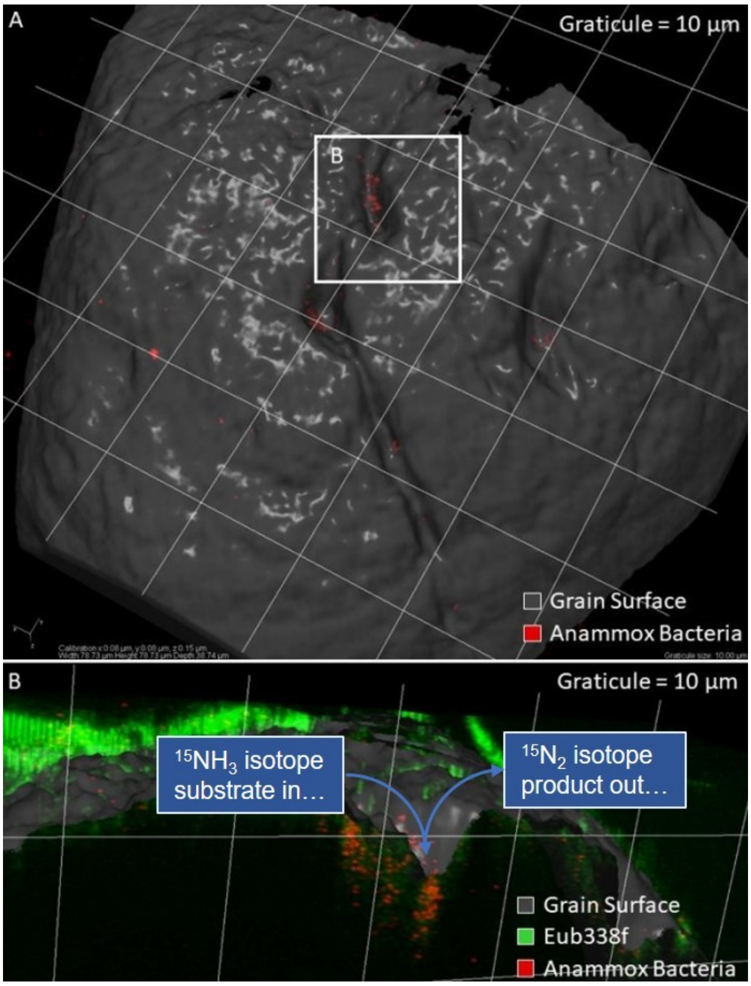
How we use isotopes to study microbes and their activities

We cannot even see microbes – how can we possibly understand what they do!?



Is it a meteor hurtling towards Earth from outer space!? No, it's a tiny grain of riverbed sand covered in bacteria. **A,** anammox bacteria (coloured red) "hiding" in the crevices away from oxygen with **B,** many other bacteria (coloured green) living on the oxygen rich surface. We can add isotope substrates to trace microbial activity, here measuring respiration by anammox as $^{15}N_2$ gas – how cool is that!? Image Philippe Laissue of the University of Essex.

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Storyline

If you look in the mirror in the morning you may reasonably assume that you are the same person as you were yesterday – true, in terms of your character, but not strictly true for what you are made of. All life on Earth is made up of elements that are constantly being cycled between the environment (air, soil, water) and life – key to that cycling (e.g. carbon cycle, nitrogen cycle) are the microbes and we can measure their cycling activities using isotopes.

How we use isotopes to study microbes and their activities

1. Isotopes – not all bad. You may already be familiar with the word "isotope" but perhaps not so familiar with how we can use them to study microbes. Some people get concerned when they hear the word "isotope" as it is often negatively associated with radioactive isotopes, atomic decay and the emission of radiation e.g. neutrons – sounds exciting! Rest assured, the isotopes we are looking at today belong to the family of stable isotopes: stable because they do not decay and do not therefore emit radiation. But, before we get too far ahead of ourselves let us first consider some fundamentals…

2. Elements and life You, your friends, all the animals, plants and even microbes (on land and sea) – that is, all living organisms on Earth – are made up of **elements** created in the stars before our solar system even existed. No wonder you feel tired sometimes, your elements are already billions of years old!

Life is a bit choosy however and while there are 94 naturally occurring elements in the **periodic table**, just 6 dominate the structural organic molecules that make a living organism – these 6 are the **macro-elements** (Figure 1).

