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Comer-Warner, S, Knapp, JLA, Blaen, P et al. (19 more authors) (2020) The method controls the story - Sampling method impacts on the detection of pore-water nitrogen concentrations in streambeds. Science of The Total Environment, 709. 136075. ISSN 0048-9697

https://doi.org/10.1016/j.scitotenv.2019.136075

The method controls the story - sampling method impacts on the detection of pore-water nitrogen concentrations in streambeds

Author list

Sophie Comer-Warner, School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

Julia Knapp, Center for Applied Geoscience, University of Tübingen, Tübingen, Germany, now Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland

Phillip Blaen, School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

Megan Klaar, School of Geography and Water, University of Leeds, Leeds, U.K.

Felicity Shelley, School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London E1 4NS, UK

Jay Zarnetske, Department of Earth and Environmental Sciences, Michigan State University, East Lansing, MI, USA

Joseph Lee-Cullin, Department of Earth and Environmental Sciences, Michigan State University, East Lansing, MI, USA

Silvia Folegot, School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K., now Faculty of Science and Technology, Free University of Bozen-Bolzano, Universitätsplatz 5 - piazza Università, 5 39100, Bozen-Bolzano

21 Marie Kurz, Department of Hydrogeology, Helmholtz Centre for Environmental Research-
22 UFZ, Leipzig, Germany, Patrick Center for Environmental Research, The Academy of
23 Natural Sciences of Drexel University, Philadelphia, Pennsylvania, USA

24 Jorg Lewandowski, Leibniz-Institute of Freshwater Ecology and Inland Fisheries,
25 Department of Ecohydrology, Müggelseedamm 310, D-12587 Berlin, Germany

26 Judson Harvey, National Research Program, U.S. Geological Survey, Reston, Virginia, USA

27 Adam Ward, School of Public and Environmental Affairs, Indiana University, Bloomington,
28 Indiana

29 Clara Mendoza-Lera, IRSTEA, UR MALY, Centre de Lyon, 5 rue de la Doua BP 32108,
30 69616 Villeurbanne Cedex, France, now Department of Freshwater Conservation
31 Brandenburg University of Technology BTU Cottbus–Senftenberg Bad Saarow Germany

32 Sami Ullah, School of Geography, Earth and Environmental Sciences, University of
33 Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

34 Thibault Datry, IRSTEA, UR MALY, Centre de Lyon, 5 rue de la Doua BP 32108, 69616
35 Villeurbanne Cedex, France

36 Nicholas Kettridge, School of Geography, Earth and Environmental Sciences, University of
37 Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

38 Daren Gooddy, British Geological Survey, Maclean Building, Wallingford, Oxfordshire,
39 OX10 8BB, UK

40 Jennifer Drummond, Integrative Freshwater Ecology Group, Center for Advanced Studies of
41 Blanes (CEAB-CSIC), Blanes, Girona, Spain, now School of Geography, Earth and
42 Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

Eugènia Martí, Integrative Freshwater Ecology Group, Center for Advanced Studies of Blanes (CEAB-CSIC), Blanes, Girona, Spain

Alexander Milner, School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

David Hannah, School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

Stefan Krause, School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

Abstract

Biogeochemical gradients in streambed environments are steep and can vary over short distances often making adequate characterisation of sediment biogeochemical processes challenging. This paper provides an overview and comparison of different streambed pore-water sampling methods, highlighting their capacity to address gaps in our understanding of streambed biogeochemical processes. This work, therefore, reviews and critiques available techniques for pore-water sampling to characterise streambed biogeochemical conditions, including their respective characteristic spatial and temporal resolutions, and associated advantages and limitations. A field study comparing three commonly-used pore-water sampling techniques (multilevel mini-piezometers, diffusive equilibrium in thin-film gels and miniature drivepoint samplers) was conducted to assess differences in observed nitrate and ammonium concentration profiles. Pore-water nitrate concentrations did not differ significantly between the respective sampling methods (p-value = 0.54, Kruskal-Wallis rank sum test, Table 2) with mean concentrations of 2.53, 4.08 and 4.02 mg l^{-1} observed with the multilevel mini-piezometers, miniature drivepoint samplers and diffusive equilibrium in thin-film gel samplers, respectively. Pore-water ammonium concentrations, however, were

significantly higher in pore-water extracted by multilevel mini-piezometers (3.83 mg l^{-1}) and significantly lower where sampled with miniature drivepoint samplers (1.05 mg l^{-1}, p-values < 0.01, Kruskal-Wallis rank sum test followed by Dunn Tests, Table 2). Differences in observed pore-water ammonium concentration profiles between active (suction: multilevel mini-piezometers) and passive (equilibrium; diffusive equilibrium in thin-film gels) samplers were further explored under laboratory conditions. Results showed that measured pore-water ammonium concentrations were significantly greater when sampled by diffusive equilibrium in thin-film gels than with multilevel mini-piezometers (all p-values ≤ 0.02, Wilcoxon signed rank or paired t-test, Table 4).

The findings of this study have critical implications for the interpretation of field-based research on hyporheic zone biogeochemical cycling and highlights the need for more systematic testing of sampling protocols. For the first time, the impact of different active and passive pore-water sampling methods is addressed systematically here, highlighting to what degree the choice of pore-water sampling methods affects research outcomes, with relevance for the interpretation of previously published work as well as future studies.

1. Introduction

Ecohydrological and biogeochemical processes in streambed environments have recently received increasing attention by the hyporheic research community, regulators, policy makers, restoration organisations and utility companies (Boano et al., 2014; Harvey & Gooseff, 2015; Krause et al., 2011a; Krause et al., 2014). This is due in part to the observation of 'hotspots' and 'hot moments' of biogeochemical reactivity in the hyporheic zone (HZ), where surface water and groundwater mix (Krause et al., 2011a; Krause et al., 2017; Lautz & Fanelli, 2008; McClain et al., 2003; Ward, 2016). 'Hotspots' are zones of increased biogeochemical reactivity whereas 'hot moments' are temporal periods of increased

biogeochemical reactivity (McClain et al., 2003). These functions arise because hyporheic zones are characterised by high rates of microbial activity, enhanced nutrient cycling and steep redox gradients relative to surface water, leading to descriptions of HZ's and riparian corridors as the "river's livers" (Boulton et al., 1998; Brunke & Gonser, 1997; Fischer et al., 2005; Harvey et al., 2013; Harvey & Gooseff, 2015; Pinay et al., 2018).

The investigation of streambed biogeochemical processes relies upon the extraction and analysis of interstitial pore-waters, often over multiple depths and horizontal patterns and over varying timescales. However, despite the growing volume of interdisciplinary research in the HZ, there remains a lack of systematic protocols for sampling methodologies to facilitate transferability between studies (Krause et al., 2011a; Ward, 2016). Sampling, as well as data interpretation, therefore, can be challenging (Kalbus et al., 2006; Rivett et al., 2008). Current sampling techniques have had varying success with capturing nutrient conditions adequately across the respectively relevant spatial and temporal scales (Boano et al., 2014; Krause et al., 2011a), ranging from short-term (minutes to hours) and small-scale (mm-m) to intermediate-term (up to several years) and medium-scale (up to several km). As a result, selecting a pore-water sampling methodology remains non-standard and likely relies on the experience of the practitioner rather than systematic selection that is well-matched to study objectives.

Several pore-water sampling methodologies have been developed over the last couple of decades to best address application-specific challenges in identifying spatial patterns and temporal dynamics of streambed biogeochemical processes. In consequence, we now have at our disposal a wide range of different pore-water sampling tools and methodologies, with variations of how these methods are deployed and applied in practice. Depending on the application, the chosen methods may be based on permanent (e.g. piezometers) (Lee & Cherry, 1979; Rivett et al., 2008) or temporary (e.g. United States Geological Survey (USGS)

Minipoint samplers, Minipoints from here onwards) (Duff et al., 1998; Harvey & Fuller, 1998) installations (Figure 1). Although some samplers can extend several metres in depth the majority of sampling techniques developed for extracting pore-water samples for biogeochemical analysis predominantly focus on the upper metre of the streambed, often targeting the top 0.2 m at a higher spatial resolution (Berg & McGlathery, 2000; Duff et al., 1998; Harvey & Fuller, 1998; Krom et al., 1994; Rivett et al., 2008; Sanders & Trimmer, 2006), with the vertical scale achievable depending heavily on the technique used, and the volume and rate of pore-water extraction. There are various technical differences between the most commonly used pore-water sampling methods, with respect to their spatial and temporal resolution, sampling volume and rates (few millilitres to several litres) (Bou & Rouch, 1967; Conant et al., 2004; Duff et al., 1998; Hunt & Stanley, 2000; Kalbus et al., 2006; Krause et al., 2013; Palmer et al., 2006; Rivett et al., 2008), maximum sampling depths (mm's to 2 m) and sampling intervals (Bou & Rouch, 1967; Duff et al., 1998; Hunt & Stanley, 2000; Krause et al., 2011a; Krom et al., 1994; Metzger et al., 2016; Palmer et al., 2006; Rivett et al., 2008; Sanders & Trimmer, 2006).

