

Hyporheic Network



The Hyporheic Handbook

A handbook on the groundwater–surface water interface and hyporheic zone for environment managers

Chapter 6 Microbial and invertebrate ecology

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Acknowledgements

The Hyporheic Handbook is a product of the Hyporheic Network.

The Hyporheic Network is a Natural Environment Research Council (NERC) funded Knowledge Transfer Network on groundwater – surface water interactions and hyporheic zone processes.

The authors wish to acknowledge the support and assistance of many colleagues who have contributed to the review, production and publishing of the Handbook: Joanne Briddock, Mark Cuthbert, Thibault Datry, John Davis, Rolf Farrell, Richard Greswell, Jan Hookey, Tim Johns, Dave Johnson, Arifur Rahman.

We are also very grateful for the support and efforts of the Environment Agency Science Communication department, in particular, Stuart Turner and our editor Hazel Phillips.

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6 Microbial and invertebrate ecology

6.1 Introduction

Microbial ecology processes underpin the key functional biogeochemical components of the hyporheic zone. Therefore the microbial ecology is crucial to the understanding the role of the HZ in larger ecosystems, observing change and assessing the potential for natural attenuation.

This review examines the microbial ecology of the HZ in the context of global processes and cycles as well as specific characteristics within the HZ. It discusses the applications and limitations of them modern methods available to investigate HZ ecology and finally contributes to gaps in knowledge that may be relevant to management policies as discussed in Chapter 2. This complements the chapter on 'Biogeochemistry and hydroecology of the hyporheic zone' (Chapter 5) which considers the HZ as a habitat and refugia for a range of organisms and as an area of biogeochemical cycling of nutrients and contaminants.

6.2 Microbial ecology of protozoa, fungi and bacteria: global scale

Microbial ecology is the relationship of microorganisms with one another and with their environment. It concerns the three major domains of life - Eukaryota, Archaea, and Bacteria — as well as viruses. Microorganisms, are present in virtually all of our planet's environments, including some of the most extreme and hence impact the entire biosphere. Microbes, especially bacteria, often engage in relationships with other organisms, and these relationships affect the ecosystem. Microbes are the backbone of all ecosystems (Falkowski et al., 2008), but even more so in the zones without light where energy cannot come from photosynthesis. In these zones, chemosynthetic microbes provide energy and carbon to the other organisms. Other microbes are decomposers, with the ability to recycle nutrients from others waste products. These microbes play a pivotal role in biogeochemical cycles - the nitrogen cycle, the phosphorus cycle and the carbon cycle all depend on microorganisms. In addition, microbes exhibit high degrees of genetic flexibility due to the high level of horizontal gene transfer among microbial communities which means they can adapt according to the prevailing conditions. As a result they exhibit through their diversity and genetic acquisition mechanisms, a high degree of functional redundancy that gives a high degree of functional stability (Torsvik and Øvreås, 2002). Microbial ecology is underpinned by its inherent morphological, structural, metabolic, behavioural and ecological diversity.
(http://www.biotechnology.uwc.ac.za/teaching/BTY327/bty327_lec3_07.ppt).

6.3 Global scale biogeochemical processes and cycles

The Earth is essentially a closed system for matter and all the elements continually cycle through Earth's systems - the atmosphere, hydrosphere, biosphere, and lithosphere - on time scales that range from a few days to millions of years. These biogeochemical cycles comprise biological, geological, and chemical processes and each takes many different pathways and has various reservoirs, or storage places, where elements may reside for short or long periods of time. Each of the chemical, biological, and geological processes varies in their rates of cycling depending on its chemical reactivity.

There is no scope for reviews on individual biogeochemical cycles (McClain et al., 1994) but an appreciation of the N cycle provides an oversight into the cyclical nature of the other cycles. Nitrogen exists in a variety of forms in natural systems and its compounds are involved in numerous biological and abiotic processes. Nitrogen, in its gaseous form of N_2 , makes up almost 80 percent of the atmosphere, which constitutes the major storage pool in the complex cycle of nitrogen through ecosystems. Some of this gas is converted in soils and waters to ammonia (NH_3), ammonium (NH_4^+), or many other nitrogen compounds. The process is known as nitrogen fixation, and, in the absence of industrial fertilisers, is the primary source of nitrogen to all living things. Biological nitrogen fixation is microbially-mediated. Once nitrogen has been fixed it can either be oxidised for energy (nitrification) or assimilated by an organism into its biomass (ammonia assimilation). Nitrogen, fixed as proteins, eventually returns via the nitrogen cycle to its original form of nitrogen gas in the air. The process of decomposition through denitrification generates mainly N_2 with nitrous oxide (N_2O) in much smaller quantities (<10%). The disruption of the nitrogen cycle by human activity plays an important role in a wide-range of environmental problems including the contamination of groundwater when nitrogen oxides are chemically transformed back to either N_2 or to nitrate or nitrite compounds causing river management problems.

6.4 Hyporheic zone as a biological entity

In the HZ biogeochemical cycling, microbial ecology and the ecology of higher animals should not be considered as discrete compartments but rather as an interactive system. This is often best described as the microbial loop (Figure 6.1) showing flow of carbon from the microbial level and its release to higher trophic levels (Figure 6.1). Feris et al. (2003) describe the hyporheic zone as a spatially and temporally dynamic ecotone which provides connectivity between terrestrial, groundwater, and lotic habitats. It lies beneath the channel of a stream, often extending great distances laterally in the subsurface, and is an essential part of lotic ecosystems. The microbial transformations of dissolved and particulate nutrients taking place in the hyporheic zone have been shown to influence both macro-invertebrate and algal assemblages and may play a role in the productivity of riparian vegetation. This zone supports an active microbial community involved in nutrient cycling and nutrient retention and this community constitute the majority of the biomass and activity in lotic ecosystems (Craft et al., 2002, Fischer and Pusch, 2001, Pusch et al., 1998) and may contribute up to 96% of the ecosystem respiration (Naegeli and Uehlinger, 1997). Therefore, Feris et al. (2003) noted that the microbial transformations in the HZ of dissolved and particulate nutrients influence both the macro-invertebrate and algal communities and furthermore influence the productivity in lotic systems and beyond (Barlocher and Murdoch, 1989, Jones et al., 1995a, Pusch et al., 1998).

