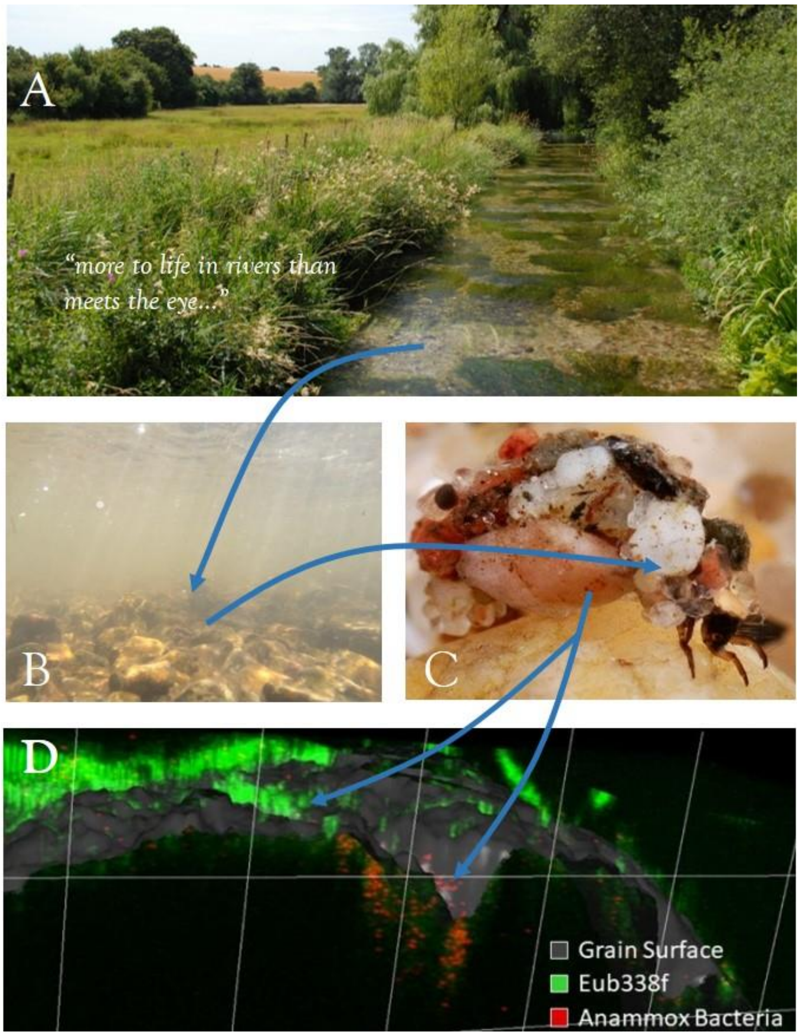
Microbial life in rivers: more than meets the eye

I thought that only fish lived in rivers, why do we need to care about microbes?



Looking at a river from eye-height you can see plants growing on the riverbed **A**. Look a little closer **B**, and you will see the bed is made of small stones and gravels (others rivers may be clay, sand or cobbles). Closer still, some insects, snails and molluscs and here, **C**, a young caddis fly *Agapetus fuscipes* has made its home out of gravel. Even closer, and you will see **biofilm** on the gravel comprising a multitude of microbes **D** – all bacteria are coloured green and the specialist anaerobe, anammox, red. **What are these microbes doing?** Images Jon Grey, University of Lancaster; Ian Sanders, Queen Mary University of London; Philippe Laissue of the University of Essex.

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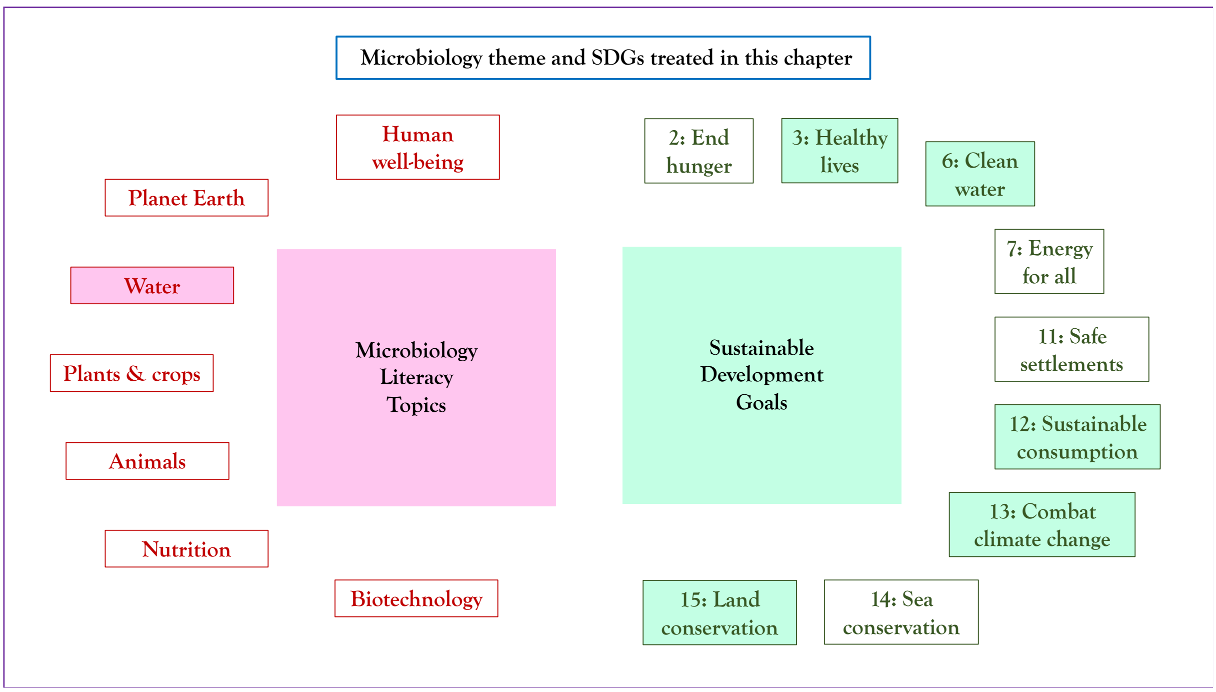
Life in rivers: more than meets the eye

