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Event-transport of beta-D-glucuronidase in an agricultural headwater stream: Assessment of seasonal patterns by on-line enzymatic activity measurements and environmental isotopes

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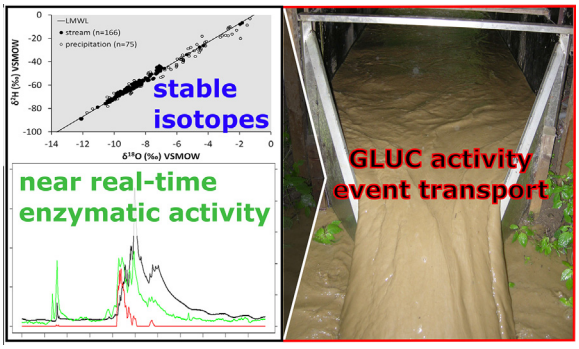
HIGHLIGHTS

- Environmental isotopes were used to estimate event water fraction in an agricultural stream.
- High resolution GLUC measurements of stream water were conducted on-site.
- Streambed and field sediments were analyzed for *E. coli* and GLUC.
- Resuspended streambed sediments are a significant source of fecal indicators.

GRAPHICAL ABSTRACT



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ABSTRACT

Understanding the fate of fecal pollution in the landscape is required for microbial risk analysis. The aim of this study was to assess the patterns and dynamics of beta-D-glucuronidase (GLUC), which has been suggested as a surrogate for fecal pollution monitoring, in a stream draining an agricultural headwater catchment. Automated enzymatic on-site measurements of stream water and sediments were made over two years (2014–2016) to quantify the sources and pathways of GLUC in a stream. The event water fraction of streamflow was estimated by stable isotopes. Samples from field sediments on a hillslope, streambed sediment and stream water were analyzed for GLUC and with a standard *E. coli* assay. The results showed ten times higher GLUC and *E. coli* concentrations during the summer than during the winter for all compartments (field and streambed sediments and stream water). The *E. coli* concentrations in the streambed sediment were approximately 100 times those of the field sediments. Of the total GLUC load in the study period, 39% were transported during hydrological events (increased streamflow due to rainfall or snowmelt); of these, 44% were transported when the stream contained no recent rainwater. The results suggested that a large proportion of the GLUC and *E. coli* in the stream water

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Fecal pollution
Surface water

stemmed from resuspended streambed sediments. Moreover, the results strongly indicated the existence of remnant populations of GLUC-active organisms in the catchment.

1. Introduction

Compared with nutrient fluxes, microbial fluxes in the landscape have received much less attention from research and policy institutions; however, their health implications can be enormous (Kay et al., 2007; "Nutrients in the Nation's Waters: Identifying Problems and Progress, USGS Fact Sheet FS218-96", n.d., "WHO|Engaging with the water sector for public health benefits," n.d., "WHO|Water quality assessments," n.d., (Farnleitner et al., 2010; Reischer et al., 2011). The high spatial heterogeneity of microbial sources and the complexity of transport processes make the study of microbial landscape–scale fluxes challenging. While laboratory experiments, such as column tests, have significantly contributed to the theoretical understanding of microbial transport (Harvey et al., 2010; Scholl and Harvey, 1992; Stevenson et al., 2015; Wang et al., 2012), their time scales tend to be much shorter than those of microbial processes in the landscape driven by hydrological processes. Prior research on *E. coli* in stream water has typically focused on individual events (Jamieson et al., 2005; Kim et al., 2010; Pandey et al., 2012; Wilkinson et al., 1995). However, the "Rotorua Declaration" (adopted at a joint meeting of the IWA Health Related Water Microbiology Symposium and the Diffuse Pollution Conference held in Rotorua, NZ in Sept. 2011) highlighted the need for exploring microbial processes at high temporal resolution over seasons and periods of years.

A number of methods have been developed to measure microbiological parameters in near real-time. These include on-site flow cytometry (Besmer et al., 2016, 2014), optical detection of suspended particles, including the differentiation between bacteria and particles (Højris et al., 2016), indirect indicators of bacterial activity, such as adenosine triphosphate (ATP) (Vang et al., 2014) and the sensing of bacteria by direct contact (Geary, 2009; Ji et al., 2004). Instruments using these technologies are commercially available; however, at present, most instruments are not suited for the real-time monitoring of specific bacterial targets, such as *E. coli*, that serve as indicators of fecal pollution (Deshmukh et al., 2016). For specific, automated and near-real time assessment of bacteria in water, the detection of enzymatic activities has been proposed as a rapid surrogate method (Cabral, 2010; Farnleitner et al., 2001, 2002). Several studies have demonstrated the indicator applicability of beta-D-glucuronidase (GLUC) activity measurements in determining the abundance of the fecal indicator bacteria, *E. coli*, in rivers (Ender et al., 2017; Farnleitner et al., 2001, 2002; Stadler et al., 2017, 2016), ponds (George et al., 2000) and coastal waters (Fiksdal et al., 1994).

To gain insight into the event transport of GLUC at high temporal resolution and over different seasons in a small agricultural headwater catchment, we combined conventional isotope-hydrology with a novel rapid enzymatic on-site assay. Events with increased streamflow and suspended solids mobilization typically dominate the transport of fecal pollution in streams (Muirhead et al., 2004; Pachepsky et al., 2006; Kay et al., 2007). We identified stream water originating from recent precipitation events by stable isotopes and explored the role of isotopic flow-separation in explaining GLUC variability in streams as an indicator of fecal pollution. Specifically, the aim of this study was to address the following questions: (a) What fraction of the transported GLUC in a stream during events originated from resuspended stream bed material? (b) Does this fraction of remobilized GLUC change seasonally? Regarding the proposed fecal indicator applicability of GLUC and *E. coli*, (c) Are they solely surface associated, or can a persistence of GLUC and *E. coli* in the hyporheic zone be observed throughout the seasons?

2. Methods and materials

2.1. Test site and monitoring location

This study has been conducted in the HOAL - Hydrological Open Air Laboratory (Blöschl et al., 2015, 2011) in Lower Austria (Fig. 1). The HOAL catchment is 0.66 km^2 in size and is drained by a stream 620 m in length. Twelve point discharges contribute to the stream, including tile drains, springs and surface tributaries (Exner-Kittridge et al., 2013). During the study period (January 2014 to January 2016) the mean annual precipitation was 823 mm/yr, and the mean discharge was 2.7 l/s (Table 1). The hydrogeology is characterized by porous and fissured aquifers consisting of clay, marl and sand. The soils exhibit medium-to-limited infiltration capacities (Eder et al., 2014, 2010). The annual sediment erosion is approximately 1 t/0.1 km^2 (Eder et al., 2014, 2010). The land use of the catchment is dominated by agriculture, consisting of 87% arable land, 5% grassland, 6% forested area and 2% paved land. The main source of fecal input into the catchment is swine manure applied periodically to the fields. The stream has high discharge dynamics (Table 1, Fig. 2) with a rapid response to rain events, causing significant peaks in the concentration of *E. coli*, GLUC and suspended sediments (TSS) in the stream water.

The instrumentation of the HOAL included on-line measurements of water level for discharge estimation, electrical conductivity (EC), turbidity and water temperature at a stream monitoring station MW (Table 1) at the catchment outlet (Fig. 1) (see Blöschl et al., 2015, for details). At the same location, instrument prototypes for on-site measurement of GLUC were operated, and samples for stable isotope analyses were automatically extracted from the stream during events. Precipitation samples for stable isotope analyses were collected regularly at a station approximately 500 m from the catchment outlet.

