A child-centric microbiology education framework

Rethinking chocolate for the future

Mummy, why don't you allow me to eat more chocolate?

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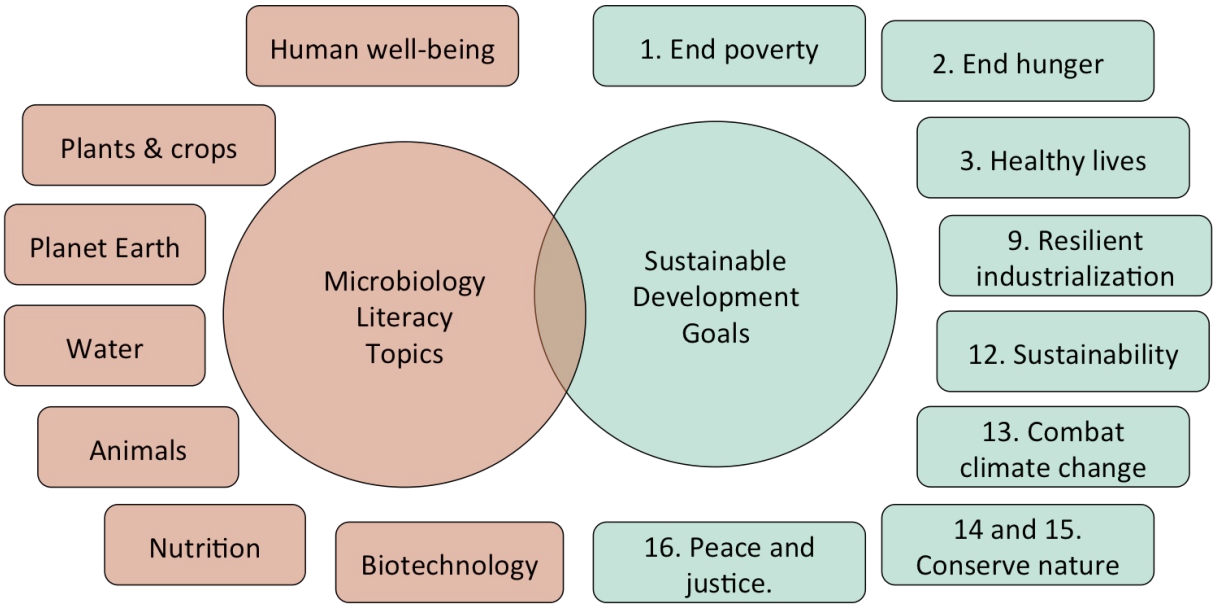
Storyline

Most people enjoy eating chocolates and especially children have a sweet tooth. While cocoa contains compounds that can provide health benefits, its mix with sugars can influence human health negatively. Nutrition-based health effects are often times mediated through the gut microbiome, which also responds in changes in its composition based on our diets. The production, transportation and processing of chocolate touch upon socio-economic as well as environmental problems. Here, we summarize these issues especially in light of microbiology.

The Microbiology and Societal Context

The microbiology: soil biodiversity and its role in agriculture; flavor formation through microbial activity; fermentation; effect of nutrition on the human gut microbiome; pollution; eutrophication and toxic algal blooms. *And, peripherally for completeness of the storyline*: value generation in chocolate production *Sustainability issues:* health; food and energy, economy and employment; environmental pollution; global warming.



Microbial Literacy topics and Sustainable Development Goals covered in this chapter.

Chocolate: the Microbiology

Theme 1: Agricultural practices and their impact on cocoa yield and biodiversity

1. ***Monocultures reduce overall biodiversity and deplete nutrients in the soil.*** A healthy soil is able to sustain life of microorganisms, plants and animals. It does so by uptake and storage of (rain)water, filtering pollutants, cycling of nutrients, and through providing physical support. Soil is the most biodiverse habitat on earth and many of the functions above are in part or entirely taken care of by the organisms living in the soil.

