Microbial communities in UK aquifers: current understanding and future research needs

S. P. Gregory[1*], L. D. Maurice[2], J. M. West[1] & D. C. Gooddy[2]

[1]British Geological Survey, Keyworth, Nottingham NG12 5GG, UK
[2]British Geological Survey, Wallingford OX10 8BB, UK
*Corresponding author (e-mail: simongr@bgs.ac.uk)

Abstract: The presence and activity of microorganisms in aquifers can affect, amongst other things, nutrient cycling, contaminant degradation and water flow. The introduction of a pollutant or other changes in water chemistry can alter the microbial community composition and affect aquifer functioning. To understand the microbial response to anthropogenically induced changes, a better knowledge of baseline microbial communities in uncontaminated aquifers is needed. Here, we review the information on microorganisms in UK aquifers together with examples of research from other countries on this topic, and discuss how these communities might respond to disturbance. Research into microbial communities in UK aquifers has mostly been limited to bacteria and often reveals a community dominated by Proteobacteria. The community composition is influenced by factors such as mineralogy and water chemistry, and the natural baseline community may be altered by aquifer contamination. A UK-wide survey of aquifer microbes, similar to one recently carried out in New Zealand, would provide valuable information about the current state of UK aquifer microbiology. This would lead to a greatly improved understanding of the ecosystem services provided by the microbial communities present in aquifers, allow future monitoring and assessment of the effects of pollution, and assist in groundwater resource management.

It is well established that aquifers provide suitable environments for microbial activity (e.g. Novarino *et al.* 1997; West & Chilton 1997; Goldscheider *et al.* 2006; Pronk *et al.* 2009). There has been a shift from considering microorganisms only when they cause problems (such as disease outbreaks, pipeline corrosion, or blockage of water flow), to a realization that the natural ecology of groundwater is important in determining biogeochemical cycling and water quality, and may provide a means of restoring the quality of contaminated aquifers (Chapelle 1993; Humphreys 2009). The role of groundwater ecosystems is now considered in some groundwater monitoring programmes (Danielopol *et al.* 2004; Goldscheider *et al.* 2006; Danielopol & Griebler 2008), but groundwater ecosystems are generally not considered in groundwater management. This is partly because microbial processes and microbial diversity in aquifers remain poorly understood. Many questions remain about the make-up of microbial communities in groundwaters, the differences in community composition in polluted and uncontaminated aquifers, the contribution of microorganisms to contaminant attenuation, and the longer term changes that occur in microbial communities.

A first, important step towards a better understanding of the role of microorganisms in UK aquifers would be to assess current or 'baseline' communities in a range of geologies, along the lines of the baseline groundwater chemistry survey initiated by Edmunds *et al.* (2003), and similar to the UK-wide survey of soil bacteria (Griffiths *et al.* 2011). It is unlikely that there are truly natural microbial communities in aquifers, entirely undisturbed by anthropogenic impacts (Goldscheider *et al.* 2006). However, a survey of UK aquifers would provide a useful assessment of current conditions, representing a baseline for future comparisons, and would provide new understanding of the impact of pollution, climate change and human intervention on the ecosystem services that aquifers provide. Here, the term 'uncontaminated' aquifer is used to mean aquifers that are not affected by severe point source pollutants but may be affected by diffuse pollutants, which occur fairly ubiquitously.

Generally, a systematic approach to groundwater microbiology monitoring (except for specific pathogens) has been overlooked. The exception is a recent nationwide survey of groundwater in New Zealand, which revealed that hydrochemistry had the greatest influence on bacterial diversity of groundwater samples, with geological factors and human impact having a secondary influence (Sirisena *et al.* 2013).

In this paper, following an introduction to the characteristics of groundwater microbial communities, an overview of what is known about natural aquifer microbiology in the UK, supplemented with data from global studies, is presented. Finally, some microbiological issues that are current and potential future concerns for UK aquifers are discussed.

Introduction to aquifer microbial communities

The following section describes the aspects of microbiology that are specific to, or important to, aquifer environments. Many of these features are not specific to a UK setting and examples are drawn from other countries to illustrate general features or concepts. Throughout this paper, organisms are grouped on the basis of evolutionary relationships, or in terms of their functional ability. The choice depends on the usage, approach and techniques used in the study being described. For example, much DNA-based work lends itself well to a phylogenetic approach; therefore organisms are grouped according to their

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Table 1. *Examples of different types of microorganisms present in aquifers*

Microorganism	Phylum or subphylum	Examples of species	Potential role of interest in aquifers
Prokaryote (Bacteria)	β-Proteobacteria	*Rhodoferax ferrireducens*	Iron reduction
Prokaryote (Bacteria)	β-Proteobacteria	*Gallionella* spp.	Iron oxidation and creation of iron deposits that inhibit flow
Prokaryote (Bacteria)	γ-Proteobacteria	*Escherichia coli*	Pathogenic bacteria
Prokaryote (Bacteria)	Actinobacteria	*Rhodococcus* spp.	Degradation of hydrocarbons and polychlorinated biphenyls
Prokaryote (Archaea)	Crenarchaeota	*Nitrosopumilus maritimus*	Ammonia oxidation
Prokaryote (Archaea)	Euryarchaeota	*Methanosaeta* spp.	Methanogenesis
Eukaryotic (Protists)	Rhizaria	*Cercomonas* spp.	Grazing bacterial biofilms
Virus	Group IV virus (N.B. not a phylum)	Hepatits A virus	Pathogenic virus

evolutionary relationships. So, for example, descriptions of broad phyla such as Proteobacteria, Actinobacteria, Firmicutes, etc. may be given. Each of these major groups can be subdivided; for example, the Proteobacteria can be divided into classes such as α-, β-, γ- and δ-Proteobacteria. Again these classes can be divided into further groups of increasingly related organisms until single species are described. This approach groups organisms by evolutionary history, but does not imply that all organisms within any group will have all the same characteristics. For example, pathogenic species of *Escherichia, Vibrio, Salmonella* and *Pseudomonas* are all Proteobacteria but this class also contains many non-pathogenic organisms, such as, methane-oxidizing bacteria (e.g. *Methylobacter*), ammonia oxidizers (e.g. *Nitrosomonas*) and sulphur-reducing bacteria (e.g. *Desulfovibrio*). The alternative way of grouping organisms is by an aspect of their behaviour; for example, pathogenicity, denitrification, ammonia oxidization or methanogenesis. This does not imply any degree of phylogenetic relationship between members of a group. For example, heterotrophic denitrification is widespread across many different phyla but autotrophic nitrification is restricted to a few closely related groups within the Proteobacteria. Table 1 is provided to illustrate the range of organisms that might be found in aquifers, their phylogenetic classification and the important roles they can play in aquifers. For further information about the classification and diversity of microorganisms, the reader is referred to a general microbiology textbook such as that by Madigan (2005).