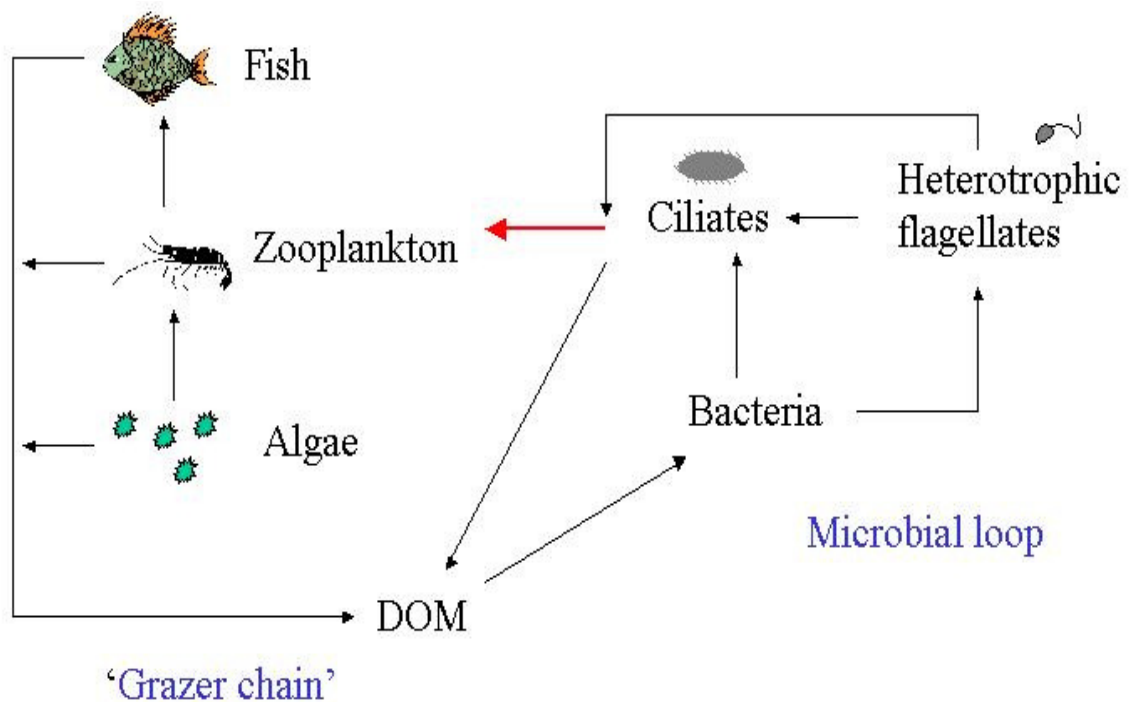


Figure 6.1 Microbial loop (omitting viral component). The microbial loop is important as it reintroduces dissolved organic carbon back into the food web.

6.5 Microbial ecology in the hyporheic zone

Little is known about the microbial activities in sediments of, for instance, large lowland rivers, despite their potentially high influence (Fischer et al., 2005, Boulton et al., 1998). Fischer and co-workers suggest that their presence causes the sediment to act like an animal's liver, as a detoxifier through the nitrogen and carbon cycles (Fischer et al., 2005). Therefore the microbial ecology is crucial to the understanding the role of the HZ in large ecosystems.

6.6 Physical location of microbes

Microbial diversity can be morphological, structural, metabolic, behavioural or ecological, but this is driven by the habitat. In most environments certain groups or species dominate, usually in response to prevailing conditions such as temperature or redox potential, however, other organisms for whom the environment is not optimal can still grow there. The community structure may change in terms of the total number of species but the relative proportions of species may also change, with a few species (often linked by functionality) becoming numerically dominant as they benefit from the prevailing chemical environment. If the environment changes again then other species

will become dominant, some may be 'new' arrivals' but the population often still reflects the earlier dominant species. Most microbes in the environment are substrate limited and respond by size reduction, lower activity and increased cell division time (when they divide they do so by reductive cell division, hence the smaller size). Increases in nutrient levels reverses this process (apart from culture on high nutrient media) (Torsvik and Øvreås, 2002).

The microbes in HZ can be found in plankton form (in free flows larger flows), in interstitial areas (that allow both settling and movement) and in biofilms (a complex matrix of polysaccharide, cell products and a diversity of microorganisms). In planktonic form numbers of free 'swimming' bacteria are often related to the sediment load in that water, and are typically in the range of 10^6 - 10^8 bacteria per ml river water. For bacteria in interstitial water, for example, it was shown that in the Töss River (gravel-bed stream, Switzerland) bacterial abundances ranged between 1.6×10^5 to 4.8×10^8 cells/ml interstitial between depths (Brunke and Fischer, 1999). The upper range was two orders higher than most lake waters. This change with depth was significantly modulated by the type of hydrological exchange. The bacterial carbon portion of total Particulate Organic Carbon (POC) varied between 0.06 % and 5.3 % and tended to decrease with depth. Bacteria were most numerous at sediment depths where inflow of stream water occurred, but had been attenuated. Bacterial production was highest in hyporheic interstices dominated by surface water inflow. Bacterial abundance and production were strongly correlated to interstitial particulate organic matter; the best predictor for both was the content of particulate nitrogen, explaining 75 % and 72 % of the variation, respectively. Abundance of several hyporheic invertebrate taxa, taxa richness and total invertebrate density were positively correlated to bacterial abundance and production. The hyporheic fauna exhibited a gradient between interstitial positions influenced by surface water and those dominated by phreatic ground water. The coupling of sediment depth and hydrological exchange type revealed flow path connections as being superimposed vectors in determining hyporheic abiotic and biotic gradients.