Storyline

Lots of people on Earth live near a river and we have been exploiting rivers for water, food, transport and energy since the dawn of civilisation. Given their utility, you might think we would treat them with respect, but we use them directly for washing away our waste – sewage and industrial outflow etc., and they are also indirectly impacted by farming by the vast quantities of fertilizers, pesticides, herbicides and fine sediment that find their way from farmland into rivers. These negative pressures on rivers may reflect the fact that few people fully appreciate what a river really does and for a long time rivers were merely seen as pipes connecting the land to the sea. The benefits of rivers to humanity i.e., the ecosystem service of clean water, along with their diverse habitats are gaining appreciation and, beyond that, the role rivers play in the life-sustaining bio-element cycles (e.g. carbon and nitrogen) is taking centre stage – key to these vital ecosystems are the microbes.

The Microbiology and Societal Context

The microbiology: river ecosystem services; bio-element cycling; biofilms; metabolism; anaerobic respiration and greenhouse gases; biodegradation; water-borne infections, natural and human augmented. *Sustainability issues:* over-abstraction of river water; poor-sanitation and health; pollution from sewage, industrial food-processing and intensive farming.



Rivers: The Microbiology

1. ***How can something as small as microbes possibly affect rivers?*** You might think from looking at the picture of the river on the cover page that it is just some water flowing through the landscape, with the bottom of the river (i.e., the riverbed) merely serving to "hold" the water in place – a bit like water simply flowing through a pipe. In reality, however, the riverbed is very much alive – teaming with microscopic (and larger) organisms – and *what is not so obvious at all* is that the flowing water and riverbed are intimately connected in an **ecotone,** a transitional zone between two distinct environments with contrasting ecologies. What we mean by connected is that the flowing water and riverbed are actively exchanging, i.e. "sharing", materials such as solutes – substances dissolved in the water – like oxygen and nitrate or organics from the breakdown of **detritus**. Unlike the microbes on the riverbed that are hard to see, we can quite easily see the effect they imprint on the flowing water (Figure 1).

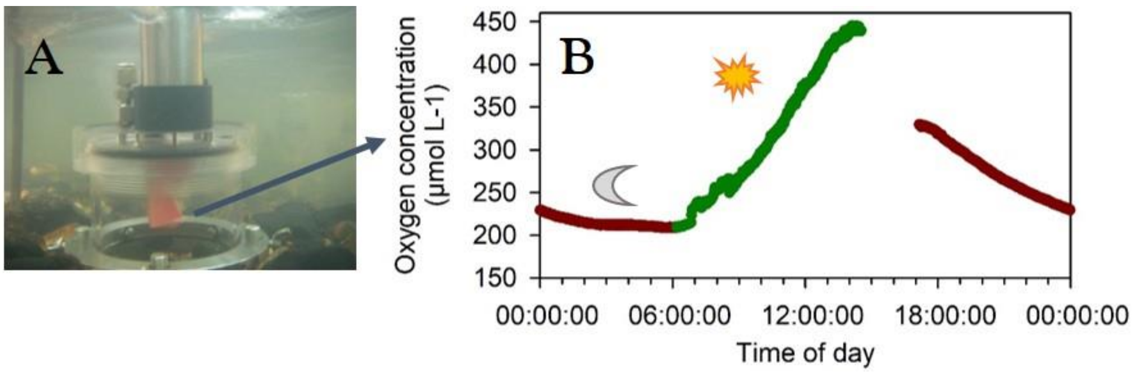


Figure 1 | Here in **A,** we have used a small **benthic chamber** to isolate a sample of pebbles and gravels on a riverbed that enables us (using underwater electrodes) to measure changes in oxygen over 24 hours **B**. During the **day**, when it is **light**, microscopic algae on the riverbed photosynthesise (equation 1) to produce sugars (CH_2O) and oxygen (O_2) and O_2 in the water goes up. When it is **dark**, the microscopic algae[†] – along with many other microbes – respire sugars by consuming O_2 from the water and O_2 goes down. Oxygen is a fantastic integrator of **whole ecosystem metabolism** that can tell us a lot about the "health" of our rivers. Do not worry about the gap in the afternoon data (Trimmer, unpublished) – our batteries ran out! [†]Algae are respiring all of the time and the changes in **B**, reflect net differences in equations 1 and 2.