In each gram of soil we find 4,000-50,000 different species – the majority of which are microorganisms. In natural ecosystems, many different microorganisms, plants and animals live together in and on soil, forming a rich community with many interdependencies. Plant roots are associated with specific microorganisms, like fungi that grow inside the roots of the plants and extend into the soil. These microbes often form important symbiotic relationships with the plants and help them acquire key nutrients like nitrogen (N) and phosphorus (P). Nitrogen is an essential element of all life but is present in a useful form in only limiting amounts in most soils. Some microorganisms are capable of fixing atmospheric nitrogen into a useful form, like ammonium, and exchange it with the plants to receive other nutrients in turn. The microbiomes of different plants can be connected allowing plants to communicate with each other and to exchange nutrients across a wide web of actors and species.

The creation of artificial agricultural systems, such as monocultures of cocoa trees, is not only associated with a reduction in the biodiversity of plant species but also with a reduction in microbial biodiversity in the soils and reduced biodiversity of animals that may require diverse types of plants and structures as habitats. The overall loss of biodiversity is associated with numerous challenges including

- **a.** nutrient depletion in soils, due to the loss of important species that can fix, cycle or exchange nutrients with each other,
- **b.** erosion of the soil, as it is no longer protected from wind and water through a rich vegetation and diverse microbiome,
- **c.** reduction in insect biodiversity and abundance that is crucial for pollination in cocoa production

2. Fertilisers can be used to increase cocoa yield but come with an environmental cost.

Today, 90% of the cocoa is produced by small-scale, family-owned farms in Western Africa and South America. Most farms rely on monocultures but suffer from low productivity. One reason is depletion of N and P nutrients in the soils. As many family-owned farms do not have access to fertilisers, the nutrients in the soils are not replenished over time. While fertilisers may help to increase productivity and restore soil nutrients, they are also associated with negative environmental effects such as eutrophication of water ecosystems: excessive growth of microorganisms and algae, resulting in oxygen depletion for other organisms, and finally a total reduction in biodiversity and destabilization of the aquatic ecosystem.

3. *Pathogenic microorganisms infect cocoa plants and result in production losses.*

Another factor that reduces the productivity of cocoa are diseases of the cocoa plant. While some diseases are caused by insects or other animals, most of the production loss is derived from fungal pathogens infecting the cocoa plants. Monocultures of genetically identical or very similar plants are highly susceptible to pests which transmit very easily between plants. Many fungal pathogens also have a stage in their lifecycles within the soil, which makes them difficult to eliminate in annual production systems.

Healthy cocoa pod Black pod disease Witches broom disease

The two major diseases of cocoa are called black pod and witches broom diseases and they result in black/brown lesions on the cocoa pod. They are caused by members of the oomycete *Phytophthora* genus and the fungus *Moniliophthora perniciosa*, respectively.

One solution is pesticides that either kill or repel pests. Pesticide use is often linked to a temporary increase in production yield but can be associated with many challenges:

a. Farmers famers might not know which pesticide to use;

b. pesticides might not be available or are expensive;

c. pesticides may contain substances that are also bioactive in or against other organisms including humans (e.g. heavy metals);

d. many pesticides result in acidification of soils and consequently a reduction in soil fertility and biodiversity;

e. pesticides can deplete the ozone layer and consequently increase the effects of climate change;

f. in the long run, pesticides are rarely successful because resistance quickly develops.

4. Soil microbiome management can help to mitigate agricultural challenges in a probiotic way. The microbiomes of the cocoa plant depend in part on the genetics of the cocoa plant, the environmental conditions such as the soil type and climate, but also on the agricultural practices employed. Many interventions such as monocultures, fertilisers and pesticides are aimed at controlling and reducing biodiversity, in order to increase yields of an isolated species/product.