6.7 Biofilms

When assessing microbial activity at a process level or microbial diversity at a community level, biofilms shouldn't be ignored over ease of analysing water samples.

Biofilms vary in nature from larger visible streamers (such as those found in hot springs) to microscopic mucus coating around sediment particles. These biofilms will account for the majority of microbial biomass and comprise a diversity of microorganisms arranged within a complex extracellular polysaccharide often with a thickness that isolates the bulk of the matrix from the immediate environment, limits gaseous and chemical exchange and consequently will generate redox gradients within the structure. Biofilms are dynamic, they undergo recruitment and loss (usually through sloughing processes), and they often have sufficient 3D structure to create flows in micro-channels. A number of cycles co-exist within biofilms (e.g. sulfur and nitrogen (Ramsing et al., 1993)). The redox potential within is sufficient to allow methanogenesis to occur despite external flows being oxygenated. Often synergies develop as specific niches are created by producers and consumers e.g. methane, methanogenesis, and methane oxidation.

6.8 Microbial diversity (functional groups)

Microbial diversity can also be considered in the form of functional groups. Microbes, and bacteria in particular, show considerable functional and metabolic diversity which enables them to derive energy from sources other than organic carbon and to use other electron acceptors other than oxygen, hence their cosmopolitan abundance in diverse and extreme habitats. Heterotrophic bacteria, tend to dominate systems, particularly when oxygen is available. These utilise organic carbon directly and can consume simple to complex compounds. However, once oxygen is consumed and anaerobic conditions below the surface layers prevail, carbon cycling processes depend on the redox environment (see Figure 6.2). Decomposition is faster under aerobic conditions than that occurring in anaerobic zones.

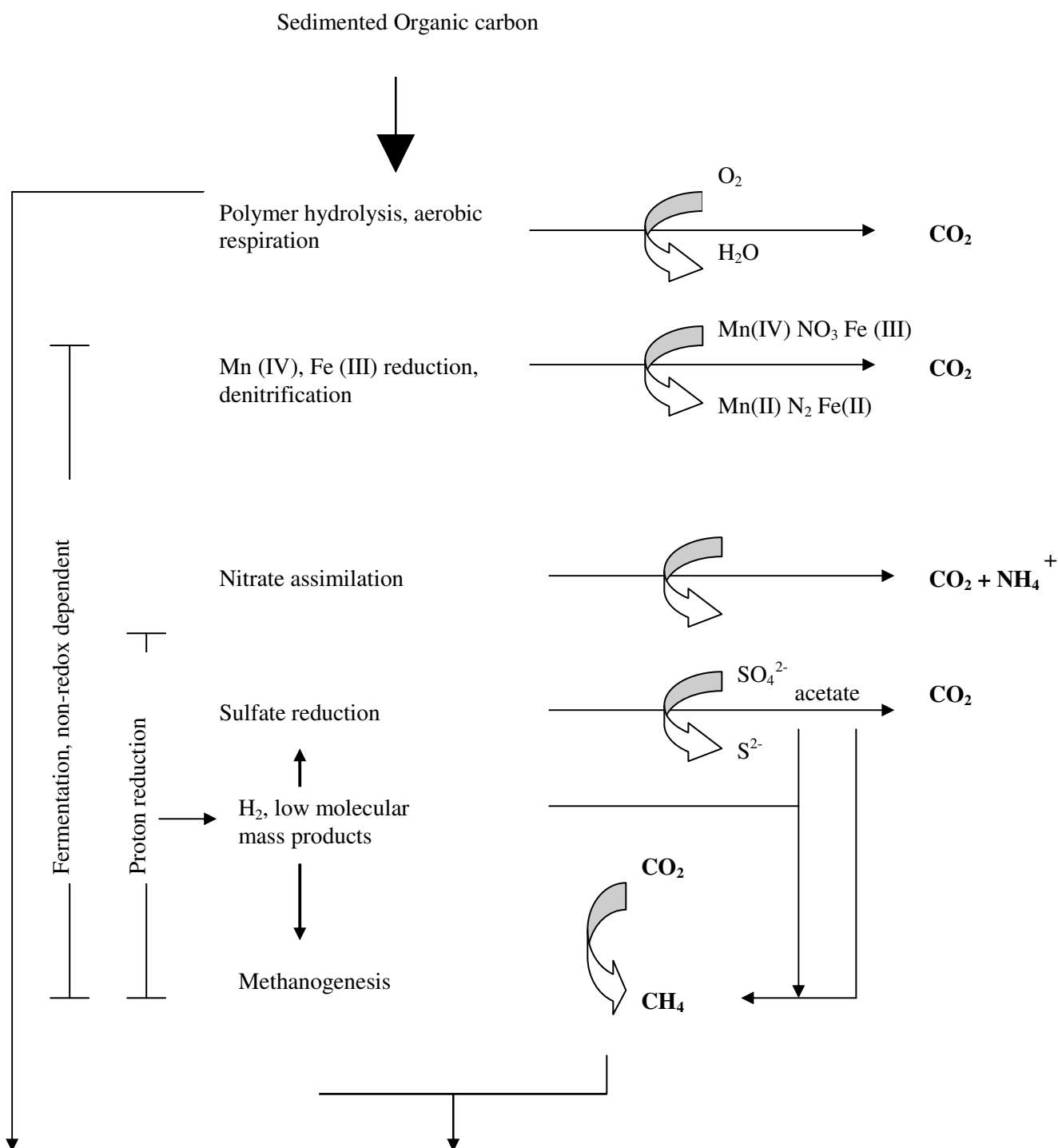


Figure 6.2 Depth distribution and interactions of decomposition process in buried sediments (from Jones (1985)).