Each sampling technique may be better suited for different sampling conditions. The ease of installation of samplers in soft, sandy or silty sediments results in these streambeds being the easiest to sample (Dahm et al., 2007). Although gravel and clay sediments provide challenges to sampler installation both single-depth and multilevel mini-piezometers can be deployed after hammering or pre-drilling (Baxter et al., 2003; Geist et al., 1998; Grimm et al., 2007). Miniature drivepoint samplers are less suitable for gravel, cobble and clay-rich sediments but have been successfully deployed in coarser sediments (Harvey et al., 2013; Ruhala et al., 2018), and although DET gels are less suitable gravel sediments a device for their use in armoured streambeds has been developed (Ullah et al., 2012). If river flow is too high then the use of DET gels may not be appropriate and single-depth piezometers made of

rigid pipes may become dislodged during storms (Rivett et al., 2008). The temporary nature of miniature drivepoint sampler installation may also limit their use as they may be easily disturbed.

Pore-water sampling methods may be active, requiring pore-water samples to be withdrawn through actively applying pressure by suction via a syringe or pumping (e.g. piezometers), or passive through diffusion where solutes are sampled without actual pore-water extraction but rather through the transfer of solutes into the respective sampler (e.g. Diffusive Equilibrium in Thin-film (DET) gels), which may influence the sampling outcomes.

Streambed sediments contain pores of varying sizes and connectivity, resulting in different pore-water residence times, redox conditions and nutrient concentrations (Briggs et al., 2014, 2015; Harvey, 1993; Harvey et al., 1995). Active samplers tend to preferentially sample from macropores as the zone of sediment sampled ranges from the largest pores to those of the size related to the applied pressure (Harvey & Gorelick, 1995; Harvey, 1993; Harvey et al., 1995). In contrast, passive samplers preferentially sample micropores or matrix pores (Harvey, 1993; Harvey et al., 1995) as they do not rely on extraction of mobile pore-waters. The mechanical difference between active and passive sampling may have a large effect on nutrient concentrations in the obtained samples. Additionally, the sampling duration can vary between sampling methodologies, with active samplers typically representing a snapshot in time, whereas passive equilibrium samplers represent an integration over the time of diffusive equilibrium (Berg & McGlathery, 2000; William Davison et al., 1994; González-pinzón et al., 2015). If slow pumping is used with an active sampler, however, this can result in an integrated signal over a similar time period to passive techniques. There are, therefore, substantial differences between sampling techniques. How these differences affect resulting nutrient concentrations remains insufficiently understood.

166 Here this work aims to ascertain whether there are differences in the results obtained
167 between different pore-water sampling methodologies to enable researchers to easily select
168 the most appropriate technique and to enable cross-study comparisons of biogeochemical
169 processes in streambed environments. There are three main objectives to meeting this aim: 1)
170 To provide technical information on pore-water sampling techniques to aid in sampler
171 selection, 2) to investigate the differences in pore-water nutrient profiles and subsequent
172 streambed characterisation obtained from three common pore-water sampling methodologies
173 and 3) to investigate differences in porewater ammonium profiles from the use of active
174 versus passive samplers.

175 A literature review of the most common pore-water sampling techniques, discussing
176 their specific advantages and limitations for specific applications is presented. Subsequently,
177 the outcomes of a selection of common pore-water sampling methodologies were compared
178 in a comparative *in-situ* field study, assessing the ability of multilevel mini-piezometers and
179 Minipoints (as examples of active samplers), and DET gel probes (as examples of passive
180 samplers) (Byrne et al., 2015; Krause et al., 2011a; Ullah et al., 2012) to capture nutrient
181 patterns in streambed pore-waters across a stream reach at varying spatial resolutions. These
182 methods all allow pore-water nutrient concentrations to be determined at multiple depths
183 within the streambed and cover a variety of spatial resolutions and both active and passive
184 sampling. The more common multilevel mini-piezometer setup, with a coarser resolution and
185 a greater depth range than Minipoints and DET gels, was used here to compare techniques as
186 they are widely applied in field-based research. Data was, therefore, compared within the top
187 0.15 m of the streambed, where the sampling zones of all three techniques overlap. A
188 laboratory control experiment comparing NH_4^+ pore-water concentrations gained from
189 multilevel mini-piezometers and DET gels was conducted to determine whether differences
190 observed in the *in-situ* study were due to sampler differences or field-specific conditions.

2. Literature review: Comparison of sampling techniques

Various literature reviews have previously provided comparative analyses of the performance of experimental methods for streambed characterisation, however, these have either predominantly focussed on methodologies to determine hydrological properties of streambeds or on only active or passive sampling (e.g. Davison et al., 2000; González-Pinzón et al., 2015; Kalbus et al., 2006; Landon et al., 2001; Scanlon et al., 2002). This study focusses on the comparison of streambed sampling methodologies developed to analyse vertical profiles of nutrients, which enable ecohydrological investigations across surface water-groundwater interfaces. A summary of the following literature review can be found in Table 1.

2.1 Active Samplers

2.1.1 Single-depth piezometers and mini-piezometers

Single-depth piezometers are used to sample pore-water at depths of up to several metres and are typically constructed from a steel, polyvinyl chloride (PVC) or high-density polyethylene (HDPE) pipe, which is screened at the bottom end over the desired vertical range; the bottom of the pipe is then blocked (Figure 1) (Argerich et al., 2011; Baxter et al., 2003; Conant et al., 2004; Dahm et al., 2007; Geist et al., 1998; Grimm et al., 2007; Lee & Cherry, 1979; Lewandowski et al., 2015; Rivett et al., 2008). A screened section varying between tens and hundreds of millimetres is utilised depending on whether depth-specific or depth-integrated sampling is required (Baxter et al., 2003; Dahm et al., 2007; Geist et al., 1998; Winter et al., 1998). An alternative design, using porous (20 μm mean pore diameter) HDPE pipe, which does not require a screened section has also been used (Wondzell & Swanson, 1996). Whilst piezometers sample water at a single depth, multiple piezometers may be nested to allow sampling at multiple depths, covering a larger horizontal instrument footprint, which are

typically sampled consecutively (Battin et al., 2003a; Baxter et al., 2003; Käser et al., 2009; Krause et al., 2009). The instrument footprint of a single piezometer is typically 10-50 mm in diameter (Argerich et al., 2011; Baxter et al., 2003; Blume et al., 2013; Conant et al., 2004; Dahm et al., 2007; Geist et al., 1998; Krause et al., 2009; Rivett et al., 2008; Valett et al., 1994; Wondzell & Swanson, 1996), which can result in a relatively large instrument footprint when a nested design is utilised. Piezometers are deployed in the streambed usually for longer time scales of several weeks to years (Argerich et al., 2011; Dahm et al., 2007; Lee & Cherry, 1979), and the extracted pore-water sample represents a snapshot of the conditions at the time of sampling (González-Pinzón et al., 2015). Prior to sampling, piezometers have to be purged of water by pumping until dry or until multiple times the water volume has been removed if complete purging is not feasible (Johnson et al., 2004; Krause et al., 2009; Lapworth et al., 2009). Pore-water is sampled from the piezometer with a pump or syringe once it has refilled, hence, the pore-water is not extracted through suction from the sediment, but through ambient pore-water flow into the piezometer (Dahm et al., 2007), and is, therefore, affected by the hydrological conditions of the stream i.e. gaining or losing and surface water level.

Advantages:

Information on exchange fluxes between stream and subsurface, and properties such as hydraulic gradients and hydraulic conductivity can be obtained in the piezometer at the depth of sampling (Argerich et al., 2011; Baxter et al., 2003; Dahm et al., 2007; Datry et al., 2015; González-pinzón et al., 2015; Grimm et al., 2007; Kalbus et al., 2006; Lee & Cherry, 1979; Valett et al., 1994), allowing hydrological and chemical information to be gained at the same location and through the same sampling device. The wide diameter of the piezometer also enables permanent installation of loggers to measure a variety of parameters including temperature, electrical conductivity, turbidity and pressure. The design, with water flowing into

240 the piezometer (Dahm et al., 2007), allows larger volumes of water to be extracted than is
241 attainable with other sampling methods. Furthermore, piezometer installation is
242 straightforward in sandy and silt sediments, and if a wider spatially-integrated signal is required
243 the relatively large sampling footprint may be advantageous.