2.2. Automated on-site GLUC measurements

The rapid, on-site GLUC assay was based on the specific bacterial hydrolysis of 4-methylumbelliferyl-β-D-glucuronide (MUG) and a fully automated fluorescence detection (excitation: 365 nm, emission: 455 nm) of the resultant enzymatic reaction product 4-methylumbelliferone (MU) (*Enzymatic Assay of β-Glucuronidase (EC 3.2.1.31) from E. coli [WWW Document]*, n.d.; Fishman and Bergmeyer, 1974). The automated measurements were performed in batches using a 6.5-ml sample per measurement. A flow-through photometric measurement-chamber enabled a high-resolution fluorescence analysis of the enzymatic reaction product MU. The measurement step required 15 min, and the assay was calibrated to Modified Fishman Units (MFU/100 ml) based on the enzyme unit definition for beta-D-glucuronidase activity (Fishman and Bergmeyer, 1974). During this study, the instrument operated at location MW (Fig. 1) was programmed to conduct measurements every 60 min (Fig. 2), which included an automated cleaning procedure suitable for long-term on-site operation. The measurement results were transmitted automatically via GPRS modem for on-line data availability. The prototype was installed in a weatherproof and air-conditioned housing. The construction and function of the same prototype design have been described in detail by Koschelnik et al., 2015, and Stadler et al., 2016. Beta-D-glucuronidase (GLUC) activity is a specific indicator for the abundance of *E. coli* in surface waters (Farnleitner et al., 2001, 2002; Fiksdal et al., 1994; George et al., 2001, 2000; Morikawa et al., 2006). The correlation between GLUC and *E. coli* was found to be especially strong for waters impacted by municipal

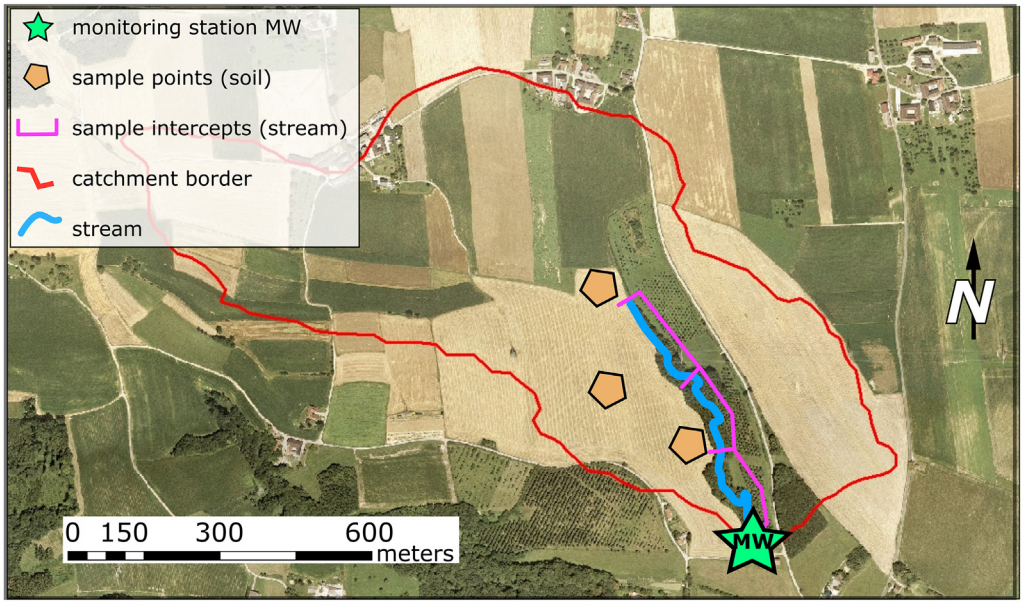

Fig. 1. Map of the HOAL, a 0.66 km^2 experimental catchment with predominantly agricultural land use. Drained by a 620 m stream (blue line), the stream monitoring station MW (green star) is located at the catchment outlet. Sample areas for field sediments (brown pentagons) and streambed sediments (yellow intercepts) are marked. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

sewage (Farnleitner et al., 2001, 2002) and manure (Stadler et al., 2016). GLUC measurements in various aquatic habitats (Ender et al., 2017; Koschelnik et al., 2015; Stadler et al., 2016) showed the suitability of this on-site assay to indicate *E. coli* at high temporal resolutions (>1 h), especially during runoff events (Stadler et al., 2017, 2016).

2.3. Environmental isotopes

Event monitoring by environmental isotopes (Clark and Fritz, 1997; Mook and Rozanski, 2000; Stadler et al., 2008) was chosen in this study because the stable isotopes of hydrogen and oxygen are conservative tracers (Clark and Fritz, 1997) and thus, provide information about aquifer response characteristics, storage dynamics and run-off characteristics (Goller et al., 2005; Huth et al., 2004; Stadler et al., 2008). Stream water samples (Fig. 2) were taken automatically at the monitoring station MW (Fig. 1) during runoff events. Two sampling devices (ISCO sampler 6712) were connected to a pressure transducer and triggered once a water level threshold was exceeded. Each sampler contained 24 bottles. The first device sampled at 15-minute intervals. Once the first device was filled, the second device started sampling every hour, achieving a total sampling period of 30 h. During the study period, 799 samples of stream water were taken during events. Additionally, 106 grab samples were taken manually at monitoring station MW (Fig. 1) on a weekly basis during base flow conditions. A total of 285 precipitation samples (Fig. 2) were taken automatically by a precipitation sampler located some 500 m from the stream monitoring location MW. The precipitation sampler switched bottles every 5 mm of precipitation, filling bottles with 100-ml volumes. Stream water and precipitation samples were collected within 24 h following an event and stored in closed vessels until analyses. Isotopic compositions ($\delta^{18}O$ and $\delta^{2}H$) of the water samples were measured using cavity ring-down spectroscopy (Berden et al., 2000) with a WS-CRDS (Wavelength-Scanned Cavity Ring-Down Spectroscopy) instrument (Picarro, Inc.). The instrument setup was similar to a system described by Gupta et al., 2009.

The event contribution of precipitation to streamflow Q_p [l/s] was estimated by a two component hydrograph separation:

$$Q_p = Q_s(C_s - C_b) \times (C_p - C_b)^{-1} \quad (1)$$

where Q_s is the discharge [l/s] of the stream, C_s is the isotope signal [$\delta^{18}0$ in ‰] of the stream water (from automated event sampling, n = 799), C_b is the isotope signal [$\delta^{18}0$ in ‰] of the stream base flow (from weekly grab samples, n = 106) and C_p is the isotope signal [$\delta^{18}0$ in ‰] of precipitation water (derived from automated rain sampling, n = 285). The event water fraction F_s (%) of total flow (Fig. 3) was calculated as:

$$F_s = \left(Q_p \times Q_s^{-1}\right) \times 100 \quad (2)$$

Table 1
Range of key parameters in the HOAL stream during the study period (2014–2016, n = number of measurements). Table includes *E. coli* concentrations in streambed sediment and soil.

			Min	Max	Median	Mean
Discharge	[l/s]	n = 105,120	0.4	79	2.3	2.7
Suspended solids	[TSS mg/l]	n = 52,560	0	5862	8	19
Electrical conductivity	[µS/cm]	n = 52,560	195	856	769	765
Water temperature	[°C]	n = 8760	0.2	20.0	10.7	10.3
Air temperature	[°C]	n = 7099	−8.7	34.9	12.2	11.6
E. coli in stream water	[MPN/100 ml]	n = 54	<1	3730	134	424
GLUC in stream water	[mMFU/100 ml]	n = 12,209	0.8	123	5.5	9.0
E. coli in stream sediment	[MPN/g]	n = 12	81	1181	189	321
E. coli in field sediment	[MPN/g]	n = 12	<1	16	<1	6

