et al. 2017). Its sensitivity relative to other microbial water quality metrics lends itself for its use as a discriminatory indicator.

Recognizing that potentially dynamic aquifer water quality could be confounded with measurable changes in ATP concentrations attributable to purging, the concurrent purging of two adjacent wells situated in the same aquifer (here, 5 m apart) was necessary. Although the mean differences in ATP concentrations between the wells were never significantly different from zero, their variability decreased by nearly an order of magnitude during the latter stages of purging (Table 1). The results from other microbial parameters evaluated (i.e., BART™ results, microbial community analysis through 16S rRNA gene amplicon sequencing) were largely consistent with this interpretation. Accordingly, these multiple lines of evidence suggest that purging times on the order of 2 h or more are likely necessary to move beyond contributions from biofilms in the immediate vicinity of the well. These observations are in alignment with the recommended sampling times proposed by Cullimore (2007): at 1 h, samples taken would be from the outer edge of the biofilm biomass and partially reflect microbial loadings from the groundwater; at 1.5 h, samples should indicate the outer edge of the subsurface biofilm biomass surrounding the well; and at 2 h, the sample should be from beyond the biomass but may still be subject to lingering impacts of detached biofilms. Although ATP stabilization occurred during some events to suggest that the outer edge of the biomass can be reached prior to 2 h, a site-specific—arguably even well-/season-specific—understanding of the subsurface biofilm community's behavior may still need to be developed prior to attempting to minimize purging time. This work is the first to document the behavior of ATP concentrations throughout purging in a shallow, unconfined, and highly aerobic aquifer and demonstrate that this biochemical measurement generally aligns with the behavior expected of other microbial water quality metrics.

It is commonly acknowledged that extended purging time at rates consistent with the goals of low-flow purging will likely yield increasingly representative indications of aquifer microbial water quality void of unwanted well-related biofilm artefacts. Purging times of over several hours per monitoring well are seldom pragmatic due to constraints such as purge water disposal and the need for most microbiological parameters to be evaluated within 24 h of sample collection. Indeed, extended purging times of at least 24 to 48 h have been recommended so that the sample obtained is most representative of the "natural flows of [suspended] microorganisms through the well" (i.e., not unduly influenced by biofilm artefacts from pumping) (Cullimore 2007); some studies have suggested that even longer periods are necessary (Kwon et al. 2008; Roudnew et al. 2014). From this perspective, the additional effort to track ATP concentrations beyond several hours to minimize purging time for representative aquifer water quality sampling is not likely warranted and beyond the scope of the current work. However, in circumstances where extended purging is not practical or possible, the relative (in)stability of ATP concentrations may still be useful for contextualizing the possible influence of subsurface biofilms on microbial results.

Possible Insights Related to the Spatio-Temporal Scales of ATP Patterns and Associated Subsurface Biofilm Behavior

The heterogeneity of subsurface microbiology has been well documented at a range of spatial and temporal scales (Young et al. 2008). Due to the complexity of these spatial patterns that result from environmental controls at multiple scales and over time (Ettema and Wardle 2002), much of the existing work has been qualitative in nature (e.g. Young et al. 2008) and does not fully exploit the information inherent to the acquired data (such as that available from sequential measurements). In fact, spatial patterns of microbial biomass have been shown to be more complex than those of other soil/porous media properties based on fractal dimension (Oline and Grant 2002). In this work, to facilitate the systematic examination of the patterns emanating from purge water ATP measurements, DTW was applied. This approach overcomes limitations of irregular sampling time intervals for which parametric time series analysis approaches would not be possible. Moreover, the reliance upon a measure of microbial activity to characterize subsurface biofilm expanse requires consideration of its sensitivity to systematic differences between wells (e.g., steady-state flow rate achieved and inherently higher ATP concentrations in WO78). This taken into consideration, the differences—and remarkably, similarities—exhibited by the normalized ATP concentration sequences (i.e., *relative* microbial activity) throughout purging can be used to gain further insights related to subsurface biofilm dynamics. This may have more specific implications for applications such as remediation of biofouled wells and studies of subsurface microbial ecology in well environments.