244 *Limitations:*

245 Single-depth piezometers must be installed with sufficient time prior to sampling for the
246 natural conditions of the streambed to re-establish, this time can be long (hours to days),
247 especially when installing into clay, silt or shale sediment (Lewandowski et al., 2015; Ohio
248 EPA, 2012). Piezometer installation in gravel and clay sediments can be difficult, and
249 requires substantial hammering or pre-drilling of the sediment (Baxter et al., 2003; Geist et
250 al., 1998; Grimm et al., 2007). The time taken for the piezometer to refill after purging can be
251 long, in some cases prohibiting sampling, exposing pore-water to exchange with the
252 atmosphere affecting dissolved gases. Additionally, the horizontal instrument footprint of the
253 piezometer is relatively large, and the achievable vertical resolution is low compared to other
254 techniques. Although hyporheic pore-water fluxes can be estimated, this assumes vertical
255 flow is present, which is not always the case (González-pinzón et al., 2015), and reaction
256 rates cannot be determined with this technique. Additionally, if the larger piezometer design
257 is used (up to ~ 50 mm) this may alter the hyporheic flow at the sampling location (Ward et
258 al., 2011).

259 ***2.1.2 Multilevel mini-piezometers***

260 Multilevel mini-piezometers consist of a number of small Tygon© or PTFE tubes of different
261 lengths, which are fitted around a larger diameter central steel, PVC or HDPE tube (acting as
262 a more traditional piezometer, Figure 1) (Krause et al., 2013; Lewandowski et al., 2011,
263 2015; Rivett et al., 2008; Shelley et al., 2017). The piezometer design allows the extraction of

pore-water at multiple discrete sampling depths and intervals, with minimal lateral spacing, which are defined by the user (Rivett et al., 2008). Sampling depths are typically between 0.1 and 2 m (Gooddy et al., 2014; Heppell et al., 2013; Krause et al., 2011b; Krause et al., 2013; Lansdown et al., 2015; Rivett et al., 2008; Shelley et al., 2017), with a vertical sampling interval of 0.1 m (Lansdown et al., 2015; Rivett et al., 2008; Shelley et al., 2017), although a vertical spatial resolution up to 50 mm is achievable with a low pore-water extraction rate (Rivett et al., 2008). The horizontal instrument footprint of the multilevel mini-piezometer setup is small, usually ~ 30 mm in diameter due to a relatively small diameter central piezometer tube, allowing depth profiles to be sampled over a small horizontal area of the streambed (Krause et al., 2013; Rivett et al., 2008; Shelley et al., 2017). Multilevel mini-piezometers are deployed into the streambed to usually remain for time periods between several days to years (Rivett et al., 2008), and the extracted pore-water sample represents a snapshot of the conditions at the time of sampling. Sample volumes are typically small and collected slowly with a syringe or with a peristaltic pump at a low flow rate, which limits disturbance to the hyporheic flow, as well as allowing a higher vertical resolution to be achieved (Krause et al., 2013; Lewandowski et al., 2015). If low pumping rates are used then the time taken for sampling may integrate a changing nutrient signal if sampling under rapidly changing environmental conditions. The multiple depths of the multilevel mini-piezometers may be sampled simultaneously or consecutively. A pore-water sampler combining attributes of the single-depth piezometer and the multilevel mini-piezometers has recently been developed, using a relatively large central piezometer (32 mm outer diameter) up to 4 m depth (Gassen et al., 2017). Sampling ports are connected to the central tube so that the sampling resolution varies from 0.05 to 0.5 m, depending on which zone is being sampled at that depth. Although this affords high-resolution sampling at critical zones with a large

depth profile, this sampling methodology retains the issues associated with a large horizontal instrument footprint.

Advantages:

Hydraulic gradient, hydraulic conductivity and hyporheic exchange can be determined in the central piezometer tube provided its internal diameter is large enough to be manually dip-metred (Baxter et al., 2003; Dahm et al., 2007; Grimm et al., 2007; Lee & Cherry, 1979), while residence times and hyporheic water fluxes may be determined in the multilevel tubes, therefore, reaction rates can also be calculated using this technique (Shelley et al., 2017). Multilevel mini-piezometers allow pore-water samples to be extracted from discrete depths, enabling vertical solute profiles to be captured (Krause et al., 2013; Rivett et al., 2008). Their design, which is both compact and user-defined, leads to easy installation in soft sediment (Dahm et al., 2007) and a small sampling diameter (Krause et al., 2013; Rivett et al., 2008; Shelley et al., 2017), as well as a flexible vertical depth and resolution (Rivett et al., 2008), to target focus areas based on the specific research questions. The central piezometer tube is flexible and so bends with surface water flow resulting in a more storm-resilient piezometer, less likely to be displaced or contaminated during storms, than more traditional, rigid single-well piezometers (Rivett et al., 2008). The flexible design also causes less visual disturbance; therefore, these samplers are also less prone to vandalism. Furthermore, the larger range of sampling available when using multilevel mini-piezometers allows streambed biogeochemistry to be investigated at a higher spatial (vertical) resolution and depth. Sampling with syringes or pumping into syringes prevents contact with the atmosphere eliminating issues of exchange of dissolved gases.

Limitations:

311 The hydrological information gained via hydraulic gradients is difficult to determine in the
312 discrete depths of the multilevel mini-piezometers, due to the small diameter of the multilevel
313 sampling tubes (Rivett et al., 2008). Only the central piezometer tube, therefore, can provide
314 information on hydraulic gradients (Krause et al., 2013; Rivett et al., 2008). Hence, it is not
315 possible to ascertain this information for each sampling depth and only information at the
316 deepest location of the piezometer is available. Additionally, the central tube is usually too
317 small to allow installation of continuous monitoring devices for hydraulic heads, electrical
318 conductivity, turbidity or different solute chemical parameters. There is a risk of disrupting
319 the vertical solute profile during sampling, as drawing samples at too high flow rate or at too
320 great a vacuum may cause overlap in the sample area between depths or alter preferential
321 flow (artificially increasing horizontal or vertical flow) in the streambed (Krause et al., 2013).
322 The sampling interval achievable using multilevel mini-piezometers is relatively coarse
323 (typically 50-100 mm's) compared to other discrete depth-sampling techniques (Berg &
324 McGlathery, 2000; Duff et al., 1998; Harvey et al., 2013; Rivett et al., 2008; Sanders &
325 Trimmer, 2006). The piezometers are usually installed several days in advance of sampling to
326 allow the sediment to re-settle around the piezometer and for the ambient flow conditions to
327 re-establish (Lewandowski et al., 2015). In gravel or clay sediments, installation can be more
328 difficult and may require pre-drilling of a hole or substantial hammering to install the
329 piezometer into the streambed (Baxter et al., 2003; Grimm et al., 2007). Although hyporheic
330 fluxes can be estimated, this assumes vertical flow is present, which is not always the case
331 (González-pinzón et al., 2015).

332 ***2.1.3 Miniature Drivepoint Samplers***

333 Miniature drivepoints have been developed to sample streambed chemistry at high vertical
334 resolution with minimal disturbance caused at the streambed (Berg & McGlathery, 2000;
335 Duff et al., 1998; Harvey & Fuller, 1998; Sanders & Trimmer, 2006). Several variations and

336 design adaptations have been developed over time, including: 1) six ~3 mm diameter,
337 stainless steel drivepoints fixed in a 0.1 m diameter circle on a plastic disk (USGS Minipoint
338 sampler, shown as example in Figure 1) (Duff et al., 1998; Harvey & Fuller, 1998), 2) nine 8
339 mm diameter drivepoints held in a PVC or stainless steel ring (Sanders & Trimmer, 2006)
340 and 3) a single 2.4 mm diameter, stainless steel drivepoint, which is deployed successively
341 for spot sampling through six guiding holes in a 47 mm diameter circle on an acrylic plate
342 (Berg & McGlathery, 2000).

343 Water is sampled through a screened section near the tip of the drivepoint, which typically
344 comprises of slots (Duff et al., 1998; Harvey & Fuller, 1998) or holes (Berg & McGlathery,
345 2000; Sanders & Trimmer, 2006). The drivepoint samplers are installed to discrete, user-
346 defined depths to enable the upper 0.4 m of the streambed to be sampled at high vertical
347 resolution, between 10 and 30 mm (Berg & McGlathery, 2000; Duff et al., 1998; Harvey et al.,
348 2013; Harvey & Fuller, 1998; Sanders & Trimmer, 2006). The horizontal instrument footprints
349 of miniature drivepoint samplers are relatively large resulting in pore-water samples collected
350 from different depths over a wider area than those from a multilevel mini-piezometer. These
351 samplers are usually installed shortly before sampling, enabling them to be used as roaming
352 samplers, with extracted samples representing a snapshot of the conditions at the time of
353 sampling (González-Pinzón et al., 2015; Sanders & Trimmer, 2006). Due to the usually low
354 pumping rates used for sampling, however, this time can be long. Samples collected using
355 miniature drivepoint samplers tend to be of relatively small volume (1.5-70 ml) (Berg &
356 McGlathery, 2000; Duff et al., 1998; Harvey & Fuller, 1998; Sanders & Trimmer, 2006) and
357 are extracted slowly using a syringe or a peristaltic pump with very low flow rates (Berg &
358 McGlathery, 2000; Duff et al., 1998; Harvey & Fuller, 1998). This prevents the ambient
359 hyporheic flow from being disturbed, as well as maintaining a high vertical resolution (Duff et
360 al., 1998; Harvey & Fuller, 1998). The discrete sampling depths may be sampled

simultaneously (Duff et al., 1998; Harvey et al., 2013; Harvey & Fuller, 1998) or consecutively. Sampling with syringes or pumping into syringes prevents contact with the atmosphere eliminating issues of exchange of dissolved gases.