Subsurface habitats are generally characterized by the absence of light, limited nutrient availability and by limited space, dictated by the pore and fracture size in the aquifer (Ghiorse 1997; West & Chilton 1997). However, in common with other habitats, life in aquifers has certain minimum requirements: liquid water, and sources of carbon, nitrogen, phosphorus and sulphur, certain trace elements and both electron donors and electron acceptors. The electron donors (substances that can be oxidized) may be organic matter or (in the case of chemoautotrophs, which derive their energy requirements from inorganic sources) substances such as molecular hydrogen, ammonia, sulphides or ferrous iron. The electron acceptors (substances that can be reduced) include molecular oxygen, sulphate, nitrate, ferric iron, carbon dioxide and simple organic compounds (West & Chilton 1997).

The absence of light means that photosynthesis will not occur and any primary production occurring within aquifers is carried out by chemoautotrophs. Photosynthetic microorganisms may be present if they have been transported from the surface (Sinclair & Ghiorse 1989), but they are only transient members of the microbial community. Groundwater ecosystems supported primarily by autotrophy are unusual, but have occasionally been described in cave systems, such as Ayyalon cave, Israel (Por 2007), Movile cave (Vlasceanu *et al.* 1997) and Frasassi cave, Italy (Dattagupta *et al.* 2009) (although no such systems have been reported in the UK). Therefore, in most aquifers the basis of the food web is mainly carbon transported into the aquifer from near-surface environments, combined with nutrient inputs from mineral weathering within the aquifer (Rogers & Bennett 2004; Uroz *et al.* 2009). Dissolved organic carbon is typically low in aquifers; for example, it is often less than 3 mg l^{-1} in most UK aquifers (Gooddy & Hinsby 2008). Most microbially available carbon is oxidized in soil before reaching aquifers (Goldscheider *et al.* 2006). The low-carbon environment thus results in a low biomass and a low abundance of microorganisms, which have a patchy and uneven distribution (over space and time), determined by the local availability of nutrients (Goldscheider *et al.* 2006). Addition of dissolved organic carbon to groundwater has been shown to increase microbial metabolism and alter the microbial community composition (Baker *et al.* 2000; Findlay *et al.* 2003). The scarcity of carbon and other sources of chemical energy in uncontaminated aquifers makes the microbial community particularly susceptible to change following high-energy inputs from organic pollutants because indigenous microorganisms are adapted to live in low-nutrient conditions (West & Chilton 1997).

The majority of microbiological research has been focused on the bacterial community. Estimates of bacterial density range from 10^2 to 10^6 cells per cm^3 water or from 10^4 to 10^8 cells per cm^3 sediment (Pickup *et al.* 2001; Johnson *et al.* 2004; Griebler & Lueders 2009; Sorensen *et al.* 2013). However, the microbial community also includes archaea, fungi, other microeukaryotic organisms and viruses (see Table 1 for examples). These are less well studied but it is estimated that archaea may make up 20% of cell counts, and protozoa density may be up to 10^8 cells per cm^3 (but are likely to be restricted to the upper aerobic portions of aquifers; Griebler & Lueders 2009). The pathogenic bacteria shown in Figure 1 represent the approximate size of most bacteria. Archaea are of a similar size, typically 0.1–10 μm. Protozoa and fungi range from around 1 μm to 1 mm (although multicellular fungi can grow much larger), and are therefore excluded from some areas where bacteria and archaea may be present. Each of these groups comprises both indigenous organisms and those that are introduced to the aquifer (which may be transitory or take up residence). The indigenous organisms are the residents that metabolize, grow and replicate in the low-nutrient conditions of the aquifer. The transient organisms are present only through transport from the surface layers (e.g. during periods of high water flow). This group may not be metabolically active and includes, for example, enteric organisms, which can be transported down into aquifers during the infective stage of their life cycle; or photosynthetic cyanobacteria (Sinclair & Ghiorse 1989), which are unable to photosynthesize in a dark subterranean environment.

Aquifers have been assumed to have stable physico-chemical conditions, buffered by a combination of overlying soil and saturated and unsaturated sediments. However, there is growing evidence that in shallow or karst systems seasonal changes affect the composition of microbial communities (Lin *et al.* 2012; Zhou *et al.* 2012). Zhou *et al.* (2012), studying a Quaternary sand aquifer in

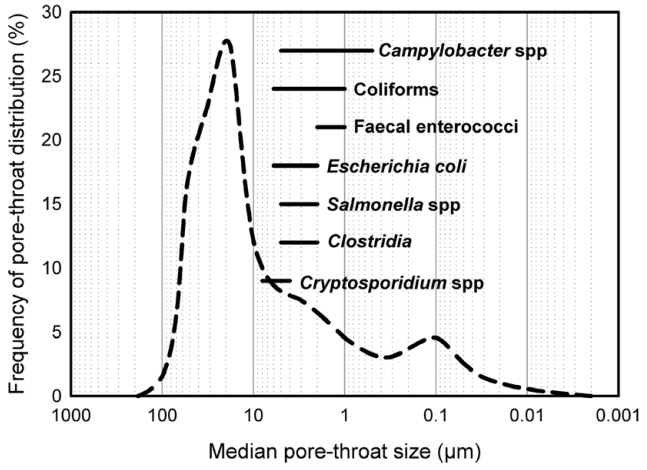

Fig. 1. Experimentally derived distribution of mean pore-throat sizes in Permo-Triassic sandstones, with the range of sizes of some pathogenic bacteria and protozoa (*Cryptosporidium*) of concern in aquifers superimposed for comparison (modified from Bloomfield *et al.* 2001).

south Germany, noted that planktonic bacterial numbers and diversity showed significant seasonal changes in response to autumn recharge (correlated to increases in available organic carbon), although the attached bacterial community showed relatively little change. Similarly, Pronk *et al.* (2009) found that a core group of organisms were seasonally present in Swiss karst aquifers. This core was supplemented by seasonal changes in the communities caused by transient organisms flushed down from the upper layers during high input flow events. Therefore, any sampling strategy that aims to understand baseline microbial community characteristics in shallow or karst aquifers needs to take into account these temporal patterns.