The recurring ATP patterns characteristic of each well (i.e., double peaks after an hour of purging in WO77, a single peak less than an hour of purging in WO78) during consecutive events was supported by the systematic evaluation of their (dis)similarities using the approach proposed in this work. Spatial heterogeneity clearly underpins the distinctive patterns exhibited in each well despite exhibiting practically equivalent physical and chemical water quality characteristics (i.e., patterns in each well during the same sampling event were generally dissimilar). The proximity of WO77 to two monitoring wells installed within 2 m may exert considerable influence on the double ATP peaks observed in WO77. We suspect the visually similar ATP patterns (i.e., sampling

events 5, 8, and 12) within each well that follow consecutive sampling events during which the recurring characteristic ATP patterns were observed may reflect a maturation of subsurface biofilm in the vicinity of the well screen at relatively stable hydrogeological conditions. We believe that the similar ATP patterns *between* the wells on January 30 coinciding with the first substantial increase in groundwater levels observed over the 1-year period reflect a hydrogeological perturbation to both wells that overwhelms underlying spatial biofilm heterogeneity normally characteristic of each well. Seasonal fluctuations of the water table have been suggested to promote multidirectional flow (i.e., vertical and horizontal flow), which can result in a greater degree of mixing in the aquifer (Smith et al. 2018). Collectively, the ATP patterns observed at this site supports previously documented hydrogeological influences on microbial water quality changes at field scale manifested as changes to seasonal groundwater elevations (Lin et al. 2012), groundwater flow velocities, and/or hydraulic gradients (van Driezum et al. 2018).

Conclusions

- The use of ATP concentrations as a sensitive, field-ready tool for indicating microbial water quality stability during well purging activities was evaluated in a shallow, unconfined, and highly aerobic aquifer. ATP observations were supported by the results of other microbial water quality parameters measured concurrently (i.e., microbial densities through flow cytometry, relative abundance/activity of biofilm-related groups of microorganisms) and reflected time-limited (bio)particle transport processes rather than that of dissolved solutes throughout purging. However, the extrapolation of these results to other environments—such as anaerobic subsurface environments where ATP concentrations and microbial densities may not exhibit the same degree of correlation—must be further investigated.
- Whereas physical and chemical water quality characteristics appeared to stabilize as anticipated within 10 to 15 minutes (approximately three to five well volumes), fluctuations in ATP concentrations occasionally persisted beyond 2 h of purging (>30 well volumes). Assessments of microbial quality of drinking water sources originating in the subsurface must therefore be conducted using approaches that are designed to adequately reflect these differences in temporal scale. For example, this may involve relying on pumping tests of several days duration rather than relying on a limited number of well purge volumes for analysis.
- Extended purging time at rates consistent with the goals of low-flow purging is commonly acknowledged to yield increasingly representative indications of aquifer microbial water quality. However, purging times of over several hours per monitoring well are seldom pragmatic. In circumstances where extended purging is not practical or possible, the (in)stability of ATP concentrations may be used to contextualize the possible influence of subsurface biofilms on microbial results.
- Patterns based on the relative microbial activity, as captured using normalized ATP fluctuations throughout purging, are suggestive of subsurface biofilm behavior. Such patterns can be systematically examined using the nonparametric time series analysis approach applied. The recurrence of characteristic patterns in each well during multiple sampling campaigns appears linked to seasonal hydrogeological conditions (specifically, groundwater level fluctuations). Disruptions of these patterns are speculated to be attributable to sufficiently large hydrogeological perturbations, which may overwhelm ATP patterns normally characteristic of each well. These dynamics warrant further investigation and should be considered in the design and interpretation of subsurface microbial water quality investigations.

Acknowledgments

We acknowledge the support of the Natural Sciences and Engineering Research Council of Canada (NSERC), [funding reference number EGP 507411-16] and the Queen Elizabeth II Graduate Scholarship in Science and Technology. We would also like to thank LuminUltra Technologies Ltd. for providing the equipment and reagents necessary for this investigation. Oxford County and Dr. Dave Rudolph kindly provided access to the study site. Terry Ridgway, Mark Sobon, Benjamin J.M. Beelen, Kaitlyn E. O'Sullivan, and Caitlin Wong are acknowledged for their help throughout the sampling campaigns. We thank Dr. William B. Anderson, Dr. Maria M.F. Mesquita, Kaitlyn E. O'Sullivan, Dr. Inge H. van Driezum, Celine M. Vandenberg, and two reviewers for their insightful comments on an earlier version of this manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article. Supporting Information is generally not peer reviewed.

Appendix S1: Supporting Information

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