Advantages:

Residence times, hyporheic fluxes and hyporheic exchange can be determined at multiple depths using miniature drivepoint samplers (González-pinzón et al., 2015), providing measurements that allow calculation of reaction rates (Harvey et al., 2013; Knapp et al., 2017). The combination of small sample volumes and low extraction rates enables sampling with minimal disturbance to the ambient hyporheic flow, allowing high-resolution pore-water extraction, which is difficult to achieve with other piezometer methods (Harvey & Fuller, 1998). The small diameter of miniature drivepoint samplers (Berg & McGlathery, 2000; Duff et al., 1998; Harvey & Fuller, 1998; Sanders & Trimmer, 2006), enables easy and rapid installation with minimal disturbance to the streambed. This allows the drivepoints to be sampled shortly after deployment and used effectively as roaming samplers where probes are installed, sampled and then removed, before installation at a new location. The short deployment time also enables unstable and unconsolidated sediments, which may move frequently between events, to be sampled. Pore-water samples can be pre-filtered at the tip of the probe through its design (Berg & McGlathery, 2000) or glass wool (Sanders & Trimmer, 2006), or filtered in-line during pumping (Harvey et al., 2013).

Limitations:

Given the temporary nature of the installation of miniature drivepoint samplers, they cannot be installed for long periods and so longer temporal studies would not be conducted in exactly the same location, additionally, their ease of deployment and removal for roaming surveys means these samplers may be more easily disturbed than permanent installations, and

so the depth of sampling could be compromised. The success of miniature drivepoint sampler installation can be heavily dependent on sediment type as deployment in gravel, cobble or clay-rich sediments is challenging (Ruhala et al., 2018), despite this, samplers have been successfully used in coarser sediments (Harvey et al., 2013). The relatively large horizontal instrument footprint (Berg & McGlathery, 2000; Duff et al., 1998; Sanders & Trimmer, 2006), resulting in samples from different depths not being vertically aligned where drivepoints are held in sampling arrays as is the designs of many drivepoints, may result in inaccurate vertical profiles where small-scale heterogeneity in sediment properties occurs. Pore-water samples must be extracted from miniature drivepoint samplers at a low rate to prevent pore-water being drawn from outside of the intended sampling depth, and to prevent changes in preferential flow, to preserve the high spatial resolution (Berg & McGlathery, 2000; Harvey et al., 2013; Harvey & Fuller, 1998; Sanders & Trimmer, 2006). The screening or filter at the base of miniature drivepoint samplers is prone to clogging in silt, clay or organic-rich sediments, which may disrupt sampling and reduce the lifetime of the filter (which tends to be difficult to change) if one is used with the drivepoint design. It is not possible to determine information on hydraulic gradients from these samplers due to the small inner diameter of sampling tubes. Hyporheic fluxes can be estimated under the assumption that vertical flow is present, which is not always the case (González-Pinzón et al., 2015).

2.2 Passive Equilibration Samplers

2.2.1 DET gel probes

DET gel probes (Davison et al., 1991; Harper et al., 1997) are passive samplers consisting of a polyacrylamide hydrogel (Davison et al., 1994; Krom et al., 1994; Mortimer et al., 1998; Ullah et al., 2012), which contains ~95% water, is between ~0.4 to 1.8 mm thick, and housed

409 in a plastic probe (Davison et al., 1991; Harper et al., 1997; Krom et al., 1994; Ullah et al.,
410 2012). DET gels are available in either $NaNO_3$ or NaCl buffer, with the buffer dependent on
411 the type of solutes to be analysed (DGT Research Ltd; www.dgtresearch.com). Rather than
412 extracting pore-water actively from the streambed, solutes in the investigated substrate
413 diffuse across the DET gel membrane, into and out of the gel, until equilibrium with the pore-
414 water is reached (Davison & Zhang, 1994; Davison et al., 1991; Davison et al., 1994; Harper
415 et al., 1997). The gel probes are then removed from the sediment, the gel sliced at the
416 required vertical resolution, and back-equilibrated with a known volume of ultrapure water
417 (Krom et al., 1994; Mortimer et al., 1998). The concentration of solute in the DET gel slices
418 and hence, the pore-water is determined from this eluate (Harper et al., 1997).

419 Commercially available DET gels are typically 0.15 m in length and so this vertical range is
420 usually sampled, however, they have also been modified and used for streambed pore-water
421 sampling at depths up to 0.3 m (Figure 1) (Ullah et al., 2012). The vertical resolution attained
422 by the DET gel is determined by the interval at which the gel is either partitioned within the
423 probe or immediately sliced at upon removal from the sediment (Davison et al., 1994;
424 Mortimer et al., 1998). Vertical sampling resolutions in the mm range are possible if slicing
425 occurs fast enough after removal to avoid vertical diffusion within the gel or if the DET gel is
426 constrained at the desired resolution (Dočekalová et al., 2002; Harper et al., 1997; Krause et
427 al., 2013; Krom et al., 1994; Ullah et al., 2012). Recently, DET gels have been combined
428 with colorimetry and hyperspectral imagery, which enables two-dimensional nitrite and
429 nitrate distributions to be simultaneously measured at millimetre scale (Metzger et al., 2016).
430 The horizontal instrument footprint of the DET gel probe is ~5 mm x 40 mm, however, the
431 exposed membrane of the gel is only 18-20 mm wide (Krause et al., 2013; Krom et al., 1994;
432 Mortimer et al., 1998). DET gel probes are usually deployed into the sediment for at least 72
433 hours prior to retrieval to allow ambient flow conditions to re-establish after installation and

equilibrium with the pore-water to be reached (Byrne et al., 2015a; Mortimer et al., 1998; Ullah et al., 2012). Due to the DET gel being an equilibration technique the samples collected represent an average of the biogeochemical concentrations dynamics over the time of diffusive equilibration within the sediment i.e. the time for solute concentrations to equilibrate between pore-water and gel rather than deployment time (Berg & McGlathery, 2000; Davison et al., 1994). The nature of this technique means that all depths are sampled simultaneously and environments which are diffusion-dominated with low solute velocities are most suitable for sampling with diffusion equilibrators (Duff et al., 1998).

Advantages:

The passive sampling of solutes through diffusion into the sampler prevents potential issues associated with streambed pore-water extraction preventing crossover between depths as long as diffusion within the gel is minimum (Dočekalová et al., 2002; Harper et al., 1997). Installation in soft sediment is quick and easy, requiring only pushing into the sediment by hand. The DET gel sampler has a very high vertical resolution (Harper et al., 1997; Krom et al., 1994; Ullah et al., 2012), and the horizontal instrument footprint is small minimising the lateral distribution of the vertical profile (Krause et al., 2013; Krom et al., 1994; Mortimer et al., 1998). Despite the potential for the highest spatial resolutions of all analysed methods any biogeochemical patterns lesser or equal to the gel slicing resolution cannot be resolved (Harper et al., 1997).

Limitations:

Difficulty can arise in deployment of DET gel probes in gravel sediments, although Ullah et al. (2012) developed a stainless-steel installation device and successfully deployed the DET gel probes in an armoured gravel bed. As the DET gel probe is not a piezometer, no hydrological information, such as hydraulic gradients or hyporheic flow can be ascertained

458 from the device, therefore, information is limited to pore-water solute concentrations. The
459 long time required for DET gel deployment prior to sampling requires careful planning
460 (Mortimer et al., 1998; Ullah et al., 2012). Furthermore, the vertical resolution may be
461 compromised by vertical diffusion within the DET gel, which is dependent on gel thickness
462 and time between removal and slicing (Davison et al., 1994; Harper et al., 1997). The 40 mm
463 wide plastic frame of the gel bears the risk of altering the hyporheic flow at the sampling
464 location (Ward et al., 2011).

465 3. Comparative study of sampling methodologies

466 The literature review indicated key differences between the common streambed sampling
467 technologies available, most notably in sampling technique (active versus passive), spatial
468 and temporal resolution, and sampling range. Here we explore these differences through a
469 comparative experimental analysis using some of the most frequently used sampling
470 methodologies with important differences. These methodologies include active and passive
471 sampling techniques and span a range of vertical resolutions and sampling scales.

