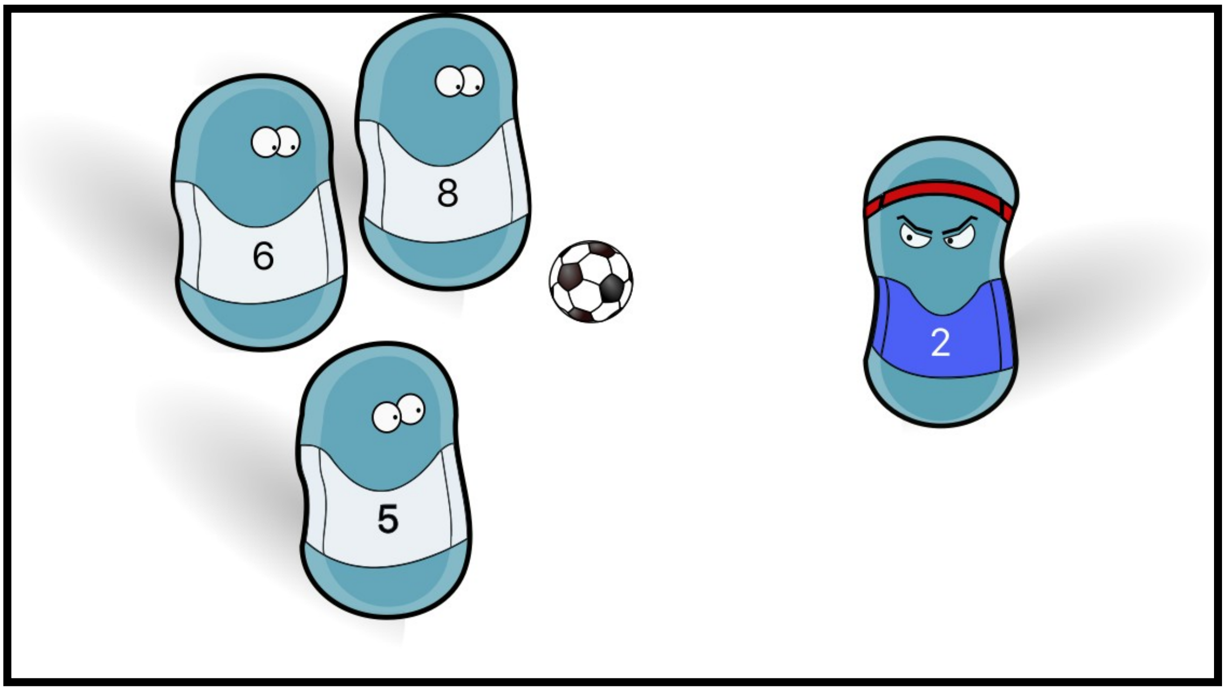
Microbial Communities and Social Behaviour

Miss: Our class is the best of our year and the sports teacher says it is because we play well together as a team: do microbes form teams?



