Evaluating a robust and easy-to-use biological-activity-based method to assess the presence of potentially adverse bacteria at two riverbank filtration sites along the Danube river: A case study

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Abstract The *Laboratory Biological Activity Reaction Test* (LAB-BART) is an easy-to-use assay that utilizes metabolic capabilities to process an array of substrates to semi-quantitatively assess the presence of potentially adverse bacteria in a groundwater sample. Here, we evaluated LAB-BART for the assessment of groundwater samples obtained under real-life conditions from two riverbank filtration (RBF) sites in Austria. Samples were taken monthly for an overall experimental period of six months and analyzed following the manufacturer's recommendations for measuring iron-related, sulfate-reducing, slime-forming and denitrifying bacteria. Additional measurements were done for analyzing chemical water composition, as well as bacterial community structure to evaluate the suitability of LAB-BART by identifying relevant bacteria. Results imply that while LAB-BART could not give detailed information on bacterial concentrations, it might be able to indicate hydrologically induced changes in biogeochemical processes in a subsurface system, thus allowing operators to determine an adequate response to a potential influx of undesired bacteria. Despite its limitations, LAB-BART might therefore be a valuable tool for monitoring purposes due to its ease of use, but more research is necessary to determine its accuracy in measuring bacterial activity.

Bewertung einer robusten und benutzerfreundlichen biologisch-aktivitätsbasierten Methode zur Evaluation potenziell schädlicher Bakterien in zwei Uferfiltrationsanlagen entlang der Donau

Zusammenfassung Der Laboratory Biological Activity Reaction Test (LAB-BART) ist eine leicht durchzuführende Methode, die es erlaubt anhand der metabolischen Fähigkeiten von Bakterien eine Reihe von Substraten zu verarbeiten, das Vorhandensein potenziell schädlicher Mikroorganismen in einer Grundwasserprobe semi-quantitativ zu bestimmen. In dieser Studie wurde LAB-BART für die Bewertung von Grundwasserproben unter realen Bedingungen in zwei Uferfiltrationsanlagen in Österreich untersucht. Proben wurden über einen Zeitraum eines halben Jahres monatlich entnommen und gemäß Herstellerangaben zur Messung von eisenmetabolisierenden, sulfatreduzierenden, schleimbildenden und denitrifizierenden Bakterien genutzt. Um die grundlegende Eignung des LAB-BART Systems zu bewerten, wurde zusätzlich die physio-chemische Zusammensetzung des Wassers sowie die bakterielle Zusammensetzung des Grundwassermikrobioms per Sequenzierung untersucht. Die Ergebnisse deuten darauf hin, dass LAB-BART zwar keine exakte Bestimmung der Bakterienkonzentrationen erlaubt, jedoch reproduzierbar und mit simplen Mitteln hydrologisch bedingte Veränderungen biogeochemischer Prozesse in einem Untergrundsystem anzeigen

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kann. Zusammen mit der einfachen Handhabung von LAB-Bart kann dies Betreibern von Uferfiltrationsanlagen erlauben, angemessen und zeitnah auf einen potenziellen Zustrom unerwünschter Bakterien zu reagieren und somit Betriebsabläufe zu sichern. Trotz der Einschränkungen könnte LAB-BART daher aufgrund der einfachen Anwendung ein wertvolles Instrument für Überwachungszwecke von Uferfiltraten sein, weitere Forschung ist jedoch erforderlich, um die Genauigkeit der Messung der bakteriellen Aktivitäten zu bestimmen.

1 Introduction

Microbial water quality measurements have been shown to be crucial to ensure consumer safety in the last century (World Health Organization 2017). Besides public health benefits, knowledge about the presence or absence of microorganisms associated with nontoxic but undesirable biofouling and microbially induced corrosion allows for timely interventions by the operator of groundwater wells, drinking water production facilities and in the overall drinking water distribution infrastructure (Abdullah et al. 2014). While simple and reliable culture-based assays such as heterotrophic plate counts have long been implemented by policy makers worldwide (e.g., AWWA, DIN TS ISO and AGES, the Austrian Agency for Health, and Food Safety) to ensure biological stability in drinking water, they are known to have certain limitations in indicating viable but noncultivable (VBNC) bacteria. Advanced assays based on the detection of known and unknown genome sequences associated with bacteria of interest such as (quantitative) polymerase chain reaction (PCR) or genome sequencing (e.g., 16S gene metabarcoding) have been proposed to compensate for this, but they are cost- and labor-intensive and therefore seldom applicable for routine operations. Furthermore, the time between taking the samples and the availability of the result ranges between several days (PCR) and multiple weeks (genome sequencing), which can be problematic for consumer safety.

Ready-to-use assays targeting bacterial metabolism such as LAB-BART (Droycon Bioconcepts Inc., Regina, Canada), on the other hand, promise to show results within a few days if bacterial activity is high, and can be used at the sampling site without laboratory equipment or trained personnel (Cullimore 1999).