Many studies have shown that the microbial communities attached to solid material in aquifers are different from suspended or planktonic bacteria (e.g. Holm *et al.* 1992; Lehman *et al.* 2001*a*,*b*; Griebler *et al.* 2002; Flynn *et al.* 2013). This has also been observed in a study of a contaminated sandstone aquifer in the UK (Rizoulis *et al.* 2013). In that study, there were marked differences between the diversity and composition of planktonic and attached bacterial communities in a phenol-contaminated sandstone aquifer. Rizoulis *et al.* suggested that the higher diversity in the attached biofilm samples may be because the heterogeneous biofilm structure offers ecological advantages to different types of bacteria. They also suggested that the analysis of biofilm samples captures the established and recently colonized organisms, whereas the planktonic samples capture only the transient populations that were present at the time of sampling. The attachment of microbes (bacteria, archaea and fungi) in biofilms is widespread and allows organisms to survive, metabolize and reproduce in a relatively well-protected environment; and microscale heterogeneities and chemical gradients offer numerous niches that can be exploited by a larger range of species (Hall-Stoodley *et al.* 2004; Stewart & Franklin 2008). Numbers of planktonic bacteria may be two orders of magnitude lower than those of attached bacteria (Goldscheider *et al.* 2006, and references therein) and this ratio may be affected by contamination. A lower proportion of planktonic bacteria and species richness has been reported in uncontaminated microcosms from UK sandstone aquifers than in polluted ones (Elliott *et al.* 2010). Other microcosm experiments using material from the Upper Chalk aquifer in Hampshire demonstrated that the presence of the solid matrix was necessary for isoproturon degradation (Johnson *et al.* 1998, 2000), which was attributed to the need for bacteria to be attached in order to be able to metabolize the herbicide. Considered together, these studies suggest that microbiological investigation using groundwater only may not fully represent *in situ* microbiological activity or community composition, and ideally the attached communities should be sampled to determine the true microbial composition and capability (Flynn *et al.* 2013; Rizoulis *et al.* 2013).

There are different types of subsurface habitats available for organisms, and the physical space available in aquifers limits their presence and distribution. In many consolidated rocks, pore spaces in the rock matrix may be too small for microbes, therefore groundwater ecosystems are predominantly developed in the more permeable voids. In granular aquifers, organisms live in the void space between sand and gravel grains. In hardrock aquifers, organisms live in fractures within the rock, whereas in carbonate (karst) aquifers larger voids are formed by dissolutional enlargement of fractures to form fissures, conduits and caves. All these void types form a habitat for larger invertebrates (e.g. Amphipoda, Isopoda, Syncarida, Copeopoda) as well as microbial organisms (Gibert & Deharveng 2002). This macrofaunal community is also thought to contribute to biogeochemical cycling, and will affect the microbial communities present in groundwaters, although the interactions between the invertebrate community and the microbial community are poorly understood (Boulton *et al.* 2008).

Introduction to UK aquifers

The most important aquifers in the UK are the Cretaceous Chalk and the Permo-Triassic sandstones, but very little is known about the microbial communities that are present. The fractures, fissures and conduits within the Cretaceous Chalk are likely to provide a good habitat for microorganisms and often also contain macro-invertebrates (Robertson *et al.* 2009). The well-connected network of voids provides a physical habitat and typically receives relatively frequent inputs of nutrients, carbon and oxygen to sustain these communities. Although the Chalk has a high matrix porosity, the median pore diameters of 3–4 μm and pore throats of *c*. 0.5 μm (Price 1987) are likely to exclude all but the smallest microorganisms from the rock matrix itself. Bacteria are found in chalk aquifers in the UK (Whitelaw & Rees 1980; Parker & James 1985; Johnson *et al.* 1998) and it is usually assumed that all bacteria that have been found reside in fractures. For example, Whitelaw & Rees (1980) reported nitrate-reducing and ammonia-oxidizing bacteria from throughout the unsaturated section of two English Chalk cores, but as pore size excludes bacteria from within the matrix, they suggested that organisms attach themselves to fissure walls and obtain the nutrients they need by diffusion of water from the pores. Similarly, Kimblin & Johnson (1992) were able to detect sulphate-reducing bacteria in fractured, but not in unfractured, samples of the Chalk aquifer from the Lee Valley in the London Basin, again indicating size exclusion from the matrix.

Within the Permo-Triassic sandstones, the degree of consolidation in the aquifer varies, and intergranular flow and storage occurs in the less consolidated sediments, whereas groundwater flow and storage is within fractures in the consolidated bedrock. Microorganisms are likely to move through both the fractures in consolidated sandstones and the pore spaces in the less consolidated areas. Figure 1 (modified from Bloomfield *et al.* 2001) shows how the sizes of different types of bacteria relate to the distribution of median pore throat sizes in unconsolidated areas of the UK Permo-Triassic sandstones. The dominant pore-throat sizes in these sandstones are 0.1–90 μm (with a range of 0.01–427 μm) (Bloomfield *et al.* 2001). The bacteria species described are between 0.5 and 6.5 μm, and are therefore likely to be able to move through these unconsolidated sandstones.

A study of sediments overlying a section of Sherwood sandstone in Yorkshire indicated that the distribution of sulphate-reducing bacteria was influenced by grain size, suggesting that physical size exclusion of bacteria occurs in fine-grained sandstone. Evidence of biological sulphate reduction ceased when the d10 value of the sand grains dropped below 1.6 μm (where d10 is the estimated 10th percentile grain size based on the grain-size distribution) (Bartlett *et al.* 2010). However, geochemical conditions also have a strong influence on the distribution of sulphate-reducing bacteria, further restricting their distribution (Edmunds *et al.* 1982, 1984). A number of studies carried out on polluted sandstone aquifers have contained data from uncontaminated controls and found these uncontaminated samples to be dominated by Proteobacteria (Table 2).

There have been few microbial studies of other aquifers in the UK. However, it is likely that in many consolidated strata microbes may be largely absent from the rock matrix because pore sizes may be too small. A study of the Lincolnshire Limestone found pore sizes of 1–2 μm in diameter, but pore throats were typically only 0.1–0.3 μm, suggesting that the size of the pore throats prevented the colonization of the matrix by sulphate-reducing bacteria (Bottrell *et al.* 2000). However, microbial communities are likely to be present in the fractures, fissures and conduits within the Lincolnshire Limestone and other carbonate aquifers, and also within fracture networks within igneous, metamorphic and sedimentary consolidated strata. It is these habitats and the high-permeability intergranular aquifers that would be most usefully studied in a future systematic survey, although it would also be useful to obtain more information to confirm the extent to which microbes are absent from the rock matrix of consolidated strata.

It is frequently assumed that aquitard materials, such as clays and low-permeability fractured rocks, offer protection against contamination (Foster 1998; Smith 2005) by pollutants and pathogens. However, these deposits may not completely exclude microorganisms. Microbial communities have been detected within clays in non-UK settings (Lawrence *et al.* 2000; Takeuchi *et al.* 2011). On the other hand, there is evidence to suggest that biological sulphate reduction is inhibited in clays within Sherwood Sandstone (Bartlett *et al.* 2010). Perhaps, even if the small pore size (<1 μm) in clays restricts microorganism transport, fractures and other features may provide suitable habitats. Investigating a UK aquitard, White *et al.* (2008) identified that palaeo-rootholes within a Holocene lagoonal clay provided preferential pathways for pollutant flow to the underlying sand aquifer, and found evidence of pyrite framboids indicating microbial presence and activity within the rootholes. The presence of microorganisms in clay material has potential impacts on natural attenuation and pathogen transport and implications for risk assessment models, but more research is needed on the presence and activity of microorganisms within aquitards to determine under which circumstances they provide a protective barrier and when they might provide a habitat for microorganisms.