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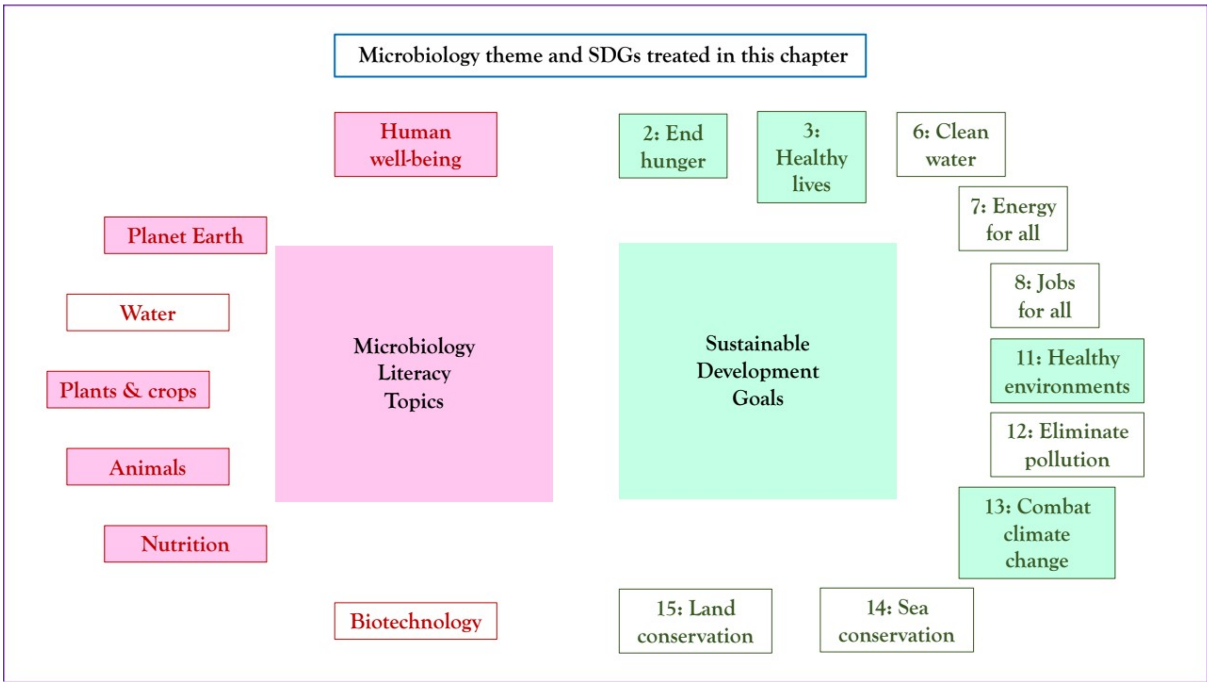
Storyline

Bacteria are rarely solitary creatures. They live in dense and diverse communities all around the world, from forests and oceans to inside us in our microbiomes. Whilst these communities may be extremely different to our own communities, bacteria within them can have surprisingly social interactions. But in the microbial jungle are bacteria working together? Or are they only looking out for themselves? The answer is a bit of both. Bacteria rapidly divide to make miniature clone armies and these work together. Cells produce what are known as 'public goods', helpful molecules that are shared with their clonemates in the local environment. These shared compounds help bacteria scavenge for nutrients in the environment or they can act to poison other bacteria. Bacteria also produce protective layers of slime called biofilms to surround and protect themselves. To help accomplish these complex behaviours, each clone will communicate by signalling to each other, synchronizing behaviours and allowing the group to work together. However, they are not alone in these slime cities. Each army of clones is surrounded by others, and they are all in intense competition over nutrients and space. And each army invests in an amazing array of weapons that are used against one another. Cells release toxins, stab each other with tiny spears, release viruses and more in the struggle for supremacy. Both competition and cooperation then are powerful forces shaping microbial communities and are often happening at the same time. Cooperation between bacteria allows big groups of very small cells to perform astonishingly complex tasks and compete in a fierce environment where a single bacterium could not manage alone.

See the online computer game: http://www.oum.ox.ac.uk/bacterialworld/gutwars/ which accompanies this chapter for use as a teaching aid.



The Microbiology and Societal Context

The microbiology: Public goods in bacterial communities; cheating on bacterial cooperation; bacterial signalling and communication; motility; cross-feeding; biofilm formation; the microbiome; division of labour and horizontal gene transfer. *Sustainability issues*: human health; improving agriculture; environments and microbial communities in climate change.

Microbial Communities and Social Behaviour: the Microbiology

1. ***Cooperation and competition.*** Bacteria can be very social organisms, and like any society, they face a mixture of competition and cooperation. In human society we help others and, occasionally, fight each other. But how can an organism as simple as bacteria be social? A social behaviour is simply a behaviour that has evolved to affect another cell, and bacteria have evolved complex behaviours that affect the cells around them. This can either be in a positive way (cooperation) or a negative way (competition). For instance, bacteria may secrete a molecule to help break down food in the environment, which can then be used by other neighbouring cells, or bacteria can secrete a toxin to which it is itself immune, but which harms bacteria that do not share immunity.