If you look carefully at Figure 1B you can see that between first thing in the morning (dawn, 6:00) and early afternoon (14:00) the microbes on the riverbed have *more than doubled* the concentration of oxygen in the water and then pretty much *consumed it all* again over night – how can microbes be that greedy!? There are quite simply a lot of microbes: whereas I'm guessing there might be around 30 students in your classroom, the riverbed is far more crowded: collect 1 gram of sand from a riverbed and you are likely to find a billion or so microbes ($1x10^9$ microbes per gram). *Think about that for a minute*: even in the very small area (~52cm^2) of the riverbed we isolated in Figure 1, you could find several billion microbes all either producing and/or consuming oxygen through equations 1 and 2:

Photosynthesis (*P*)	$CO_2 + H_2O \rightarrow CH_2O + O_2$	(equation 1)
Respiration (*R*)	$CH_2O + O_2 \rightarrow CO_2 + H_2O$	(equation 2)

Where CO_2 is carbon dioxide (**inorganic** carbon (C) as a gas in the atmosphere that dissolves readily in water); H_2O is water (plenty in the river!); CH_2O represents organic C as a simple sugar (that provides the energy to drive whole ecosystems) and O_2 is oxygen that many organisms need to live. Note, even though it is night-time in equation 2, microbes (and all other life) are respiring all of the time, this is simply to illustrate the net effect of ***R*** and ***P*** on oxygen in the river water overnight, as in Figure 2b. For example, at night, if ***R*** > ***P*** then O_2 in the river will go down and, vice versa, during the day, when ***P*** > ***R***, O_2 will go up.

2. ***Sediments and riverbed textures.*** The number of microbes found on a riverbed, along with how readily the bed exchanges materials with the overlying river water, will depend strongly on what the riverbed is made of. Depending on the type of geology dominating the land that our river is flowing through, the riverbed could be a hard mix of cobbles (64 to 256mm), pebbles (4 to 64mm) and gravels (2 to 4mm), or softer with sand (0.06 to 2mm) and fine silt and clay (<0.004 to 0.06mm) particles. We can refer to all of these different sized particles as sediment and different amounts of each sediment type creates different riverbed textures. Many riverbeds around the world are dominated by gravel and sand sized particles.

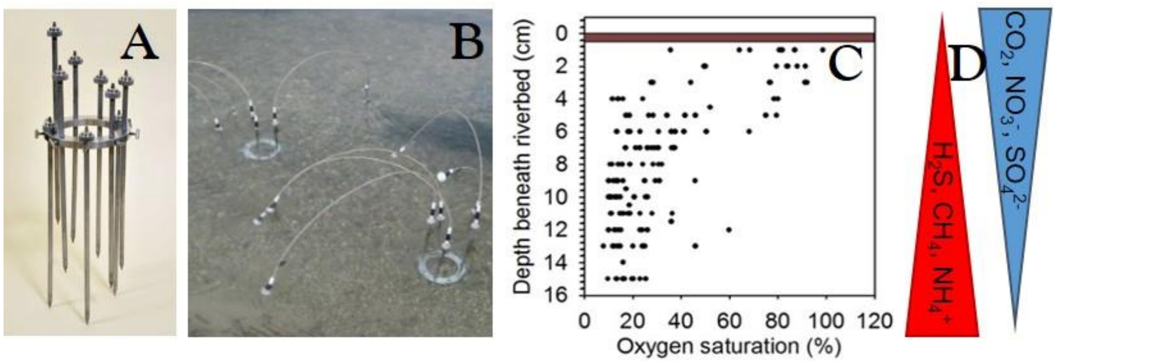


Figure 2 | Here we have made some small (40cm) stainless steel probes **A,** that we can push into a riverbed **B,** to recover porewater to measure for oxygen **C.** Oxygen saturation simply expresses the amount of dissolved oxygen measured as a percentage of how much we would expect *if there were no microbes* either respiring or photosynthesising. Here, just below the surface of our gravel riverbed (0cm to 2cm) any oxygen consumed is rapidly replaced by exchange with the flowing river, but, deeper (>6cm to 7cm), the microbes are consuming more oxygen than can be delivered and oxygen saturation declines. The brown represents oxygen penetration in clay riverbeds. It is deeper in the riverbed where anaerobic microbial life thrives and their metabolites e.g. H_2S, CH_4 and NH_4^+ can accumulate **D,** which, in turn, are re-oxidised by aerobic microbes nearer the surface back to NO_3^-, SO_4^{2-} and CO_2, for example. This microbial cycling of different elements are key riverbed functions that – combined – help deliver the ecosystem service of clean water. Photographs Katrina Lansdown.

3. ***Life without oxygen?*** At this point it is worth appreciating that, unlike me and you and other multicellular forms of life (i.e., metazoans, animals) that need O_2 to respire (**aerobic** respiration, equation 2), many microbes are perfectly happy without it and some are even killed by O_2! Depending on the texture of our riverbed, oxygen will penetrate to different depths – from a few mm in muddy clay, to 10s to 100s cm in larger gravels and cobbles (Figure 2). Where oxygen is limited and /or completely depleted (Figure 2), **anaerobic** microbes still respire, but instead of using free O_2 they use the $\mathbf{O_2}$ bound within larger molecules such as nitrate (NO_3^-) and sulfate (SO_4^{2-}).