6.9 Decomposition under anaerobic conditions

It is very important to view the HZ environment as a series of interacting processes rather than a single process such as methanogenesis (see Figure 6.2).

Carbon turnover in the presence of electron acceptors involves the interaction of the carbon, nitrogen and sulfur cycles. In the absence of electron acceptors other methods of energy conservation become significant (Triska et al., 1993). Inter-species hydrogen-transfer permits the use of otherwise energetically unfavourable reactions. Conservation of the energy in polyphosphate bonds and reduction of iron (II) and manganese (IV) to produce more energetically favourable end-products are mechanisms available in benthic environments. The reduction of carbon dioxide to acetate is a potential hydrogen sink in some circumstances (Figure 6.2). Methanogenesis occurs at the lowest redox potential (e.g. -200mV compared with methane oxidation at +250mV), with previous processes having depleted oxygen, iron (III), sulfate, nitrate and manganese with the concomitant accumulation of carbon dioxide (a by-product of most catabolic processes) and hydrogen (Figure 6.2).

Denitrification processes have been identified in the HZ (Triska et al., 1993), this process reduces nitrate is converted to nitrogen gas by a number of steps allowing the coupled oxidation of organic compounds in the presence of depleted oxygen. The source of nitrate may be through inflowing water but *in situ* nitrification is also possible thus the N cycle is coupled directly and can occur in anaerobic pockets within a predominantly aerobic zone or within biofilms. It is best observed, as a process, in a hypereutrophic pond (Finlay et al., 1997) where processing at times is so fast that nitrate is not detected. The contribution of denitrification in HZ has not been determined.

Little information exists on methanogenesis in HZ. The process was measured in anaerobic sediments beneath a stream channel and found that it dominated the anaerobic sediment but also was active in the aerobic sediment (Jones et al., 1994, Jones et al., 1995b). This highly contradictory finding suggests anaerobic pockets still exists in the sediment or within biofilms despite methanogens being extremely oxygen sensitive.

6.10 Chemolithotrophy: energy via inorganic compounds

It is apparent that the HZ is biologically active with the potential to carry out processes which occur across the full redox gradient but it is important to assess processes as part of the whole system rather than in isolation.

The HZ is not considered an extreme environment but does differ from its surrounding environment as it is the interface between reduced groundwater and oxidised surface waters. Therefore it is an ideal environment for functionally active chemolithotrophic bacteria which derive their energy from oxidation of inorganic materials like iron, sulfur,

ammonia and nitrite (Storey et al., 1999). However, the most well characterised functional group of bacteria in the HZ are those involved in nitrification converting ammonia via nitrite to nitrate; (Triska et al., 1993). Their activity depends on prevailing ammonia concentrations in the HZ and this is reflected in their relatively low abundance compared with heterotrophic bacteria where dissolved organic carbon is often 100 fold higher than ammonia (Storey et al., 2004). Jones et al. showed chemoautotrophic production was approximately 1% of the total and of that less than 30% was nitrification (Jones et al., 1994). Reliance on these figures is compromised by the degree to which the DOC is microbially available as this has not been defined (Storey et al., 1999). Despite their relatively low abundance nitrifiers can have a significant effect on oxygen consumption sometimes demanding 50% of the available oxygen for productivity which is significantly less than that of the heterotrophs (Storey et al., 1999).

In the absence of ammonia, other bacteria are able to utilise iron (II), manganese (II) or reduced sulfur (e.g. elemental sulphur or sulfide) as their sole energy source. Bacterial oxidation of iron is favoured at a reduction potential of around 300mv (see figure 6.2) under slightly acidic conditions (Hacket and Lehr, 1985). These conditions are critical to the process but exist within the HZ (Hacket and Lehr, 1985). Manganese oxidation is a bacterially mediated process. Wielinga and co-workers showed that a high proportion of culturable bacteria from the HZ had this capacity yet their numbers, as with nitrifiers and ammonia, are influenced by the prevailing manganese concentration (Wielinga et al., 1994). Manganese and iron oxidation yield less energy than ammonia oxidation and their presence is greatly influenced by redox state of the feeding waters (McClain et al., 1994). Sulfur often occurs in groundwater at high concentrations, most commonly as sulfate. Reduced sulfur requires a low redox potential (see Fig 2) not commonly seen in HZ although possible in the near stream zone. Sulfide oxidation is more energetically favourable than nitrification, with higher sulfur than nitrogen in groundwater, sulfide oxidisers may contribute more to HZ productivity if conditions are favourable (Storey et al., 1999).

Hlaváčová et al. (2005) examined the HZ in a holistic manner. They studied the distribution of dissolved oxygen, nitrate, sulfate, carbon dioxide and dissolved organic carbon (DOC), acetate and lactate in the stream and interstitial water along the subsurface flow path in the hyporheic zone of a small lowland stream (Hlavacova et al., 2005). Sediments were found to act as a source of nitrous oxide and methane. Interstitial methane concentrations were significantly higher than those from surface water, and were significantly lower in the relatively well oxygenated down-welling zone than in the rather anoxic upwelling zone. The interstitial concentrations of oxygen, nitrate and sulfate showed significant decline along the subsurface flowpath, while concentrations of carbon dioxide, nitrous oxide, DOC, acetate and lactate remained unchanged. Nitrous oxide production potential reached 71-100% of denitrification potential. This demonstrated that that respiration of oxygen, nitrate and sulfate and methanogenesis may coexist within the hyporheic zone and that anaerobic metabolism is an important pathway in organic carbon cycling in the Sitka stream sediments (anaerobic microbial metabolism in hyporheic sediment of a gravel bar in a small lowland stream (Hlavacova et al., 2005).