2. ***Cooperative and competitive nutrient uptake.*** Bacteria rely on specific nutrients for their survival, and when an essential nutrient – for example iron in a form useful to bacteria – is scarce in nature it leads to intense competition with the bacteria best at securing the scarce nutrient outcompeting those that are less effective. To compete for iron, many bacteria produce dedicated molecules called “siderophores” that help them scavenge for iron. These siderophores are released into the environment and can often be shared between cells in a cooperative manner. This allows all cells to have access to a large, shared pool of resources, which makes the process of iron-scavenging more efficient for each cell. Siderophores can also be thought of as method of competition. Bacteria may secrete a siderophore that another species cannot utilize, therefore sequestering – taking into ownership – all the iron from the environment for itself and limiting the growth of other bacteria. In this sense, bacteria that can utilize the same siderophore are cooperating, but competing for iron against bacteria that are not able to use that siderophore.

3. ***Signalling and quorum sensing.*** The cost of engaging in cooperative behaviours is often high. When a population is at low density, the benefits of cooperation may not outweigh the cost. We can face this situation when there are too few people to achieve something as a team, whether it be carrying something heavy, building a house, or winning at football. Bacteria need a method of sensing the density of the population to know when they are in a sufficiently high number of cells to engage in efficient cooperative behaviours. Quorum sensing is a cell-cell signalling mechanism that allows bacteria to trigger population-density dependent changes in behaviour. In a typical system, bacteria produce and secrete a signal molecule into the environment. As the population density increases, the concentration of the signal molecule increases until it reaches a threshold where it causes a coordinated response among the population. Quorum sensing is used by a wide range of bacteria. Quorum sensing can control many important aspects of bacterial biology, from the formation of biofilms to behaviours that help them cause infections.

4. ***Cheaters.*** When bacteria cooperate, they provide benefits to the community as a whole. However, this cooperation is vulnerable to exploitation by bacteria which do not cooperate but still reap the benefits of cooperation. For example, if as described in Section 1 a group of bacteria produces public goods, such as iron-scavenging molecules, all members of that group pay the cost of producing the public good, but also reap the benefit. If a member of this community stops producing the public good, it will not pay the cost of siderophore production but can cheat and use siderophores from neighbouring cells to scavenge iron. In theory, as these cheaters pay no cost in siderophore production, they increase in the population, and eventually so many cells in the population will not be producing the public good that the entire community suffers. However, the extent to which this occurs in nature is disputed and bacteria have evolved many ways to maintain cooperation and prevent cheaters from invading a population.