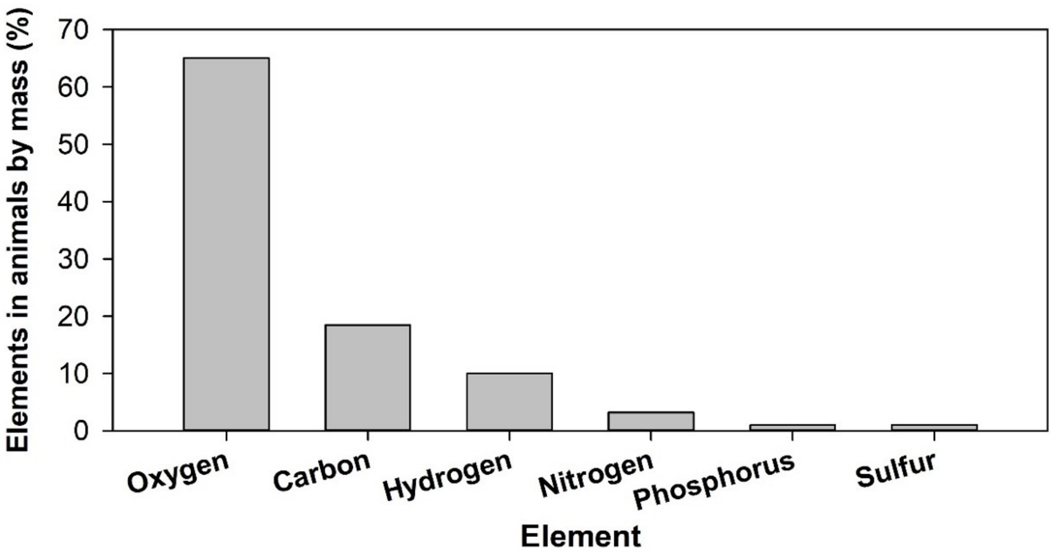

Figure 1 | Of the 94 naturally occurring elements on Earth i.e., what you'll see in the periodic table, just 6 dominate the structural organic molecules "building blocks" of life – here for animals. A lot of the oxygen is combined with hydrogen in water. As long as water - and therefore hydrogen - is available, life is said to be limited by carbon (e.g. for energy), nitrogen (to build protein and muscle) and phosphorus (cell membranes and DNA). We can study carbon and nitrogen cycling through life quite easily using their isotopes.

First, life contains a lot of water (i.e., di-hydrogen oxide, H_2O) which is a **molecule** made up of the 2 elements, hydrogen (H) and oxygen (O), with the remainder – that is the "stuff" that makes life work - being a mix of carbon (element C – energy in sugars), nitrogen (N, rich in proteins and DNA), phosphorous (P, energy store in **ATP** and cell membranes) and sulfur (S, amino acids and vitamins).

So, if you study biology, or even become a microbiologist, you are going to be working with life that principally contains – C, H, O, N, P, and S. *I use the pneumonic "HiC NOPS" to remember them, though the "i" is only there for the HiC!* One important point to remember is that life is not just a random jumble of the "HiC NOPS" elements, as the building blocks of life (amino acids, proteins, sugars, lipids etc.) need these elements in quite carefully balanced quantities and that's why it's important to have a balanced diet. For life to work properly e.g. for your blood to carry oxygen, it also needs very small quantities of a wide variety of other elements i.e., the **trace-elements,** like iron (Fe) from green vegetables and other trace-elements including copper (Cu), cobalt (Co) and zinc (Zn).

3. Isotopes – same element, just a bit heavier! Before we look at how isotopes are used to study microbes we need to "deconstruct" an element. All elements are atoms and all atoms have atomic structure. At the core of the atomic structure we have a nucleus made up of both protons and neutrons (you can go even further and look "inside" a proton or a neutron but we'll leave that to the Large Hadron Collider at CERN), and around the outside of the nucleus - "orbiting" - are the electrons (Figure 2). So, each element is made up of just 3 fundamental units – neutrons, positively-charged protons, and negatively-charged electrons.