6.11 Bacterial Community identity

Limited information shows that bacterial communities have a high resilience and respond to changing conditions.

Very little information exists on community structure or dynamics of bacteria that carry out processes in the HZ. This may be related to limitations of methodological strategy and or lack of defined research programmes. Where processes are identified, it is not unrealistic to assume bacteria identified with similar function are active in the HZ. For example, 18 genera of iron oxidising bacteria have been identified in wetlands springs, identified in nature by their orange coloration (Hacket and Lehr, 1985) so there is an expectation that they will occur in HZ receiving reduced groundwaters. where the absence of oxygen has preserved the iron valency (Storey et al., 1999) . Similarly nitrifying bacteria identified by (Whitby et al., 1999; 2001) may be present depending upon prevailing conditions and ammonia concentration which is known to drive the local diversity of ammonia oxidisers. The most extensive information of HZ bacterial structure is provided by Feris (Feris et al., 2003a; 2003b; 2004a;2004b) who used molecular methods for analysis. They identified bacterial communities in a perturbed HZ system exposed to heavy metal where the overall biomass showed no correlation with metal content. The community structure did respond to exposure with positive shifts in gamma-proteobacteria, beta- proteobacteria responding negatively, and alpha and cyanobacteria both unaffected. They therefore showed that the community responded in structure but not overall biomass to the presence of heavy metals. Furthermore HZ microbial communities responded rapidly to exposure with heavy metals (Feris et al., 2004b) and the response was greatest during the seasons when growth potential was highest (Feris et al., 2004a)

6.12 Fungi

Fungi play an important but as yet unquantified role in the HZ.

Both fungi and bacteria are metabolically very versatile yet fungal contribution to productivity is universally understudied as in the case of HZ. Early work by Barlocher and Murdoch (1989) reported the occurrence of fungi but not their distribution. Later Barlocher examined fungi from the hyporheic zone of a springbrook in southern Ontario, Canada (Barlocher et al., 2006). The number of identified species significantly decreased with depth, and was highest on deciduous leaves and lowest on wood. Season had no significant effect on species numbers. Molecular analysis showed phylotypes were significantly affected by season but not by depth. Both season and section level significantly affected the relative frequency of the 10 most common phylotypes; and consistently raised temperature lowered diversity (Barlocher et al., 2008). It was suggested that aquatic hyphomycetes and other fungi readily disperse within the hyporheic zone, and that their relative scarcity in this habitat is due to a lack of suitable substrates. Bacterial and fungal numbers decrease with decreasing particle size (Sinsabaugh and Findlay, 1995). Therefore fungal numbers and activity may be linked to presence of coarse matter. Fungi are often associated not only with coarse particles but have also been found in close association with bacteria in biofilms (some species can utilise dissolved organic matter. (Barlocher and Murdoch, 1989)

6.13 Protozoa

Protozoa are relatively better studied than bacteria and fungi and play an important role in the continuity of the food web.

Protozoa usually range from 10–50 μm , but can grow up to 1 mm. They exist throughout aqueous environments and soil, occupying a range of trophic levels. As predators, they prey upon unicellular or filamentous algae, bacteria, and microfungi and they play a role as both herbivores and consumers in the decomposer link of the food

chain. Protozoa also play a vital role in controlling bacteria populations and biomass. As components of the micro- and meiofauna, protozoa are an important food source for microinvertebrates. Thus, the ecological role of protozoa in the transfer of bacterial and algal production to successive trophic levels is important. Few data exist on protozoa and the HZ but there is some knowledge about enumeration, distribution, and grazing.

A number of studies have examined depth distribution and showed that species richness varies both spatially and temporally (Andrushchyshyn et al., 2007). They examined ciliated protozoans (phylum Ciliophora collected from five sites in a shallow groundwater system in southern Ontario, Canada) and showed that species richness was high with 170 ciliate species belonging to 89 genera identified. Highest species richness (86) occurred between 20 and 60 cm, and typically decreased below 60 cm. Leven et al. showed ciliate numbers and biomasses were greatest at the sediment surface and declined significantly with increasing sample depth with mean abundances varied between 0 and 895 cells per ml of sediment, and the mean ciliate biomass ranged between 0 and 5.3 mg of carbon per ml of sediment. Similarly Packroff and Zwick investigated Ciliata in sandy bed sediments (Packroff and Zwick, 1998). Abundance varied greatly, the observed maximum being about 4000 per ml sediment. There was no longitudinal gradient of ciliate abundance. Seasonal variation was apparent. Andrushchyshyn et al. (2007) showed ciliate densities were also seasonally and spatially variable with densities lowest in winter. Packroff and Zwick showed no clear seasonal pattern at one site; but at the other three sites it peaked in spring and early summer. Cleven (2004) and, Cleven and Konigs (2007) showed abundance and biomass varied seasonally, with maximum values in late autumn and early winter and minimum values in early summer.