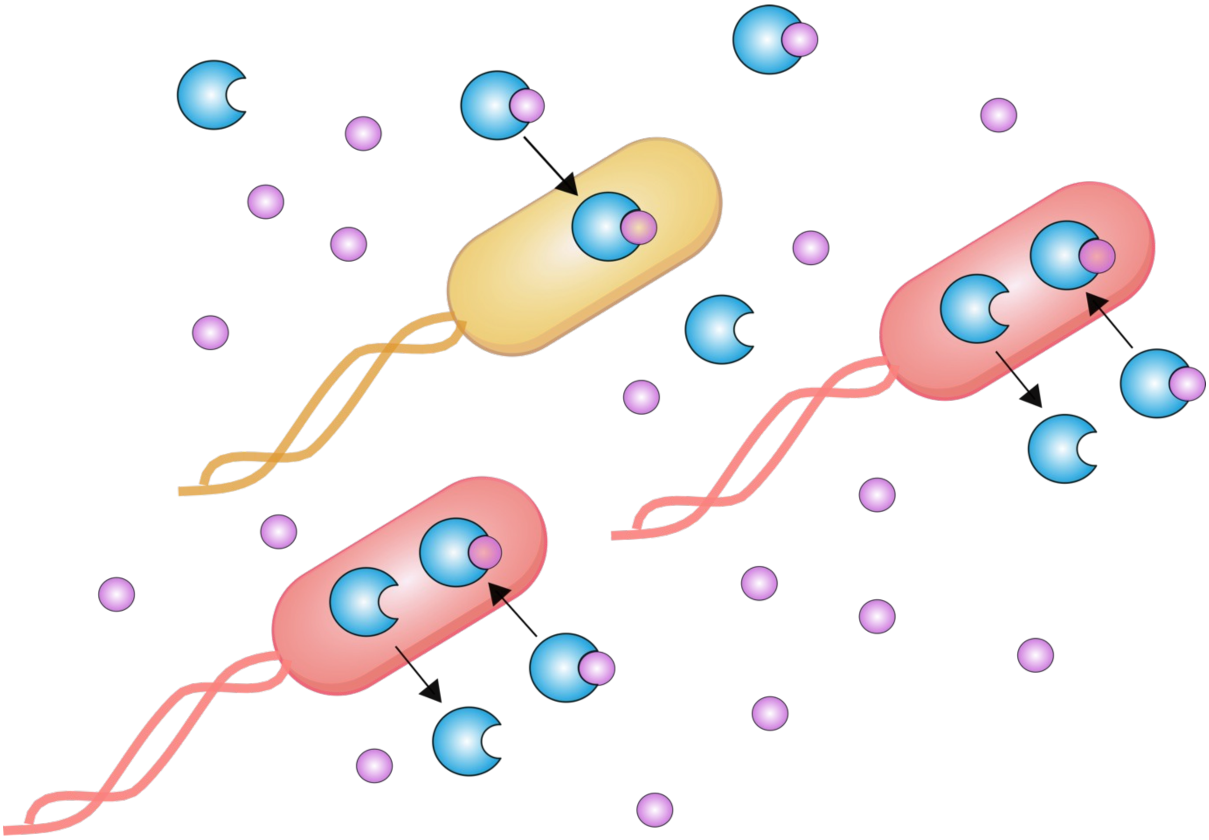


Figure 1. Bacteria produce and secrete public goods which can be utilized by neighbouring bacteria. Here, bacteria are producing siderophores (blue) which bind iron (purple) and are transported back into the cell. Some bacteria are cheaters (yellow). They do not expend any energy producing the siderophore but can use siderophores from their neighbours to acquire iron.

5. ***Cross-feeding.*** Different species of bacteria that co-exist in the same environment may have very different types of metabolism and nutritional requirements. This can result in the different species preferentially taking up different molecules *from* the environment, but also secreting different molecules *into* the environment. Sometimes, this can lead to a phenomenon called "cross-feeding," where the excreted metabolic product of one bacterium can be a useful nutrient for another bacterium. Here, one species is managing to make good use of the waste or excesses of another. For some combinations of bacteria, this can be highly beneficial for both parties as they both benefit from each other's presence and help each other grow faster and survive better. This situation starts to resemble trade or bartering in some human societies where two people exchange things to get what they need the most, such as a baker swapping

bread with a farmer for milk. In bacteria, if the two species evolve together for a long time, they can even become nutritionally completely dependent on one another. The downside of this co-dependency is that they would starve if they ever became physically separated from their cross-feeding partner. It is maybe because of this risk that most cross-feeding interactions in bacteria are not obligatory, but rather opportunistic – bacteria can benefit from them when they occur, but most of the time they do not rely on them for survival.