472 3.1 Method comparison experiment

473 *3.1.1 In-situ* Experiment

474 An *in-situ* field study was performed to compare the impact of applied pore-water sampling
475 methods on observed streambed nutrient patterns, using multilevel mini-piezometers and
476 Minipoints (as examples of active samplers), and DET gel probes (as examples of passive
477 samplers) (Byrne et al., 2015; Krause et al., 2011a; Ullah et al., 2012).

478 *3.1.1.1 Study site*

479 The study was conducted in the Hammer stream in West Sussex, UK (Figure 2), which is
480 typical of lowland rivers experiencing increased nitrate loading. The Hammer is a sandy

481 stream, which drains a 24.6 km^2 catchment with bedrock predominantly made up of
482 Greensands and Mudstones (Blaen et al., 2018; Shelley et al., 2017; BGS, 2016). Land-use
483 within the catchment is predominantly agricultural, with smaller patches of deciduous broad-
484 leafed woodland, with the Hammer stream flowing through a deciduous forested valley at the
485 experimental site (Blaen et al., 2018; BGS, 2016), and the mean annual precipitation is 790
486 mm (UK Met Office, 2016).

487 The application of the different field sampling methods focussed on an approximately 60 m
488 meandering reach of the stream (Figure 2), where the streambed was dominated by spatially-
489 homogeneous, sandy sediment (Shelley et al., 2017). The study reach is characterised by
490 multiple bedforms including pools and bars, and has extensive woody debris. Stream discharge
491 at the experimental site typically ranged between 70 and 120 l s^{-1}, however, discharge may
492 exceed 1000 l s^{-1} during storm events that typically occur in winter (Blaen et al., 2018). The
493 river valley is underlain by expansive, low conductivity peat deposits and clay lenses at 1-2 m
494 depth, which inhibit groundwater upwelling, therefore, the regional groundwater contribution
495 is not expected to cause significant inputs (Shelley et al., 2017).

496 *3.1.1.2 Multilevel mini-piezometers*

497 Pore-water samples were collected on the 9th July 2015 from 40 multilevel mini-piezometers
498 (Figure 2c), installed more than one year in advance of the experiment. Pore-water samples
499 (10 ml) were manually collected from the multilevel mini-piezometers at depths of 0.1, 0.2,
500 0.3, 0.5 and 1 m using a syringe.

501 Pore-water samples were immediately filtered (0.45 μm Whatman) into acid-washed (10%
502 HCl) vials, stored cool and in the dark in the field, and frozen once returned to the laboratory
503 until laboratory analysis. Pore-water samples were analysed for nitrate and ammonium
504 concentration using a continuous flow analyser (San++, Skalar, Breda, The Netherlands),

505 with a limit of detection and precision of 0.01±5% and 0.001±1% mg N l^{-1} for ammonium
506 and nitrate, respectively.

507 *3.1.1.3 Minipoint Samplers*

508 Pore-water samples were collected twice between the 16th and 18th June 2015 from 16
509 Minipoint samplers (Figure 2c), installed on the day of sampling. Pore-water samples (50 ml)
510 were slowly pumped from the Minipoint samplers using a multi-channel peristaltic pump at
511 depths of 25, 50, 75, 100, 125 and 150 mm. Surface water samples were also taken at this
512 time. Pore-water samples collected from Minipoint samplers were immediately filtered (0.45
513 μm Whatman) into acid-washed (10% HCl) vials, stored cool and in the dark in the field, and
514 frozen once returned to the laboratory until laboratory analysis. Pore-water samples were
515 analysed for nitrate and ammonium concentration using a continuous flow analyser (San++,
516 Skalar, Breda, The Netherlands). A different Skalar instrument was used for the samples from
517 each method resulting in Minipoint sampler samples analysed with an accuracy and precision
518 of 0.1 and ±0.02 mg NH_4^+-N l^{-1} and 0.14 and ±0.01 mg NO_3^--N l^{-1}, respectively, and a limit of
519 detection of 0.02 mg N l^{-1} for ammonium and nitrate, using three mg N l^{-1} standards.

520 *3.1.1.4 DET gels*

521 The DET gels were deployed on the 10th and 11th June 2015, so that they were co-located
522 with 21 of the multilevel mini-piezometers. The DET gels were removed on the 17th June
523 2015 and sliced at 50 mm intervals (ultrapure water-rinsed blade on an acid-washed (10%
524 HCl) board) within 5 minutes of removal. The DET gel slices were stored in acid-washed
525 (10% HCl) centrifuge tubes at 4°C until laboratory analysis within four months.

526 Elution of DET gels

527 The gels were weighed to determine the volume of water within the DET gel slice (assumed
528 water content of 95%) and 5 ml of ultrapure (18.2 MΩ) water added to each tube. The gels
529 were back-equilibrated by shaking, on ice, for 20 hours, after which, the gels were removed,
530 and the eluate frozen for storage until analysis. Eluate samples were analysed for nitrate and
531 ammonium concentration using a continuous flow analyser (San++, Skalar, Breda, The
532 Netherlands), with an accuracy and precision of 0.1 and ±0.02 mg NH_4^+-N l^{-1} and 0.14 and
533 ±0.01 mg NO_3^--N l^{-1}, respectively, and a limit of detection of 0.02 mg N l^{-1} for ammonium and
534 nitrate, using 0.61 and 1.01 mg N l^{-1} standards, respectively. The concentration within the gel,
535 and hence the pore-water, was then calculated using the volume of water within the gel slice.

536 ***3.1.2 Laboratory Experiment***

537 Fine, sand-dominated stream sediment was collected from the Mill Brook at the Birmingham
538 Institute of Forest Research, Staffordshire, UK in May 2016, see Blaen et al. (2017) for site
539 information. Moist sediment was sieved (16 mm), homogenised and placed into three 10 L
540 containers. Solutions of varying ammonium concentrations (0.0, 4.9 and 10.0 mg NH_4^+ l^{-1})
541 were made from a stock of NH_4Cl and 10 L of solution was added to each of the three
542 containers resulting in saturated sediment, and DET gels and multilevel mini-piezometers,
543 with sampling depths of 25, 75 and 125 mm, were installed into the sediment. After three
544 days, the DET gels were removed and sliced at 50 mm intervals, and the multilevel mini-
545 piezometers were sampled. Three additional DET gels were equilibrated in ultrapure water
546 (18.2 MΩ) for 24 hours for quality control purposes. The DET gels were processed as
547 detailed in chapter 3.1.1.4, and all samples were stored frozen until analysis.

548 ***3.1.3 Statistical Analysis***

549 The nitrate and ammonium data obtained from each technique in the field and laboratory
550 studies were checked for normality and equality of variances, and the appropriate parametric

551 or non-parametric test applied to determine whether differences between methods were
552 significant. In the field study, assessment of any differences (p-value <0.05) in measured
553 nitrate and ammonium from the three sampling methods were determined using the non-
554 parametric Kruskal-Wallis rank sum test. If significant differences between the groups were
555 identified, a Dunn test was performed to identify which groups were statistically different. In
556 the laboratory study, significant differences (p-value <0.05) in ammonium between sampling
557 methods were determined using a paired t-test or the equivalent non-parametric Wilcoxon
558 rank sum test.

559 **3.2 Results**

560 ***3.2.1 Field Study***

561 *3.2.1.1 Pore-water Nitrate*

562 *Vertical concentration profiles in the top 1 m of the streambed*

563 The comparison of the techniques in this section, and all subsequent sections, refers to
564 the precision of the techniques, as the actual pore-water nutrient concentrations are unknown.
565 The nitrate depth profiles observed varied depending upon which sampling technique was
566 used (Figure 3); the greatest individual porewater nitrate concentrations were observed in the
567 DET gel samples, however, more samples taken with the Minipoints had relatively high
568 concentrations. The concentrations in the multilevel mini-piezometer samples were
569 predominantly lower than those found during sampling with either the DET gels or the
570 Minipoints. Mean pore-water nitrate concentrations were determined at each sampling depth
571 used for each method and were typically highest in the data from the DET gels (3.78 to 4.34
572 mg l^{-1}), although the highest mean pore-water concentrations in the shallowest depths were
573 found using the Minipoints (10.22 and 5.86 mg l^{-1} at 2.5 and 5 cm, respectively). The largest
574 range of mean pore-water nitrate concentrations per depth was observed in the Minipoint data

575 (9.67 mg l^{-1}, Figure 4). There was no statistically significant difference (p-value = 0.54, Table
576 2) in nitrate concentrations between the methods used. The clearest trend in mean pore-water
577 nitrate concentration with depth was observed in the Minipoint data (Figure 4), where mean
578 pore-water nitrate concentrations decreased non-linearly with depth, from 10.2 to 0.54 mg l^{-1}
579 over a depth interval of 25 to 150 mm below the streambed interface. The small range in
580 mean concentrations per depth captured by the DET gels and multilevel mini-piezometers
581 (3.78 to 4.34 mg l-1 and 0.73 to 2.53 mg l-1 for DET gels and multilevel mini-piezometer
582 samples, respectively) prevented such a clear trend from being observed, although the vertical
583 concentration profile from the multilevel mini-piezometer data was similar to the one
584 observed in the Minipoints (Figure 4).

