# *The Apple Microbiome*

## *Miss: shouldn't the phrase 'an apple a day' be changed to 'an apple and its microbiome a day'?*


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# The Apple Microbiome

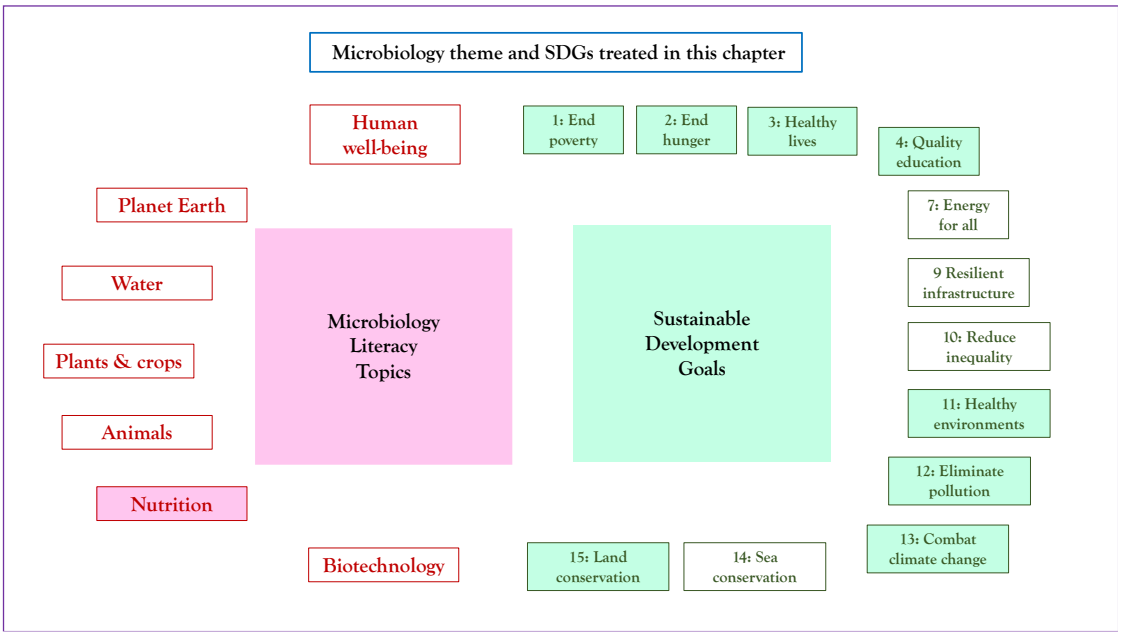
## The Storyline

"An apple a day keeps the doctor away" – this old proverb is known all over the world. Apples are often the first fresh fruit which babies are allowed to eat. Apples can be eaten in so many forms: as a whole, cut, mashed, cooked, as jam, juice or wine. Besides nutrients, vitamins, minerals, fiber, and flavonoids, apples harbor millions of microorganisms. Apples provide children with health benefits that include microbiome enrichment, considered to be important for healthy immune system development and minimization of allergies. Apples are considered to be a healthy superfood. However, they can be dangerous food as well, if mycotoxins produced by fungi get into the food chain, e.g.in apple juice. In addition, apples can cause allergic reactions.

## The Microbiology and Societal Context

Apple trees originated from Asia and have been accompanying humans over the past 4,000-10,000 years. During that time, they were selected and bred: now there are more than 7,500 known cultivars of apples – all of them with different shapes, colors and flavors. This cultivar diversity is particularly high when compared to other highly domesticated plants, such as banana. Each year more than 80 million tons of apple from approximately 100 cultivars are produced world-wide (FAO 2019), and globally $ 7.53 billion worth of apples are traded each year (OEC 2018). These apples originated from home gardens, organic orchards and industrial agriculture. Especially the latter is associated with the application of frequent and high doses of pesticides. However, there are also environmentally friendly possibilities to control apple pests and pathogens, e.g. by use of pest sexual attractants or antagonistic counterparts. Long-term storage and transport changes not only impact the quality and taste of an apple, but also its associated microbiota. So, it is important to know where the apple comes from and how it is produced.