Microbial communities in UK aquifers

Traditional culture-based microbiology has been supplemented by newer molecular techniques, and recent developments in techniques such as next generation DNA sequencing have made the characterization of aquifer communities on a large scale available to many laboratories (e.g. Lin *et al.* 2012; Pilloni *et al.* 2012; Gray & Engel 2013; Wilkins *et al.* 2013). Although there are many examples of molecular techniques applied to contaminated aquifers (e.g. Fahy *et al.* 2008; Aburto & Ball 2009; Rizoulis *et al.* 2013) these techniques have not been applied to a systematic study of the microbial composition of uncontaminated UK aquifers. Studies of contaminated UK aquifers generally focus on the impacts of remediation on water chemistry, but direct monitoring of the microorganisms involved in bioremediation is uncommon. The exception is the extensive body of literature on the occurrence of microorganisms in mine drainage waters and their use in bioremediation and bioleaching (e.g. Johnson 2012; Norris *et al.* 2012). Similarly, there is a body of work on *in situ* bioremediation, which is used to assist with the clean-up of contaminated groundwaters. The principle is to use bacteria to attenuate contamination of groundwater by creating the conditions to allow microorganisms to break down specific pollutants. An example of such an approach is the use of dehalorespiring bacteria to remediate dense non-aqueous phase liquid (DNAPL) chlorinated solvents. In the UK there has been extensive work on this type of *in situ* bioremediation at the SABRE (Source Area *in situ* BioREmediation) industrial site, where both laboratory and field studies demonstrated the effectiveness of electron donors such as linoleic acid to increase the degradation of chlorinated solvents through the activity of dehalorespiring organisms such as *Dehalococcoides* (Cai *et al.* 2012; Harkness *et al.* 2012; Harkness & Fisher 2013). Work carried out on acid mine drainage and *in situ* bioremediation may provide insights into microbial remediation processes, and a framework for investigating microbial communities in aquifers.

There are two notable features of work on the microbiology of UK aquifers. First, most molecular data from UK studies on uncontaminated aquifers come from control or reference sites in studies where the main focus is the microbiology of contaminant plumes. Second, there do not appear to be any studies of the occurrence of microorganisms other than bacteria (e.g. archaea, fungi, protozoa). The exception to this is the eukaryotic pathogen *Cryptosporidium*, which has been studied extensively because of the health risks associated with it (Morris *et al.* 2005)

Table 2 summarizes microbial studies undertaken in the UK that present data from uncontaminated sites. These studies have used community profiling techniques such as Terminal Restriction Length Polymorphism (T-RFLP) and Denaturing Gradient Gel Electrophoresis (DGGE) combined with Sanger DNA sequencing to describe microbial communities. In many cases these data are from control sites during studies of contaminated aquifers. There is one example of a UK study containing molecular analysis that does not specifically focus on contaminated aquifers (Sorensen *et al.* 2013). This study investigated ecosystems in the Chalk aquifer in southern England using inflatable packers to isolate single fractures at different depths in boreholes. The samples from the borehole water columns had higher bacterial cell counts than samples from the surrounding aquifer and were not biologically or chemically representative of the aquifer itself. There were also differences in both the planktonic and attached community composition in samples from different depths in the aquifer.

The uncontaminated control samples in the other studies provide information on natural bacterial communities and give an insight into how these communities might change in response to contamination. The characterization of uncontaminated aquifer samples comes largely from a series of studies on the effect of benzene pollution in a BTEX-contaminated sandstone aquifer at the Site for Innovative Research in Natural Attenuation (SIReN) UK. At this site, the bacterial community in samples from currently uncontaminated sites, which had previously been exposed to BTEX, was more diverse than the community in contaminated samples. The communities in uncontaminated samples were dominated by β-Proteobacteria (and to a lesser extent Firmicutes) (Fahy *et al.* 2005; Aburto & Ball 2009). When uncontaminated samples were exposed to benzene the indigenous population shifted from one dominated by β-Proteobacteria to one dominated by Gram-positive Actinobacteria (especially *Arthrobacter*) (Fahy *et al.* 2008). The ability to degrade benzene may be linked to a shift from

Table 2. *Microbial composition of communities in uncontaminated aquifers or control sites in studies of polluted aquifers in the UK*

Aquifer type	Location	Microbiology	Experimental details	Reference
Sandstone	SIReN site, UK	Communities were different in contaminated and clean sites: bacterial communities from clean sites had high diversity of taxa, whereas highly contaminated sites were dominated by only a few species. (No attempt was made to identify community members)	T-RFLP on groundwater samples in two years (2001 and 2002)	Fahy *et al.* (2005)
Sandstone	SIReN site, UK	A shift from *Proteobacteria* to *Actinobacteria* was observed following BTEX application. Bacterial communities from clean sites are predominantly composed of Proteobacteria, particularly β-Proteobacteria. After application of BTEX a shift to a community composed mainly of Actinobacteria was observed. The community composition in the original clean mesocosms at the end of the experiment was not similar to either the starting or finishing communities of the contaminated mesocosms	Groundwater sampling and laboratory mesocosms T-RFLP	Fahy *et al.* (2008)
Sandstone	SIReN site, UK	Shift in bacterial composition might be necessary for benzene degradation. A shift from *Rhodoferax* dominated community to a *Rhodococcus* and *Hydrogenophaga* spp. dominated community may be necessary for degradation of benzene	Groundwater microcosm analysed by T-RFLP	Fahy *et al.* (2006)
Sandstone	SIReN site, UK	Degradation of benzene was faster in uncontaminated than in contaminated samples	Laboratory mesocosms analysed by DGGE	Aburto & Ball (2009)
Sandstone	English Midlands	Total bacterial communities and enterobacterial community structure were variable between depths and sites, but no consistent differences were associated with phenol concentration. However, increasing phenol concentration was associated with a decrease in enzyme activity, cell counts and cultivability	TGGE, enzyme activity assay and ERIC-PCR on groundwater samples	Pickup *et al.* (2001)
Chalk, limestone and sandstone	Various sites in the UK	Addition of isoproturon reduced the dominance of *Pseudomonas*. All samples dominated by *Pseudomonas* (γ-Proteobacteria), with all other identifiable organisms representing >10% of the community being either α-Proteobacteria or β-Proteobacteria. Addition of isoproturon reduced the dominance of *Pseudomonas*, but only in one instance did another genus become more dominant	Groundwater mesocosms analysed by FAME	Johnson *et al.* (2004)
Chalk and sandstone	Bridget Farm, Winchester; Gleadthorpe, Mansfield (sandstone)	Culturable aerobic and anaerobic bacteria had highest numbers and activities near the soil surface and in the saturated zones, although there was evidence of microbes at all points between. There was no evidence of a major role for autotrophic denitrification	Plate counts from core samples, ^{14}C-labelled acetate utilization, acetylene block method	Kinniburgh *et al.* (1999)
Chalk	Berkshire, UK	Borehole samples were not representative of water from within the aquifer for planktonic or particle-attached bacteria	Microbial cell counts and T-RFLP on groundwater samples	Sorensen *et al.* (2013)