585 *Vertical concentration profiles in the top 0.15 m of the streambed*

586 Descriptive statistics were calculated individually for each method from all of the data
587 collected in the top 0.15 m of the streambed as this represents the overlap of the window of
588 detection for the sampling methods. The highest mean pore-water nitrate concentration was
589 observed in the Minipoint samples (4.08 mg l^{-1}) and DET gel samples (4.02 mg l^{-1}), in
590 comparison the mean pore-water nitrate concentration measured in the multilevel mini-
591 piezometer samples was only 2.53 mg l^{-1}. The highest coefficient of variation and range were
592 observed with the DET gels (173.36 and 34.23 mg l^{-1}, respectively), however, the lowest
593 coefficient of variation was found in the Minipoint samples (135.05) and the lowest range in
594 the multilevel mini-piezometer samples (15.00 mg l^{-1}, Table 3). The coefficient of variation
595 of the multilevel mini-piezometer data and the range of the Minipoint data were intermediate
596 of these values (151.78 and 17.62 mg l^{-1}, respectively). There was, however, no statistically
597 significant difference (p-value = 0.27, Table 2) in nitrate concentrations in the top 0.15 m
598 between the methods used.

599 *3.2.1.2 Pore-water Ammonium*

600 *Vertical concentration profiles in the top 1 m of the streambed*

601 The observed pore-water ammonium depth profiles varied between the three
602 techniques (Figure 3); with the largest values and range observed in samples from multilevel
603 mini-piezometers, and the lowest concentrations observed with the Minipoints. Mean pore-
604 water ammonium concentrations were determined at each sampling depth used for each
605 method and the largest mean concentrations (3.83 to 5.73 mg l^{-1}) and range (1.90 mg l^{-1}) were
606 observed in the multilevel mini-piezometer samples, and the smallest mean concentrations
607 (0.50 to 1.56 mg l^{-1}) and range (1.06 mg l^{-1}) were observed in the Minipoint data (Figure 4).
608 Differences in pore-water ammonium concentrations between the three methods were
609 statistically significant (p-value < 0.01, Table 2), with significant differences between all
610 sampling methods (all p-values <0.01, Table 2). The most pronounced trend in mean pore-
611 water ammonium concentration with depth was observed in the Minipoint data, where
612 concentrations increased linearly with depth from 0.50 to 1.56 mg l^{-1} (Figure 4), and the
613 multilevel mini-piezometer data indicated a maximum in pore-water ammonium
614 concentration of 5.73 mg l^{-1} at 0.2 m.

615 *Vertical concentration profiles in the top 0.15 m of the streambed*

616 Descriptive statistics were calculated individually for each method from all of the data
617 collected in the top 0.15 m of the streambed as this represents the overlap of the window of
618 detection for the sampling methods. The highest mean pore-water ammonium concentration
619 was observed in the multilevel mini-piezometer data (3.83 mg l^{-1}), whereas the lowest was
620 observed in the Minipoint sampler data (1.05 mg l^{-1}). The mean pore-water ammonium
621 concentration observed with the DET gels was intermediate of these values (2.32 mg l^{-1}). The
622 coefficient of variation was highest in the Minipoint samples (188.57) and lowest in the

623 multilevel mini-piezometer samples (74.67), whereas, the range was highest in the multilevel
624 mini-piezometer data (11.64 mg l^{-1}) and lowest in the Minipoint data, with a similar range
625 observed with the Minipoint samplers and DET gels (10.02 and 10.18 mg l^{-1}, respectively,
626 Table 3). For the top 0.15 m, the differences in pore-water ammonium concentrations
627 between the three methods were statistically significant (p-value <0.01, Table 2), and were
628 significant between all sampling methods (all p-values <0.01, Table 2).

629 *3.2.1.3 Surface water concentrations*

630 Mean surface water nitrate concentrations were high (14.27 mg l^{-1}), whereas surface water
631 ammonium concentrations were low (0.10 mg l^{-1}).

632 ***3.2.2 Laboratory Experiments***

633 A comparison of the mean pore-water ammonium concentration at each depth showed that
634 the concentration in the DET gel samples was higher than in the multilevel mini-piezometer
635 samples at all depths (Figure 6). It should be noted, however, that pore-water ammonium
636 concentrations were slightly higher in the multilevel mini-piezometer data than in the DET
637 gel data in two samples (0.14 and 0.08 mg l^{-1} higher, high concentration solution, 25 mm
638 depth). The differences in pore-water ammonium concentrations obtained by the two methods
639 were statistically significant at all depths (p-value = 0.02, 0.02 and <0.01 for 2.5, 7.5 and 12.5
640 cm depths, respectively, Table 4). Pore-water nitrate concentrations were not measured
641 during these laboratory experiments as no nitrate was detectable in the DET gel samples after
642 processing. The ammonium concentrations in the DET gel samples, which were equilibrated
643 in ultrapure water (as quality control), were below the limit of detection, and so were
644 effectively zero.

645 **3.3 Discussion**

646 ***3.3.1 Field Study***

Despite the variations in pore-water concentrations observed using the different sampling techniques discussed in detail below, these differences were not statistically significant with respect to nitrate (p-value > 0.54), suggesting that the choice of sampling techniques did not have a significant effect on the outcome of analysed pore-water concentrations. This would be expected given that the samplers do not all sample the same depths of the streambed and that they were not co-located hence the variability between different locations was greater than the variability between techniques. Even though the differences were not statistically significant, there were differences observed and these affected biogeochemical classification of the streambed (see detailed discussion below), therefore, the methods used should be carefully chosen to capture the data required to address experimental hypotheses.

On the other hand, there was a statistically significant difference in pore-water ammonium concentrations (p-value < 0.01) obtained by the different pore-water sampling techniques, indicating that the selected sampling technique can have wide implications for experimental results. It is somewhat surprising that there was no statistically significant difference in the pore-water nitrate concentrations, given that pore-water nitrate concentrations have been shown to be sensitive to active versus passive sampling techniques (Briggs et al., 2015). Although significant differences between these methodologies were observed, care should be taken when comparing results gained from differing sampling techniques.

The differences in concentrations measured with the three pore-water sampling techniques may be explained by some key differences in sampler principles and setup. The Minipoint samples revealed mean pore-water concentrations at the first sampling depth that were higher in nitrate and lower in ammonium concentrations than samples obtained from the multilevel mini-piezometers. However, as both techniques use active sampling methods, similar concentrations would be expected. The difference may be explained by the common multilevel mini-piezometer setup used, where pore-water is sampled at a coarser resolution

672 over a larger depth range (Krause et al., 2013; Rivett et al., 2008). Here the shallowest depth
673 sampled with the multilevel mini-piezometers was 100 mm, therefore, any downwelling
674 surface water, which is high in nitrate and low in ammonium at this site, would already have
675 been affected by streambed processes occurring at shallow sampling depths (Battin et al.,
676 2003b; Knapp et al., 2017; O'Connor & Harvey, 2008), whereas the Minipoint samples at 25
677 mm would capture this surface water signature more efficiently. This is furthermore
678 evidenced by other research at this study site, which found that nitrate entering the streambed
679 in surface water was immediately reduced (Shelley et al., 2017). The depth of sampling, with
680 most of the multilevel mini-piezometer samples extracted from greater than 0.3 m depth, may
681 also explain why this technique resulted in the lowest pore-water nitrate concentrations and
682 the highest pore-water ammonium concentrations, as a different section of the streambed is
683 being sampled. The results here correspond with previous observations of significant rates of
684 denitrification between depths of 50 mm and 0.7 m in streambed sediments (Stelzer et al.,
685 2011), however, previous research at this site found low rates of nitrate reduction at depths
686 greater than 0.60 m (Shelley et al., 2017). It is important to note that multilevel mini-
687 piezometers may be designed to sample at a finer resolution in the top 0.2 m of the
688 streambed, with an achievable sampling resolution of 50 mm (Rivett et al., 2008).