Nitrate and sulfate are examples of **alternative electron acceptors**. For example, whereas O_2 accepts the electrons (from the sugar being oxidised) to form H_2O in equation 2, here, in its absence, SO_4^{2-} accepts the electrons – *as an alternative to* O_2 – and gets reduced to sulfide (S) in the process:

For sulfate reducing bacteria $CH_2O + SO_4^{2-} \rightarrow CO_2 + S + H_2O$ (equation 3)

and

For denitrifying bacteria $CH_2O + NO_3^- \rightarrow CO_2 + N_2 + H_2O$ (equation 4)

For simplicity, both equations 3 and 4 are presented as unbalanced and can be written more accurately with $0.5SO_4^{2-}$ and $0.5S^{2-}$ and $0.66NO_3^-$ and $0.33N_2$. Note that whereas N_2 gas is inert – harmless to life – sulfide (S, as hydrogen sulfide gas, H_2S) is highly toxic to *all* life – *but the riverbeds microbes can help with this*.

4. ***Multiple types of riverbed microbes drive multiple functions.*** Before we can appreciate more fully the different types of microbial activities happening in riverbeds we need to extend equation 2. Currently equation 2 depicts a simple sugar ($\mathbf{C}H_2O$) being respired to $\mathbf{C}O_2$ with O_2, showing the organic **C** in the sugar being **remineralized** back to inorganic $\mathbf{C}O_2$, where organic C could come from any organism in the river – dead or alive – plant, animal or microbe.

Clearly life is not just made of sugar and we can represent **organic matter** more completely by, for example, adding in nitrogen (N) to represent proteins, here simply as NH_3:

$CH_2ONH_3 + O_2 \rightarrow CO_2 + NH_3 + H_2O$ (equation 5)

We could increase the complexity of our organic matter (CH_2ONH_3 and in turn equation 5) even further by adding in the phosphorus (P) and sulfur (S) that are essential for building life's organic molecules – but we will leave it as it is. Notice how the N is bound in with the sugar (CH_2O) to form organic N on the left of equation 5, but how it is free as simple ammonia (NH_3) on the right? Just as the microbes remineralize organic C in organic matter back to inorganic CO_2, they remineralize organic N back to inorganic N as NH_3 (ammonia).

While this remineralization of organic matter to CO_2 and NH_3 by riverbed microbes is a perfectly normal riverbed function, ammonia is potentially toxic to the wider river ecosystem (more on this below). Also, remember the toxic anaerobic product sulfide produced where oxygen is scarce in the riverbed (equation 3)? Sulfide, coupled to any ammonia, could drive an **ecotoxicological** disaster – *luckily other riverbed microbes are at hand!*

5. ***Chemolithoautotrophic microbes.*** Whereas many microbes generate their energy to live by oxidizing organic substrates (CH_2O), either with or without O_2 (equations 2, 3 and 4), others – the **chemolithoautotrophs** – generate energy by oxidizing inorganic substrates such as sulfide and ammonia:

For sulfide oxidizing microbes $H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+$ (equation 6)

For ammonia oxidizing microbes $NH_3 + 2O_2 \rightarrow NO_3^- + H_2O$ (equation 7)

You may be interested to know that equation 6 – to the best of my knowledge – represents the earliest scientific understanding of microbes in rivers (ok, small springs and streams!) that established the existence of an entirely new form of life on Earth - in the 1880s! Sergei Nikolaievich Winogradsky was a Ukrainian microbiologist and the forefather of **microbial ecology**. Winogradsky showed how the abundance of microbes in sulfur rich springs increased in the presence of sulfide (i.e., they grew by "eating" sulphide), proving life could be driven by chemical energy (**chemolithotrophy**) rather than sunlight (photosynthesis). Winogradsky's subsequent work with ammonia oxidising microbes (equation 7), proving that inorganic CO_2 could be fixed into organic microbial biomass, marked the complete birth of **chemolithoautotrophy**. Other examples include microbes which oxidise methane (CH_4) – a powerful greenhouse gas – back to CO_2.

The chemolithoautotrophs drive key riverbed functions, reducing the accumulation of toxic products generated during the natural breakdown of organic matter, and mitigating greenhouse gas emissions. The chemolithoautotrophic microbial activities, coupled to the removal of nitrate (NO_3^- to N_2 gas equation 4) by the denitrifying bacteria, help deliver the ecosystem service of clean water (Figure 2).

6. ***Rivers and humans.*** As a river flows through the land, it gains water and materials from the catchment it drains. For example, it is perfectly natural for soil, often rich in organic matter, to be washed off the land which, along with leaves and other detritus – including whole trees – adds organic matter to a river. Rivers are also natural sources of human pathogens through the animals that live there (vectors of disease) such as *Leptospira* bacteria from rodents and numerous protozoa causing cryptosporidiosis, giardiasis and microsporidiosis from ducks (Figure 4). Human activities can amplify these natural processes, however, with intensive farming increasing the loss of soil from land into rivers, along with fertilizer (nitrate, ammonia, phosphate) and other farming chemicals e.g. pesticides and herbicides (Figure 4).

You may also know about humans discharging sewage into rivers which – depending on where you are in the world – may have been very well treated "cleaned" prior to being discharged into a river or it may still be very raw. The latter obviously has health issues in relation to human borne pathogens such as various pathogenic strains of *Streptococcus* and *Escherichia coli* and you may also have heard recently about Covid-19 being detected in sewage.