T-RFLP, Terminal Restriction Fragment Length Polymorphism; TGGE, Temperature Gradient Gel Electrophoresis, ERIC-PCR, Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction.

a *Rhodoferax* (β-Proteobacteria) dominated community to a *Rhodococcus* (Gram-positive Actinobacteria) and *Hydrogenophaga* (β-Proteobacteria) dominated community (Fahy *et al.* 2006). In this and an earlier study (Fahy *et al.* 2005), the effect on the microbial community structure was spatially heterogeneous, with different contaminated samples diverging in different ways when challenged with the same pollutant. The uncontaminated control (undetectable BTEX since 1996, and only 0.1 mg l^{-1} at that time) had the biggest shift in community composition and the longest lag time before degradation occurred (Fahy *et al.* 2006). A lag in response for previously unexposed communities appears to be typical. For example, it has also been observed in samples of the Chalk aquifer in SE England challenged with methyl tertiary butyl ether (Shah *et al.* 2009).

Studies from other countries confirm that a shift in species composition is a common response to pollution, and a similar shift from Proteobacteria-dominated communities to Actinobacteria has been seen in fuel-contaminated areas of aquifers (e.g. Shi *et al.* 1999). However, other studies report decreasing numbers of Actinobacteria and increasing γ-Proteobacteria (Hendrickx *et al.* 2005) and mesocosm studies report a shift in the dominating classes of Proteobacteria (from β- and γ-Proteobacteria to α-Proteobacteria) in response to fuel contamination (Shi *et al.* 1999). These observations are based on a limited number of studies so it is difficult to make clear predictions about the changes that are likely to be observed in other aquifers. However, the diverse findings in these studies suggest that microbial communities may have varied and site-specific responses to pollution.

More work is needed to determine how these changes affect contaminant degradation and biogeochemical cycling, but initial results suggest that there is the capacity within uncontaminated communities to degrade pollutants after a period of adjustment (Fahy *et al.* 2006; Shah *et al.* 2009). However, there are mixed data from UK aquifers regarding the effect of previous exposure to pollutants on biodegradation. Some findings suggest no correlation (e.g. Johnson *et al.* 2000) whereas others identify enhanced biodegradation when there has been previous exposure to, for example, isoproturon (Johnson *et al.* 1998, 2000, 2004) or BTEX (Fahy *et al.* 2006). The key factors determining whether a bacterial community can degrade pollutants appear to be the makeup of the community itself and the groundwater chemistry (Johnson *et al.* 2004). Johnson *et al.* (2004) found that the presence of isoproturon changed the community structure, and degradation was accompanied by an increase in the proportion of a few dominant species, although different degrading taxa were found in different samples. This suggests that there is some flexibility in the community composition (functional redundancy) and that some functions can be carried out by different organisms. Water chemistry was demonstrated to play a role in providing the right environment for degradation (in chalk, limestone and sandstone aquifer samples), as addition of filtered groundwater from a fast degrading site was able to stimulate the degradation of isoproturon by bacterial populations from slow degrading limestone samples, and vice versa (Johnson *et al.* 2004).

The type of aquifer is also likely to be a key factor in the changes seen in the bacterial community in response to pollution. Johnson *et al.* (2004) examined the microbial response to isoproturon in different rock types and found that uncontaminated samples were all dominated by *Pseudomonas* (γ-Proteobacteria) (ranging from 93% of the isolates in sandstone to 49% and 43% in chalk and limestone). All other common organisms (making up more than 10% of the isolates) were either α- or β-Proteobacteria or unidentifiable. The addition of isoproturon reduced the proportion of *Pseudomonas* in sandstone and chalk but increased it in limestone. Only in chalk did another genus become more dominant (*Brevundimonas*, α-Proteobacteria) following addition of isoproturon. To summarize, these studies suggest that uncontaminated communities are often dominated by Proteobacteria, and pollution often results in a shift in the proportion of different proteobacterial groups as well as reduced bacterial diversity. This is a common finding across the small number of studies conducted, and more research is required to verify that a proteobacterial dominance is widespread. The Johnson *et al.* (2004) study is important because a range of UK aquifer rocks were investigated, although further work is required to determine whether the observed differences between these specific examples can be extrapolated to all chalk, limestone or sandstone aquifers. Overall there are relatively few studies on microbial communities, most are focused only on bacteria, and a much wider examination of all types of microbes in UK aquifers is required to understand these microbial communities.

Global studies of uncontaminated aquifer microbiology

Table 3 shows a summary of studies from other countries that have investigated the community composition of uncontaminated aquifers (mostly using molecular methods) as well as some studies that compared uncontaminated and contaminated areas of aquifers. These studies provide a context for interpreting the UK data. As with the UK studies, communities are typically dominated by Proteobacteria (usually the β- and γ-classes; e.g. Shi *et al.* 1999; Detmers *et al.* 2004; Hendrickx *et al.* 2005; Boyd *et al.* 2007; Flynn *et al.* 2012). However, in one case a community equally dominated by Proteobacteria (α- and β-) and Actinobacteria was reported (Hendrickx *et al.* 2005). Boyd *et al.* (2007) found that the most abundant class of Proteobacteria was different on different types of substrate, indicating that mineralogy influences the community structure and composition. This supports the observation of Johnson *et al.* (2004), and suggests that the matrix mineralogy influences the microbial community composition.

Other microbial groups, such as archaea, are even less well studied than bacteria in uncontaminated aquifers. One study sequenced DNA from archaea in a single borehole in the Doñana National Park, Spain. The archaeal community at a depth of 15 m had a low diversity composed only of Group 1 Crenarchaeota, whereas the community at 80 m was composed of a diverse range of members of the Euryarchaeota (López Archilla *et al.* 2007). In sulphate-reducing zones, archaea can form a significant proportion (Detmers *et al.* 2004) or even the majority of the biofilm microbial community (Probst *et al.* 2013). Flynn *et al.* (2013) reported that distinct differences could be detected between attached and suspended archaeal communities in the Mahomet Aquifer, Illinois, and that sulphate concentration had a role in influencing the community structure.