6. ***Bacterial warfare.*** As explained in Section 2, bacteria can compete by monopolising vital resources in the environment, such as iron. Bacterial clone armies can also fight other competing clones by producing toxic – poisonous – molecules to kill them or to stop them from growing. In response, bacteria have evolved strategies to avoid these toxic molecules. This had led to an evolutionary arms race, where bacteria are constantly involving new and diverse toxins and defence mechanisms. Though a wide variety of toxins exist, they can be split into two categories, diffusible toxins or molecular spears. Diffusible toxins are secreted by cells into the environment, and when they reach a certain concentration they make the environment inhospitable for bacteria which are not immune to the toxin. Molecular spears need the target cell to be next to the attacker cell, which stabs into the victim cell and delivers a toxin. Bacteria can defend against these attacks by numerous methods, such as becoming very slimy to prevent attacking bacteria from stabbing them, growing incredibly fast to overwhelm attackers, by encoding an immunity mechanism such as mutating the target of the toxin, encoding an immunity protein which neutralizes the toxin, or bacteria can produce their own toxin (see Gut Wars in Further Reading).

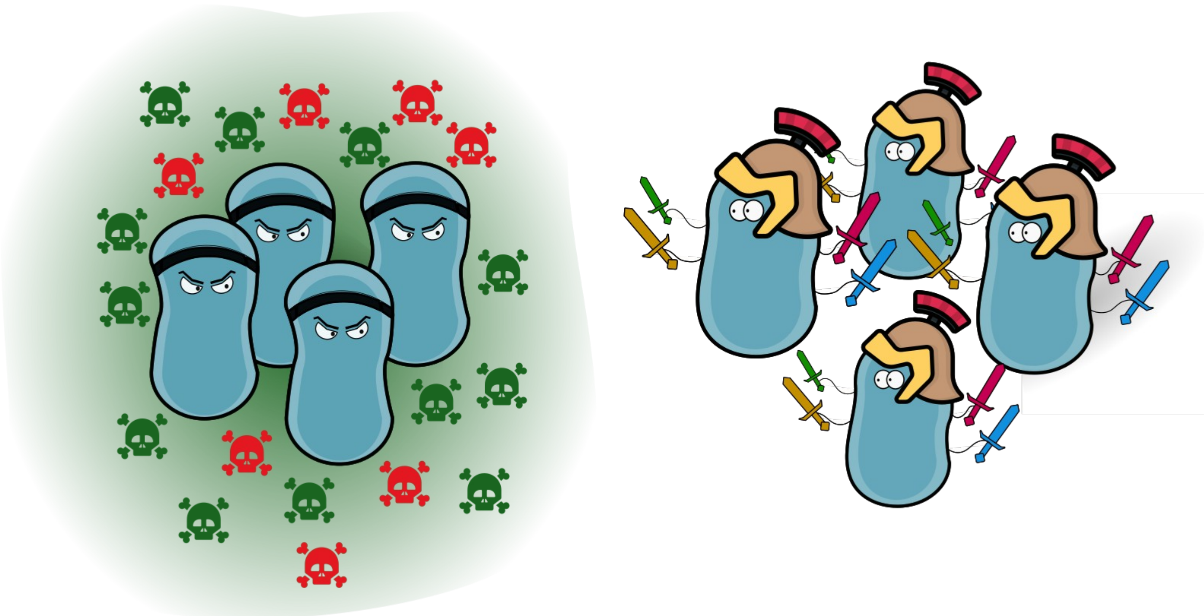


Figure 2 Bacteria can directly compete which each other by producing toxins. Bacterial warfare can be categorised into contact-independent, where toxins are secreted into the environment, and molecular spears, where the attacker cell needs to be in contact with the prey.

7. ***Biofilm formation.*** Biofilms are groups of bacteria stuck to a surface (or sometimes freely floating) and covered in slime. They are ubiquitous in nature, from rocks, forests and oceans, to plant roots and animals. Biofilms are also ancient, with biofilms identifiable in fossils from ~3 billion years ago. Biofilms are bacteria encased in a slimy matrix of sugars, DNA and proteins. This makes biofilms very resistant to environmental stresses such as ultraviolet

(UV) light and extreme temperature. The biofilm matrix also helps bacteria resist attack from the immune system and antibiotics, which makes biofilms a common feature of bacterial infections. Within a biofilm, bacteria can engage in many social behaviours, such as production of components to break down and scavenge nutrients, production of sticky molecules to help it stay on the surface and structural components that help build and stabilise the biofilm, and engaging in warfare to exclude other bacteria from the biofilm.