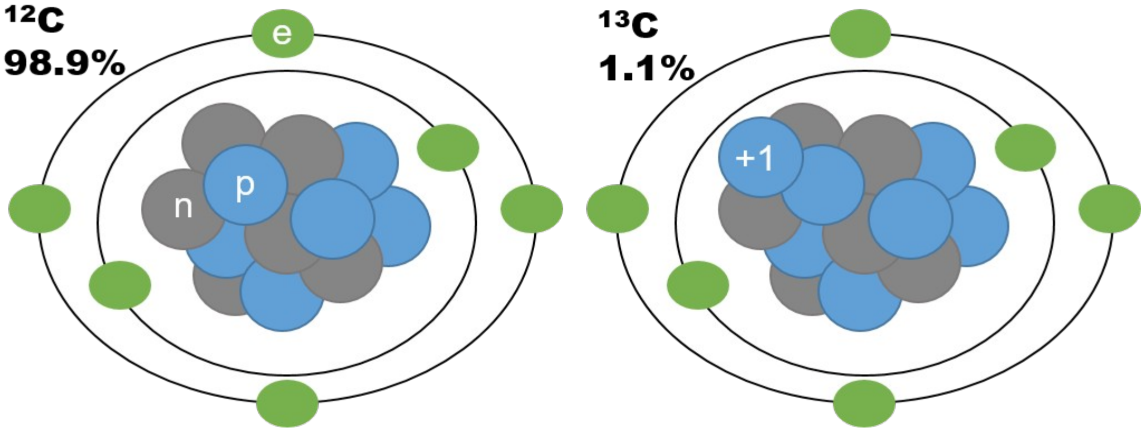

Figure 2 | All elements are atoms with a common atomic structure. The atomic nucleus comprises protons 'p' and neutrons 'n', with the number of each, here 6 for carbon C, being matched by electrons 'e' in the orbital shell – different elements have different configurations of 'p', 'n' and 'e'. 98.9% of C atoms have 6 'p' and 6 'n' giving C an atomic mass of 12 i.e., ^{12}C, left. A small fraction (1.1%) has 1 extra neutron which increases the mass to 13, giving the stable isotope ^{13}C. As neutrons have no charge i.e., they are neutral, the 1 extra 'n' has no significant[†] effect on biological reactions. There are stable isotopes of N, S, O and H but not of P. Note, quantum theory tells us that we cannot actually be sure where the 'p', 'n' or 'e' are in either space or time and the diagrams are merely illustrative. ([†]biological reactions do actually discriminate against heavy isotopes by a small fractionation factor but it is not important here)

Different elements "are different" to each other simply by how many protons, neutrons and electrons they have. For example, helium (He) has **2** protons and **2** neutrons in its nucleus, with 2 electrons in its orbital shell – it is very simple and very light i.e., it has a low atomic mass (or weight) of only **4** (unit is Dalton, Da). Note, we only add together the protons and neutrons to get the atomic mass. Carbon (C) is heavier than He, with 6 protons, 6 neutrons and 6 electrons giving C an atomic mass of 12 (well, 12.0096 – but hey!).

Almost at the isotopes... *The important point to appreciate is that it is the electrons that give the different elements their individual chemistries and control how they interact with each other to make molecules.*

Now – isotopes. For example, most (98.9%) of the carbon on Earth, be it in the **organic** compounds of life (carbohydrates, protein, DNA) or as the **inorganic** CO_2 in our atmosphere, has an atomic mass of 12 - what we call ^{12}C (Figure 2). A very small fraction (1.1% to a close approximation), however, has 1 extra neutron (**7** in total) but still the typical **6** protons and 6 electrons which give it an atomic mass of **13** – what we call ^{13}C. Remember, it is the electrons that determine an element's chemistry, but as ^{13}C still has 6 electrons, just like ^{12}C, the chemistry of the ^{13}C isotope is the *same* (to close approximation) as the chemistry of ^{12}C – it's just a little bit heavier (~8%).

4. How is this useful for studying microbes? Carbon is the backbone of all organic life and life is *driven* by a multitude of **bio-chemical** reactions – respiration, photosynthesis, protein synthesis, etc. If we picture those life sustaining biochemical reactions using ^{12}C – *and the chemistry of ^{13}C is the same as ^{12}C* – then it is not too hard to also imagine the biochemistry of life working happily with ^{13}C too!

$^{12}CH_2O + O_2 \rightarrow {}^{12}CO_2 + H_2O$ at a rate of 10 (e.g. fmol 10^{-15}) units $^{12}CO_2$ per microbe per hour (1)

$^{13}CH_2O + O_2 \rightarrow {}^{13}CO_2 + H_2O$ at a rate of 10 (e.g. fmol 10^{-15}) units $^{13}CO_2$ per microbe per hour (2)

Here a simple carbohydrate (CH_2O) made with either light ^{12}C or heavy ^{13}C is being respired to CO_2 at the same rate per hour by an aerobic microbe – *that is none-the-wiser to the presence of either ^{12}C or ^{13}C.*

5. Isotopes as tracers we can follow. You may have heard the expression of "looking for a needle in a haystack"; well, if we could paint the needle a very bright colour then it would be easier to spot. We can think of all the hay in the stack as the ^{12}C and the ^{13}C-heavy isotope as the brightly coloured needle – isotopes add "colour" that enable us to trace microbiological activities more easily. When we add isotopes to experiments we say we are adding a tracer and how a tracer is changed by microbes can be measured using a **mass-spectrometer** (Figure 5).

Returning to the "HiC NOPS" macro-elements of life: there are heavy isotopes readily available to trace microbial activities for carbon (^{13}C vs ^{12}C), nitrogen (^{15}N vs ^{14}N) and oxygen (^{18}O vs ^{16}O; though ^{18}O is ~20x more expensive than ^{13}C or ^{15}N). There is also a stable heavy isotope of hydrogen (^{2}H vs ^{1}H) and multiple heavy stable isotopes of sulfur ^{33}S, ^{34}S and ^{36}S vs ^{32}S (and even ^{17}O), but these are rarely – if at all – used in the settings described here. Note, there are no stable heavy isotopes of phosphorus – with 100% of P being normal ^{31}P. *This is why we know far more about the cycling of C and N by microbes compared to the other macro-elements because - quite simply - we have the isotopic "tools" to study them.* You could substitute ^{14}N- or ^{15}N-ammonia into similar equations to 1 and 2 or even ^{16}O or ^{18}O, as exactly the same principle applies.