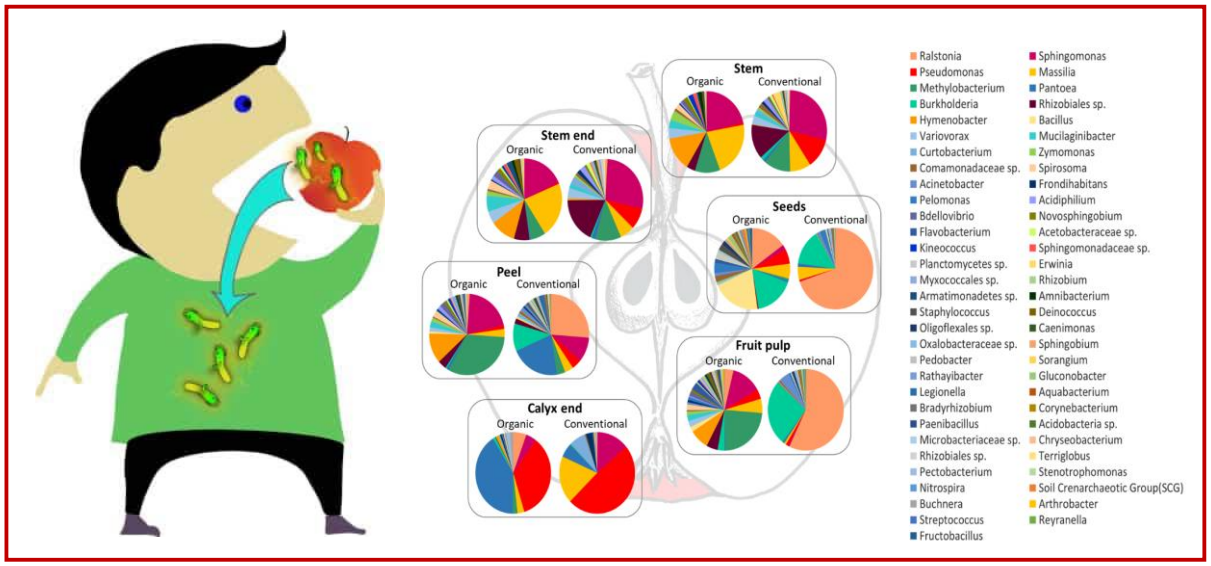
In school, apple flowers and bees are the used to explain pollination. Apple is an important symbol in fairy tales and historical and religious myths. Like Eve, each year we are waiting for the first fresh apples to pick and eat directly from the tree, when we feel again that a little bit of paradise still remains for us.



## The Apple Microbiome: the Microbiology

***1. Apples are superfood.*** Apples represent one of the most important dietary sources of nutrition, antioxidants, vitamins and dietary fiber. Apples are particularly rich in flavonoids, which possess powerful anti-oxidative, anti-allergic, anti-melanogenic, anti-aging, anti-cancer and anti-Alzheimer properties. Besides that, apple nutrients directly influence our gut microbiome in a positive sense. Gut-associated microorganisms are able to utilize the apple pectin and, as a result, protect from diet-induced obesity. Furthermore, the microbial population in the gut can be modified towards higher numbers of bacteria that are described for beneficial health impacts. An apple is an example of plant food that is eaten raw, so also the microorganisms that colonize the outer and inner parts of the apple fruit can affect the consumer in a positive or, when contaminated with human pathogens, in a negative sense.

***2. Apples host a diverse microbiome.*** The apple microbiome contains a wide range of microorganisms, including fungi, bacteria, archaea, protists, etc. Each apple fruit harbors up to 100 million microorganisms. Different parts of the apple plant, such as leaves, shoots, roots, flowers, and fruit, harbor distinct microbial communities. Even within the same fruit we can find varying microbial diversity (number of different microbial species) and composition (abundance and distribution of different microbes) in different parts, like the pulp, peel, the stem-end (fruit top), and calyx-end (fruit bottom). Even the kernels (seeds) inside the apple core are also densely colonized by microbes, which can be highly important for the next generation of plants. Here, the microorganisms are passed on from the mother plant to the seeds and, in this way, seeds are furnished with the important microbes for the new plant seedling to germinate and develop in a healthy way.