However, "probiotic" approaches seem much more promising and sustainable. Managing the microbiome of cocoa plants may have many advantages as

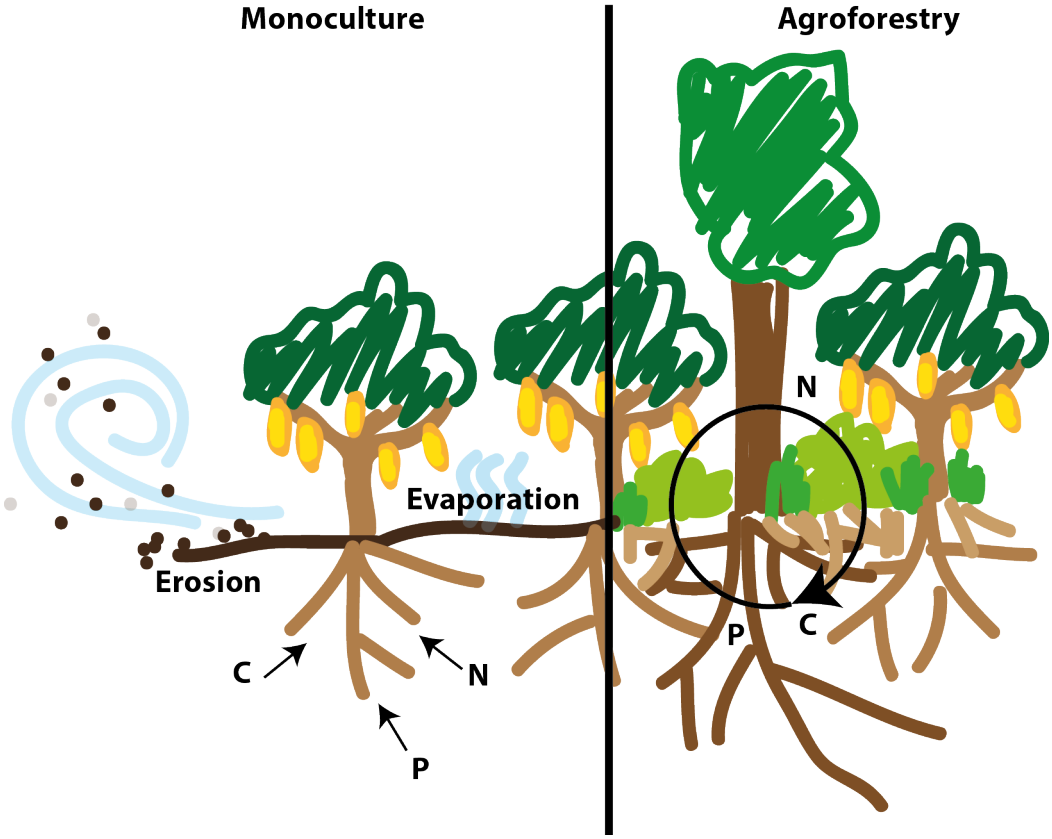
a. specific microorganisms or conditions that favor the growth of certain microorganisms, can promote plant growth and cocoa yield;

b. the cocoa plant microbiome can be used to control diseases naturally as specific species can be used to suppress the growth of pathogens (bioprotection);

c. the cocoa plant microbiome can play an active role in nutrient cycling to allow degradation of organic matter into carbon and nitrogen sources for the plant, or by fixing atmospheric nitrogen;

d. the microbiome can remediate soil pollution, for example of heavy metals or pesticides.

5. ***Agroforestry and increased biodiversity can be a way to sustainably grow cocoa.*** Today, the cocoa farming industry is encouraged and supported to transition towards agroforestry. This means that cocoa plants are grown together with other plants, especially large shade-providing trees and in-between patches or intact forest. The increase in biodiversity and creation of different niches, microclimates and conditions allows the biodiversity to increase, especially the microbial soil biodiversity, and has been associated with more robust systems that are cheaper to maintain, as they do not require artificial fertilisers or pest control and are associated with a lower loss due to pests or low nutrient content. Simultaneously, these systems are higher in productivity as well as in the biodiversity of plants, animals and microorganisms.



Comparison between monoculture and agroforestry systems in regards to soil health (erosion, water capacity, stability and nutrient cycling) as well as overall biodiversity. Plant diversity is also linked to animal diversity like birds, insects and worms that are important for pollination, pest control and soil health. Macrobiodiversity is linked to microbiodiversity of the microorganisms, which are also crucial for soil and plant health.