689 Analysis of the DET gel samples yielded different concentrations than samples obtained from
690 Minipoints, despite these two techniques sampling similar depths within the streambed. Both
691 samplers, however, are mechanically different; DET gels are passive samplers (Byrne et al.,
692 2015; Krause et al., 2011a; Ullah et al., 2012) whereas the Minipoints are active samplers,
693 hence Minipoints are likely to sample pore-water from more mobile macropores and the DET
694 gels from micropores or matrix pores (Harvey, 1993; Harvey et al., 1995). The Minipoints
695 may, therefore, predominantly sample mobile water (often downwelling surface water in the
696 near-surface sediment), which primarily flows through the macropores, whereas, the DET

gels should predominantly sample less mobile micropores less likely to reflect surface water concentrations. Macropores and micropores have differing characteristics with shorter residence times, more oxygenated conditions, lower rates of denitrification and higher rates of nitrification typically observed in macropores than micropores (Briggs et al., 2015), which may explain the higher pore-water nitrate and lower pore-water ammonium concentrations found in the Minipoint data.

Similar differences in ammonium concentrations in active versus passive samplers have been observed previously where larger ammonium concentrations were observed in DET gel samples than in multilevel mini-piezometer samples (Mortimer et al., 1998; Ullah et al., 2012), however, no differences have also been observed (Krom et al., 1994; Mortimer et al., 2002). This may also have affected the vertical profiles obtained from the Minipoints and the DET gels, with a non-linear decrease in pore-water nitrate and a linear increase in pore-water ammonium observed with depth in the Minipoint data, which was not seen with the DET gels. Despite the hypothesis presented here more rigorous testing of the pore space sampled by active versus passive samplers is required to determine whether this accounts for the differences in ammonium concentrations observed between DET gels and active samplers.

Furthermore, as porewater was extracted using Minipoints the samples for laboratory analysis were extracted *in-situ*, however, as the DET gel only samples solutes into the polyacrylamide gel a solution has to be created for analysis in the laboratory using back-equilibration. This process could produce differences in pore-water concentrations between the two sampling techniques, especially given that here gel slices were back-equilibrated on ice for 20 hours. The time required for back-equilibration was not tested here and so the time used (20 hours) may have been unnecessarily long, and is sufficient for potential changes in resulting pore-water concentrations to occur. Additionally, the difference in sampling resolution (25 mm in the Minipoints and 50 mm in the DET gels), may have had some effect on the vertical profile,

however, it is difficult to interpret the effect due to the multidirectional nature of hyporheic flow (Bencala, 1993; Mulholland & DeAngelis, 2000).

These differences in sampler principles and setup may also have affected the vertical trends of nitrogen species observed, with the clearest trend observed in the Minipoint data. Minipoint samplers were able to sample the mobile pore-waters in the most biogeochemically variable upper zone of the streambed (Battin et al., 2003b; Knapp et al., 2017; O'Connor & Harvey, 2008; Shelley et al., 2017), allowing for influences of downwelling surface water and biogeochemical processes to be observed in the profile. The lack of trend in the DET gel data was unexpected, especially given that DET gels have previously been used to capture biogeochemically active zones within sediment (Comer-Warner et al., 2017; Ullah et al., 2012, 2014).

The samples collected using the investigated methods were not ideally co-located nor sampled simultaneously. Samples were collected from multilevel mini-piezometers at a different time (9th July 2015) than those from the DET gels (17th June 2015) and Minipoint samplers (16-18th June 2015), and the Minipoint samplers were not co-located with the DET gels and multilevel mini-piezometers (see Figure 2c). Despite the sampling variations we believe the discussion remains valid due to co-located samplers requiring sufficient distance between them to prevent interference, therefore, even co-located samplers may not sample the same parcel of water. This is particularly important where there is large variability in nutrients at small-scales, which has been observed in the Hammer Stream (Shelley et al., 2017). The techniques were utilised individually to gather insight into the reach-scale streambed biogeochemistry inferred from nutrient profiles obtained from each method, therefore, all data from each sampling technique were compared rather than individual nutrient profiles. We believe the presented results are crucial observations of wider relevance, since outcomes from different sampling techniques are often used interchangeably without

747 considering effects inherent to the technique. The quantitative comparison presented here,
748 therefore, provides valuable information on the validity of assumptions that different
749 sampling techniques provide comparable results.

750 The differences in results from the streambed samplers utilised in this case study may have
751 resulted from variations in the window of observation, vertical resolution and sampler
752 principles (active versus passive) between the methods. These differences may lead to
753 conflicting characterisation of the biogeochemical conditions influencing streambed pore-
754 water concentrations within the study reach; therefore, potentially different conclusions could
755 be drawn based on the analysis of results from studies that apply only one method.

756 For the field case study presented here the streambed characterisation did vary between the
757 methods used. The multilevel mini-piezometer samples indicated a stream reach
758 characterised by reduced conditions and anoxia, leading to a decrease in pore-water nitrate
759 and increase in pore-water ammonium (Dahm et al., 1998; Duff & Triska, 2000; Lansdown et
760 al., 2016; Lansdown et al., 2014; Naranjo et al., 2015). This was reflected in the vertical
761 profiles of mean pore-water concentration values obtained with the multilevel mini-
762 piezometers, which indicated surface water high in nitrate and low in ammonium penetrating
763 the subsurface. There was then a decrease in pore-water nitrate and increase in pore-water
764 ammonium with depth (Figure 4c). The DET gel data indicated a stream reach characterised
765 by areas of oxygenated sediment, leading to a few points of high pore-water nitrate
766 concentration (Dahm et al., 1998; Duff & Triska, 2000; Holmes et al., 1994; Jones et al.,
767 1995; Naranjo et al., 2015; Seitzinger, 1994), within a streambed similar to that described in
768 chapter 3.1.1 for the multilevel mini-piezometer data. This perhaps contributed to the lack of
769 trend in mean pore-water nitrate and ammonium concentrations with depth in the DET gel
770 samples, with little vertical variation in mean pore-water concentrations making it difficult to
771 infer biogeochemical process information (Figure 4a).

In contrast, the Minipoint data indicated a stream reach characterised by oxidising conditions, leading to high pore-water nitrate and low pore-water ammonium concentrations (Dahm et al., 1998; Duff & Triska, 2000). The mean pore-water concentration profiles obtained from the Minipoints indicated a decrease in pore-water nitrate coupled with an increase in pore-water ammonium with depth (Figure 4b). This is likely due to surface water, which is high nitrate and low ammonium concentration here, entering the streambed, before a decrease in pore-water nitrate and increase in pore-water ammonium at greater depths resulting from the majority of biogeochemical processing occurring in the upper few centimetres of sandy or fine-grained sediments (Battin et al., 2003b; Knapp et al., 2017; O'Connor & Harvey, 2008; Shelley et al., 2017), which are characteristic of the study site (Shelley et al., 2017).

The streambed characterisation was likely affected by differences in sampler set-up and principles. The window of detection and vertical resolution varied between sampling methods with multilevel mini-piezometers sampling at greater depths and over a wider range (0.1 to 1 m) than the Minipoints (0.025 to 0.15 m) and the DET gels (0.035 to 0.135 m), while the Minipoint samplers had the highest vertical resolution (25 mm) compared to the DET gels (50 mm) and the multilevel mini-piezometers (0.1 to 0.5 m, depending on depth). This resulted in the majority of the multilevel mini-piezometer data originating outside the top, biogeochemically reactive layer of the streambed, whereas all of the data from the Minipoints and DET gels were collected from within the top 0.15 m. Additionally, the higher vertical resolution of the Minipoint data, and to a lesser extent the DET gel data, allows small-scale pore-water concentration dynamics to be observed. These combined may explain why pore-water nitrate was lower and pore-water ammonium was higher in the multilevel mini-piezometer samples, as these concentration dynamics are often also observed with increasing depth below the sediment surface. Typically due to increased anoxia and therefore, an accompanying increase in denitrification and decrease in nitrification (Dahm et al., 1998;

797 Duff & Triska, 2000). The difference in sampling resolution utilised in the top 0.15 m of the
798 streambed enabled clearer trends in nutrient depth profiles to be determined in the Minipoint
799 data than in the DET gel data.

800 As discussed in chapter 3.3.1 the difference in sampler principles between Minipoints and
801 DET gels, i.e. active versus passive sampling, likely also influenced the streambed
802 characterisation, resulting in DET gels preferentially sampling different pore-waters to the
803 Minipoints. This explains the higher pore-water ammonium concentrations and the lower
804 pore-water nitrate concentrations in the top sampling depths observed in the DET gels than
805 the Minipoints. Additionally, the variability in observed concentrations may be enhanced by
806 the upwelling that was observed locally with the Minipoint samplers at three locations,
807 whereas surface water was downwelling at all other locations.

808 The differences in behaviour between pore-water nitrate and ammonium profiles observed
809 are expected due to the fundamental differences in biogeochemical processes that each
810 nutrient experiences. Ammonium and nitrate are involved in many redox reactions but are
811 predominantly affected by differing redox conditions in streambeds and will, therefore, be
812 present at varying concentrations depending on oxygen availability (Bollmann & Conrad,
813 1998; Davidson, 1991; Heppell et al., 2013; Lansdown et al., 2012, 2015; Quick et al., 2016;
814 Well et al., 2005). Furthermore, the sorption of ammonium to clay sediment produces
815 additional controls on the availability and fate of ammonium (Duff & Triska, 2000), which do
816 not directly affect nitrate.