The main reason to investigate the contamination/colonization of ground water wells for adverse microorganisms is the occurrence of plugging/clogging due to microbial fouling, which in turn can cause production failure and consumer hazards. Next to manganese metabolizing bacteria, this plugging is often caused by the presence or influx of iron-related bacteria (IRB), whose build-up in the well can lead to losses in groundwater flow of as much as 90% (Cullimore and McCann 1977). Microbially induced corrosion, during which microorganisms residing in pipe-adherent biofilms affect their integrity is another common cause of operational concern, which is commonly associated with sulfate-reducing bacteria (SRB) (Cullimore and Johnston 2004).

Here, we assessed the ready-to-use LAB-BART assay to evaluate the presence of undesired bacteria in two well fields in Austria over the duration of six months under real-life conditions. The results were compared to chemical measurements of the same samples. To assess the suitability of the chosen assays, microbial communities in the samples were further characterized using 16S gene metabarcoding, a sophisticated genome-based method that gives detailed insights into the composition of groundwater microbiomes (Shaw et al. 2015).

2 Methods

2.1 Study sites

Samples from two drinking water production sites situated in Lower Austria (Site A and Site B) were utilized to investigate the suitability of the LAB-BART system to identify relevant microorganisms. Both sites use riverbank filtration (RBF) for drinking water production and are equipped with on-site oxygen enrichment.

Site A consists of three sampling points at 8–11 m depth from the ground surface: piezometers A1 and A2, as well as a pumping well: Well A (Fig. 1). The groundwater sourced from Well A is continuously fed by water from a nearby, stagnant backwater. After infiltration into the riverbank, the groundwater travels through the gravel aquifer northwards towards Well A. Oxygen enrichment wells are situated northwest of Well A, but because of their location, have little effect on the groundwater in the well. The time between infiltration and pumping is estimated to be 2 months. The operator of Site A reported that Well A is clogged, leading to reduced yield. These issues are associated mainly with manganese but also with iron.

Site B also consists of three groundwater sampling points, one of which is a pumping well. Unlike Site A, successful in-situ oxygen enrichment is employed in between piezometers B1 and B2 to improve water quality, especially to oxidize unwanted metals such as iron and manganese. The groundwater at Site B originates mainly from the Kamp River, a tributary to the Danube River, but also from ambient groundwater sources.

Samples from both sites were taken monthly at the two piezometers and the respective pumping well for a total
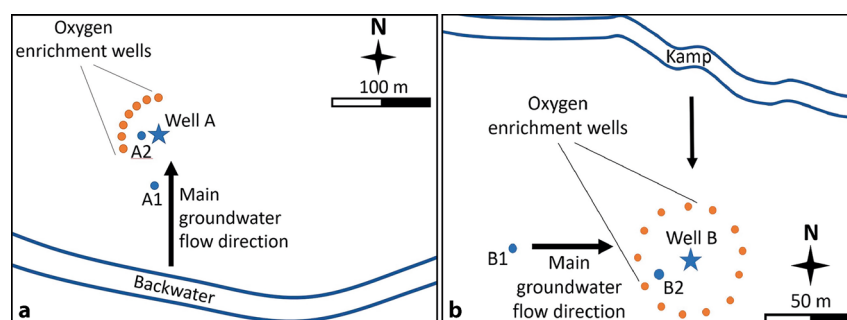
Fig. 1 Overview of Site A (**a**) and Site B (**b**), with oxygen enrichment wells in *orange*, and sampling wells in *blue*

Table 1 The corresponding concentrations (cfu/ml) for the day of color-change (time lag) for each utilized test kit, according to the LAB-BART protocol. Between brackets is the qualitative assessment of the sample, where A. = Aggressive, M. = Moderate, N. A. = Not Aggressive

Days	IRB	SRB	DN	SLYM
1	570,000 (A.)	2,200,000 (A.)	1,800,000 (A.)	1,750,000 (A.)
2	140,000 (A.)	500,000 (A.)	215,000 (A.)	440,000 (A.)
3	35,000 (A.)	115,000 (A.)	25,000 (M.)	67,000 (A.)
4	9000 (A.)	27,000 (A.)	3000 (M.)	13,000 (M.)
5	2200 (M.)	6000 (A.)	350 (N. A.)	2500 (M.)
6	500 (M.)	1400 (M.)	<50 (N. A.)	500 (M.)
7	150 (M.)	325 (M.)	<50 (N. A.)	100 (N. A.)
8	25 (M.)	75 (M.)	<50 (N. A.)	<20 (N. A.)
9	8 (N. A.)	20 (N. A.)	<50 (N. A.)	<20 (N. A.)
10	<1 (N. A.)	5 (N. A.)	<50 (N. A.)	<20 (N. A.)
11	<1 (N. A.)	<1 (N. A.)	<50 (N. A.)	<20 (N. A.)

period of six months in 2019. Samples were immediately stored at 4 °C and transported to the laboratory for analysis within 24 h.