6. Application of isotopes – ^{13}C examples If you collect a sample of soil from your garden, seal it in a bottle (scientifically a gas-tight vial, see Exercises) and incubate it for a few days, you would find the amount (concentration) of CO_2 in the bottle increases over time (Figure 3, Top-row). While this might be nice to demonstrate in class, as young scientists you might want to ask - *why* is the CO_2 increasing and *where* is the CO_2 coming from?

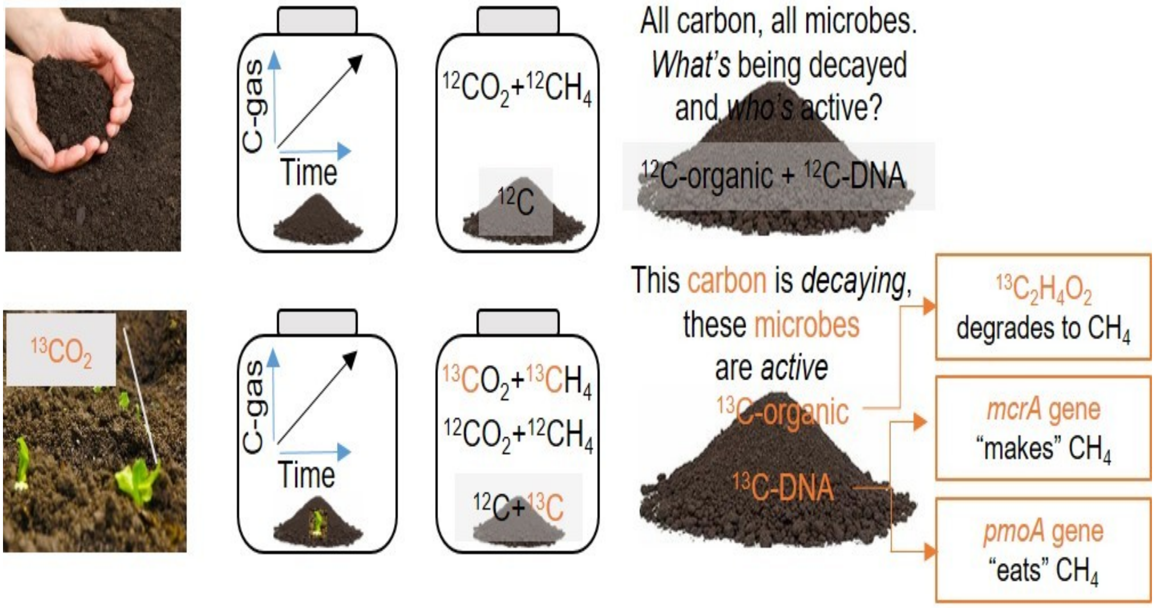

Figure 3 | Soil collected from a garden or forest floor comprises a complex mix of organic carbon compounds and billions upon billions of microbes all of which - combined - will respire and ferment carbon to CO_2 and CH_4. While that would tell us that the soil is alive, we would not know what types of carbon were being decayed or which microbes were active (Top-row). By adding a ^{13}C-tracer as organic carbon (here plant leaves grown with $^{13}CO_2$) we can follow its decay through simpler sugars and fatty acids (e.g. $^{13}C_2H_4O_2$, acetate) to $^{13}CO_2$ and $^{13}CH_4$ and identify which microbes are active by the ^{13}C in their DNA. Here, specifically, for two **functional genes** involved in both the production (*mcrA*) and oxidation (*pmoA*) of CH_4 (Bottom-row).

I am guessing you are sitting in a classroom with around 30 other students and the student concentration is therefore 30 students per classroom. The first thing to appreciate about your sample of soil is that it is far busier than your classroom – the concentration of microbes in 1 gram of soil alone could easily be a billion! i.e. $\sim 10^9$ microbes per gram of soil. Whereas you might recycle your plastic bottles and tin cans at home, soil microbes are largely responsible for breaking down and recycling dead organic C e.g. dead leaves, grass, worms and bugs back to atmospheric CO_2 to complete the carbon cycle.

By recycling, we actually mean the organic C is either being respired (typically by **aerobic** microbes using oxygen) or fermented by **anaerobic** microbes in the absence of oxygen (wet soil often contains little oxygen, for example) back to CO_2. The point to appreciate is that little of the organic C actually goes straight to CO_2 with the full recycling involving a complex consortium of microbes all doing slightly different "jobs" i.e., they have different ecological niches. For example, fungi use extra-cellular enzymes to solubilise the long, structural polysaccharide cellulose ($[C_6H_{10}O_5]_n$ where *n* indicates repeat units of a type of glucose) in plants into simpler polysaccharides, and proteins into simpler peptides and amino acids. Once solubilised, other microbes can get to work creating even simpler sugars (e.g. single molecules of glucose $C_6H_{12}O_6$), fatty acids (e.g. propionate $C_3H_6O_2$) and alcohols (e.g. ethanol C_2H_6O) that can then be fully respired and fermented to CO_2, again by different microbes.