Theme 2: The creation of chocolate flavor through microbial activity

1. Impact of the soil microbes on cocoa flavor. Besides impacting the productivity of the cocoa farming, soil microorganisms can directly impact the nutritional composition and flavor of crops. While the terroir – the local environmental factors that determine the taste of the cocoa– has been found to vary significantly, it has not been researched in detail how the climate, soil and microbiome affect the cocoa qualities. Yet, scientific studies found that strawberries and wheat have different nutritional composition and flavor profiles depending on

the microorganisms associated with them in the soil. It can be assumed that the same holds true for cocoa.

2. Creation of cocoa flavor through microbial fermentation. After harvesting, the cocoa fruits are collected, cut open and tossed into boxes or onto banana leaves. Microorganisms that are naturally present on the cocoa plant and fruit, in the boxes or banana leaves, as well as in the environment will ferment the cocoa fruit. The cocoa beans do not have a cocoa-like flavor at the beginning of the fermentation; they are bitter and highly astringent. The microorganisms, especially yeasts, lactic and acetic acid bacteria, as well as some *Bacillus* and fungal species, grow on the fruit pulp and create flavor precursors for chocolate. Further, the microbial activity leads to the degradation of bitter and astringent compounds. Additional, oftentimes floral flavors are the result of the microbial metabolism and add to the flavor profile.

The fermentation takes place over a period of 3 to 7 days. Differences in flavor profiles have been observed which may be attributed to local differences in fermentation conditions and length, but may also be driven by different plant microbiomes that are important to start the spontaneous fermentation process.

Food fermentation

1. **Definition (Rotten vs. fermented):** Fermented foods are produced through controlled microbial growth and the associated transformations through microbial metabolic activity. While all organic matter decays, decay is a spontaneous uncontrolled process that might also result in the creation of toxins or growth of pathogenic microorganisms. Food fermentations are usually controlled by humans through salt content, sugar content, pH, access/limitation of oxygen, temperature, humidity and time. Many food-safe microorganisms can thrive under these conditions and they can further avoid spoilage by altering the pH, creation of alcohol, competition for nutrients or production of compounds with antimicrobial properties.
2. **History and co-evolution:** Apes have been observed to purposefully ferment fruit and it is debated whether humans have used fermentation for all of their existence. Genetic adaptations have been observed in humans that speak for a co-evolution of fermented foods and humans (alcohol metabolism and sensing of organic acids often found in fermented foods). Yeast and filamentous fungi have been domesticated by humans since the stone age for the creation of safe and delicious foods and beverages. It is speculated that beer and bread (two fermented products) have incentivized humans to settle down and to adopt an agricultural lifestyle with huge consequences for our species and culture.
3. **Use and potential of fermented foods:** Fermented products are generally safe to consume (often safer than their unfermented counterparts like water (stone age) vs. low-alcoholic beer or raw cassava vs. fermented cassava). The fermentation process creates products that can be stored over longer periods of time, the exploitation of new food sources through the degradation of anti-nutrients or toxins, and fermentation decreased processing efforts (no need for fire/energy) and improved digestibility and increased nutrient content. In contrast to cooking, where only roasting can create new flavors, fermentation is capable of creating new flavors and textures (e.g. milk vs. cheeses).

Theme 3: Processing of cocoa – social and environmental implications

After the initial fermentation step, cocoa beans are usually shipped in containers to a new destination. Large cocoa processing industries can for example be found in the Netherlands. The cocoa beans are roasted to kill off all microorganisms that remain on the cocoa beans. Afterwards, the cocoa is further processed for the production of raw materials for cocoa powder or the chocolate industry. These raw materials are further shipped, processed and sold in various places around the world. The long distances between production, processing, refinery and sales require shipping and an associated environmental footprint. In addition, the value generation in the production chain increases over time, often leaving the farmers and countries of production with low incomes. While fair trade initiatives try and help to reallocate the revenues back to the farmers, it is important to rethink this production system to ensure a fair treatment of all stakeholders and to equitably account for resources such as nutrients and water that went into the production, but that currently are taken for granted.