2.2 LAB-BART analysis

The LAB-BART test kits consist of tubes containing a pellet of substrate that, in the presence of the defined group of bacteria, is metabolized into a visible color compound. The approximate concentration as colony forming units per ml of the target bacteria is then determined by the duration until the color change is observable (time lag). Unlike plate-based assays, LAB-BART also accounts for VBNC bacteria and can in theory provide a more realistic estimate of the quantity of viable microbes present. The LAB-BART system is available in nine different kits, each assessing distinct physiological groups of bacteria or algae. Here, we employed the IRB-BART targeting iron-related bacteria, SRB-BART targeting sulfate-reducing bacteria, DN-BART targeting denitrifying bacteria, and SLYM-BART targeting slime-forming bacteria. Detailed information on these bacterial communities, as well as protocols for the specific test kits can be found at the manufacturer's homepage (www. dbi.ca).

For each sample taken at the well fields, 15 ml of groundwater was aseptically transferred into LAB-BART test tubes, which were then kept in the dark and at room temperature. Tubes were observed for a total of 11 to 15 days, and any change in color was documented. In case of the SLYM-BART test kits, additional documentation with fluorescent light was necessary to monitor for the occurrence of fluorescent bacterial

metabolites in the tubes. Changes in color and appearance of foam in the tubes corresponded to an approximate bacterial population as colony forming units per milliliter (cfu/ml) according to Table 1.

2.3 Chemical analysis

In addition to the LAB-BART test, 35 chemical elements were determined in all samples by high resolution inductively coupled (sector field) mass spectrometry (HR-ICP-MS) with a Finnigan Element 2 mass spectrometer (Thermo Fisher Scientific, Germany) at the IFA-Tulln. All unfiltered samples were acidified with ca. 1% ultrapure HNO_3 (ROTIPURAN Supra, 69%, Carl Roth, Karlsruhe, Germany) to reach a pH of 1–2 in order to avoid precipitation and minimize biological activity within 24 h of sampling. Scandium, indium, and thallium were added as internal standards for quality control, in concentrations of 20 µg/l, 10 µg/l, and 10 µg/l, respectively. Temperature and dissolved oxygen were measured at the sampling sites, while other basic parameters such as pH, electrical conductivity (EC), chloride, nitrate, nitrite, sulfate, phosphate, iron and dissolved organic carbon (DOC) were determined in a commercial ISO 17043 accredited laboratory. Parameters unlikely to directly influence the LAB-BART results are not shown in this publication.

Samples with a high amount of iron related bacteria (IRB) were expected to contain either high concentrations of insoluble ferric forms of iron (e.g., $Fe(OH)_3$) or soluble ferrous forms of iron (e.g., Fe^{2+}), as these bacteria can either reduce or oxidize iron in groundwater. High amounts of sulfate reduc-

ing bacteria (SRB) were expected to lead to the presence of hydrogen sulfide (H_2S) under anoxic conditions. SRB use hydrogen rather than oxygen as the primary energy source for their metabolism, and should be inhibited by the presence of oxygen (Cord-Ruwisch et al. 1987; Krekeler et al. 1998). Their primary source of sulfur may not be only sulfate, as samples containing organic acids, alcohols and proteins could also lead to the presence of sulfides. Hence, the absence of sulfate does not necessarily mean that SRB should be absent (Plugge et al. 2011). Slime-forming bacteria (SLYM) are able to function in a large range of hydrochemical conditions but form the thickest slimes in oxygen-rich environments. Consequently, a correlation between SLYM activity and oxygen concentrations was expected. Denitrifying bacteria (DN) were expected to be present at locations with high concentrations of nitrate in the subsurface, which they utilize. However, this activity is limited to anoxic conditions in reductive environments.

2.4 Microbial community analysis using gene metabarcoding

To analyze microbial communities in the groundwater samples, gene metabarcoding via 16S rRNA gene amplicon sequencing was conducted. For this, approximately 2 l of groundwater was filtered through 0.22 µm polycarbonate membrane filters (Merck Millipore, Germany). Then, DNA extraction was performed using a bead-beating and phenol/chloroform protocol as described elsewhere (Griffiths et al. 2000; Reischer et al. 2006; Mayer et al. 2018). Microbial community composition was

determined targeting the highly conserved V3–V4 genome region present in all bacteria (Ong et al. 2013). After amplification using polymerase chain reaction and thorough quality control, the Illumina MiSeq platform (Illumina, USA) was used in accordance with the manufacturer's recommendation. Sequence data was processed in R (R Core Team, USA), using the DADA2 pipeline as described in detail by Callahan et al. (2016).

3 Results and discussion

3.1 Results of LAB-BART analysis

The results of one of the sampling campaigns are shown in Fig. 2.

Results of the LAB-BART tests showed that Site A and Site B have some distinct differences with regards to all the utilized test kits (Fig. 3).

In Well B, IRB were measured in lower approximate concentration (AC) than in Well A on most of the sampling dates. IRB further seemed to be stable over time as well as distance, especially at Site A. At Site B, the AC of these bacteria was reduced towards Well B, which was not the case at Site A. An explanation for this might be that the oxygenation at Site B reduced these bacteria in the groundwater.

Results of SRB at Site B showed a persistent increase in AC between piezometers B1 and B2. Because SRB is generally associated with low oxygen environments, it is unlikely that the oxygenation happening between these wells was the cause. However, groundwater samples from B2 were consistently turbid, implying the presence of suspended solids, which might affect the chemical and bacterial composition at that location. In Well B, SRB was not found in any of the samples. This was similar to the IRB results, where ACs in Well B were also generally lower than in B1 and B2.