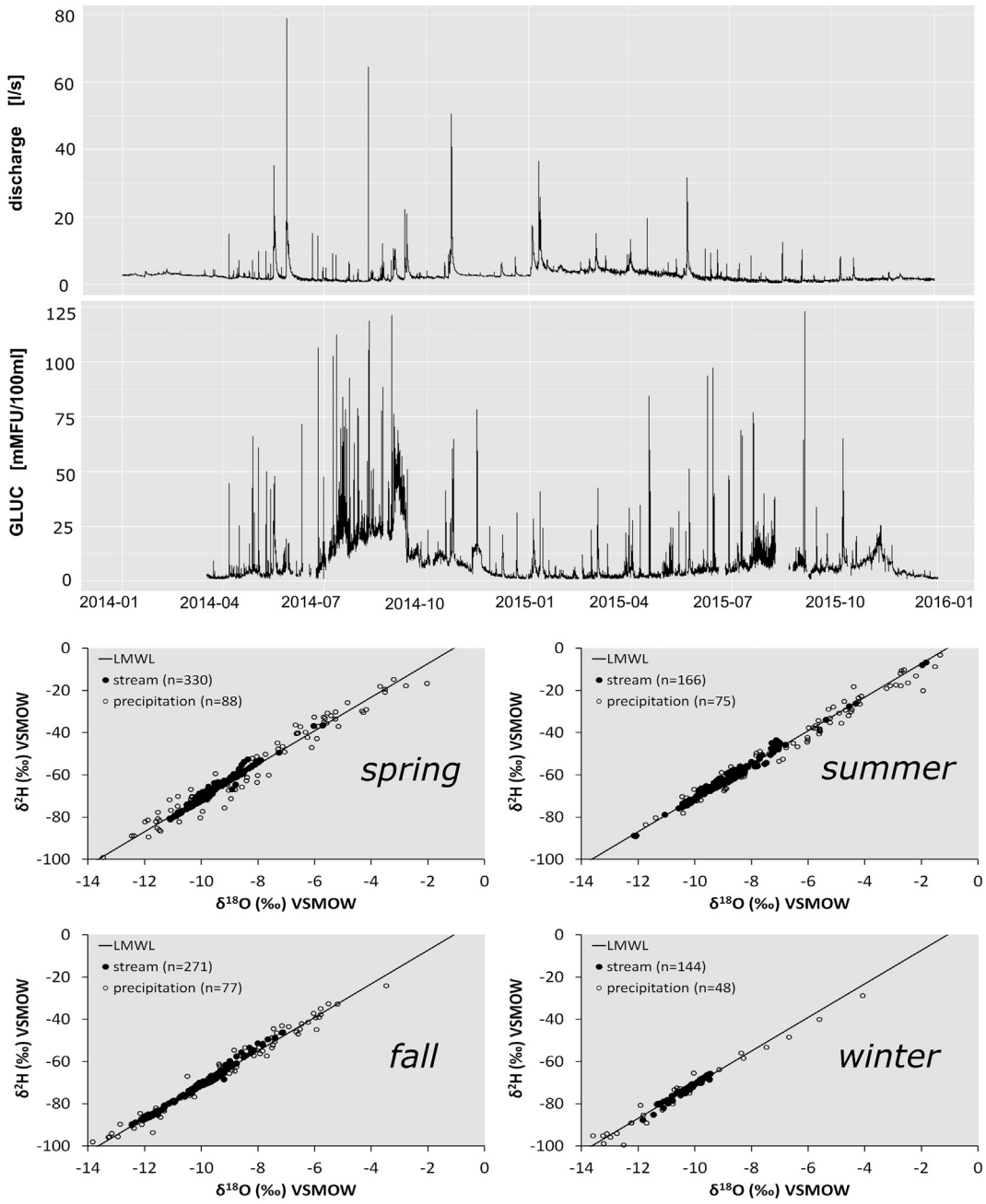

Fig. 2. Hydrograph (top), GLUC activity (second from top) and scatter plots of isotopes $\delta^{18}O$ and $\delta^{2}H$ (four bottom panels) in stream water during the study period. Full and open circles indicate the stream and precipitation samples, respectively (n = number of samples). A local meteoric water line (LMWL) of $\delta^{2}H = 7.97\ \delta^{18}O + 8.78$ ($R^2 = 0.99$, $p < 0.001$) was estimated from 681 precipitation samples for the period 2006–2017.

2.4. Event characterization

Stream flow was measured at 1-minute intervals and averaged every 10 min (Fig. 2). Based on findings from previous studies in the HOAL (Eder et al., 2014; Exner-Kittridge et al., 2013), hydrological conditions were flagged as events when streamflow changed by at least 0.1 l within 60 min. Precipitation data and electrical conductivity of stream water [μS/cm] were used to check this threshold. To define the ascending and descending limbs of the event (Fig. 3), slopes of the hydrograph were calculated from the moving average of the discharge within a 240-minute period. A positive slope of the hydrograph defined an ascending phase, whereas a negative slope of the hydrograph defined a descending phase (Fig. 3). Information about the ascending and descending limbs of the hydrograph were used to interpret the GLUC-discharge relationship (Fig. 4).

2.5. Soil and sediment sampling

To quantify the seasonal fluctuations of *E. coli* in surface-associated matter originating from the crop fields and the resuspended material from the streambed, soil and streambed sediment samples were collected and analyzed during dry weather conditions in spring (April 18th, 2017), summer (August 29th, 2016), fall (October 23rd, 2017) and winter (February 20th, 2017). During each sampling campaign, soil samples were taken from three sampling areas of 25-m diameters in crop fields adjacent to the stream (Fig. 1). Five samples per sampling area were drawn from the top layer of the soil (top 5 cm) and merged to one composite sample per area, yielding three composite samples for the crop field. Sediment samples from the streambed were drawn with a sampling cylinder from three stream reaches, each being approx. 200 m in length (Fig. 1). Five samples per stream reach were drawn

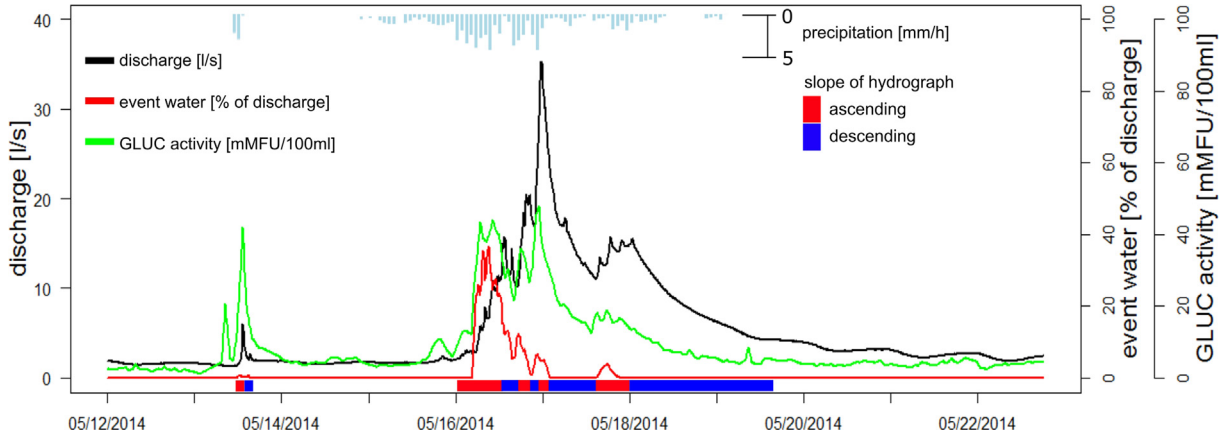

Fig. 3. An event in May 2014 as an example for event water estimation and event characterization: hydrograph (black), event water fraction (red) and GLUC activity (green) show distinct event dynamics. The red and blue bars at the bottom indicate ascending and descending phases of the event hydrograph. Precipitation (mm/h) is plotted at the top (light blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

from the top layer of the stream bed (top 5 cm) and merged to one composite sample per reach, yielding three composite samples for the stream. Each composite sample was homogenized, and one part was used to determine dry mass m_d [g]:

$$m_d = m_w \times (1-\varphi) \quad (3)$$

where m_w is the wet mass of the sample [g] and φ is the water content. The remaining portions of the composite samples were weighed and suspended in sterile water. The suspension was then analyzed with the ISO 9308-2:2012 assay (IDEXX Colilert18®) for *E. coli*. The *E. coli* concentration c_s [MPN(100 ml ∗ g)] per g dry soil was calculated as:

$$c_s = c_f \times (f \times m_d)^{-1} \quad (4)$$

where c_f is the concentration of bacteria in the suspension [MPN/100 ml], f is the dilution factor and m_d is the dry mass of the sample [g]. The mean *E. coli* concentration $\overline{c_s}$ [MPN/(100 ml ∗ g)] per dry weight for each compartment (soil and streambed sediment) was calculated as:

$$\overline{c_s} = \frac{1}{n}\sum_{i=1}^{n} c_s \quad (5)$$

where n is the number of composite samples and c_s is the *E. coli* count [MPN/g] per dry weight of each composite sample. The GLUC activity of the suspended sediments in the stream water per dry mass, $GLUC_{TSS}$ [mMFU/g], at location MW was estimated as:

$$GLUC_{TSS} = \left(10\, GLUC_S \times TSS^{-1}\right) \times 1000 \quad (6)$$

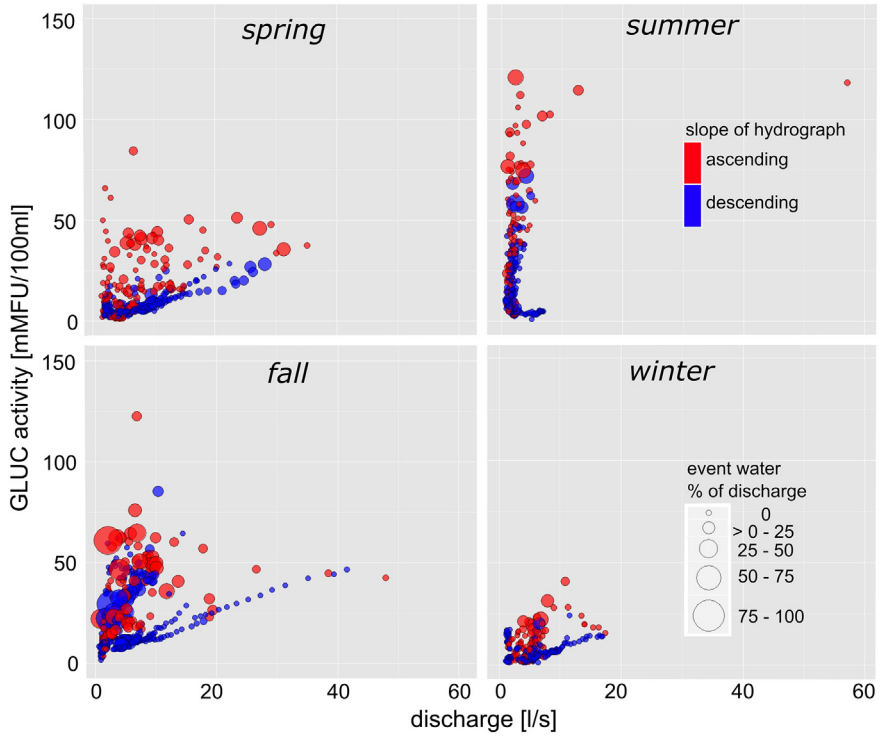

Fig. 4. Seasonal clustering of the GLUC-discharge relationship at MW. Colors indicate the slopes of the event hydrographs (red = ascending phase, blue = descending phase). Point size indicates the event water contribution (% of discharge) in the stream. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

where $GLUC_S$ is the GLUC activity of the stream water [mMFU/100 ml] and TSS_S is the concentration of suspended sediments in the stream water [mg/l].

2.6. Data processing and interpretation

Linear correlation and principal component analyses (PCA) were performed to assess the relationship between stream discharge, GLUC, total suspended solids (TSS) and event water. For further visual interpretation of the associations among GLUC, discharge, event water and hydrograph slope, bubble charts and correlation plots were generated for each season. The data were separately analyzed for the following four seasons: spring (March–May), summer (June–August), fall (September–November) and winter (December–February). On-site measured GLUC and discharge were used to calculate GLUC loads [mMFU/s] for event and non-event conditions in each of the seasons and for the total study period.

3. Results

3.1. GLUC – discharge relationship

The GLUC–discharge association exhibited distinct seasonal clusters (Fig. 4, isochronal measurements of GLUC and discharge at temporal resolution of 1 h, not all short discharge peaks were isochronal with GLUC measurements). In spring, discharges of up to 79 l/s, with a median of 2.9 l/s and GLUC peaks of up to 80 mMFU/100 ml, with a median of 3.0 mMFU/100 ml were recorded. Increased GLUC values during discharges below 5 l/s were related to streamflow not containing any event water (small circles in Fig. 4) and occurred during the ascending limb of the hydrograph (red circles in Fig. 4), suggesting a remobilization of remnant bed sediments in the early phase of events. Increased GLUC values during discharges above 5 l/s were related to significant event water contributions (between 10 and 35% of total discharge) and occurred exclusively during the ascending phase of events. During the descending phase of the event-hysteresis, GLUC decreased linearly with decreasing discharge, and event water contributions were <25%.

Discharges in summer were generally low with a median of 1.4 l/s, but one intense precipitation event caused a discharge peak of 65 l/s. GLUC values during both years of monitoring reached a maximum during the summer seasons with values of up to 121 mMFU/100 ml (median 10.9 mMFU/100 ml) even though discharges were below 10 l/s. The highest GLUC values were recorded during the ascending phases of events with event water contributions of approximately 35%. Similar to the other seasons, there was a linear but less pronounced relationship between discharge and GLUC during the descending limb of the events. A significant amount of descending phase data points with high GLUC values of up to75 mMFU/100 ml was observed during the summer.

The stream discharge during the fall had a median of 4.7 l/s and reached a maximum of 51 l/s. GLUC had a median value of 17.0 mMFU/100 ml and values up to 123 mMFU/100 ml. The highest GLUC values occurred during the ascending phase of the events. Event water contributions reached 75% during discharges below 10 l/s. There was a pronounced linear relationship between discharge and GLUC during the descending phase of events with low event contributions, although several descending phase data points with both high GLUC values (>50 mMFU/100 ml) and increased event water contribution (>75%) were recorded.

Winter was characterized by a median discharge of 2.6 l/s (maximum 37 l/s) and GLUC values below 41 mMFU/100 ml with a median of 3.1 mMFU/100 ml. The highest GLUC values again occurred during the ascending hydrograph limbs with event water contributions of up to 25%. There was an apparent linear relationship between discharge and GLUC during the descending phase of events.

While all seasons showed a clear clustering of the GLUC-discharge relationship (Fig. 4) reflecting the changing hydrological and microbiological conditions in the catchment throughout the year, there were similarities between the seasons. The maximum GLUC values in all seasons tended to occur during the ascending event phases and when event water contributions were larger than 10% (Fig. 4). However, in all seasons significant amounts of increased GLUC values were also recorded during the ascending phases of events with no event water contributions (Fig. 4). In all seasons, linear relationships between discharge and GLUC during the descending phases of events were observed, which were most pronounced in spring and fall (Fig. 4). The majority of these data points were characterized by little (below 25%) to no event water and relatively low GLUC values (Fig. 4).

3.2. Transport of GLUC loads during events

Over the entire study period, 71% of the total stream discharge volume occurred during non-event conditions and 29% during events (Table 2). The GLUC loads reflected these hydrological conditions with 61% of the total GLUC load transported during non-event conditions and 39% transported during events (Table 2). Overall, 44.4% of the GLUC load transported during events were mobilized by stream water not containing any recent precipitation water (Table 2). Stream flow containing up to 25% event water transported 45.2% of the GLUC load during events. Stream flow containing >25% event water occurred infrequently (only 6.3% of the total discharge during events) and transported 10.6% of the GLUC load during events (Table 2).

3.3. E. coli and GLUC in the soil and the stream bed sediments

The field and streambed sediment samples showed seasonal variations in *E. coli* concentrations with maximums during the summer and minimums during the spring and winter in both compartments (Fig. 5). During spring, *E. coli* concentrations in the field sediment were <1 MPN/g and 81 MPN/g in the streambed sediment. During the summer, they were 16 MPN/g and 1186 MPN/g, respectively, and during the fall, they decreased to 6 MPN/g in the field sediment and to 385 MPN/g in the streambed sediment. During the winter, they were <1 MPN/g in the field sediment and 89 MPN/g in the streambed sediment. Overall, the *E. coli* concentrations in the streambed sediment (Fig. 5) were almost 2 log higher in all seasons than those in the field sediment (Fig. 5). The grab samples of stream water (sample location

Table 2
The first two rows show the percentage of streamflow volume during non-event and event conditions in various seasons, and during the whole study period. The rows below show the event water contribution to discharge of all events separately for time steps with event contributions of different magnitudes (0% to >75%). The two middle rows show the percentage of GLUC loads transported during non-event and event conditions. The five lowest rows show the percentage of GLUC loads transported during event conditions by different event water contributions (0% to >75%) during the various seasons and during the whole study period.