Theme 4: Health considerations for the consumption of chocolate

1. ***Cocoa flavonoids and polyphenols improve the human gut microbiome and health.*** Multiple small molecules, so called polyphenols and flavonoids, in cocoa have been found to improve human health. While these compounds may also be present in other foods, they reach the highest density in cocoa. They act through multiple pathways and modes to improve human health:

a. Flavonoids have antioxidant potential, which is associated with decreased risk of heart diseases and cancer;

b. they improve the cardiovascular system;

c. they have anti-inflammatory properties;

d. cocoa compounds can protect nerve cells from injury or inflammation;

e. these compounds can protect the skin from UV induced oxidation;

f. they can reduce insulin resistance and consequently the risk for type 2 diabetes;

g. cocoa can improve cognitive function;

h. it can increase satiety and consequently help reduce obesity;

i. cocoa can improve mood,

j. polyphenols can act as prebiotics and help to establish a balanced gut microbiome.

2. ***Nutritional value of different cocoa derived products***. While cocoa *per se* is associated with many health benefits, the majority of products that can be bought in supermarkets and that contain cocoa also contain other ingredients. While cocoa powder or nibs contain pure cocoa, chocolate milks, chocolate spreads, baking chocolate or milk chocolate usually contain also high amounts of fats and refined sugar. This can be illustrated by the ingredient list of a chocolate bar: sugar (58%), cocoa butter, skimmed milk powder, cocoa powder, butterfat, soj lecithin, aroma.

3. ***Sugar consumption has a negative impact on the human gut microbiome, inflammation and overall health.*** Simple sugars such as refined table sugar can be taken up by the human body very quickly without the help of the human microbiome in the gut. A diet that is mainly based on simple sugars can negatively impact the human gut microbiome. Many species in our gut rely on the digestion of complex carbohydrates and fibers found in unprocessed vegetables or grains. If these are lacking due to an unbalanced diet, microbes with the ability to degrade complex carbohydrates can be depleted from the gut microbiome. If large amounts of sugar are consumed over a long period of time, a reduction in microbiome diversity can be seen. Further, the microbes present are starved and start to metabolize the thin mucus layer lining the gut wall and separating human cells from the microbes. A thinner and incomplete mucus layer allows direct contact between microbes or their proteins and metabolites and human cells, which often results in inflammation. In general, a reduction in biodiversity in the gut is linked to increased risk for multiple diseases including cancer, autoimmune diseases, metabolic diseases like type 2 diabetes and many more.

Theme 5: Rethinking chocolate for the future

Currently, gastronomic chefs and scientists like Kim Wejendorp or Johnny Drain have developed products that mimic the taste and texture of chocolate by utilizing side streams, like brewers spent grain, that are abundant locally across the world. Chocolate flavors are created through fermentation steps to recreate the chocolate experience. Using side streams to replace

products with a high environmental footprint may allow us a more efficient use of our food production system and generation of products with increased sustainability profiles. However, effects on cocoa farmers and health aspects need to be considered when advocating for alternative products.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 1. End poverty.** Using probiotic resilient agricultural practices for cocoa farming is likely to generate higher and stable yields and to protect the plants and farmers from pests. Resilient and reliable production coupled with fair trade principles will ensure better incomes for cocoa farmers.
- **Goal 2. End hunger.** Implementation of agroforestry or agroecological approaches for resilient cocoa farming might also mean intercropping with other species that in turn might feed the farmers families. In addition, increase cocoa yields and fair payment for cocoa would reduce poverty among cocoa farmers and thereby decrease hunger.
- **Goal 3. Healthy lives.** Consumption of chocolate products should be carefully assessed: consumption of products high in cocoa and low in sugar and added fats should be preferred in order to benefit from cocoa while minimizing negative side effects from the sugar intake.
- **Goal 9. Resilient infrastructure, industrialization, innovation.** It will be important to innovate in the value and processing chain of cocoa to ensure that value is created for the people and countries that actually grow the cocoa. Circularity principles need to be considered more to reduce food waste production and to increase the amount of food, fertilizer or feed that can be generated from cocoa farming and processing. Reduction of supply chains and transport should also be a goal.
- **Goal 12. Sustainable consumption and production.** Production should be done in a way that increases rather than decreases biodiversity. Reduction of fertilizer and pesticide and use of bioprotection agents will be important to create a balanced and healthy agricultural system in future. The production chain will need to be redesigned to be as sustainable and fair as possible. Due to the environmental footprint of chocolate and current injustice in value generation and distribution, a conscious consumption of chocolate is required. Additionally, a conscious choice of chocolate-containing products is needed to ensure good health.
- **Goal 13. Combat climate change.** Increasing biodiversity in agricultural systems, allowing for intra-cropping and increased growth of plant materials including large shade-providing trees will help to create agriculturally useful areas with still high biodiversity. Trees and crops in general also promote the fixing of atmospheric carbon dioxide. Bioprotection through biodiversity may also aid the reduction of fertilizer and pesticide use, resulting in improved ozone layers and reduced pollution of aquatic ecosystems. Improving the supply chain of cocoa products will further help to reduce the environmental footprint.
- **Goal 14. Conserve marine systems.** Reduction in fertilizer and pesticide use can help to protect marine and fresh water environments and avoid eutrophication and pollution.
- **Goal 15. Protect terrestrial ecosystems.** Increase in biodiversity will allow more animal, plant and microbial species to thrive.
- **Goal 16. Peace, justice and strong institutions.** Rethinking the cocoa production system will be needed to generate a just and fair system that generates the value where it is created.