While SLYM was present in samples from piezometers at both sites, it was barely shown in Well A and B. One explanation for this might be that slime-forming bacteria are more active in slower-flowing waters, and towards the well the groundwater flow rate increases drastically. The mechanical strain from fast-flowing groundwater in the relatively conductive gravel aquifers can inhibit the formation of slimes (Stoodley et al. 1999; Tsai 2005). At Site B, ACs in B2 were usually the highest (with the notable exception of 24.09.2023, when the AC was zero), which could be an effect of the oxygenation just before this sampling location.

Analysis of the DN test kits mostly resulted in an AC of <50 cfu/ml, the lowest value that these tests can determine (Table 1), implying that at these study sites, DN bacteria were of too low concentration to be accurately assessed. While most samples did not show presence of DN, the few samples that did were all taken in late summer or autumn, possibly signifying a temperature dependency.

The results at Site A showed fewer fluctuations over time than at Site B. Firstly, this might be due the source of the water; at Site A it comes from a nearby backwater, while at Site B the groundwater infiltrates both from the Kamp River, a more dynamic waterway, as well as from ambient groundwater flows (Fig. 1). Secondly, oxygen enrichment was more successful at Site B, which might affect microbial communities. Lastly, no temporal or seasonal trends could be discovered, implying that the groundwater temperature did not play a significant role in these analyses. However, the sampling period was short (May to October), and therefore these trends might have been missed.

Analysis of Variance (ANOVA) was used to carry out a statistical comparison between the two sites. Assuming a p-value of 0.05, the results showed that the mean ACs at Site A and B were the same. However, as the variance was larger than the mean values, and because of the small sample size and semi-quantitative nature of the LAB-BART system, this result might not be meaningful.