	Spring	Summer	Fall	Winter	2014–2016
% of total discharge during					
Non-event conditions	21	13	11	26	71
Event conditions	13	3	6	7	29
% of total discharge during event conditions with:					
0% event water	25.7	7.9	8.6	16.3	58.5
>0–25% event water	16.4	2.6	9.6	6.6	35.2
25–50% event water	2.6	0.3	2.7	0.2	5.8
50–75% event water	0	0	0.4	0	0.4
>75% event water	0	0	0.1	0	0.1
% of total GLUC load transported during:					
Non-event conditions	15	13	13	20	61
Event conditions	14	6	14	5	39
% of GLUC load during event conditions transported with:					
0% event water	17.2	7.5	11.5	8.2	44.4
>0–25% event water	15.4	6.5	19.8	3.5	45.2
25–50% event water	3.9	0.6	4.9	0.5	9.9
50–75% event water	0	0.1	0.5	0	0.6
>75% event water	0	0	0.1	0	0.1

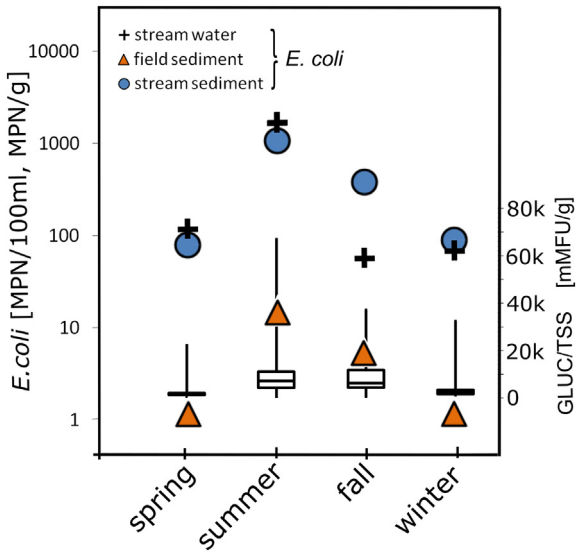

Fig. 5. Seasonal variation of *E. coli* concentrations c_s in different compartments: *E. coli* concentrations in field sediments [MPN/g] are shown as orange triangles, *E. coli* concentrations in the streambed sediment [MPN/g] are shown as blue circles and *E. coli* concentrations in the stream water [MPN/100 ml] are shown as black crosses. Box plots show the GLUC-TSS ratio (GLUC concentration in total suspended sediments, mMFU/g). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

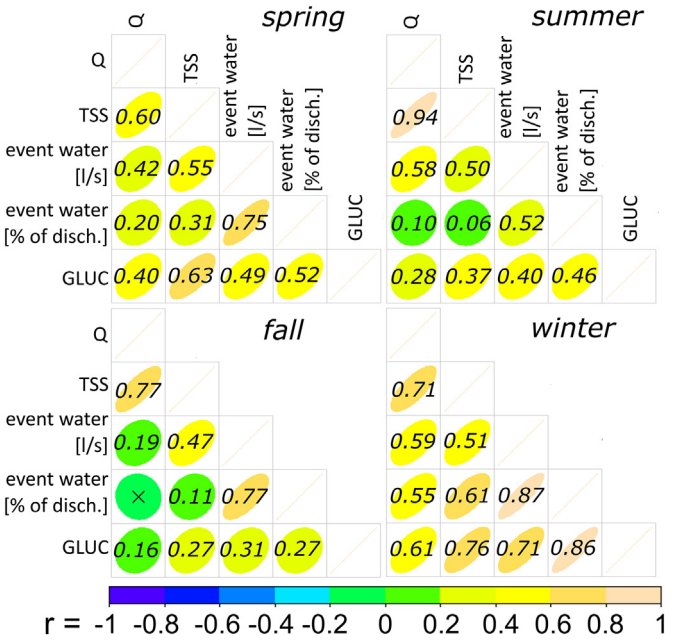

Fig. 6. Pearson correlation coefficients, *r*, between GLUC, discharge (Q), TSS, event water discharge (l/s) and event water fraction (% of discharge) throughout the seasons of the study period. All p-values < 0.001 except for event water (%) and Q in fall ($p > 0.001$ marked with X). The correlation of GLUC with other in-stream variables varies between the seasons. The correlations between GLUC, TSS and event water fraction are the strongest.

MW, mean values 2012–2017, n = 43) showed similar patterns with maximum *E. coli* concentrations during the summer (1713 MPN/100 ml), lower values during spring (122 MPN/100 ml) and minimum concentrations during the fall (59 MPN/100 ml) and winter (69 MPN/100 ml). The GLUC/TSS ratio, reflecting the GLUC activity in suspended sediments also followed the seasonal pattern with a maximum in the summer ($67 * 10^3$ mMFU/g) and minimum values in the spring ($22 * 10^3$ mMFU/g) and winter ($32 * 10^3$ mMFU/g) (Fig. 5).

3.4. Comparison of GLUC with E. coli

Comparisons of automated measurements of GLUC with ISO 9308-2:2012 analyses (IDEXX Colilert18®) of grab samples (n = 54) for *E. coli* and physical in-stream parameters showed that GLUC was more significantly correlated with *E. coli* ($R^2 = 0.52$, $p < 0.001$) than with the observed physical in-stream parameters (e.g., TSS, $R^2 = 0.22$, $p < 0.001$). The correlation between these two parameters was significantly higher during precipitation-induced run-off (event conditions) with an R^2 of 0.80 (n = 13, $p < 0.001$, data not shown). *E. coli* concentration also showed significant correlations with discharge ($R^2 = 0.63$, $p < 0.001$) and TSS ($R^2 = 0.51$, $p < 0.001$).

3.5. Drivers of GLUC concentrations in stream water

Linear correlations among GLUC concentration, discharge, TSS and event water contributions (both in l/s and % of total discharge) showed distinct seasonality (Fig. 6). Correlations among all variables were significant ($p < 0.001$), except between event water fraction and discharge during the fall. During the spring, GLUC showed the strongest correlation with TSS (r = 0.63) and event water fraction (r = 0.52). During the summer, GLUC showed the highest correlations with event water fraction (r = 0.46) and event water discharge (r = 0.37). During the fall, the correlations of GLUC with the other in-stream variables were low (all $r < 0.4$). During the winter, GLUC was strongly correlated with TSS (r = 0.76), event water discharge (r = 0.71) and event water fraction (r = 0.89).

Principal component analysis (Fig. 7) of GLUC concentration, discharge, TSS and event water fraction (both in l/s and % of total discharge) showed that the first two factors (Dim1 and Dim2) accounted for 71% (winter) to 74% (fall) of the variation. While the linear correlations (Fig. 6) among the observed variables, i.e., GLUC concentrations and event water fraction, were not consistent over the seasons, the PCA results (Fig. 7) showed that these two variables were similar during all seasons. This joint variation in response to an unobserved, latent variable indicated similar drivers of GLUC and event water fraction throughout the study period.

4. Discussion

Previous studies on fecal indicator bacteria (FIB) in streambed sediments (Cho et al., 2010; Foppen and Schijven, 2006; Garzio-Hadzick et al., 2010; Kim et al., 2010) have highlighted the important role of resuspension with respect to the event transport of *E. coli*; these studies have reported extensive survival times of enteric bacteria in alluvial systems (Jamieson et al., 2004). Studies on the event transport of *E. coli* have been typically based on monitoring campaigns (Kim et al., 2010), modeling (Pandey et al., 2012) or artificial flooding experiments (Muirhead et al., 2004). The findings of this study showed that remobilization of streambed sediment was a significant component for GLUC dynamics in streamwaters and were in accordance with Kim et al., 2010, Pandey et al., 2012, and Muirhead et al., 2004, who focused on flood-induced streambed release of fecal indicator bacteria. Additional insights into the seasonality of enzymatic activity event transport are provided here due to the continuous and high resolution GLUC measurements over two years and isotopic flow separation.