7. Can we "see" that? As we said, 98.9% of the organic carbon in the soil will be ^{12}C and, in turn, 98.9% of the recycled CO_2 will also be ^{12}C (Figure 3). This large background of ^{12}C makes it difficult to distinguish where the CO_2 is coming from. If, however, we were to grow plants in the presence of ^{13}C-labelled $^{13}CO_2$, by **fixing** that $^{13}CO_2$ they would make ^{13}C-organic C e.g. $[^{13}C_6H_{10}O_5]_n$ cellulose. The ^{13}C-labelled plants (or even simple algae) can then be mixed with soil to enable us to trace the recycling of the organic ^{13}C through the different organic fractions (^{13}C-sugars, ^{13}C-fatty acids) back to $^{13}CO_2$.

Whereas we need oxygen to survive, many microbes thrive in its absence by fermenting simple fatty acids, such as propionate. Some of the ^{13}C-propionate ($^{13}C_3H_6O_2$) in our soil can be broken down further to ^{13}C-acetate ($^{13}\mathbf{C}_2H_4O_2$) – notice how we are now left with just **two C** atoms from the original **six** in each segment of cellulose $[^{13}\mathbf{C}_6H_{10}O_5]_n$? Acetate can be further metabolised by very specialised anaerobic microbes (the **methanogens**) that use one of those last **two ^{13}C** atoms to make **one** molecule $^{13}\mathbf{C}O_2$ and the other **one** molecule of the far more powerful greenhouse gas methane, $^{13}\mathbf{C}H_4$.

Fortunately, for our planet, another group of specialised microbes (the **methanotrophs**) can live on just CH_4 – not like the "greedy" microbes that need the two C atoms in acetate ($C_2H_4O_2$), the methanotrophs make do with just the one C atom in CH_4!

Methanotrophs convert (oxidise) most (typically >90%) of the CH_4 back to CO_2 before it leaves the soil: *they are called the methane filter that reduces the amount of methane reaching our atmosphere*. We can add ^{13}C-labelled CH_4 directly to soil and trace the production of ^{13}C-labelled CO_2 to determine what fraction of total soil CO_2 is due to the activity of the methanotrophs. Such ^{13}C-isotope-tracer techniques are being used to study how climate change is affecting the balance of the methanogens and the methanotrophs in the methane cycle.

8. Can we see who does that? When you grow you "simply" add new cells to your existing cells i.e., you grow muscle by adding more muscle cells to your existing muscle cells. Microbes are only single cellular organisms and when they grow, they grow by doubling the whole cell i.e., 1 becomes 2, then 4, then 8, etc. As each microbial cell contains vital DNA, each new cell needs a new copy of DNA.

Let us imagine a population of microbes growing by metabolising some of the simpler ^{13}C-acetate ($^{13}C_2H_4O_2$). Some of the ^{13}C is used to generate energy but some is also used to synthesise new ^{13}C-labelled DNA. Remember how ^{13}C is a little bit heavier than ^{12}C? Now the DNA in the microbes which are growing with the ^{13}C-acetate will be a little bit heavier than the ^{12}C-DNA in microbes that are *not* growing (Figure 3, Bottom-row). We can then extract DNA from the soil, separate the light, ^{12}C-DNA, from the heavy, ^{13}C-DNA (using an ultra-centrifuge). By analysing the microbial genetic signatures in the heavy DNA, we can identify which microbes are active in our soil sample.

Similarly, when methanotrophs oxidise $^{13}CH_4$ some of the heavy ^{13}C gets incorporated into their DNA and we use that to identify which methanotrophs are actively oxidising methane.

9. More advanced applications using ^{15}N isotopes. Nitrogen (N) exists largely as a gas (di-nitrogen, N_2) in our atmosphere, with only a small fraction being found in the organic-N of life (animals, plants, microbes). After death and decay, organic N (proteins, amino acids) decomposes to ammonia (NH_3 / NH_4^+) which is then recycled back to atmospheric N_2 by couplings between distinct aerobic (grey) and anaerobic microbes (blue, red – Figure 4).

The complexity of the N cycle reflects the multiple redox states that N can exist in – from fully reduced as ammonia (NH_3), to fully oxidised as N_2 gas – and different microbes exploit the energy potentials between these different redox states to grow. In oxygen-rich habitats, such as dry grassland soils, aerobic microbes use oxygen to oxidise ammonia to nitrite and nitrate (NO_2^- + NO_3^-) to gain energy. Separately, where oxygen is scarce, such as in water-logged soils (and see below), anaerobic microbes respire that nitrite and nitrate to N_2 gas to also gain energy.