3.2 Results of chemical analysis

Chemical analysis showed that concentration fluctuations were generally low, and no real temporal trends could be seen in river water nor groundwater, which was similar to the LAB-BART results (Table 2). Notable exceptions are the temperature at Site A, which fluc-
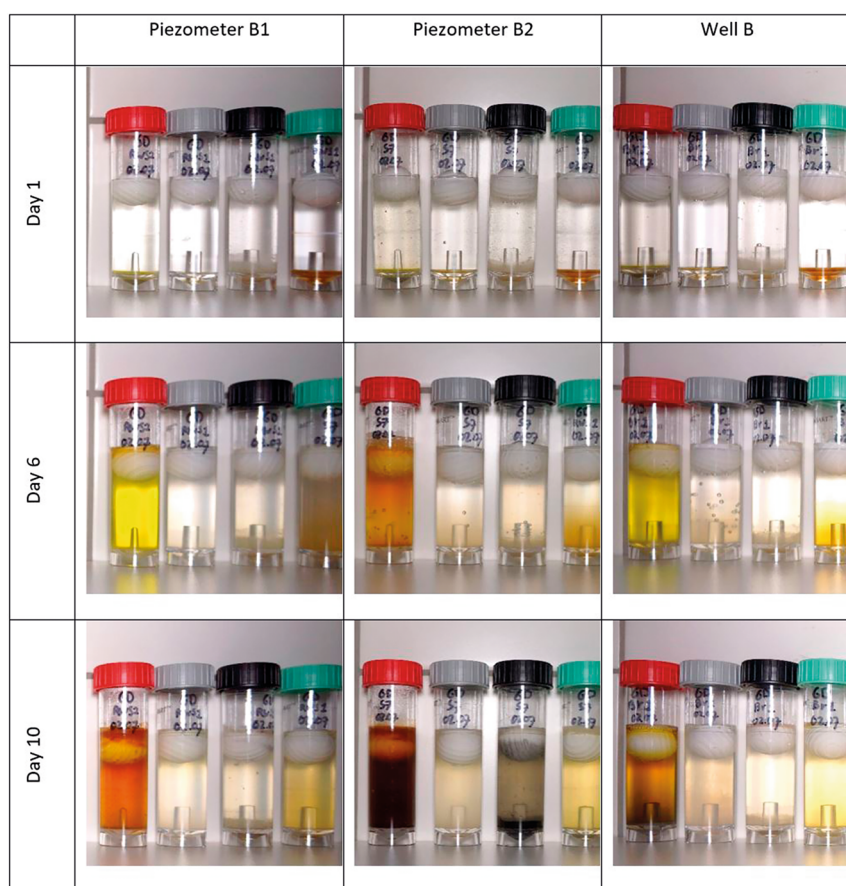
Fig. 2 Photos of the analysis of the 02.07.2019 sampling campaign at Site B. Vials with *red caps* are IRB-BART, with *grey caps* DN-BART, with *black caps* SRB-BART and with *green caps* SLYM-BART
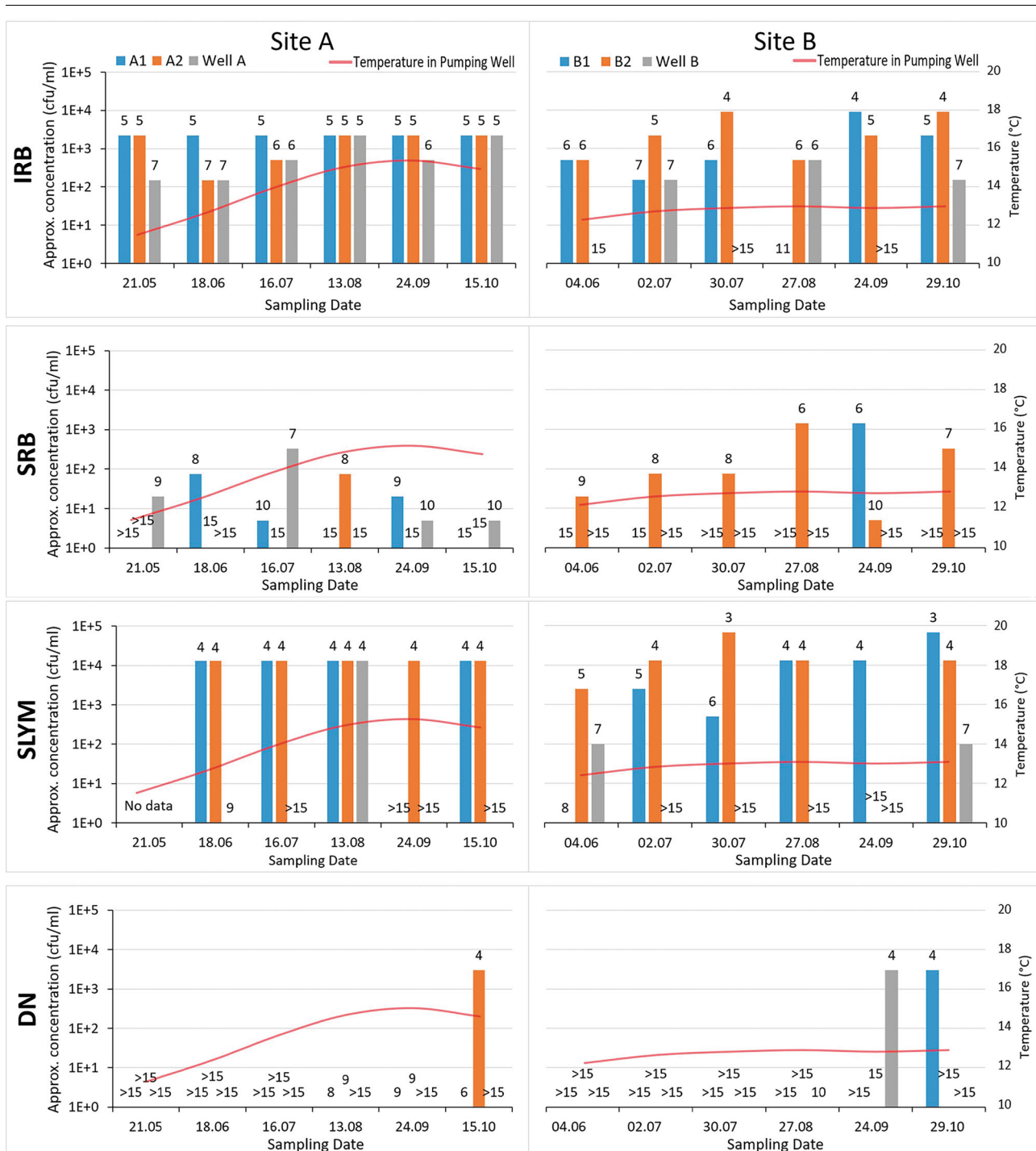
Fig. 3 LAB-BART results. The number above the bars signifies the number of days before the color change took place (time lag). Note: the approximate concentration can be zero even if a color change does happen, because it has been too many days (see Table 1)

tuated by a maximum of 7 °C over the study period, while at Site B the temperature was more stable. This did not seem to influence LAB-BART results. Surface waters at both sites showed an increase in EC with increasing temperatures, but this was not reflected in the groundwater EC, and therefore the

effect on LAB-BART results would be minimal. At Site A, oxygenation had only a limited effect on the groundwater, as shown by the fact that oxygen concentrations remained stable throughout the transect, while at Site B, oxygenation did increase the oxygen concentrations between B1 and B2,

after which concentrations decreased again towards Well B.