Eukaryotic microorganisms have also not been well studied, with the exception of some pathogens such as *Cryptosporidium* and *Giardia*. Laboratory studies indicate that organic contamination tends to increase protozoal population densities, but the relationship between pollution and diversity remains poorly understood (Novarino *et al.* 1997; Yagi *et al.* 2009). A more detailed understanding of protozoal communities in uncontaminated aquifers would be beneficial because these organisms could influence the response of aquifers to pollution events as they have been shown to influence various aspects of aquifer chemistry, water flow and bacterial communities. For example, in laboratory studies, inhibition of the growth of protozoa has been shown to have a positive effect on degradation of trichloroethane (Cunningham *et al.* 2009), but to have a negative effect on BTEX removal (Kota *et al.* 1999). Protozoa also have the ability to decrease clogging in wells in some circumstances (DeLeo & Baveye 1997; Mattison *et al.* 2002), and affect nitrogen cycling (Strauss & Dodds 1997) and carbon cycling

Table 3. *Summary of research into community composition in uncontaminated aquifers outside the UK*

Aquifer	Location	Microbiology	Experimental details	Reference
Quaternary fluvioglacial and aeolian materials	Doñana National Park, Spain	Most common class of bacteria at two depths was β-Proteobacteria. Almost all of the bacteria identified in the deepest sample were from the phylum Proteobacteria; the shallowest sample contained a greater diversity of organisms. Archaeal diversity was lower in the shallow sample (only Crenarchaea compared with both Crenarchaea and Euryarchaea in the deeper sample). Sulphur-oxidizing bacteria, nitrifiers, denitrifiers and methanotrophs were all identified	Groundwater sample, DNA sequencing	López Archilla *et al.* (2007)
Weathered shale (unconsolidated clay-rich saprolite)	Oak Ridge Reservation, TN, USA	Type of substrate influences bacterial community structure and diversity and which class of Proteobacteria is numerically dominant	*In situ* growth on emplaced materials, T-RFLP, DNA sequencing	Boyd *et al.* (2007)
Glacial sands and gravels	Mohamet aquifer, IL, USA	Planktonic and attached bacterial communities were distinct (only 15% of species were common to both communities). There was no significant difference in the total number of species detected in each community. IRB *Geothrix* and *Geobacter* formed >20% of total in most wells, but no more than 1% of planktonic bacterial community. Predominantly δ-Proteobacteria community was composed roughly of equal numbers of SRB and IRB	T-RFLP carried out on *in situ* growth on emplaced aquifer material and groundwater samples	Flynn *et al.* (2008)
Glacial sands and gravels	Mohamet aquifer, IL, USA	Investigated SRB; identified that SRB (*Desulfobacter* and *Desulfobulbus*) were nearly as abundant as IRB (*Desulfuromonas, Geothrix* and *Geobacter*)	*In situ* growth on emplaced materials, T-RFLP, DNA sequencing	Flynn *et al.* (2012)
Quaternary fluvioglacial and aeolian materials	Doñana National Park, Spain	No nitrifying activity or eukaryotes detected. Iron reducers are consistently more active than denitrifiers, sulphate reducers varied more over time. There was an overall trend for higher biomass, abundance and activity in summer than in winter	Groundwater sample, microscopy and BART	Velasco Ayuso *et al.* (2009)
Not described	Fort McCoy, Sparta, WI, USA	Most dominant bacterial classes were β- and γ-Proteobacteria, followed by α-Proteobacteria, SRB and high-GC Gram-positive bacteria. Contaminated samples (nitrate, toluene or BTEX) were distinct in having a greater abundance of high-GC Gram-positive bacteria relative to α-Proteobacteria. Eukaryotes or archaea were detected only in laboratory microcosms	Groundwater, aquifer samples and laboratory microcosms, FISH	Shi *et al.* (1999)
Tertiary marine sands	Garzweiler, Germany	FISH probes positively identified the community to be composed of 51.9% bacteria and 25.7% archaea. FISH identified *Desulfomaculum* as the dominant SRB not *Desulfotomaculum* and *Desulfosporosinus*, which were the only organisms identified by culture methods	Groundwater samples, FISH	Detmers *et al.* (2004)
Not described	Northern Bohemia, Czech Republic	Pristine communities were dominated by β- and γ-Proteobacteria and Actinobacteria. The communities from a pristine well, placed in a BTEX-contaminated well, changed to become similar to the surrounding community, which was composed mainly of γ-Proteobacteria and had a lower number of detectable species than the pristine aquifer community	Fresh samples and *in situ* growth on emplaced materials, T-RFLP, DNA sequencing	Hendrickx *et al.* (2005)
Not described	Illinois, USA	Attached bacterial community dominated by δ-Proteobacteria; suspended bacterial communities were dominated by other proteobacterial classes. The attached archaeal community was a distinct subset of the suspended community	DNA sequencing of water samples and emplaced materials	Flynn *et al.* (2013)

BART, biological activity reaction tests; FISH, Fluorescence *In Situ* Hybridization; SRB, sulphate-reducing bacteria; IRB, iron-reducing bacteria; PCR, Polymerase Chain Reaction; T-RFLP, Terminal Restriction Fragment Length Polymorphism; DGGE, Denaturing Gradient Gel Electrophoresis.

(Euringer 2008). Removal of protozoa from mesocosms increases bacterial numbers and has a strong influence on shaping bacterial community structure, suggesting that protozoa may control the size and composition of prokaryotic communities in aquifers (Nagaosa *et al.* 2008; Longnecker *et al.* 2009). The role of protozoa in aquifer environments is particularly interesting from an ecological point of view because, in some aquifer environments, pore size and food supply preclude larger organisms and result in a truncated food web with few predators (Gibert & Deharveng 2002; Gibert *et al.* 2009). Therefore, microeukaryotes may be important predators or competitors for prokaryotic microorganisms (Euringer 2008). The study of microeukaryotes in aquifers might provide an opportunity to test and inform general ecological theory such as predator–prey relationships and niche differentiation in a simple natural system without the complex trophic structures that often exist in other ecosystems (Reiss *et al.* 2011).

Our knowledge of fungi in aquifers is also poor, but on the basis of the limited work, they appear to be found when targeted for analysis (Euringer 2008; Lategan *et al.* 2012; Smith *et al.* 2012). They are likely to be common in shallow, anaerobic, confined aquifers, probably utilizing cellulose transported from the surface as an energy source. Their impact on groundwater processes is not well understood, but one possible role could be mobilizing pollutant-degrading bacteria, with the hyphae acting as transport routes for bacteria (Kohlmeier *et al.* 2005).

Contemporary issues in UK aquifer microbiology

Diffuse nitrate pollution

Elevated nitrate concentrations are very common in UK groundwaters (Rivett *et al.* 2007), and this is likely to continue to be a problem in the future owing to a legacy of pollution from previous nitrate inputs. Under current agricultural practices and climate conditions, nitrate concentrations are generally predicted to continue rising (Stuart *et al.* 2007). Although nitrate concentrations may be decreasing in some areas (Environment Agency 2012; Wang *et al.* 2012), in many areas they are predicted to increase, especially where the unsaturated zone is thick; for example, parts of the Scottish Devonian sandstones, the Cretaceous Chalk, the Carboniferous Coal Measures, and the Yoredale and Millstone Grit of northern England (Stuart *et al.* 2011; Wang *et al.* 2012). In other aquifers, such as the Cretaceous greensands, Zechstein Group dolomites and Dinantian limestones, the peak nitrate loading may have passed (Wang *et al.* 2012).