Most of the N on Earth has an atomic mass of ^{14}N (99.6%) with a smaller fraction being present naturally as ^{15}N (0.4% or, more precisely, 0.366%). The complex microbial activities driving the N cycle can be studied easily using ^{15}N-isotope tracers, as scientists have synthesised a ^{15}N variant of the main forms of N in Figure 4 e.g. $^{15}NH_4^+$, $^{15}NO_2^-$, $^{15}NO_3^-$, $^{15}N_2O$ and $^{15}N_2$.

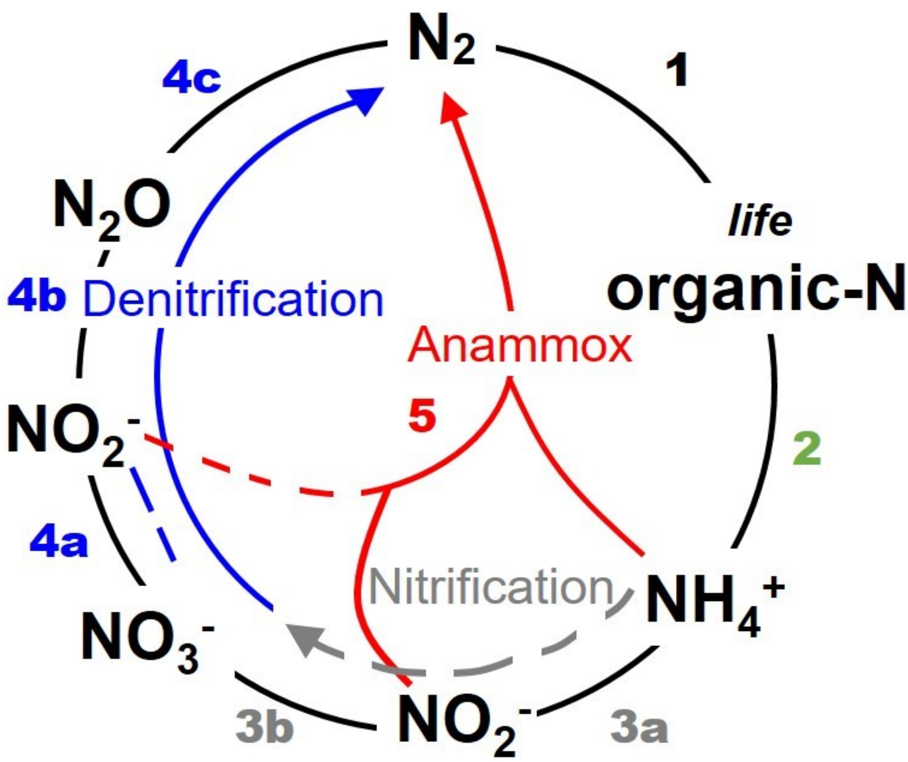

Figure 4 | 1, Specialised microbes use a lot of energy to make (fix) N_2 available so that life can build organic-N. 2, life excretes excess N as urea and uric acid and – after death and decay – all organic-N decomposes via urea to ammonium (NH_4^+ or NH_3). In environments with oxygen (i.e., they are oxic), ammonium is first (3a) oxidised by microbes to nitrite (NO_2^-) and then (3b), typically by another group, to nitrate (NO_3^-). Where oxygen is absent (i.e., truly anoxic or very limited) some microbes (including *E. coli* in your gut) start to respire by reducing NO_3^- to NO_2^- (4a) and many others (4b) can truly "denitrify" to make nitrous oxide (N_2O) and N_2 gas that completes the cycle. In contrast to the multitude of denitrifying microbes, a further specialised group known as anammox bacteria combines parts of 3 and 4 to oxidise ammonia anaerobically to N_2 gas (5). *You could actually add ^{15}N-N_2 at the top and trace it all the way through the different microbial pathways until it reappeared again as ^{15}N-N_2 gas!*

Whereas you have probably already learnt about the N cycle, you may wonder where on Earth does N actually get cycled? If you look at an atlas you can quite easily identify the vast expanse of the Pacific Ocean. If you look closer you can find Hawaii and if you go east you will come to California and further down Mexico, Guatemala and finally Panama. A triangle with its corners in California, Panama and near to Hawaii (see Exercises for details) roughly marks out the Oxygen Minimum Zone in the tropical North Pacific (there is also one further south off Peru and Chile).