Generally, microorganisms need phosphorus to thrive (Bünemann et al. 2008; Widdig et al. 2019). At both sites, there was abundant phosphorus for microbiological activity in the surface water (~20 µg/l to 130 µg/l), which de-

Table 2 Results of the chemical analysis of the groundwater and corresponding surface waters

Site A									
–	Parameter	Unit	21/05/19	18/06/19	16/07/19	13/08/19	24/09/19	15/10/19	Average
Back-water	*Temp.*	°C	–	–	–	–	–	–	**–**
	pH	–	–	–	–	–	–	–	**7.8[a]**
	EC	µS/cm	291	295	350	344	353	466	**350**
	DOC	mg/l C	–	–	–	3.0	3.0	2.4	**2.8**
	Oxygen	mg/l	–	–	–	–	–	–	**–**
	Iron	µg/l	133	112	79	91	83	243	**123**
	Chloride	mg/l	18	13	19	19	18	21	**18**
	Phosphorus	µg/l	19	31	26	33	22	45	**29**
	Nitrate	mg/l	12.0	6.2	3.6	2.9	5.0	2.6	**5.4**
	Nitrite	mg/l	0.074	0.110	0.110	0.036	0.012	0.020	**0.060**
	Sulfate	mg/l	21	17	21	21	22	22	**21**
A1	*Temp.*	°C	11.4	12.9	15.1	16.7	17.3	14.8	**14.7**
	pH	–	–	–	–	–	–	–	**7.7**
	EC	µS/cm	472	436	450	463	443	449	**452**
	DOC	mg/l C	–	–	–	1.5	2.0	1.2	**1.6**
	Oxygen	mg/l	2.50	0.77	0.91	0.80	0.06	0.27	**0.89**
	Iron	µg/l	1.3	0.6	1.7	0.8	2.6	0.6	**1.3**
	Chloride	mg/l	20	14	15	17	16	15	**16**
	Phosphorus	µg/l	9.1	9.2	6.6	11.4	7.4	10.2	**9.0**
	Nitrate	mg/l	1.7	1.9	<1	<1	<1	–	**0.7**
	Nitrite	mg/l	<0.005	0.011	<0.005	0.007	<0.005	<0.005	**0.003**
	Sulfate	mg/l	22	18	13	14	11	18	**16**
A2	*Temp.*	°C	10.5	11.8	13.9	17.7	16.2	15.3	**14.2**
	pH	–	–	–	–	–	–	–	**7.2**
	EC	µS/cm	511	489	509	505	485	492	**499**
	DOC	mg/l C	–	–	–	1.3	1.3	1.1	**1.2**
	Oxygen	mg/l	1.21	2.06	2.58	4.20	3.35	1.84	**2.54**
	Iron	µg/l	0.2	1.4	0.7	0.4	0.4	0.9	**0.7**
	Chloride	mg/l	18	15	17	17	16	17	**17**
	Phosphorus	µg/l	7.1	6.5	5.5	9.8	6.0	6.9	**7.0**
	Nitrate	mg/l	1.2	2.0	<1	<1	<1	<1	**0.5**
	Nitrite	mg/l	<0.005	0.010	<0.005	<0.005	<0.005	<0.005	**0.002**
	Sulfate	mg/l	24	22	23	21	21	22	**22**
Well A	*Temp.*	°C	11.2	12.5	14.0	15.2	15.6	15.1	**13.9**
	pH	–	–	–	–	–	–	–	**7.7**
	EC	µS/cm	477	464	472	475	463	469	**470**
	DOC	mg/l C	–	–	–	1.3	1.2	1.1	**1.2**
	Oxygen	mg/l	3.87	3.60	3.49	3.08	3.32	3.56	**3.49**
	Iron	µg/l	3.9	2.7	3.2	5.5	9.9	9.4	**5.8**
	Chloride	mg/l	20	16	17	17	17	16	**17**
	Phosphorus	µg/l	10.2	10.0	9.2	11.9	10.8	11.0	**10.5**
	Nitrate	mg/l	2.7	2.6	1.800	1.000	1.000	1.6	**1.8**
	Nitrite	mg/l	<0.005	0.010	<0.005	<0.005	<0.005	<0.005	**0.002**
	Sulfate	mg/l	24	19	20	20	20	21	**21**

creased slightly after infiltration into the subsurface. This could be an indication of bacterial metabolism during subsurface flow, showing that LAB-BART should detect some activity. Both phosphorus and nitrite concentrations showed high temporal fluctuations in the Kamp River at Site B. Interestingly, phosphorus stabilized after infiltration (except for the 04/06 B2 sample, possibly due to the same reason as the iron concentration increase, see below), while the nitrite fluctuations increased in B1. Nitrite concentrations only stabilized after the oxygenation between B1 and B2 and were notably (approx. 2 logs) reduced. At Site A, these fluctuations were much lower, possibly due to the stagnant surface waters at that site. The other parameters were more stable at both sites, besides some smaller (<1 log) fluctuations.

At both sites, the conditions in the subsurface were suboxic, leading to significant reduction of iron concentrations after infiltration into the riverbank. However, in piezometer B2 at

Table 2 (Continued)

Site B[b]