The GLUC-discharge relationship (Fig. 4) along with the event water contribution and the slope of the event hydrograph can be interpreted with respect to the potential sources and pathways of fecal pollution in different parts of the year as follows. Linear relationships with small slopes were found for the descending event phase, usually containing fewer than 25% event water. The linearity of these baseline relationships (Fig. 8) and a consistent slope during all seasons suggested that this pattern was caused by a postrainfall drainage of a large reservoir in the catchment. We interpreted this pattern as a signal of remnant GLUC

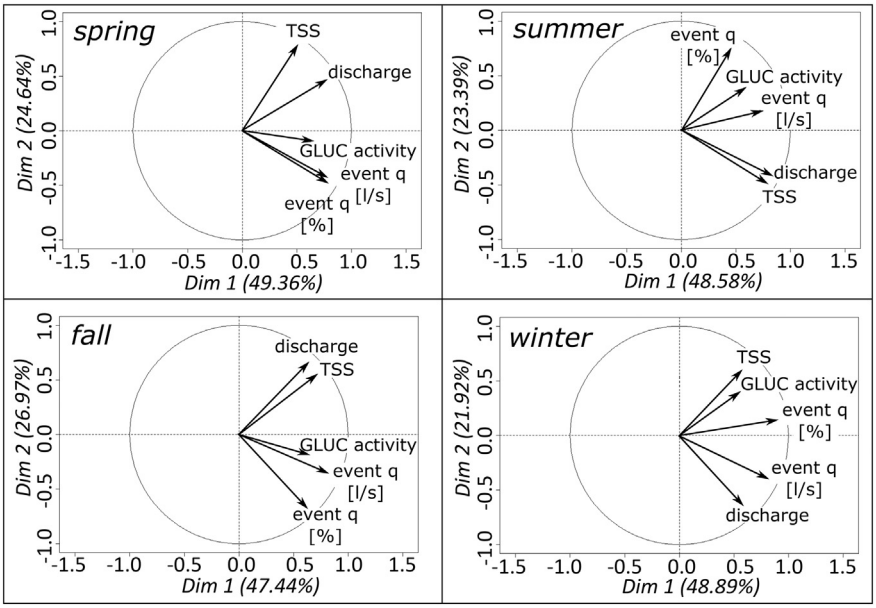

Fig. 7. Principal Component Analysis of the variables GLUC, discharge, TSS event water volume (event q, l/s) and event water contribution (in l/s, % of discharge) in the seasons of the study period. GLUC and event water contribution (%) in the stream plot in the same sector in all seasons, indicating similar drivers.

active organisms mobilized from the aquifer during events. Above this baseline relationship, two additional clusters were observed: (a) Data points with the highest GLUC values in streamflow containing event water predominantly occurred during the ascending event phase. Because of the high event water fraction, these data points were interpreted as signals of surface-associated GLUC input due to recent rain water (Fig. 8) that reached the stream via overland flow or preferential flow paths.

(b) Increased GLUC values in the stream water without event water contribution occurred at discharges below 10 l/s and predominantly during the ascending event phases. Because of the absence of recent rain water in stream flow, no surface-associated input was assumed; these data points were interpreted as signals of resuspended matter in the stream causing increased GLUC (Fig. 8). Flushing experiments conducted in the same stream by Eder et al., 2014, showed higher

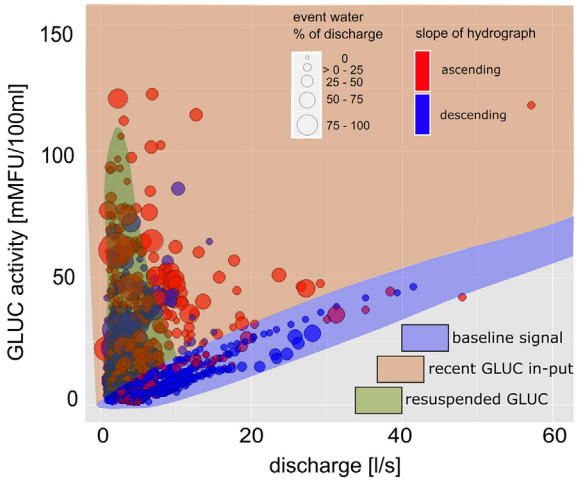

Fig. 8. Interpretation of the clustering within the GLUC-discharge relationship: Baseline (blue zone) is interpreted as the signal of a remnant population of GLUC active organisms within the catchment. Recent input of GLUC due to event water (red zone) and GLUC sourced from resuspended matter (green zone) are plotted above the baseline. Clusters of recent input and resuspended GLUC overlap but are diminished by different event water contributions (size of points). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

resuspended sediment loads during the first flush compared with subsequent induced floods. We believe that the same mechanisms relevant for the streambed release of *E. coli* described by Kim et al., 2010, and Muirhead et al., 2004, effected an increase of hydraulic shear stress, remobilizing and "washing-out" GLUC active organisms from the hyporheic zone at the beginning of events (Figs. 3, 8).

The majority (61%) of the GLUC load was transported during non-event conditions (Table 2) supporting the interpretation of remnant populations of GLUC active organisms in the aquifer or the hyporheic zone. Overall, 44% of the GLUC loads transported during events were measured when no recent precipitation water contributed to stream flow, suggesting that these GLUC loads stemmed from remobilized matter, most likely from the stream bed.

The linear correlation analysis (Figs. 6, 7) showed that GLUC concentrations in the stream water were mainly correlated with TSS and event water fraction; these correlations were not particularly strong and changed seasonally. However, in the PCA analysis, the GLUC concentration was consistently close to the event water fraction (Fig. 7) during all seasons, indicating similar drivers of the two variables. We interpret these drivers to be induced by precipitation events because event water fraction in stream flow and GLUC dynamics are controlled by hydrologic catchment conditions (e.g., soil moisture and groundwater level), precipitation depth and intensity.

The comparison of GLUC measurements with *E. coli* analysis from grab samples extracted during the whole test period ($n = 54$, both event conditions and low flow) showed an R^2 of 0.52. An $R^2 > 0.95$ between these two variables would be required to consider GLUC as a quantitative proxy parameter for E. *coli* (Stadler et al., 2016). However, exclusively during event run-off, the correlation tended to be significantly higher with an R^2 of 0.80. Moreover, GLUC showed the strongest correlation with *E. coli* compared with the other evaluated stream parameters. This was interpreted in terms of indicator applicability of GLUC for fecal indicators. The data shown in Table 3 indicated that *E. coli*, compared with GLUC, had a higher correlation (R^2) with physical stream parameters, such as discharge and TSS. This was interpreted as a likely result of the association of *E. coli* with particles and the respective transport mechanisms. GLUC measurements were sensitive to all enzymatic activity, including extracellular enzymes produced by GLUC active organisms. This supported the assumption of remnant populations of GLUC active organisms within the catchment and may partly

Table 3
Linear correlation (R^2) between GLUC (mMFU/100 ml), *E. coli* (MPN/100 ml) and physical in-stream parameters. Asterisks show the significance level (***: p-value ≤ 0.001, **: p-value ≤ 0.05, for $R^2 > 0.1$), n = number of measurements).

	GLUC [mMFU/100 ml]	*E. coli* [MPN/100 ml]	Discharge [l/s]	Sediment concentration (TSS) [mg/l]
E. coli [MPN/100 ml]	0.52*** n = *54*			
Discharge [l/s]	0.22*** n = *3792*	0.63*** n = *54*		
Sediment concentration (TSS) [mg/l]	0.24*** n = *3558*	0.51*** n = *53*	0.47*** n = *6571*	
Water temperature [°C]	0.11*** n = *3792*	0.14*** n = *54*	0.01 n = *6917*	0.00 n = *6571*

explain the fairly poor correlations between GLUC and *E. coli* during low flow conditions.

5. Conclusions

The combination of isotope-hydrology with on-site and rapid biochemical monitoring provided insight into the seasonality of event transport of beta-D-glucuronidase. This study estimated the contribution of resuspended GLUC active organisms in an agricultural headwater stream for a period of two years. The fraction of resuspended GLUC loads was shown to change between seasons. Increased GLUC values during event run-off occurred despite the absence of recent precipitation water in stream flow, suggesting that a significant part of the GLUC load in the study period originated from remobilized matter. The majority of the GLUC load was transported during nonevent conditions, and a linear relationship between discharge and GLUC during the descending phases of events was observed during all seasons. This strongly supports the presence of remnant populations of GLUC-active organisms in used small head water catchments.

The combination of isotope hydrology and novel microbial methods has the potential for assessing microbial fluxes at large scales. This applied approach is not restricted to enzymatic assays. Other emerging microbial assays, such as on-site flow cytometry, could also be used in similar studies. Process studies, such as this one, contribute toward microbial transport and risk assessment. Molecular research, such as genetic community analyses of stream water or in the catchment (Savio et al., 2018, 2015), may complement the findings of this work.