There is also a lot of talk in the news currently about excess sewage – often rich in organic matter – finding its way into rivers and, while this is a long-standing problem, the frequency with which this is happening appears to be increasing along with wider flooding. We can simplify all of this complexity by thinking about the soil and sewage going into rivers as organic C i.e. the CH_2O in equation 1. Going back to Figure 1, if the amount of oxygen needed to sustain all the respiration in the river ecosystem (equation 2) can be matched by that produced through photosynthesis in equation 1 – the river's oxygen budget will be in balance (This is a simplification that ignores oxygen replenished through re-aeration). If, however, the amount of organic C entering the river *externally* from excess soil and sewage requires more oxygen to be respired (ecosystems are always hungry and will always eat more) than can be produced in the river – the river's oxygen budget will go into debt. In effect, there will not be enough oxygen to go around, oxygen sensitive organisms (fish and invertebrates such as stoneflies) will either migrate away or die and harmful products (e.g. NH_3) can start to accumulate and exacerbate the problem (Figure 4).

In addition, fertilizer coming in from the land (nitrates and phosphates) can further "fuel" the growth of *all* plants i.e., increasing primary production through equation 2 by microscopic algae and the visible plants in the cover picture. Notice how the product of equation 2, CH_2O, is the organic C needed to drive respiration in equation 1 and consume oxygen? Hence, whether the organic C enters the river directly, or results from the stimulation of primary production by fertilizers, it will be respired and strip oxygen from the river. This overall syndrome is called **eutrophication** and it is driving down **biodiversity** in rivers and coastal seas around the world. Microbes can help clean our rivers but only to a certain extent, humans are overloading this ecosystem service and we need to take better care of these valuable resources.