–	Parameter	Unit	04/06/19	03/07/19	31/07/19	27/08/19	24/09/19	29/10/19	Average
Kamp river	Temp.	°C	–	–	–	–	–	–	**–**
	EC	µS/cm	219	352	283	304	417	395	**328**
	DOC	mg/l C	–	–	–	5.1	4.7	4.6	**4.8**
	Oxygen	mg/l	–	–	–	–	–	–	**–**
	Iron	µg/l	338	201	170	595	190	63	**259**
	Chloride	mg/l	18	33	21	16	27	35	**25**
	Phosphorus	µg/l	74	132	71	52	28	52	**68**
	Nitrate	mg/l	16	12	12	11	10	11	**12**
	Nitrite	mg/l	0.060	0.110	0.024	0.099	0.018	0.470	**0.130**
	Sulfate	mg/l	20	28	20	17	24	28	**23**
B1	Temp.	°C	13.8	13.1	13.7	11.5	12.4	12	**12.8**
	EC	µS/cm	367	372	365	375	320	369	**361**
	DOC	mg/l C	–	–	–	2.3	2.2	2.1	**2.2**
	Oxygen	mg/l	1.11	1.93	1.94	0.12	0.17	1.37	**1.11**
	Iron	µg/l	0.5	1.0	37.1	0.3	1.0	0.3	**6.7**
	Chloride	mg/l	26	25	24	24	25	25	**25**
	Phosphorus	µg/l	70	65	72	71	67	69	**69**
	Nitrate	mg/l	15.0	16.0	15.0	12.0	10.0	6.7	**12.5**
	Nitrite	mg/l	0.006	0.017	0.630	<0.005	0.440	0.460	**0.259**
	Sulfate	mg/l	23	22	22	21	21	22	**22**
B2	Temp.	°C	15.3	12.8	13.3	12.3	12.6	12.2	**13.1**
	EC	µS/cm	440	440	448	461	366	450	**434**
	DOC	mg/l C	–	–	–	1.8	1.5	1.5	**1.6**
	Oxygen	mg/l	7.89	8.70	8.88	10.39	11.40	11.29	**9.76**
	Iron	µg/l	3759	423	292	194	207	298	**862**
	Chloride	mg/l	28	28	29	29	28	27	**28**
	Phosphorus	µg/l	998	81	51	87	44	33	**215**
	Nitrate	mg/l	9.9	13.0	12.0	10.0	11.0	11.0	**11.2**
	Nitrite	mg/l	0.012	0.019	<0.005	0.014	<0.005	<0.005	**0.008**
	Sulfate	mg/l	24	25	24	25	25	24	**24**
Well B	Temp.	°C	11.8	12.3	12.5	12.6	12.5	12.6	**12.4**
	pH	–	7.6	6.0	6.1	6.4	6.3	6.2	**6.4**
	EC	µS/cm	437	449	452	463	478	434	**452**
	DOC	mg/l C	–	–	–	1.6	1.6	1.5	**1.6**
	Oxygen	mg/l	6.25	3.80	6.80	6.67	5.93	6.95	**6.07**
	Iron	µg/l	0.4	0.3	0.1	0.2	0.3	0.1	**0.2**
	Chloride	mg/l	28	27	29	29	28	28	**28**
	Phosphorus	µg/l	47.6	48.5	54.8	57.3	54.2	57.2	**53.3**
	Nitrate	mg/l	11.0	13.0	12.0	11.0	11.0	10.0	**11.3**
	Nitrite	mg/l	0.006	<0.005	<0.005	<0.005	<0.005	<0.005	**0.001**
	Sulfate	mg/l	24	22	24	24	24	24	**24**

[a]pH values were not measured in this study period, so an average of the two years before is given
[b]No pH values available for Site B

Site B, iron concentrations increased over 2 orders of magnitude (2 logs) after oxygenation, which was only reflected moderately in the IRB results, and only at the dates 02.07, 30.07 and 29.10 (Fig. 3). This might have been an effect of suspended solids in the samples, as mentioned earlier. Possibly these suspended solids were present due to the oxygenation happening close to the sampling location, or suboptimal sampling due to problems with the piezometer. Towards Well B, iron concentrations decreased again, possibly due to the mixing of water from different sources (Fig. 1), which might explain the decrease of IRB bacteria at that location.

Sulfate concentrations did not change after oxygenation at Site B, even though concentrations of SRB bacteria increased between B1 and B2 (Fig. 3). Unlike iron, sulfate was very stable at ~20 mg/l across all sampling sites, including surface waters. This might explain the overall lower ACs measured by SRB-BART as opposed to IRB-BART, as seemingly there were no biogeochemical processes happening that consumed sulfate, at least not in mea-

surable amounts, while this did seem to be the case for iron.

DOC was determined, among other reasons, because the SLYM bacteria's excreted polymer substance (i.e., "slime") consists of a multitude of organic matters. DOC decreased slightly towards the pumping wells at both sites, which might explain the lower concentrations of SLYM bacteria at Well A and B compared to the piezometers. However, the bioavailable part of DOC would be relevant in this case, but this was not determined. Oxygenation between B1 and B2 did seem to increase activity of SLYM bacteria, even though it was only observable during the first 3 sampling dates. The formation of slimes is a complex, multi-parameter occurrence, and is very difficult to be predicted from the presence or absence of one or two parameters. Still, as discussed above, Site B had a less stable chemistry than Site A, which might explain the difference in SLYM bacteria concentrations between the two sites.