Denitrification is uncommon within the unconfined Chalk, Sherwood Sandstone and Jurassic limestone aquifers (Hiscock *et al.* 1991; Wilson *et al.* 1994; Feast *et al.* 1998; Rivett *et al.* 2007, 2008). Where it occurs it is mainly limited to saturated, oxygen-depleted areas (less than 1–2 $mg\,l^{-1}$ oxygen), particularly those with sufficient organic carbon to drive the reductive process (Rivett *et al.* 2008). The limited carbon supply in most aquifers means that natural processes may not be able to remediate the increasing nitrate levels typically found in the UK (Edmunds *et al.* 1987; Hiscock *et al.* 1991). Denitrifying microorganisms occur in subsurface ecosystems, but are generally restricted to anaerobic conditions (with appropriate pH, temperature, nutrients and trace minerals; Rivett *et al.* 2007, 2008). However, it must be remembered that within biofilms, anaerobic microzones may develop, which could allow anaerobic processes such as denitrification to occur even in otherwise aerobic environments (Costerton *et al.* 1995). *In situ* bioremediation strategies, such as stimulating denitrification by the injection of a carbon source (ethanol or methanol) have been proposed to assist natural attenuation, but there are unanswered questions about the biological, chemical and hydrological processes that control denitrification when using these strategies, which need to be addressed before such techniques are viable (Hiscock *et al.* 1991; Tompkins *et al.* 2001). Compared with nitrate attenuation, the pathways for ammonia removal in aquifers (biological nitrification and ion exchange) are less well studied (Buss *et al.* 2004). Biological removal is likely to be most significant in aerobic sections of aquifers with low cation exchange capacities (i.e. clay-poor aquifers or those with low metal oxides), and in particular at NH_4^+ plume margins (Buss *et al.* 2004). Although it is likely that most nitrification will occur in aerobic conditions, there is some evidence for the occurrence of anaerobic nitrification in UK aquifers (Buss *et al.* 2004; Heaton *et al.* 2005; Lee *et al.* 2006; Erguder *et al.* 2009).

Emerging contaminants

There is a growing awareness of the increase in new organic groundwater contaminants including nanomaterials, pharmaceuticals, personal care products, and caffeine and nicotine, which may affect groundwater quality (Lapworth *et al.* 2012). The full impact of these is not yet known, but because these emerging contaminants include antimicrobial or bacteriostatic compounds such as triclosan and parabens in personal care products, and benzotriazole in industrial contaminants, the impact on microbial communities and their ability to carry out ecosystem services deserves further study. These xenobiotic substances do not appear to be efficient growth substrates for microorganisms, but they can often be degraded by a process of co-metabolism where a microorganism breaks down one substrate (the pollutant) without deriving energy from it as long as another substrate is present that can be used as an energy source (Nzila 2013). This process suggests that bioremediation of these emerging pollutants may be possible and an improved knowledge of the microbial community may assist in developing new approaches to bioremediation of these emerging contaminants.

Climate change

Wilby *et al.* (2006) identified some ways in which climate change might affect the ecosystems of surface bodies in relation to the Water Framework Directive (WFD) (WISE 2012). A number of the effects may also be relevant for groundwater. These include altered metabolic rates of organisms, altered ecosystem productivity and biodiversity, and altered species assemblages. The effect of altered plant and animal distributions, altered fish migration and dispersal, and increased eutrophication and algal blooms at the surface could potentially have an indirect effect on groundwater, through changing nutrient inputs or cycling. There is considerable uncertainty about how climate change will affect aquifers, but it seems likely that minimum groundwater levels will decrease (Bloomfield *et al.* 2003), long-term pesticide accumulation will occur in Chalk aquifers (Bloomfield *et al.* 2006) and nitrate concentrations may increase (Stuart *et al.* 2011). The effects on microbial communities have not been established, but microbes may respond to different aspects of climate change such as changes in wetting and drying cycles altering nitrogen and carbon cycling (Bardgett *et al.* 2008; Borken & Matzner 2009). In addition, intensification of aquifer exploitation may alter the prevalence of pathogens in aquifers and lead to an increase in *Cryptosporidium* outbreaks (Khaldi *et al.* 2011). Although the extent of the effects that climate change will have on the hydrology or geochemistry of aquifers is still not clear, the direct and indirect effects of climate change on microbial communities could have an impact on water quality and health.

Table 4. *Some potential pathogens that may been found in aquifers (after Environment Agency 2002; Powell et al. 2002; Pedley et al. 2006)*

Organism	Occurrence/host	Disease	Notes on survival
Aeromonas spp.	Natural inhabitants of freshwater environments	No clear evidence of disease, but in high numbers can be an indicator of faecal contamination	Freshwater as habitat. Different species usually found in pristine water and sewage effluents
Campylobacter spp.	Found in livestock, birds, natural waters	Bacterial gastroenteritis	Survival in surface waters for days
Clostridium perfingens	Warm-blooded animals	Used as a faecal indicator, food poisoning	Spores are capable of surviving for longer periods than vegetative bacteria
Escherichia coli	Various warm-blooded animals	Diarrhoeal disease	Not stated
Legionella spp.	Naturally occurring in the aquatic environment	Legionnaires' disease, influenza-like illness	Grows within protozoa. Growth controlled below 20 °C
Mycobacterium spp.	Ubiquitous; present in soil, dust, water, livestock, etc.	Infections of the skin and lungs	Persistent in the natural environment
Pseudomonas spp.	Widespread in the environment	Wide range of infections. Only some species are pathogenic, others lead to odour problems	Capable of growth in relatively low-nutrient environment
Salmonella spp.	Livestock faeces	Typhoid, gastroenteritis, pneumonia, reactive arthritis, meningitis	Survival in surface waters for hours to days
Shigella spp.	Human faeces	Bacillary dysentery (shigellosis)	Hours to days
Vibrio spp.	Brackish and saline waters and sometimes freshwater, human faeces	Diarrhoea, gastroenteritis, septicaemia, *V. cholerae* causes cholera	Some species will survive in freshwater
Yersinia spp.	Natural waters, farms, meat processing plants	Mild diarrhoea, infections, septicaemia, reactive arthritis	May be able to grow in water
Cryptosporidium parvum	Many warm-blooded animals including humans. Open water often contaminated	Diarrhoeal disease	Long surviving oocytes, more than several months
Giardia lamblias	Humans, wild and domestic animals	Giardiasis: diarrhoeal disease to more serious complications	Can remain viable for several months in cold water
Toxoplasma gondii	Cats, other wild animals and livestock	Influenza-like symptoms	Water-borne infections have been linked to infected wild cats
Coxsackieviruses	Indicator of human enteric viruses	Fever, pharyngitis, diarrhoea, aseptic meningitis	Water-borne transmission not confirmed, survive 'longer than bacteria'
Enteric adenovirus	Human faeces	Gastroenteritis, mainly in infants; respiratory disease	Survive 'longer than bacteria'
Hepatitis A Virus	Human faecal origin	Fever, nausea, liver infection	No water-borne outbreak confirmed in the UK; survive 'longer than bacteria'
Norwalk-like virus	Strains pathogenic to humans come only from human contamination	Viral gastroenteritis	No water-borne outbreak confirmed in the UK; survive 'longer than bacteria'
Rotavirus A and Rotavirus C	Human faeces	Gastroenteritis especially in infants; may subsequently be asymptomatic	Rarely water-borne; survive 'longer than bacteria'