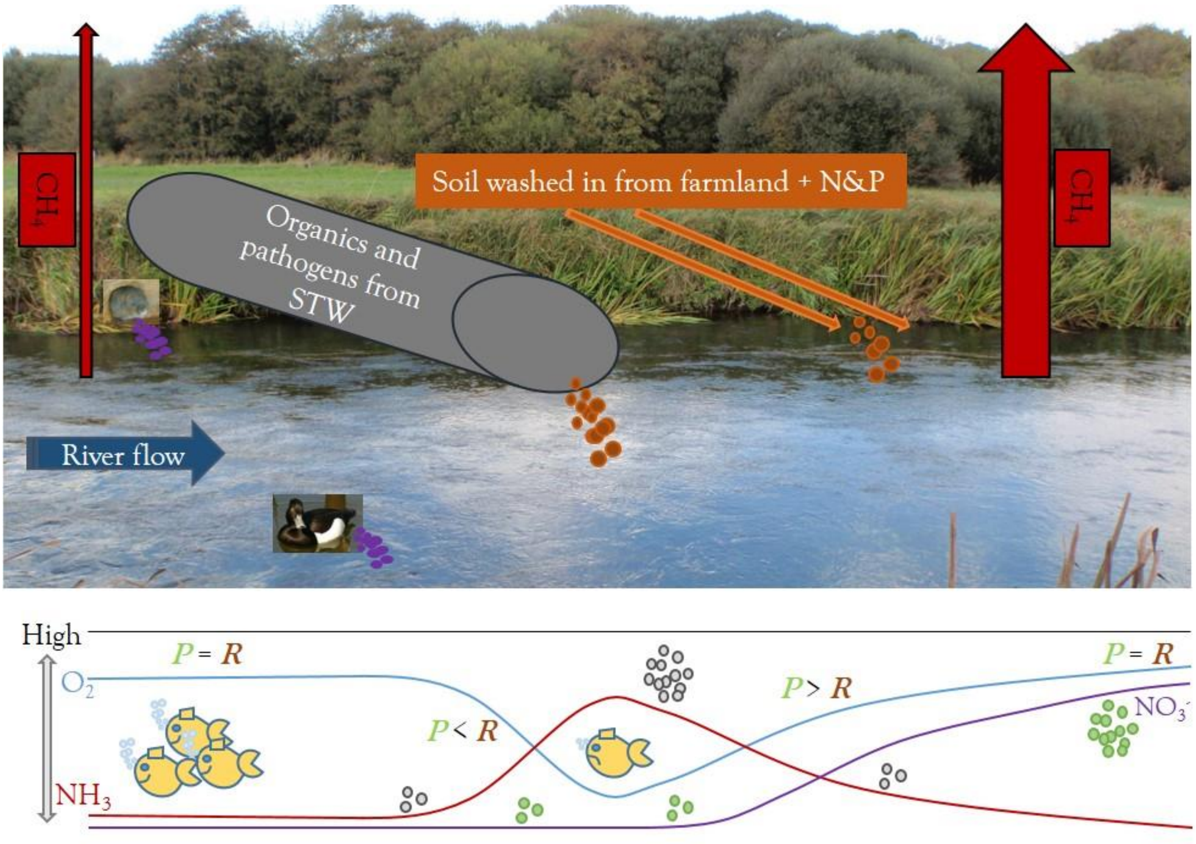


Figure 4 | Sewage treatment works (STWs) process human waste to produce effluent that can still be rich in organic matter. Soil, also rich in organic matter, can be washed off farmland and STWs and soil both add excess organic C to river ecosystems. This excess organic C can be used by microbes to grow, with their respiration (*R* equation 2) consuming O_2. If respiration exceeds the production of O_2 by photosynthesis (*P* equation 1) O_2 will decline, the river will become **hypoxic**, or even truly **anoxic**, and fish and other oxygen sensitive organisms will either migrate away or die. This microbial breakdown of organic matter can generate ammonia (NH_3) that can accumulate to harmful levels before being oxidised by aerobic ammonium oxidising microbes (grey dots) to nitrate NO_3^-. Nitrate, ammonia and phosphate (from farms and STWs) can also stimulate the growth or microscopic plants (algae, green dots) that further exacerbate the problem (not illustrated here). This excess organic C can amplify the amount of greenhouse gases (GHGs) emitted from rivers; here illustrated by methane (CH_4) but microbes oxidising NH_3 along with those anaerobically respiring NO_3^- can also produce the GHG nitrous oxide (N_2O). Note, in this simplified presentation we are ignoring oxygen exchange between air and river by reaeration and plants assimilating NH_3. Purple dots indicate potential natural sources of bacterial and protozoan pathogens from rodents (illustrated by "Ratty" the Water Vole by David Dixon) and ducks (illustrated by Tafted duck by Richard Sutcliffe), for example. Main river image Iwan Jones.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 3. Ensure healthy lives and promote well-being for all at all ages** (*improve health, reduce preventable disease and premature deaths).* Taking a walk along the riverbanks of a "healthy" river has clear mental health benefits, but as populations grow the demands we all place on river ecosystem services will increase – in addition to their basic requirement of providing more drinking water. Rivers can harbour natural diseases through their wildlife that people need to be aware of e.g. do not drink untreated river water but we all need to appreciate the effects we have on the natural river biome through over-use of antibiotics and hormonal treatments that enter our rivers through sewage discharge. All of these effects have economic consequences for health budgets.
- **Goal 6. Ensure availability and sustainable management of water and sanitation for all** (*assure safe drinking water, improve water quality, reduce pollution, protect water-related ecosystems, improve water and sanitation management)*. Human waste water disposal from sewage and industrial food processing add organic matter to rivers that can be further added to by intensive agriculture causing soil run-off to rivers, along with inputs of N and P nutrients that may cause **eutrophication** and, in the case of drinking water supplies, the need for additional purification measures and their associated costs. Population growth that requires more housing and peoples' growing demands for water (power-showers, hot-tubs, dishwashers etc.,) can lead to over abstraction of water from rivers that can further amplify the negative effects of what humans add to rivers i.e., sewage is not diluted enough by reduced river flow.
- **Goal 12. Ensure sustainable consumption and production patterns** (*achieve sustainable production and use/consumption practices, reduce waste production/pollutant release into the environment, attain zero waste lifecycles, inform people about sustainable development practices)*. There is a need for more efficient farming through the development of new crop strains e.g. wheat that make better use of N and P fertilizers and hence reduce their application rate. Recent sky-rocketing costs of fertilizers driven by the war in Ukraine will put further strain on developing countries increasing the need to farm more efficiently.

- **Goal 13. Take urgent action to combat climate change and its impacts.** The microbes of river ecosystems mediate the cycling of carbon and nitrogen, both in terms of production and consumption, and hence the fluxes of greenhouse gases. Our carbon and nitrogen inputs into rivers greatly influence these fluxes. Combatting climate change must involve the reduction of carbon and nitrogen inputs into riverine systems.
- **Goal 15. Protect, restore and promote sustainable use of terrestrial ecosystems…and halt and reverse land degradation and halt biodiversity loss.** One of the major problems relating to land degradation is intensive farming practices that lead to soil erosion. This results in the washing of carbon-carrying soil into river systems by rain and flooding, which in turn results in the microbial transformation of soil organic carbon into greenhouse gases and, in some cases, to the creation of oxygen-deprived waters and a loss of biodiversity.

Potential Implications for Decisions

1. ***Individual***
   - **a.** What are my personal water requirements? Where does my water come from and where does it go when I've finished with?
   - b. How many showers do I need to take each day? Do I need a hot-tub? Do I need to wash all of my cars, and how frequently?

2. ***Community policies***
   - **a.** Local environmental consequences from our own waste water as pollution in rivers and the provision of clean drinking water,
   - **b.** Health benefits of aesthetically pleasing river habitats and clean water

3. ***National policies relating to rivers aimed at tackling***
   - a. Climate change, increased drought intensity
   - **b.** Increasing populations, growing demands greater environmental pollution
   - **c.** Ensuring safe drinking water supplies
   - **d.** Eutrophication/algal blooms/toxic algal blooms preventing use of surface water bodies, fisheries, tourism, etc.
   - **e.** Greenhouse gas production and global warming,
   - **f.** Sequestration of agricultural land otherwise used for food and renewable production.

Pupil Participation

1. ***Class discussion***
   - ***a.*** What do you really know about rivers?
   - ***b.*** Where do they come from, where do they go?
   - ***c.*** What do humans both take from and add to rivers as they flow through the landscape?
   - ***d.*** Is a river a simple pipe with water flowing through it to the sea or is it alive and microbially active?

2. **Pupil stakeholder awareness**
   - **a.** What type of human activities put stress on rivers?

b. What are the consequences of that stress?
c. How could we as a society reduce that stress?