The low concentrations of nitrate (NO_3^-) in the backwater and Kamp River might explain why no significant ACs of denitrifying bacteria (DN) were detected by LAB-BART in any of the locations at either site (Fig. 3); the mean concentrations were 5 mg/l and 12 mg/l at Site A and B, respectively, while the acceptable limit is 50 mg/l for drinking water (EU 2020). At Site A, nitrate levels were lower in A1 than in the surface water, indicating denitrifying processes happening during bank filtration, but no subsequent changes in nitrate concentration were observed between A1, A2 and Well A, which explains low concentrations of DN bacteria at these locations. Oxygenation should inhibit the anaerobic respiration of these bacteria, but because they were not present before oxygenation, no effect was observed. The few samples where the AC of DN bacteria was higher (i.e., 15/10/19 at A2, 24/09/19 at Well B, or 29/10/19 at B1), did not show increased concentrations of nitrate or nitrite, so it is difficult to explain why in these samples DN bacteria were more active.

3.3 Results of microbial community analysis and detection of LAB-BART relevant physiological bacterial groups

Genome analysis of the samples using metabarcoding revealed the presence of Amplicon Sequence Variants (ASV) associated with approximately 900 bacteria taxa. An average of 38.8% of the taxa were classified as unknown while the remainder indicated the presence of environmental bacteria commonly found in comparable groundwater environments (Yan et al. 2021). On a phylum level, Proteobacteria and the recently defined and common groundwater superphylum Patescibacteria have been observed predominantly in all samples (Fig. 4; Tian et al. 2020).

In recent literature, SRB, IRB, and DN bacteria have been identified as particularly relevant in well fields like the ones under investigation here and are part of the Proteobacteria phylum which is abundant in all samples. As described by Wargin et al. (2007), SRB representatives like *Desulfovibrio* and *Desulfotomaculum* tend to form bacterial consortia negatively influencing organoleptic water quality in groundwater and drinking water distribution infrastructure. Ferric-iron reducers *Geobacter* and *Geothrix* have recently been identified in the USA as notable in the presence of SRB (Flynn et al. 2013) while bacteria capable of denitrification are widely present in groundwater biomes with *Woesearchaeota, Nitrospirales, Nitrosopumilales* and *Acidobacter* among the most abundant in groundwater used for agricultural purposes (Korbel et al. 2022). All indicators for SRB, IRB and DN bacteria have been observed in at least one replicate sample, and common IRB groundwater genera *Rhodoferax* and *Geobacter* have also been identified as present in the study sites (Zaa et al. 2010; Flynn et al. 2013). While gene metabarcoding does not allow for a quantification of the ASV identified, positive hits in samples that are also positive in the respective LAB-BART strongly indicates true positive results.

4 Conclusion

Based on the findings presented here, we found that the LAB-BART test kits are sufficiently robust and specific for on-site monitoring of drinking water wells. In combination with the fact that gene metabarcoding confirmed that results obtained by LAB-BART did not result in any "false negative" reads, the potential operational hinderances associated with its usage should be minimal. Because LAB-BART only detects active bacteria, it might be useful in specific situations. The kits were found to be simple to handle by professionals without a microbiological background or access to a laboratory. This ease of use is promising for water management professionals in low resources settings worldwide.

At Site B, LAB-BART results were less stable and showed higher fluctuations between sampling locations than at Site A, something that was only moderately shown by the chemical results and not by gene metabarcoding. Possibly, LAB-BART functions better in hydrologically less complicated systems. Mixing of groundwater from different sources and the biochemical instability caused by oxygenation can lead to considerable changes in the microbiological communities in the groundwater, which could be difficult to accurately determine. A loss in water production at a simple RBF site, like Site A, would be an exemplary situation where LAB-BART could be beneficial, at least for a preliminary investigation into the type of problem that is causing well clogging. Because of this, in-depth hydrological knowledge of the study site is advised, as it would be difficult to interpret the results otherwise. However, a longer time series of samples would be beneficial, especially when changes take place at the study site, e.g., changes in chemical conditions or pumping rate. This would help to understand how the bacterial communities behave under different stressors such as oxygenation or flow rate changes.

LAB-BART has its limitations. It is very difficult to measure a specific subset of bacteria accurately. Especially if particular taxa are of interest, other quick tests such as the Analytical Profile Index (API) or assays indicating the metabolic activity of fecal bacteria (i.e., *Escherichia coli* or Enterococcus via IDEXX) tests should be employed (Logan et al. 1985; Paziak-Domańska et al. 1999). For more detailed chemical information, such as determining the biochemical conditions in the subsurface, measurements such as oxygen content and photometrics are paramount. Still, as a tool to help characterize well biofouling, LAB-BART could be beneficial if not a substitute for a full microbiological analysis. Although it can give some information on the type of bacteria present in the groundwater, its strength lies in the combination of chemical and microbial processes.

Fig. 4 Relative abundance of bacterial communities at the phylum level in all the samples

Although LAB-BART results were reasonable when compared to chemical and sequencing data, no definitive verification could be made of the approximate concentration of bacteria that LAB-BART claims to be able to measure. More research is therefore needed, preferably utilizing long term sampling campaigns under more tightly controlled conditions, and with an extended array of molecular and culture-based methods so that the approximate concentration of bacteria can be verified more accurately.

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