817 ***3.3.2 Laboratory Experiment***

818 The laboratory experiment allowed further investigation of the effect of active versus passive
819 sampling on resulting ammonium concentrations that was observed in the *in-situ* data. The
820 ammonium concentrations observed in the data from the DET gels were greater than those

observed in the samples obtained from the co-located multilevel mini-piezometers in all three ammonium sediment concentrations used, (Figure 6), which has been observed previously (Ullah et al., 2012). We believe that the discrepancy between techniques, between 31 and 56% over the different depths in this experiment, is further evidence of the difference in sampling principles between active and passive samplers. DET gels equilibrated in ultrapure water resulted in ammonium concentrations below the limit of detection (0.02 mg N l^{-1}) and confirmed that the high pore-water ammonium concentrations observed in the DET gels during the *in-situ* or laboratory experiments were not introduced from the DET gels themselves.

As mentioned in chapter 2.2.1, the DET gel is a passive, diffusive equilibrium sampler (Byrne et al., 2015; Krause et al., 2011a; Ullah et al., 2012) sampling micropores, whereas, the multilevel mini-piezometers are active samplers relying on a vacuum or pumping action to sample the 'free' pore-water that occupies macropores. The DET gels preferential sampling of micropores/matrix pores (Harvey, 1993; Harvey et al., 1995) can explain the large differences in pore-water ammonium concentrations found between the two methodologies due to active and passive samplers sampling different pore-waters and therefore, different chemical signatures, as outlined in detail in chapter 3.3.1.

The difference in pore-water ammonium concentrations observed between the data from the DET gels and the multilevel mini-piezometers was statistically significant (p-values <0.05) indicating that the principles of the sampling methodology (active versus passive) used can greatly influence the resulting concentration of ammonium. When designing an experiment, the researcher should, therefore, carefully consider whether they need to target macropores or micropores to address their research questions, or if they need to utilise a combination of both active and passive sampling methods. Furthermore, the methods discussed in this paper are all *ex-situ* in nature, i.e. samples are collected from the streambed and analysed in the

laboratory. *In-situ* pore-water chemistry measurement methods are also available, and continue to be developed, these methods have the advantage of capturing the intended concentration dynamics without issues of contamination or concentration changes associated with transport, storage and laboratory analysis. These methods should, therefore, also be considered during experimental design.

4. Conclusions

As interest in hyporheic biogeochemistry continues to increase, along with the volume of interdisciplinary research conducted in the HZ, the development of standard sampling protocols and further sampling methods is required. The three samplers (multilevel mini-piezometers, Minipoint samplers and DET gels) discussed in this study mainly differ with respect to the absolute sampling depth they can reach, the achievable vertical spatial resolution and the pore sizes (and therefore mobile versus immobile water) samples are predominantly extracted from. Although samplers such as Minipoints and DET gels provide high-resolution nutrient profiles in the top few centimetres of the streambed, where the majority of biogeochemical cycling occurs, multilevel and single-depth piezometers remain a valuable tool for the investigation of deeper influences of groundwater and larger scale processes. The extent of hydrological information and the macropore versus matrix zones sampled also vary with technique, therefore, care needs to be taken when selecting a methodology. Furthermore, the sampling method used may significantly affect the resulting ammonium concentrations and may result in differing conclusions on reach-scale streambed characteristics (Table 5). The research question, and desired spatial and temporal resolution will, therefore, determine which sampling technique is most appropriate to use, with each one characterised by specific advantages and limitations (Table 1). Larger scale processes including groundwater zones of upwelling and downwelling, hydrological information and contaminant plume identification and investigation at greater depths are best investigated

using multilevel or single depth mini-piezometers, which allow chemical and hydrological information to be determined at the same point within macropores at greater depths. The ability to also sample at shallower depths allows processes within the shallow streambed to be investigated although at a coarser resolution than miniature drivepoint samplers. In-depth characterisation of hyporheic zone hydrology and biogeochemical processes in the top 0.4 m of the streambed are best investigated using miniature drivepoint samplers, which allow high-resolution investigation of chemical and hydrological information at the same depth within macropores. Fine-scale investigations of concentration dynamics within the top 0.15 m of the streambed are best investigated using DET gels, which allow very high-resolution measurements of the sediment matrix of micropores, but no hydrological information to be obtained, although the passive nature of this technique means it may be difficult to capture some events.

The differences between pore-water sampling methodologies presented here provide guidance for future studies into pore-water nitrogen cycling, improving sampler selection based on specific research questions. This has global relevance for researchers focussing on important questions of chemical cycling within saturated sediments including the hyporheic zone, moving towards a more uniformed sampling protocol and better understanding of how the selected methodology may bias results.

Future work should continue to develop sampling methodologies with focus on *in-situ* methodologies that measure nutrient concentrations without the need for sample extraction, therefore, reducing the likelihood of sampling altering results. *Ex-situ* methodologies, such as those examined here, continue to be of importance and further development of these methods including high vertical resolution samplers robust enough to sample gravels and cobbles is encouraged.

Acknowledgements

This research was funded by The Leverhulme Trust project "Where rivers, groundwater and disciplines meet: A hyporheic research network" and from the authors' institutions. Additional funding was also provided from NERC through a Central England NERC Training Alliance Studentship, NERC standard grant NE/L004437/1, and the European Union's H2020-MSCA-RISE-2016 project 734317. Jay Zarnetske and Joseph Lee-Cullin were partially supported by the US NSF Award Number 1446328. The authors would like to thank the Leverhulme project team for their help, guidance and insight.

Table Headings

Table 1. Comparison of key characteristics, advantages and limitations of most frequently used streambed pore-water sampling methodologies.

Table 2. Statistical test results from all data from the Hammer stream, UK, where the Kruskal-Wallis rank sum test indicated a significant difference between results obtained by the different pore-water sampling methods, a Dunn test was used to determine which groups of pore-water samples were significantly different. Statistically significant comparisons are indicated by bold p-values.

Table 3. Descriptive statistics for all pore-water data from the top 0.15 m of the streambed obtained from application of DET gels, Minipoint samplers and multilevel mini-piezometers sampling at the Hammer Stream, Sussex, UK

Table 4. Statistical test results from all pore-water data from the laboratory column experiments, p-values <0.05 (shown in bold) indicate a significant difference between pore-water samples extracted by DET gels and multilevel mini-piezometers at the respective depths.

Table 5. Summary results of the *in-situ* field comparison of nitrate and ammonium pore-water concentrations obtained from multilevel mini-piezometers, Minipoint samplers and DET gels, as well as suggested applications for the respective pore-water sampling techniques

Figure Headings

Figure 1. Conceptual diagram of main streambed pore-water sampling techniques for analysis of biogeochemical cycling in hyporheic zones, including (from left to right): single well piezometers, diffusive equilibrium in thin-film (DET) gels, miniature drivepoint samplers (example shown: USGS Minipoint sampler Duff et al., 1998; Harvey & Fuller, 1998), and multilevel mini-piezometers. Also shown (on the right) are the vertical ranges covered and horizontal instrument footprints of the respective pore-water sampling techniques.

Figure 2. Location of a. the Hammer stream within the UK, b. the study reach (indicated by the red section) at the Hammer Stream and c. the location of the different sampling devices used in this study

Figure 3. Vertical profiles of pore-water nitrate concentration (mg l^{-1}) observed in the streambed of the Hammer Stream, Sussex, UK using a. multilevel mini-piezometers, b. Minipoint samplers and c. diffusive equilibrium in thin-film (DET) gel probes and vertical profiles of pore-water ammonium concentration (mg l^{-1}) in the streambed of the Hammer Stream, Sussex, UK using d. multilevel mini-piezometers, e. Minipoint samplers and f. DET gels.

Figure 4. Mean pore-water nitrate concentrations (mg l^{-1}) ±1 standard deviation for each sampling depth analysed in the streambed sediments of the Hammer Stream, Sussex, UK by using a. multilevel mini-piezometers, b. Minipoint samplers and c. diffusive equilibrium in thin-film (DET) gels and mean pore-water ammonium concentrations (mg l^{-1}) ±1 standard

941 deviation for each sampling depth in the streambed sediments of the Hammer Stream,
942 Sussex, UK using d. multilevel mini-piezometers, e. Minipoint samplers and f. DET gels.

943 Figure 5. Mean ammonium pore-water concentrations (mg l^{-1}) ±1 standard deviation found
944 by multilevel mini-piezometer and DET sampling at each sampling depth in the laboratory
945 column experiments

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