3. **Exercises**

a. Make a Winogradsky column and watch the river microbes grow! See if you can organise a trip to a local river or stream (or you could even use a small pond as a substitute or soil and rainwater if easier) and collect several spoons of riverbed sediment – the muddier the better! – and a litre or so of river water. Back at class, get some cellulose filter paper (or newspaper), rip it into small pieces and mix it with your sediment (50:50 or try other ratios). Then grind up some white chalk (or cooking sodium bicarbonate), half a teaspoon should do, and mix it with some egg yolk (for sulfur) and add this to your sediment/paper mix. Scrape all of this into the bottom of a clear measuring cylinder or bottle (1/3rd to ½ full should do it), top it up with river water, cover with cling film and put it on a windowsill. You need to be patient now, but after a few weeks you will notice a whole load of different colours starting to develop where each colour is a different type of microbe you have enriched from the riverbed – just as Winogradsky did back in the 1880s. You can experiment simply with different amounts of chalk, paper, sediment and egg and just see what happens – do not worry about this, they always work! Score your Winogradsky columns – which has the most colours i.e., which has the highest microbial biodiversity? You can simply search "Winogradsky column" online to find out more.

The Evidence Base, Further Reading and Teaching Aids

The normal view of life in rivers, nice animals but where are the microbes!? You'll find lots of videos like this online.

https://www.youtube.com/watch?v=gzVYYMXH1n0

Common and easily recognised threats to rivers

https://www.nature.com/scitable/knowledge/library/eutrophication-causes-consequences-and-controls-in-aquatic-102364466/

Upstream: Microplastics in UK rivers

More advanced text on the above and concepts in figure 4

Hilton J. et al. 2006. How green is my river? A new paradigm of eutrophication in rivers. Science of the Total Environment. 365. 66-83. Doi 10.1016/j.scitotenv.2006.02.055

Global issues facing rivers

Vörösmarty CJ. et al. 2010. Global threats to human water security and river biodiversity. Nature. 467. 555-561. Doi 10.1038/nature09440

The dawning of rivers being important in the global carbon cycle and similar ideas exist of nitrogen.

Cole JJ. et al. 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosystems. 10. 171-184. Doi 10.1007/s10021-006-9013-8

Battin TJ. et al. 2009. The boundless carbon cycle. Nature Geoscience. 2. 598-600. Doi 10.1038/ngeo618

Importance of biodiversity to river function including microbes!

Cardinale BJ. 2011. Biodiversity improves water quality through niche partitioning. Nature. 472. 86-113. Doi 10.1038/nature09904

Measuring microbial activities and microbes in rivers – advanced!

Lansdown K. et al. 2014. Fine-Scale in Situ Measurement of Riverbed Nitrate Production and Consumption in an Armored Permeable Riverbed. Environmental Science & Technology. 48. 4425-4434. Doi 10.1021/es4056005

Trimmer M. et al. 2015. Riverbed methanotrophy sustained by high carbon conversion efficiency. The ISME Journal. 9. 2304-2314. Doi 10.1038/ismej.2015.98

Glossary

Aerobic respiration: production of energy (energy conservation) coupled to the reduction of oxygen to water.

Alternative electron acceptors: in the electron transport chain of aerobic respiration, oxygen serves as the final electron acceptor. In anoxic environments where there is no oxygen anaerobic microbes use alternative electron acceptors such NO_3^- or SO_4^{2-} to respire anaerobically.

Anaerobic respiration: production of energy (energy conservation) coupled to the reduction of alternatives to oxygen e.g. NO_3^- or SO_4^{2-}. While anaerobic respiration enables microbes to grow in the absence of oxygen the energy yields are lower than for aerobic respiration and the anaerobes tend to grow more slowly.

Anoxic: an environment, be it soil, sediment, river water or the deep blue ocean that contains no measurable oxygen is said to be anoxic. The opposite being oxic. Anaerobic microbes live in anoxic environments by using alternatives to oxygen to drive their anaerobic respiration.

Benthic chamber: typically a Perspex chamber attached to a collar that can be used to isolate a section of riverbed (or more widely of seabed) to enable the exchange e.g. production or consumption of solutes (e.g. oxygen, methane) by microbes to be quantified.

Biodiversity: a measure of the overall number or type of different species of organisms in an ecosystem where high biodiversity indicates many different species and low biodiversity a more limited number of species. Can be extended ecologically to consider genetic diversity and functional diversity i.e. different groups of organisms having different roles in an ecosystem.

Biofilm: a community comprising different types of microbes living on a surface (organic on plants or inorganic on sediment particles) often "stuck" together with secretions of polysaccharides i.e., mucous. Some photosynthetic microbes will live at the surface of the biofilm for better access to light while others will live deeper down to avoid oxygen. Biofilms give the characteristic slippery-slimy feel to rocks on riverbeds or to rock pools at the seaside.

Chemolithotroph, chemolithoautotrophy and chemolithoautotrophs: we can break these words down to make them easier to understand. Starting at the end, "troph" means to nourish or feed and "litho" means stone or mineral and here "chemo" is referring to chemical energy in contrast to photosynthetic energy i.e., energy from sunlight. A chemolithotroph, is a microbe that gets its nourishing energy by oxidising mineral, inorganic substrates or "chemical energy from eating stones"! If it also uses that energy to fix inorganic CO_2 into organic sugars (CH_2O) it is feeding itself and is said to be autotrophic – self-feeding. Put it all together and you have chemolithoautotrophy that is carried out by the chemolithoautotrophs which contrasts with the photoautotrophs which are the self-feeding plants.