With respect to diversity Andrushchyshyn et al. (2007) showed that at all depths, small (< 50 µm) bacterivorous ciliates typically dominated, but omnivorous and predatory species were also present (combined, up to 30% of the average density). Several ciliate genera, traditionally considered planktonic, occurred at low densities from 40 cm down to 100 cm. The main factors influencing the shallow groundwater ciliate communities were depth and temperature with dissolved oxygen also appeared to influence these communities in that they typically comprised genera that preferred either low-oxygen or anaerobic conditions; they also showed abundances of both flagellates and ciliates were higher in the hyporheic zone than in surface sediments. Flagellates were distributed at all depths over the sampling period, but densities were highest at 30-40-cm depth before a spate. Ciliate depth distribution also showed high densities from 10 to 40 cm and patches of high abundance occurred at 30-40 cm. Preliminary estimates of resilience suggested that flagellates were more resilient than ciliates and that large flagellate individuals and ciliates <50 µm in the hyporheic zone had higher resilience values than those in the streambed surface sediments. Several ciliate genera, traditionally considered planktonic, occurred at low densities from 40 cm down to 100 cm (Andrushchyshyn et al., 2007). At all depths, small (< 50 µm) bacterivorous ciliates typically dominated, but omnivorous and predatory species were also present (Andrushchyshyn et al., 2007, Packroff and Zwick, 1998).

Neubacher and co-workers showed grazing by ciliates has no influence on abundance and growth of nitrifying bacteria and nitrification as they showed no significant selective grazing or food preferences for any bacterial taxon or any size class or morphotype (Neubacher et al., 2008). On the bacterial side, neither an active defence mechanism of the nitrifying bacteria against ciliate grazing, such as changes in morphology, nor competition for resources were observed (Neubacher et al., 2008). Konigs and Cleven also suggest that interstitial ciliate grazing impact on bacteria biomass and production was too low to represent an important link in the carbon flow of the hyporheic zone

under study (Konigs and Cleven, 2007). Despite this they showed that ciliate generation times ranged between 4.8 and 9.9 days with ingestion rates for *C. margaritaceum*, other small scuticociliates and *Pleuronema* spp being 26, 50 and 86 bacteria per individual predator per hour, respectively (Konigs and Cleven, 2007).

6.14 Microbial pathogens in HZ

Microbial pathogens in HZ are understudied and this has issues for river management particularly with respect to recreational bathing and freshwater and marine environments.

There is a vast amount of information on the presence and survival of microbial quality indicators in groundwater and streams, as well as rivers and lakes (John and Rose, 2005), but limited information relating to the HZ. Of that, Halda-Alija showed the total number of bacteria, cultivated heterotrophic aerobic bacteria, and enteric bacteria showed significant differences between winter and summer. The cultivated numbers of heterotrophic aerobic bacteria and enteric bacteria were significantly more abundant in summer than in winter. The abundance of enteric bacteria was 12.9% in an upwelling zone and 9.8% in a downwelling zone in summer. Most of the enteric bacterial strains were identified as *Enterobacter cloacae* and *E. agglomerans* by in summer and fall showed significant spatial variation and were heterogeneously distributed along the stream. Temperature, inorganic nutrients, and occurrence of anoxic zones affected the distribution of enteric bacteria. Transport between groundwater and HZ depends on factors such as cell size, size of source, porosity, pore size of sediments and degree of entrapment on surfaces or in biofilms and grazing rates (Pickup et al., 2003). However, surface flows, river entry and settling as typified by *Cryptosporidium* oocysts, allows entry of pathogens into HZ. Furthermore pore water flow, particle filtration and gravitational settling, all parameters used to predict solute and colloid exchange, may be useful for 'biocolloids' such as *Cryptosporidium* oocysts (e.g. see (Searcy et al., 2006). Exchange mechanisms between flow and sediment probably regulate pathogen flow in rivers through deposition, only to create a reservoir of pathogens in the sediment that may be released in high numbers during high flow events (Searcy et al., 2006, Pickup et al., 2003).

6.15 HZ is a biological entity

As stated prior to this section, it is important that HZ should be viewed as a complex biological entity functioning at a number of trophic levels typified by the microbial loop (Figure 6.1: Falkowski et al., 2008). Leichtfried (2007) although describing lotic systems showed that the complexity and faunal-associations were still relevant in the HZ. He stated:

'Organic matter is the basic source of energy for consumers in ecosystems. Most of the organic matter is allochthonous, The energy content of unprocessed organic matter is not readily available to all consumers; it has to be processed by the microbial community. Microorganisms are most active in biofilms, comprised of fungi, bacteria, protozoa etc., and their organic excretions attached to surfaces. The colonizable surface area in sediments is negatively correlated with the grain size. Therefore, the largest amounts of organic matter are likely to occur in small grain size classes, which shows that biofilms are an important component of the organic matter pool. Most of the meiobenthic species, which play also a very important role in these processes, are

closely connected to biofilms. These and their associated communities are doubtless an important food source for benthic consumers. The main energy pathway passes from organic matter (either particulate or dissolved) to the microbial community in biofilms, which transforms the organic matter and makes it available and palatable to benthic consumers. Wherever the benthic community is living, either in bed sediments, the energy stored in biofilms or their associated communities is mostly used'

Furthermore Storey et al. (1999) predicted that the biofilm growth form of interstitial micro-organisms will create a variety of microniches, allowing coexistence of a great diversity of microbial types, and promoting the activity of some otherwise poor competitors. It is further predicted that the confluence of reduced groundwaters and aerobic surface waters will favour chemolithotrophic processes in the HZ, but that these will contribute significantly to hyporheic production only if surface water is very low in dissolved organic carbon, or the groundwater is extremely reduced, such as by the influence of riparian wetlands. A variety of anaerobic respiratory pathways, such as nitrate, iron (III), sulfate and even methanogenic respiration will be employed in the HZ, with biofilm dynamics permitting these to occur even in aerobic sediments. Anaerobic pathways may account for a significant proportion of total hyporheic organic matter mineralization.

6.16 Investigating microbial ecology of HZ

6.16.1 Overview

Molecular techniques are now available to answer hypothesis-driven HZ science. Understanding the limitations of these approaches is as important as their application.