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References

Berden, G., Peeters, R., Meijer, G., 2000. Cavity ring-down spectroscopy: experimental schemes and applications. Int. Rev. Phys. Chem. 19, 565–607. https://doi.org/10.1080/014423500750040627.

Besmer, M.D., Weissbrodt, D.G., Kratochvil, B.E., Sigrist, J.A., Weyland, M.S., Hammes, F., 2014. The feasibility of automated online flow cytometry for in-situ monitoring of microbial dynamics in aquatic ecosystems. Front. Microbiol. 5. https://doi.org/10.3389/fmicb.2014.00265.

Besmer, M.D., Epting, J., Page, R.M., Sigrist, J.A., Huggenberger, P., Hammes, F., 2016. Online flow cytometry reveals microbial dynamics influenced by concurrent natural and operational events in groundwater used for drinking water treatment. Sci. Rep. 6, 38462. https://doi.org/10.1038/srep38462.

Blöschl, G., Carr, G., Bucher, C., Farnleitner, A.H., Rechberger, H., Wagner, W., Zessner, M., 2011. Promoting interdisciplinary education – the Vienna Doctoral Programme on Water Resource Systems. Hydrol. Earth Syst. Sci. Discuss. 8, 9843–9887. https://doi.org/10.5194/hessd-8-9843-2011.

Blöschl, G., Blaschke, A.P., Broer, M., Bucher, C., Carr, G., Chen, X., Eder, A., Exner-Kittridge, M., Farnleitner, A., Flores-Orozco, A., Haas, P., Hogan, P., Kazemi Amiri, A., Oismüller, M., Parajka, J., Silasari, R., Stadler, P., Strauß, P., Vreugdenhil, M., Wagner, W., Zessner, M., 2015. The Hydrological Open Air Laboratory (HOAL) in Petzenkirchen: a hypotheses driven observatory. Hydrol. Earth Syst. Sci. Discuss. 12, 6683–6753. https://doi.org/10.5194/hessd-12-6683-2015.

Cabral, J.P.S., 2010. Water microbiology. bacterial pathogens and water. Int. J. Environ. Res. Public Health 7, 3657–3703. https://doi.org/10.3390/ijerph7103657.

Cho, K.H., Pachepsky, Y.A., Kim, J.H., Guber, A.K., Shelton, D.R., Rowland, R., 2010. Release of Escherichia coli from the bottom sediment in a first-order creek: experiment and reach-specific modeling. J. Hydrol. 391, 322–332. https://doi.org/10.1016/j.jhydrol.2010.07.033.

Clark, I.D., Fritz, P., 1997. Environmental Isotopes in Hydrogeology. Crc Pr Inc.

Deshmukh, R.A., Joshi, K., Bhand, S., Roy, U., 2016. Recent developments in detection and enumeration of waterborne bacteria: a retrospective minireview. Microbiol. Open 5, 901–922. https://doi.org/10.1002/mbo3.383.

Eder, A., Strauss, P., Krueger, T., Quinton, J.N., 2010. Comparative calculation of suspended sediment loads with respect to hysteresis effects (in the Petzenkirchen catchment, Austria). J. Hydrol. 389, 168–176.

Eder, A., Exner-Kittridge, M., Strauss, P., Blöschl, G., 2014. Re-suspension of bed sediment in a small stream—results from two flushing experiments. Hydrol. Earth Syst. Sci. 18, 1043–1052. https://doi.org/10.5194/hess-18-1043-2014.

Ender, A., Goeppert, N., Grimmeisen, F., Goldscheider, N., 2017. Evaluation of β-D-glucuronidase and particle-size distribution for microbiological water quality monitoring in Northern Vietnam. Sci. Total Environ. 580, 996–1006. https://doi.org/10.1016/j.scitotenv.2016.12.054.

Enzymatic Assay of β-Glucuronidase (EC 3.2.1.31) from E. coli [WWW Document], n. d. Sigma-Aldrich. URL http://www.sigmaaldrich.com/technical-documents/protocols/biology/enzymatic-assay-of-b-glucuronidase-from-ecoli.html (accessed 5.12.16).

Exner-Kittridge, M., Salinas, J.L., Zessner, M., 2013. An evaluation of analytical streambank flux methods and connections to end-member mixing models: a comparison of a new method and traditional methods. Hydrol. Earth Syst. Sci. Discuss. 10, 10419–10459. https://doi.org/10.5194/hessd-10-10419-2013.

Farnleitner, A.h., Hocke, L., Beiwl, C., Kavka, G.c., Zechmeister, T., Kirschner, A.k.t., Mach, R. l., 2001. Rapid enzymatic detection of *Escherichia coli* contamination in polluted river water. Lett. Appl. Microbiol. 33, 246–250. https://doi.org/10.1046/j.1472-765x.2001.00990.x.

Farnleitner, A.H., Hocke, L., Beiwl, C., Kavka, G.G., Mach, R.L., 2002. Hydrolysis of 4-methylumbelliferyl-β-D-glucuronide in differing sample fractions of river waters and its implication for the detection of fecal pollution. Water Res. 36, 975–981. https://doi.org/10.1016/S0043-1354(01)00288-3.

Farnleitner, A.H., Ryzinska-Paier, G., Reischer, G.H., Burtscher, M.M., Knetsch, S., Kirschner, A.K.T., Dirnböck, T., Kuschnig, G., Mach, R.L., Sommer, R., 2010. *Escherichia coli* and *enterococci* are sensitive and reliable indicators for human, livestock and wildlife faecal pollution in alpine mountainous water resources. J. Appl. Microbiol. https://doi.org/10.1111/j.1365-2672.2010.04788.x (no-no).

Fiksdal, L., Pommepuy, M., Caprais, M.P., Midttun, I., 1994. Monitoring of fecal pollution in coastal waters by use of rapid enzymatic techniques. Appl. Environ. Microbiol. 60, 1581–1584.

Fishman, W.H., Bergmeyer, H.U., 1974. B-glucuronidase. Methods Enzym. Anal. 2, 929.

Foppen, J.W.A., Schijven, J.F., 2006. Evaluation of data from the literature on the transport and survival of *Escherichia coli* and thermotolerant coliforms in aquifers under saturated conditions. Water Res. 40, 401–426. https://doi.org/10.1016/j.watres.2005.11.018.

Garzio-Hadzick, A., Shelton, D.R., Hill, R.L., Pachepsky, Y.A., Guber, A.K., Rowland, R., 2010. Survival of manure-borne *E. coli* in streambed sediment: effects of temperature and sediment properties. Water Res. 44, 2753–2762. https://doi.org/10.1016/j.watres.2010.02.011.

Geary, J.R., 2009. In-situ Optical Sensing for the Detection and Quantification of Pathogen Indicator Organisms. University of Notre Dame.

George, I., Petit, M., Servais, P., 2000. Use of enzymatic methods for rapid enumeration of coliforms in freshwaters. J. Appl. Microbiol. 88, 404–413. https://doi.org/10.1046/j.1365-2672.2000.00977.x.

George, I., Crop, P., Servais, P., 2001. Use of beta-D-galactosidase and beta-D-glucuronidase activities for quantitative detection of total and fecal coliforms in wastewater. Can. J. Microbiol. 47, 670–675.

Goller, R., Wilcke, W., Leng, M.J., Tobschall, H.J., Wagner, K., Valarezo, C., Zech, W., 2005. Tracing water paths through small catchments under a tropical montane rain forest in South Ecuador by an oxygen isotope approach. J. Hydrol. 308, 67–80.

Gupta, P., Noone, D., Galewsky, J., Sweeney, C., Vaughn, B.H., 2009. Demonstration of high-precision continuous measurements of water vapor isotopologues in laboratory and remote field deployments using wavelength-scanned cavity ring-down spectroscopy (WS-CRDS) technology. Rapid Commun. Mass Spectrom. 23, 2534–2542. https://doi.org/10.1002/rcm.4100.