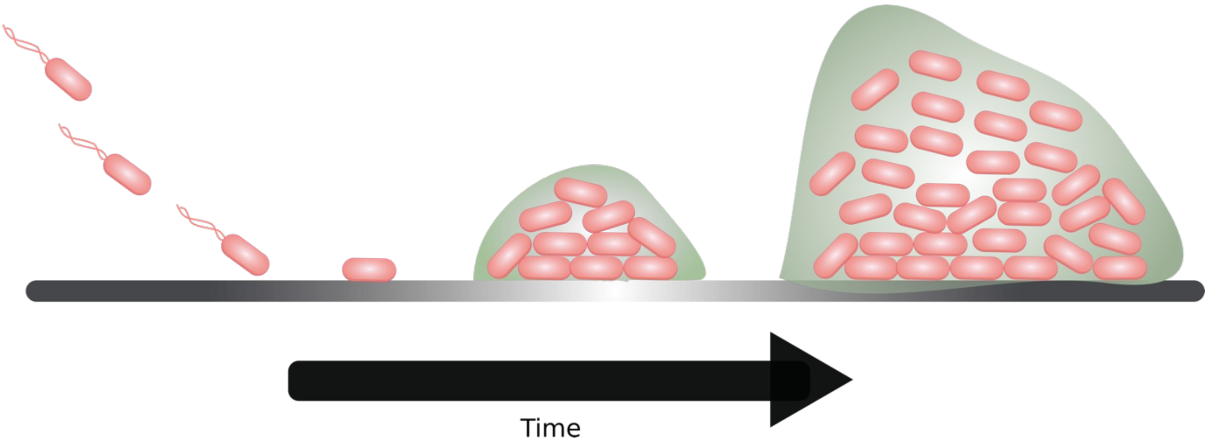


Figure 3. Bacteria form biofilms on solid surfaces. Bacteria moving in the environment attach to a solid surface and secrete polysaccharides, DNA and proteins to form a biofilm. As the cells grow and divide the biofilm matures and grows.

8. ***The microbiome.*** Some of the most dense and diverse bacterial communities on earth are the microbiomes of animals, including those of humans. In the human gut there are 10^{11} (100 billion) bacterial cells per millilitre compared to only 10^{8} (100 million) human cells. Not only is the gut microbiome dense, it is also fantastically diverse with an estimated 150-400 different species. These bacteria have a significant impact on human health, are involved in priming our immune systems and aiding metabolism, and may even affect our mood and behaviours. The exact species in the microbiome and their proportions vary from person to person and depend on the human's genetic makeup, as well as environmental factors such as diet. Even though we are constantly exposed to thousands of bacteria from the environment and our food, our microbiome communities are remarkably stable throughout our adult lives. However, the microbiome is not immune to disruption. Antibiotics, changes in diet, disease and infections can all disrupt our microbiome. Disruptions in the microbiome may result in harmful changes in gut physiology and have been linked to a wide range of serious diseases, from inflammatory bowel disease and intestinal infections, to allergies and asthma. As the microbiome is such a dense and diverse community, microbes are under intense competition for nutrients and space.

9. ***Division of labour.*** A group of genetically identical bacteria is called "clonal", and one might reasonably expect that cells in clonal groups would generally all behave in the same way. However, when a clonal group of cells is faced with a complex, collective task – such as, for example, constructing a biofilm (see above) - it can sometimes be beneficial to split up the work into complementary sub-tasks that are each executed by only a subset of cells. This is called "division of labour". In the soil-dwelling bacterium *Bacillus subtilis*, it has been observed that clonal groups of cells will segregate into sub-groups that each produce different essential components of the "biofilm matrix", a slime-like mixture of molecules that forms a protective layer around the cells. This division of labour makes the process of producing the biofilm

matrix more efficient for the clonal group, since – metabolically speaking - it is easier for individual cells to focus on just producing one component each, and not several different ones at the same time.