Oxygen Minimum Zones are a little hard to grasp at first, as although the Pacific is ~ 4,500m deep here, you only need go down 50m to 100m and 90% of the oxygen dissolved in the water will have gone – go a little deeper *and there is practically no oxygen at all i.e., the water is anoxic*. Here, microbes obviously cannot rely on oxygen and anaerobic microbes are adapted to respiring either nitrite (NO_2^-) or nitrate (NO_3^-) to N_2 gas and this anaerobic microbial activity has attracted a lot of scientific attention. Scientists use specialised equipment to collect and work with anoxic water to which they add ^{15}N-tracers e.g. $^{15}NO_2^-$ (Figure 5). The anoxic water also contains natural $^{14}NO_2^-$ and anaerobic microbes will respire both the $^{14}NO_2^-$ and $^{15}NO_2^-$ to give 3 isotopic "colours" of N_2 gas, namely $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$. This principle can be extended further to distinguish between different types of microbes that produce N_2 gas in different ways i.e., the blue and red pathways in Figure 4. Oxygen Minimum Zones are major players in the global N cycle, producing some 200 Tg of N_2 gas per year (1 Tg = 1,000,000 tonnes) – *and all by microbes!*

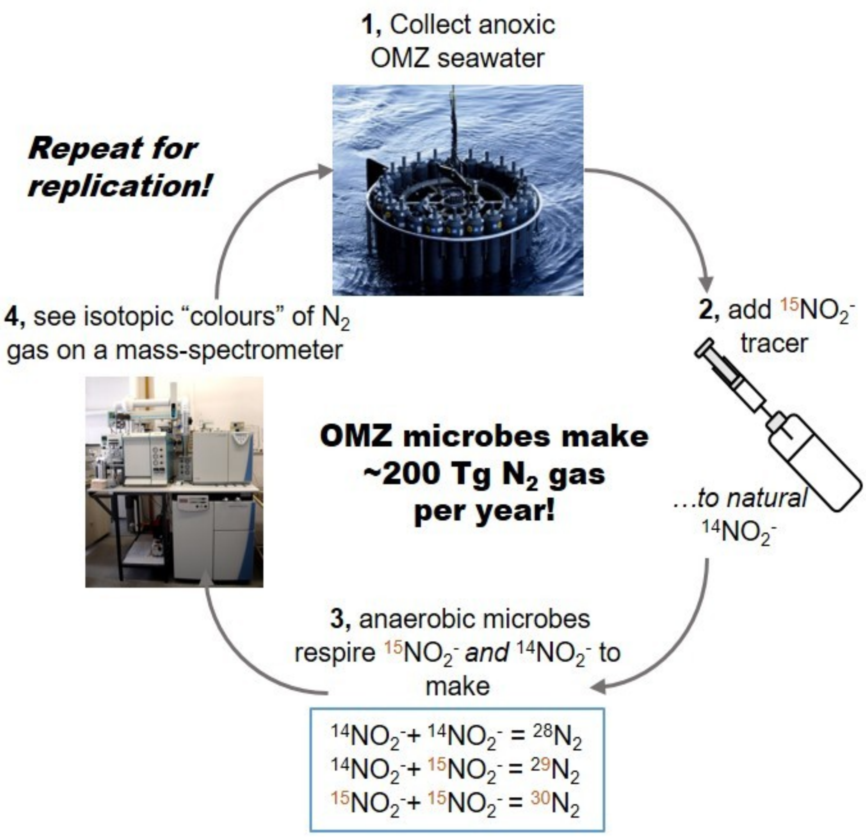

Figure 5 | 1, A CTD (conductivity-temperature-depth) rosette is used to collect discrete samples of water from any depth in the ocean and returned to the ship. **2,** Careful steps are taken to set-up anoxic incubations where, for example, a $^{15}NO_2^-$ tracer is added to a sample of water containing natural $^{14}NO_2^-$. **3,** the prepared samples are then incubated while the anaerobic microbes respire both $^{15}NO_2^-$ and $^{14}NO_2^-$ to make different "colours" of N_2-gas that can be measured **4,** using a mass-spectrometer. Incubations are repeated to replicate the findings, along with different combinations of ^{15}N-tracers used to identify different types of microbial activities (e.g. blue and red microbial pathways in Figure 4).

Pupil Participation

1. ***Class discussion***

   a. Think about what happens to your hair as it grows and then gets cut off at the barbers. Each time you get your hair cut, the barber is cutting-off *new* hair – your hair has been replaced and the same is true (though at different rates) for all your cells and for all cells in all forms of life. Your elements are cycling and you are part of global biogeochemical cycles that turnover your macro-elements (HiC-NOPS) – microbes drive that cycling.

2. ***Pupil stakeholder awareness***

   a. Microbes get a bad name as they are often only associated with causing disease and death, but they are key to maintaining Earth's biosphere where we all live. Human activities tend to cause environmental problems by "overloading" these microbial activities: flush the toilet and microbes will eventually convert some of *your* N into the greenhouse gas N_2O; send *your* organic waste to the dump and microbes will use it to make CH_4; and intensive farming for *your* food washes soils into rivers along with fertilizers that disturb the microbial cycling of C and N. We can study these problems using isotopes but we can only redress the balance if we all do our bit to be more sustainable. The point to appreciate is that *you* are not separate to the microbial cycles, *you are part of* the microbial cycles.