Biofilm clogging of boreholes

Microbial processes can alter transmission of water through accumulation of inorganic salts or organic complexes, or through gas generation, and can also cause problems through corrosion of well structures (Baveye *et al.* 1998; Cullimore 2000). It appears that in electron donor-limited aquifers microbial processes decrease porosity by the production of intergranular cements, whereas in electron acceptor-limited aquifers porosity is often increased by dissolution of mineral grains by microbial processes (Chapelle *et al.* 1997). Reduction in flow due to biofilm formation is a well-established process (e.g. Harrison *et al.* 2011; Wragg *et al.* 2012). In laboratory experiments using chalk samples, biofilm formation has been associated with bioremediation of organic contaminants (Arnon *et al.* 2005*a*,*b*). It has also been suggested that interventions to improve denitrification by supplementing the aquifer with a carbon source may result in clogging with biomass or microbially produced gases (e.g. CO_2 and N_2). Historically, clogging (through iron deposition) was thought to be primarily due to (electro)chemical processes because aquifers were assumed to be sterile, and bacteria found in wells were thought to result from contamination during drilling (Howsam 1988). The role of 'iron' bacteria and other bacteria in deposit forming as well as a host of other bacteria has now been recognized by a number of field and laboratory studies (Baveye *et al.* 1998). Brassington *et al.* (2009) suggested that iron-oxidizing bacteria (probably *Gallionella* and *Leptothrix*) can increase borehole drawdown by 20% (measured at Warrington in a Sherwood Sandstone aquifer), although reductions of up to 95% capacity have been reported for other areas of the same aquifer. Hydrogen peroxide can restore flow, probably via the biocidal effects of the hydrogen peroxide and physical scrubbing by oxygen bubbles. Although effective in reducing biofilm clogging, this approach may have other undesirable impacts on the microbial community as a whole.

A better knowledge of the microbial interactions involved in biofilm clogging is required to answer questions such as: How far beyond the well or formation screen does clogging exist? How can the pump be clogged but not the screen or vice versa? What determines which wells will clog? The answers are likely to depend upon factors such as differences in construction, design, or condition of wells (Howsam 1988), pH (Kirk *et al.* 2012), availability of organic matter (Cullimore 2000) water turbidity, and total nitrogen (Pavelic *et al.* 2007).

Pathogens in aquifers

Perhaps the most pressing concern about microorganisms in aquifers remains the potential impact of the transport of pathogens into drinking water. A summary of some of the microorganisms that could be present in UK aquifers along with their

native habitats and notes on their survival in water is shown in Table 4. Waste from farm animals and human sewage treatment are the main sources of pathogen contamination. However, many pathogens have been detected in native wild animals in the UK; for example, rats, mice or birds (Bouchier 1998; Simpson 2002). These may not be considered to be important reservoirs of disease, but it could be argued that the presence of pathogens in native animals and their inevitable transit into water courses means that pathogens (albeit in low numbers) could be considered to be part of the indigenous transient community in aquifers. The traditional view that aquifers provide a safe environment, free from potential pathogens, is being questioned (e.g. Pedley *et al.* 2006). There is evidence to indicate that faecal bacteria can travel through aquifers (e.g. to depths of up to 90 m in Triassic sandstone in Nottingham and Doncaster) and that both human enteric viruses (including pathogens) and faecal indicator bacteria are widespread at low concentrations (Powell *et al.* 2002, 2003; Morris *et al.* 2006). As well as being widespread, viruses can be persistent: in groundwater taken from Permo-Triassic sandstone in Birmingham, some viruses were detectable by polymerase chain reaction (PCR) for over 2 years after inoculation, although their infectivity based on plaque assay lasted only for 238 days (Charles *et al.* 2009). In other cases, sandstone aquifers act as filters of microorganisms, and pathogens are undetectable in all but the shallowest samples (Gooddy *et al.* 2001). Pathogen transport is a particular problem in karst aquifers with rapid flowpaths (e.g. Tranter *et al.* 1997). Because of their smaller pore spaces chalk aquifers can filter pathogenic organisms very effectively (Gooddy 2002; Gooddy *et al.* 2001), although where well-connected fissure and conduit networks are present rapid pathogen transport can occur (e.g. Dussart-Baptista *et al.* 2003).

Conclusions

Although there has been considerable work on certain areas of aquifer microbiology such as remediation of contaminated aquifers and pathogen transport through aquifers, our understanding of the indigenous microbial communities in UK aquifers and the contribution they make to maintaining good groundwater quality remains limited. It is clear that microbial communities play an important role in aquifer biogeochemical processes (Griebler & Lueders 2009), but globally these processes remain poorly understood. In England and Wales the Environment Agency carries out routine monitoring for nutrients and pesticides in aquifers, but there is currently no routine monitoring programme for microorganisms, except *Cryptosporidium*, which is routinely monitored by water companies where it has been identified as a high risk (Environment Agency 2005). There would be much to be gained from conducting a systematic nationwide survey to determine the baseline diversity that occurs at present in UK aquifers. This should allow the major drivers of microbial diversity and the factors that structure these communities to be determined, and would be of international significance because globally there have been few studies of microbial communities in aquifers not affected by serious pollution. A better understanding of the microbial diversity in aquifers and the response of microbes to pollution may also allow areas of aquifers that require greater monitoring or management input to be identified, as well as areas in which communities have greater resilience to, or protection from, detrimental anthropogenic activities. Only through an understanding of the microbiology of aquifers in their current state can we interpret the changes that result from future human impacts.

Some key areas for research include understanding the broad controls that influence microbial community structure across a range of aquifer types in the UK. This basic information should inform applied research aimed at maintaining water quality, developing strategies to improve bioremediation, monitoring pathogen transport, and preventing problems such as iron-oxidizing bacteria reducing water flow and causing biofilm clogging of boreholes. Studies of the impact of macro-invertebrates on microbial communities are also needed to improve understanding of aquifer biogeochemical processes. Additionally, there are questions about how much functional redundancy exists within microbial communities, allowing continued biogeochemical functioning following changes in community structure.

Examples of systematic surveys that have an established framework upon which future research on microbial function and local diversity can be built include the recent survey of British soil bacterial communities (Griffiths *et al.* 2011) and a national survey of New Zealand groundwater microbiology (Sirisena *et al.* 2013), the first of its kind ever undertaken. A similar survey of UK aquifers would provide a valuable contribution to understanding the biodiversity of microorganisms and the roles they play in these environments, placing the UK at the forefront of this important area of research.

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