Detritus: small fragments of dead and decaying organic matter that could be leaves, faeces or fragments of larger organisms.

Ecotone: a region where two distinct environments and ecosystem types meet e.g. flowing river water and the riverbed. Typically marked by sharp gradients in the concentrations of solutes such as oxygen or nitrate and changes in dominant organisms.

Ecotoxicological: Typically the toxic effect of chemicals is determined by exposing a single type of organism to a single type of chemical in the laboratory. Ecotoxicological studies seek to understand the behaviour and interaction of multiple toxic substances (e.g. "cocktails" of pesticides, herbicides or other chemicals) in more realistic ecological settings and how, for example, damage by toxins to one part of an ecosystem can have knock-on effects for other parts of the ecosystem.

Eutrophication: a collective syndrome, typically linked to human derived (e.g. sewage, fertilizers) excesses of macronutrients (nitrogen and phosphorus), characterised by increased growth of algae and more complex plants in rivers (or lake, estuary, coastal sea). This increased primary production (organic carbon) is in turn respired, drawing down oxygen in the water resulting in hypoxia and in extreme cases complete anoxia. Typically rivers suffering eutrophication will also have reduced biodiversity and excesses of greenhouse gases – CO_2, CH_4 and N_2O.

Hypoxic and hypoxia: a condition where the amount of oxygen dissolved in a body of water, here a river, is below that required to sustain a healthy ecosystem. If a river water is at 10°C and 100% saturated with oxygen, we would expect it to contain 352 µmol O_2 per L (or if you prefer, 11 mg O_2 per L). Hypoxia tends to affect animals after approximately 70% of the expected oxygen has been lost due to respiration of excess carbon i.e., ~100 µmol O_2 L^{-1} (3 mg O_2 per L) is remaining, but tolerances vary. Note the contrast with anoxic which denotes absolutely no oxygen whereas hypoxia is a gradient or "sliding-scale" condition.

Inorganic and organic: in a simple sense we could consider all living organisms to be organic and anything not derived from organic life to be inorganic i.e., dead animals and plants are still organic. We can make the definition more scientific by saying organic life is based on carbon – as I am sure you may have heard in school or science fiction movies. You may then ask: "Surely that means CO_2 is organic as it contains carbon?" The scientific community often say that C is only organic if it is coupled with hydrogen in a compound, which would make CO_2 *inorganic*. We then have the puzzling example of the simplest hydrocarbon methane gas (CH_4) which is *often* considered to be *organic*. I take a biogeochemical perspective and say that C as a gas (e.g. CO_2 and CH_4) is inorganic because it is unavailable to most life on Earth i.e., it is not a source of food or energy. The C in CO_2 and CH_4 only becomes organic when it is *fixed* or converted by either photosynthesis or chemosynthesis (e.g. ammonia oxidation or methane oxidation) into sugars that are then *available* as energy for other organisms in an ecosystem.

Microbial ecology: classic ecology studies the interactions between visible organisms and their environment. Microbial ecology is similar but given we cannot see microbes the techniques employed are typically more challenging than for classic ecology. The definition of a species that is central to classic ecology e.g. part of niche theory etc. does not apply in the same sense to microbes.

Organic matter: material derived from living organisms be it whole or small fragments as detritus. Riverbed sediment is comprised of both organic e.g. detritus, microbes and inorganic particles e.g. particles of sand etc. which, in addition to sediment particle size also helps shape the texture of the riverbed.

Porewater: riverbeds can have different textures depending on their particle sizes e.g. very small silt particles (<0.063mm) or pebbles (64mm to 4mm) and if you think of these particles as spheres, and those spheres packed inside a box (e.g. the riverbed), then there will be spaces between the particles filled by water - that is porewater.

Remineralized: The complex organic molecules that make life work are comprised mainly of the six macro-elements – C, N, O, P, S, H and those elements are constantly being cycled between life and the environment. Inorganic C as CO_2 in the atmosphere is fixed by plants to make organic sugars (CH_2O) which can then be consumed by other organisms. If we think of the CO_2 as the mineral, inorganic form of C, then when organic C in sugars gets respired back to mineral CO_2 - the C has been *remineralized*. This ebb and flow of C between its organic and inorganic, mineral forms, is summarised in equations 1 and 2. You can think of organic and inorganic forms of the other 5-key elements being remineralized in the same way e.g. carbohydrates have oxygen and hydrogen and amino acids, nitrogen and sulfur.

Whole ecosystem metabolism: The rivers pictured throughout this chapter will contain a multitude of organisms from microbes, through protozoa, to invertebrates and fish etc., all of which will consume oxygen (either directly or indirectly). In addition, there will be microscopic algae and other photosynthetic microbes on the riverbed, along with larger plants and all of which will be producing and consuming oxygen. The sum of all this photosynthesis and respiration by all of the organisms in the ecosystem gives us whole ecosystem metabolism and can be measured for whole river reaches (~50m to 200m) by measuring changes in oxygen i.e., for the river as a whole ecosystem and not just the small parts of the bed isolated in Figure 1.