10. ***Horizontal gene transfer.*** For the vast majority of animals and plants, reproduction involves the mixing of genetic material between different organisms, which is then passed on to the next generation, for example via sexual reproduction. Bacteria mostly divide asexually by making copies of themselves, a process that does not involve a (genetically different) partner. However, bacteria can also share genetic material with other cells through a process called "horizontal gene transfer", where DNA – and thus, genes - are physically transferred from one cell to another. The crucial difference between bacterial gene sharing and sexual reproduction is that horizontally transferred genes immediately become active in the recipient bacteria. This means that bacteria can pass useful genes between each other instantly, without the need for a future generation to realize this newly acquired genetic potential, like e.g. in human offspring. Frequent gene sharing can be enormously beneficial for bacteria when they need to adapt to a new stressful environment really quickly, for example when they encounter an antibiotic that they are not yet resistant to.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 2. End hunger, achieve food security and improved nutrition and promote sustainable agriculture.** Increasing agricultural yields without devoting more land to agricultural use is a key goal in ensuring food security for the growing world population. Bacterial communities associate with all parts of plants, engaging in social behaviours such as biofilm formation, but significant focus has been given to root-associated bacteria. Root-associated bacterial communities help plants acquire nutrients, tolerate harsh environments, and resist pests and pathogens. Examples include the bacteria that fix atmospheric nitrogen into a form plants can use, and Plant Growth Promoting Rhizobacteria, which can improve crop yields. Development of bacterial communities that increase yield will allow more food production without increased land use, while communities that increase environmental tolerance will allow agriculture in environments where it has historically not been fruitful. Globally, around 20-40% of global crops production is lost to pests each year, and many chemical pesticides used to fight pests have negative effects on human health and the environment. Manipulating the plant microbiome is a promising method to increase plant resistance to disease whilst reducing pesticide use.
- **Goal 3. Healthy lives and fighting bacterial infection.** The bacterial community of the human microbiome has been shown to have a huge protective effect against invasion from pathogens. Stimuli that cause changes in the composition of the microbiome, such as a drastic change in diet or a course of antibiotics, can reduce this protective effect and leave the individual more susceptible to gut pathogens such as *Clostridium difficile*. Understanding what makes a microbiome more resistant to pathogen invasion opens up the door for new therapies to treat and prevent infections by 'strengthening' the microbiome. Using a 'healthy' gut community to replace an 'unhealthy' community has been reported in traditional medicine throughout human history, with reports of intaking animal dung to treat stomach disorders being recorded over 3000 years ago. Recent understandings of the importance of the human microbiome has seen a resurgence in interest of Faecal Matter Transplant (FMT), where bacteria from a healthy individual are transferred to an unhealthy individual. This has already

been shown as an effective treatment for patients suffering from reoccurring *Clostridium difficile* infections. FMT is a promising therapy yet, without understanding what makes a bacterial community resistant to invasion, we cannot be sure if we are transplanting the correct bacteria. Many researchers are attempting to identify bacteria - or bacterial communities - capable of providing strong protection from pathogens. Before we can do this, however, we need to understand the ways bacteria interact with each other.

Antimicrobial resistance is a growing threat to global healthcare. As bacteria develop resistance to antibiotics at a faster rate than new treatments are developed, we face the very real possibility of infections that can no longer be treated by any of our currently available antibiotics. Understanding how bacterial communities respond to antibiotics is critical to understanding the mechanisms and spread of antimicrobial resistance (AMR). Bacteria can secrete molecules into the environment which degrade antibiotics and therefore help non-resistant bacteria survive. Biofilms also help bacteria survive exposure to antibiotics, as the drugs are partially held back by the slime that surrounds the bacteria within the biofilm. Horizontal gene transfer ("gene sharing", see above) is a major pathway for the spread of AMR. Genes which make bacteria resistant to antibiotics can spread from bacteria to bacteria and even across species. Disrupting these mechanisms could help slow or prevent the spread of antimicrobial resistance in bacteria.