The Evidence Base, Further Reading and Teaching Aids

Fundamental building blocks – elements, atoms and life

What are Atoms? The smallest parts of Elements and YOU! - YouTube

Biological Molecules - You Are What You Eat: Crash Course Biology #3 - YouTube

Mass-spectrometry and isotopes

https://www.youtube.com/watch?v=lxAfw1rftIA

Microbes and carbon in soil

Schimel J. 2013. Microbes and global carbon. Nature Climate Change. 3. 8670868. Doi: 10.1038/nclimate2015

Microbes in oceanic oxygen minimum zones

Wright J. et al. 2012. Microbial ecology of expanding oxygen minimum zones. Nature Reviews Microbiology. 10. 381-394. Doi: 10.1038/nrmicro2778

Microbes – humans and climate change

Cavicchiol R. et al. 2019. Scientists' warning to humanity: microorganisms and climate change. Nature Reviews Microbiology. 17. 569-586. Doi: 10.1038/s41579-019-0222-5

Glossary

ATP: adenosine triphosphate comprises one molecule of adenosine, bound to three phosphate groups. Energy from cellular respiration is bound in the triple phosphate bond and subsequently released to drive life when adenosine **tri**phosphate is converted to adenosine **di**phosphate, ADP.

Biochemical, biochemistry: the study of chemical reactions and structures in relation to biology. For example, many inorganic chemical reactions proceed more quickly in the presence of an inorganic catalyst. In a biological setting, enzymes (protein) serve as catalysts to facilitate bio-chemical reactions e.g. alcohol is broken down to acetyl-CoA in the liver by the enzyme alcohol dehydrogenase.
Element: a fundamental pure substance comprised of atoms with unique numbers of protons, neutrons and electrons. All matter in the universe comprises at least one element e.g. hydrogen (H), with compounds (organic and inorganic) comprising at least two elements e.g. hydrogen and oxygen in water, H_2O.
Functional genes: genes ascribed to particular microbial functions that can be used to look at relationships between microbes performing the same functions. For example, methane gas is produced by specialist microbes known as methanogens and, even though the overall biochemistry of methanogenesis is complex and different types of methanogens use different substrates to make methane, there is one final step common to all encoded by the *methyl coenzyme-M reductase* (*mcrA*) gene. The number of *mcrA* genes can be counted to infer the total number of methanogens in a soil sample or we can look at relationships between methanogens by comparing genetic variation in the *mcrA* gene.
Macro-elements: the 6 elements e.g. C, H, O, N, P, and S that dominate the organic molecules in life. Note there is nothing fundamentally different about the macro-elements – they are just elements – the prefix "macro" merely serves to distinguish them from the trace-elements that are also just elements but are found in far smaller quantities in life compared to the macro-elements. That said, the chemistries of C, N and P do make them particularly suited to their organic roles in life. Macro-element also has ecological relevance as organisms compete for macro-elements (often also termed macro-nutrient) i.e., you can think of food or prey as carbon, C.
Mass-spectrometer: a scientific piece of analytical equipment that separates a mixture of molecules depending on their mass and quantifies the abundance (amount) of each. For example, the mass-spectrometer shown in figure 5 uses a large magnet to separate three different isotopic species of N_2-gas, namely: $^{28}N_2$ which has a lower mass than $^{29}N_2$ which, in turn, is lighter than $^{30}N_2$. The N_2 molecules are first charged by having an electron knocked off and then accelerated in a magnetic field. The path of the lightest $^{28}N_2$ is deflected "bent" by the magnet more than the $^{29}N_2$ which is deflected more than the $^{30}N_2$ to create a spectrum of N_2 dependant on mass. The three separated isotope masses can then be quantified.
Molecule: a chemical compound comprising either more than one atom of a single element e.g. oxygen, O, in air exists as di-molecular O_2 or multiple elements e.g. sodium, Na, chloride, Cl, as the molecule NaCl.
Organic and inorganic: 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. Then surely that means CO_2 is organic as it clearly contains carbon? The scientific community often say that C is only organic if it is coupled with hydrogen which would make CO_2 inorganic. We then have the puzzling example of the simplest hydrocarbon methane gas (CH_4) which is *often* considered 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 forms of 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 *available* as energy for other organisms in the ecosystem.

Periodic table: the systematic listing of all natural and non-natural i.e., human-made (e.g. in nuclear reactors or the Hadron Collider) elements according to their shared characteristics e.g. metals, non-metals, Nobel gases etc., as first proposed by the Russian chemist Dmitri Ivanovich Mendeleev in 1869. The total number of natural and non-natural elements is still debated but considering 94 as natural and 24 as non-natural to give 118 elements in total is acceptable!
Trace-elements: elements that are also found in life's organic molecules but in far smaller quantities to the macro-elements. For example, chlorophyll (*a*) is a compound fundamental to photosynthesis and life on Earth that comprises 55C atoms, 72H atoms, 5O and 4N atoms i.e., lots of the macro-elements but it still needs ONE atom of the trace-element manganese (Mg) to work! The ecological point being that a plant (and life that eats plants) will run out of N *before* it runs out of Mg.