Potential Implications for Decisions

1. ***Individual***
    a. How much cocoa and derived products should I consume?
    b. Do I consider fair trade when buying cocoa?
    c. What type of cocoa containing products do I choose?

2. ***Community policies***
    a. Costs of health care associated with unhealthy diets.
    b. Promotion of sustainable alternatives or fair trade products.
    c. Education of agroecological framing practices.

3. ***National policies***
    a. Healthcare economics, especially related to gut dysbiosis and metabolic diseases
    b. Environmental pollution
    c. Eutrophication
    d. Greenhouse gas emissions
    e. Land management and biodiversity

Pupil Participation

1. ***Class discussion of the issues associated with chocolate production and consumption.***

2. ***Pupil stakeholder awareness***
    a. Chocolate production and consumption has positive and negative consequences for the SDGs. Which of these are most important to you personally/as a class?
    b. What can be done to change the cocoa food production system to make it environmentally and ethically better?
    c. Which choices do you have as an individual to impact production and health effects of chocolate?
    d. Would you be open to try chocolate-like products that are not made from cocoa? Why or why not?

3. ***Exercises***
    a. Can you think of ways to improve value generation and monetary reflection thereof in the cocoa production chain?
    b. If you were to design an alternative product for chocolate, which material and processes would you use?
    c. Should chocolate as a commodity or luxury item be eaten and to which extent? What would be the consequences if we were to increase or decrease the chocolate consumption around the world?

The Evidence Base, Further Reading and Teaching Aids

Cocoa farming and microbes:
https://www.organic-center.org/research/organic-cacao-production-improves-soil-microbiome
https://edepot.wur.nl/240014

https://link.springer.com/article/10.1007/s13593-021-00748-2
https://nationalzoo.si.edu/scbi/migratorybirds/research/cacao/greenberg.cfm
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https://www.naturefund.de/fileadmin/pdf/Studien/DAF/JJ_-_Andres_et_al._2016_Cocoa_in_Monoculture_and_Dynamic_Agroforestry.pdf
https://iopscience.iop.org/article/10.1088/1748-9326/abb053
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https://www.researchgate.net/profile/Goetz-Schroth/publication/261713726_Chocolate_forests_and_monocultures_-_an_historical_review_of_cocoa_growing_and_its_conflicting_role_in_tropical_deforestation_and_forest_conservation/links/00b49535482f8bcb1d000000/Chocolate-forests-and-monocultures-an-historical-review-of-cocoa-growing-and-its-conflicting-role-in-tropical-deforestation-and-forest-conservation.pdf
https://www.youtube.com/watch?v=jLZ0KtNx354
https://www.youtube.com/watch?v=-dywxCaGOu0

Cocoa fermentation:
https://sarahs-world.blog/bacteria-delicious-chocolate/
https://www.frontiersin.org/articles/10.3389/fmicb.2020.00650/full
https://ojs.openagrar.de/index.php/JABFQ/article/download/15422/15426

Cocoa and health:
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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6723656/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4696435/