Information on microbial ecology of the HZ is sparse and comprises observational rather than functional ecology. The availability and ease of application of classical and molecular microbiological methods is leading to more detailed studies that link the exploration of microbial community structure, with not only their function, but with their response and resilience to perturbation by a number of chemical challenges. This section explores the methods available but avoids detailed methodology and some limitations that are important to factor into larger scale interpretation of microbial responses. Molecular and classical microbial approaches that can be applied to the HZ are summarised in Figure 6.3 (Head et al., 1998) and reviewed by Pusch et al. (1998). All procedures require sampling and sample preparation which is a crucial step in any analysis and often determines the success of any down-stream analytical procedure. Classical methods include growth on solid or liquid media supplement by co-factors and single or multiple carbon sources, incubation at relevant temperatures, purification and subsequent identification; direct counts are achieved by microscopy on unstained, non-specifically stained or specifically stained cells (Pickup, 1995, Pickup et al., 2003). Non specific stains such as DNA stains allow total bacteria to be counted whilst specific stains such as viability dyes, molecular fluorescent probes or antibodies allow specific cells, at a species or group level, to be observed.

Molecular methods (Figure 6.3) based on the extraction of DNA, whether from an environmental sample or a culture, require sample processing involving lysis to generate a DNA extract that is assumed to be representative of the sample under analysis. The DNA extract can then be analysed by a number of routes. The most favoured is amplification of specific target sequences, often the 16S rRNA gene, by polymer chain reaction protocols (PCR: (Head et al., 1998). Once completed amplified DNA can be subjected to cloning and sequencing and sequences can then be

compared with DNA sequence libraries to allow the identity of the sequence relative to other clustered sequences to be determined. From this, function can be inferred if the sequences are corroborated with those from organisms of known function.

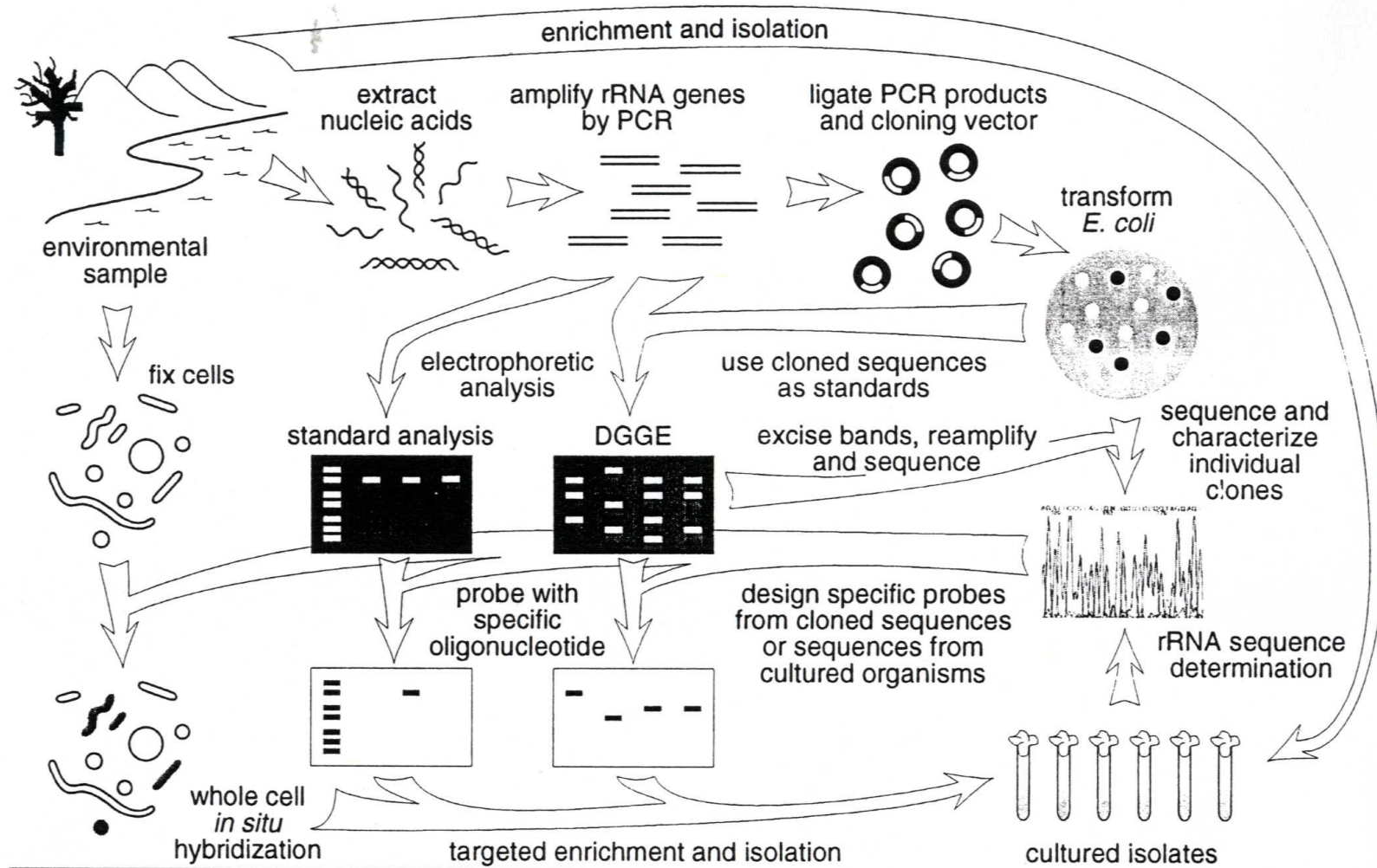


Figure 6.3 Common methodological approaches to studying the microbial ecology of the HZ (Head et al., 1999).