Harvey, R.W., Kinner Nancy, E., Dan, MacDonald, Metge David, W., Amoret, Bunn, 2010. Role of physical heterogeneity in the interpretation of small-scale laboratory and field observations of bacteria, microbial-sized microsphere, and bromide transport through aquifer sediments. Water Resour. Res. 29, 2713–2721. https://doi.org/10.1029/93WR00963.

Højris, B., Christensen, S.C.B., Albrechtsen, H.-J., Smith, C., Dahlqvist, M., 2016. A novel, optical, on-line bacteria sensor for monitoring drinking water quality. Sci. Rep. 6, 23935. https://doi.org/10.1038/srep23935.

Huth, A.K., Leydecker, A., Sickman, J.O., Bales, R.C., 2004. A two-component hydrograph separation for three high-elevation catchments in the Sierra Nevada, California. Hydrol. Process. 18, 1721–1733.

Jamieson, R.C., Joy, D.M., Lee, H., Kostaschuk, R., Gordon, R.J., 2004. Persistence of enteric bacteria in alluvial streams. J. Environ. Eng. Sci. 3, 203–212. https://doi.org/10.1139/s04-001.

Jamieson, R.C., Joy, D.M., Lee, H., Kostaschuk, R., Gordon, R.J., 2005. Resuspension of sediment-associated Escherichia coli in a natural stream. J. Environ. Qual. 34, 581–589. https://doi.org/10.2134/jeq2005.0581.

Ji, J., Schanzle, J.A., Tabacco, M.B., 2004. Real-time detection of bacterial contamination in dynamic aqueous environments using optical sensors. Anal. Chem. 76, 1411–1418. https://doi.org/10.1021/ac034914q.

Kay, D., Edwards, A.C., Ferrier, R.C., Francis, C., Kay, C., Rushby, L., Watkins, J., McDonald, A.T., Wyer, M., Crowther, J., 2007. Catchment microbial dynamics: the emergence of a research agenda. Prog. Phys. Geogr. 31, 59–76.

Kim, J.-W., Pachepsky, Y.A., Shelton, D.R., Coppock, C., 2010. Effect of streambed bacteria release on *E. coli* concentrations: monitoring and modeling with the modified SWAT. Ecol. Model. 221, 1592–1604. https://doi.org/10.1016/j.ecolmodel.2010.03.005.

Koschelnik, J., Vogl, W., Epp, M., Lackner, M., 2015. Rapid Analysis of β-D-Glucuronidase Activity in Water Using Fully Automated Technology. , pp. 471–481 https://doi.org/10.2495/WRM150401.

Mook, W., Rozanski, K., 2000. Environmental Isotopes in the Hydrological Cycle. IAEA Publ.

Morikawa, A., Hirashiki, I., Furukawa, S., 2006. Development of a coliforms monitoring system using an enzymatic fluorescence method. Water Sci. Technol. J. Int. Assoc. Water Pollut. Res. 53, 523–532.

Muirhead, R.W., Davies-Colley, R.J., Donnison, A.M., Nagels, J.W., 2004. Faecal bacteria yields in artificial flood events: quantifying in-stream stores. Water Res. 38, 1215–1224. https://doi.org/10.1016/j.watres.2003.12.010.

Nutrients in the Nation's Waters: Identifying Problems and Progress, USGS Fact Sheet FS218-96 [WWW Document], n.d. URL https://pubs.usgs.gov/fs/fs218-96/ (accessed 8.22.17).

Pachepsky, Y.A., Sadeghi, A.M., Bradford, S.A., Shelton, D.R., Guber, A.K., Dao, T., 2006. Transport and fate of manure-borne pathogens: modeling perspective. Agric. Water Manag. 86, 81–92. https://doi.org/10.1016/j.agwat.2006.06.010.

Pandey, P.K., Soupir, M.L., Rehmann, C.R., 2012. A model for predicting resuspension of *Escherichia coli* from streambed sediments. Water Res. 46, 115–126. https://doi.org/10.1016/j.watres.2011.10.019.

Reischer, G.H., Kollanur, D., Vierheilig, J., Wehrspaun, C., Mach, R.L., Sommer, R., Stadler, H., Farnleitner, A.H., 2011. Hypothesis-driven approach for the identification of fecal pollution sources in water resources. Environ. Sci. Technol. 45, 4038–4045. https://doi.org/10.1021/es103659s.

Savio, D., Sinclair, L., Ijaz, U.Z., Parajka, J., Reischer, G.H., Stadler, P., Blaschke, A.P., Blöschl, G., Mach, R.L., Kirschner, A.K.T., Farnleitner, A.H., Eiler, A., 2015. Bacterial diversity along a 2600 km river continuum. Environ. Microbiol. https://doi.org/10.1111/1462-2920.12886 n/a-n/a.

Savio, D., Philipp, Stadler, Reischer, Georg H., Kirschner Alexander, K.T., Katalin, Demeter, Rita, Linke, Blaschke, Alfred P., Regina, Sommer, Ulrich, Szewzyk, Wilhartitz, Inés C., Mach, Robert L., Hermann, Stadler, Farnleitner, Andreas H., 2018. Opening the black box of spring water microbiology from alpine karst aquifers to support proactive drinking water resource management. Wiley Interdiscip. Rev. Water 0, e1282. https://doi.org/10.1002/wat2.1282.

Scholl, M.A., Harvey, R.W., 1992. Laboratory investigations on the role of sediment surface and groundwater chemistry in transport of bacteria through a contaminated sandy aquifer. Environ. Sci. Technol. 26, 1410–1417. https://doi.org/10.1021/es00031a020.

Stadler, H., Skritek, P., Sommer, R., Mach, R.L., Zerobin, W., Farnleitner, A.H., 2008. Microbiological monitoring and automated event sampling at karst springs using LEO-satellites. Water Sci. Technol. J. Int. Assoc. Water Pollut. Res. 58, 899.

Stadler, P., Blöschl, G., Vogl, W., Koschelnik, J., Epp, M., Lackner, M., Oismüller, M., Kumpan, M., Nemeth, L., Strauss, P., Sommer, R., Ryzinska-Paier, G., Farnleitner, A.H., Zessner, M., 2016. Real-time monitoring of beta-D-glucuronidase activity in sediment laden streams: a comparison of prototypes. Water Res. 101, 252–261. https://doi.org/10.1016/j.watres.2016.05.072.

Stadler, P., Farnleitner, A.H., Zessner, M., 2017. Development and evaluation of a self-cleaning custom-built auto sampler controlled by a low-cost RaspberryPi microcomputer for online enzymatic activity measurements. Talanta 162, 390–397. https://doi.org/10.1016/j.talanta.2016.10.031.

Stevenson, M.E., Sommer, R., Lindner, G., Farnleitner, A.H., Toze, S., Kirschner, A.K.T., Blaschke, A.P., Sidhu, J.P.S., 2015. Attachment and detachment behavior of human adenovirus and surrogates in fine granular limestone aquifer material. J. Environ. Qual. 44, 1392–1401. https://doi.org/10.2134/jeq2015.01.0052.

Vang, Ó.K., Corfitzen, C.B., Smith, C., Albrechtsen, H.-J., 2014. Evaluation of ATP measurements to detect microbial ingress by wastewater and surface water in drinking water. Water Res. 64, 309–320. https://doi.org/10.1016/j.watres.2014.07.015.

Wang, D., Bradford, S.A., Harvey, R.W., Gao, B., Cang, L., Zhou, D., 2012. Humic acid facilitates the transport of ARS-labeled hydroxyapatite nanoparticles in iron oxyhydroxide-coated sand. Environ. Sci. Technol. 46, 2738–2745. https://doi.org/10.1021/es203784u.

WHO|Engaging with the water sector for public health benefits: waterborne pathogens and diseases in developed countries [WWW Document], n.d. WHO. URL http://www.who.int/bulletin/volumes/88/11/09-072512/en/ (accessed 10.13.17).

WHO|Water quality assessments: a guide to the use of biota, sediments and water in environmental monitoring, 2nd ed. [WWW Document], n.d. WHO. URL http://www.who.int/water_sanitation_health/resourcesquality/wqa/en/ (accessed 5.27.15).

Wilkinson, J., Jenkins, A., Wyer, M., Kay, D., 1995. Modelling faecal coliform dynamics in streams and rivers. Water Res. 29, 847–855. https://doi.org/10.1016/0043-1354(94)00211-O.