- **Goal 11. Healthy environments.** Bacteria are ubiquitous and play key roles in the environment. One such example is the soil, where microorganisms including bacteria are involved in the decomposition of soil organic matter, nitrogen fixation and nutrient cycling. The composition of bacterial communities, and if and how their members cooperate, can heavily influence the rates of all of these processes. Degrading soil organic matter to free carbon for use by plants, is dependent on secretion of multiple molecules which act as public goods (see above). A better understanding of how microbes interact in the soil could allow the targeted engineering of bacterial communities to improve soil nutrient levels and plant yield as mentioned in Goal 2.
- **Goal 13. Combat climate change.** The key role of bacteria in the global carbon cycle makes them a promising target for developing carbon capture technologies. Increases in atmospheric carbon increase plant growth, therefore making more material available for microbes to degrade in the soil. Whilst bacteria can affect climate change, they are also affected by climate change. Higher temperatures and atmospheric carbon could increase the release of carbon from soil by bacterial communities. Understanding how climate change effects bacterial communities will allow better modelling of climate change.

Potential Implications for Decisions

1. ***Individual***
   a. Completing the antibiotics treatment prescribed to us and not using antibiotics when unnecessary
   b. Eating a healthy diet to maintain a healthy diverse microbiome

2. ***Community policies***
   a. Participation in Antimicrobial Resistance Awareness Week
   b. Encourage Natural History Museums to include information on important microbial communities in their exhibitions

3. ***National policies***

a. Restricting the use of antibiotics to cases where they are truly needed
b. Using microbial communities for farming instead of pesticides or large amounts of artificial fertilizers
c. Improving farming practices to maintain soil integrity

Pupil Participation

1. ***Class discussion on the potential roles of bacterial communities***

2. ***Pupil stakeholder awareness***
    a. How do bacterial communities in your own body affect you?
    b. How do bacterial communities in the environment affect you?
    c. How do bacterial communities in the environment affect everyone?
3. ***Exercises***
    a. How is bacterial cooperation similar to how you cooperate with teammates?
        i. Do bacteria need to communicate?
        ii. Do bacteria work towards a common goal?
    b. How is bacterial cooperation different to how you cooperate?
    c. Where can we find bacterial biofilms? What every day activity do we do to prevent biofilms on our teeth?

The Evidence Base, Further Reading and Teaching Aids

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Drugs vs Bugs: An antimicrobial resistance boardgame.
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Gut Wars
http://www.oum.ox.ac.uk/bacterialworld/gutwars/

Glossary

Antibiotic – A substance that inhibits the growth or kills bacteria
Antimicrobial – A substance that inhibits the growth or kills a microorganism
Antimicrobial Resistance (AMR) - Resistance to the inhibitory effects of an antimicrobial
Biofilms - A group of bacteria encased in a layer of self-made slime, often adhered to a surface
Clone – Genetically identical bacteria originating from the same ancestral cell
Cooperation – An evolved behaviour which is beneficial to another at a cost to the producer
Competition - An evolved behaviour that is antagonistic towards another organism

Community - A group of people or organisms living in a defined space (in the field of ecology this means specifically a group of different *species*)
Faecal Matter Transplant – Transferring faecal matter, including faecal organisms, from a healthy donor to another individual
Gene – A sequence of DNA which encodes for a product such as a protein
Immune system – A complex system that protects the body against infections
Microbiome – The combination of all living microbial organisms and their genes in a particular environment
Pathogen – A microorganism that causes disease
Public good – Compounds released by a bacterium into the environment which can be utilized by surrounding cells
Siderophore – A molecule secreted by bacteria to bind iron and transport it into a bacterium
Sequestering – Obtaining or taking ownership of something for yourself.