Modification of the PCR procedure and subsequent separation of amplified DNA by gel electrophoresis density or temperature gradient allows analysis of complex samples, with each amplicon separated by sequence not size and representing a 'bacterial species'. The resulting 'bar-code' of electrophoretic bands allows easy comparison with other samples and the degree of similarity (hence the diversity) to be determined. Furthermore the electrophoretic bands can be excised and sequenced thus identifying novel or common 'species' from the sample.

The knowledge gained at a sequence level can be utilised in developing more primers for PCR or probes for direct detection and enumeration of cells in a sample. Oligonucleotide probes can be linked to fluorescent molecules for direct insertion into cells in a sample. The cells for which the probe is specific can then be counted by fluorescent microscope or automated analyser. One disadvantage with many molecular approaches is that they do not distinguish between live and dead cells, however use of innovative techniques such as the incorporation of radioactively labelled compounds or heavy isotopes can reveal the active community (Earl et al., 2003, Whitby et al., 2001).

Molecular techniques advance at a pace and whole sample sequencing is now a feasible but expensive option (454 sequencing), as are the 'omic technologies allowing multi-species identification from single samples (microarrays) and assessments of metabolic capability (metabolomics).

6.16.2 Limitations to methods

It is very important to optimise all procedures against known standards and controls. It has to be recognised that no single technique will satisfy all requirements and their choice depends on the question and the characteristics of the study site (Hendricks, 1993). However, any study that combines hydrology and biology in HZ will yield important information.

This section is not exhaustive, but provides an important appreciation of the limitations to even standard methods. Interpretation of data is often compromised by a lack of understanding of the inherent limitations imposed by the techniques employed (Pickup 1999, (Head et al., 1998).

6.16.2.1 Sampling strategy

HZ samples may be collected using a range of approaches (see Environment Agency Report SC030155/SR3). However, once a sample has been removed from a site it is no longer representative of that environment or micro-environment and that imposes a major and immediate limitation on any future interpretation of data derived from that sample particularly when microbial activity is a focus (Pickup, 1995). For example, a sample that undergoes active methanogenesis will show a reduced, if not zero, activity once removed from a site and exposed to oxygen (Hall et al. 1990). Even if, anaerobic conditions are restored, if there is any subsequent activity it will be reduced (Hall et al., 1990;1996). Therefore during sampling, the fewer disturbances the better which intuitively suggests that *in situ* experimentation is the preferred option. However, this is not always feasible. The least representative sample method is destructive sampling (e.g. by grab sampler). With sediments this destroys redox gradients and often disrupts intimate biological, chemical and physical associations. A feasible option is to remove the sample but maintain the sample integrity, for example using core samplers that can extract intact cores and maintain overlying water, and be manipulated and maintained at *in situ* temperatures (Hall et al., 1990, Pickup, 1995).

6.16.2.2 *Classical microbial methods*

A major limitation of population studies, particularly those based on enumeration is experimental design which requires replication of samples and the statistical treatment of the subsequent data (Hall et al., 1990). It is important to stress that an impractical degree of replication may be required to work to a given level of statistical significance. The degree of variability of microbiological data, on both temporal and spatial scales is reported by Hall et al. (1990), further emphasizing the caution which must be exercised in interpreting data.

A further limitation to research into microbial populations is an inability to isolate and culture the majority of bacteria. There has always been a discrepancy between cell numbers obtained by direct and viable counting methods and studies have concluded that culturable bacteria represented only 0.01—12.5% of the viable bacterial population in terrestrial and aquatic environments (Pickup, 1995). Furthermore, some bacteria have been shown to become unculturable but retain their viability after exposure to the environment and have been called 'non-culturable but viable' (NCBV). This complicates both the detection and enumeration of microorganisms. In addition, NCBV state is often wrongly attributed to some microorganisms, although confirmatory methods have been developed. There are two other factors which contribute to this discrepancy. The direct count cannot distinguish between cells that are viable, NCBV, or dead. Conversely, media used for the isolation of viable bacteria may actively select against growth because they are too rich in nutrients or do not supply essential co-factors (Pickup, 1995, Pickup et al., 2003).

Methods have been developed that go some way towards distinguishing viable cells under epifluorescence microscopy. However, as with bacterial isolation procedures, it is clear that all experimental conditions are not suitable for all samples. Despite some limitations, viability assessment assays represent a bridge between counting culturable bacteria and direct counts and has been termed a direct viable count PVC; (Pickup, 1995, Pickup et al., 2003).

6.16.2.3 *Molecular Microbial Ecology*

While we have undoubtedly gained much new and valuable knowledge using the molecular techniques described, as with all methods, there are important limitations that must be minimised, eradicated, or, at the very least, recognised. As an example, we focus on the limitations the extraction of nucleic acids from natural samples. However other limitations exist for other techniques and these should be investigated prior to use.

6.16.2.4 *Nucleic Acid Extraction*

A major limitation of all DNA-based methods described is the quantitative recovery of nucleic acids from environmental samples (Head et al., 1998). This is because

- a) If you do not know the total amount of nucleic acids present in a sample, then it is difficult to assess the efficiency of recovery by any extraction technique.
- b) Spores will be less readily lysed than vegetative cells.
- c) Gram-positive cells are more resistant to cell lysis than Gram-negative cells and smaller cells (0.3–1.2 μm) are also more resistant to lysis.
- d) Not all methods are suitable for all environments. It is possible that the same lysis technique may give different results with different types of

sample such as water, sediment, or soil, so the degree of cell lysis should be determined independently.

It is paramount that any extraction procedure is optimised to target DNA being extracted as different targets require different strategies. For instance relatively gentle lysis is required for ammonia oxidising bacteria in environmental samples (Whitby et al., 2001) whereas high speed agitation in the presence of glass microbeads is required for some mycobacteria pathogens where gentle lysis is totally ineffective (Pickup et al., 2006).