The different compositions of microbiota of different parts of an apple

***3. The environment and the cultivar shape the apple microbiome.*** The apple microbiome is influenced by several factors including the plant genotype, soil type, climate, agricultural management practices, and the geographical location in which the apples were grown, including the microbial community present in that area. Apples come in different shapes, color, taste, and aroma, all of which were domesticated from the same ancestor (*Malus sieversii* (LEDEB.) M.ROEM.). In the last 4,000-10,000 years, humans have domesticated, bred and selected apples to give us more than 7,500 cultivars. Interestingly, without knowing, they also changed the

microbiota. Therefore, each cultivar has not only a different genome, shape, color and taste, it also harbors specific microbes. This is what we call the genotype effect. Apple trees can also select and recruit microbes from the surrounding environment, like soil, in a symbiotic relationship in which the plant provides those microbes with few drops of sugar and in return the microbes preform various, yet crucial functions, such as resistance to diseases or drought, that the plant may suffer without.

***4. Agricultural management practices shape the apple microbiome.*** Apple trees and their microbiomes can also be affected by the management practices which the farmers use in the field. For example, organic and conventional agriculture rely on different strategies to fertilize and protect the plants against pests and pathogens. While in conventional agriculture the farmer is allowed to use synthetic fertilizers and chemical pesticides, organic farming prohibit the use of those chemicals and promote the use of environmentally friendly products. Both types of practice can have significant outcomes on both the plant and its microbiome.

**5.** ***Storage shapes the apple microbiome***. In order to provide consumers with apples throughout the whole year, apples are either imported from countries with different growing seasons, or they are long-term stored under controlled conditions. These controlled conditions also include specific post-harvest treatments that aim to reduce or kill apple pathogens (mainly microbial fungi), and thereby increase the shelf life of apples. Besides chemical and pesticide treatments, which have a serious impact on the apple microbiome, there are also more sustainable and biological ways to protect an apple from storage diseases. However, apart from the treatment, the physiological conditions (e.g. ripening processes, increasing sugar content, etc.) within the apple fruit change over time and these changes also affect the apple microbiome.

***6. Pollinators shape the apple microbiome***. Finally, pollinators, like bees which visit the flowers (blossom) of apples and other plants, are also responsible for microbiome exchange in the flower. Pollen is associated with microorganisms which are transported by bees to the surrounding apple trees. This ensures not only fertilization but also microbiome exchange.

**7.** ***All the same, apple plants share a core microbiome***. Although each genotype, or cultivar, has a different microbial community, and all the above-mentioned factors can influence the plant microbiome, all apple cultivars share some specific microbes (also known as the core microbiome) which can be considered as the typical apple microbiome.

***8. Apples and the apple microbiome can help shape the infant microbiome***. Recent investigations have revealed that, in humans, the microbiome of the new-born is inherited from the mother. The microbiome then undergoes changes for 2 to 3 years until it reaches a mature state, at which point it remains relatively stable throughout the person's lifetime. Infant exposure to high diversity of microbes, either from the environment or food, influence greatly the composition of their microbiome. Since babies are often allowed to eat apples as their first fresh fruits, or food for that matter, it is expected that apples have a great influence on the gut microbiome of infants which, in turn, can help train their immune system to ensure optimal immune function and development.

**9.** ***The apple microbiome can affect the gut microbiome***. Vegetables and fruits that are eaten raw, and hence carry a heavy load of microbes that would otherwise be killed by cooking, can be a significant source of diverse microbes that enrich and maintain the diversity of our gut microbiome.

Why do we consider microbial diversity in general as a beneficial feature for health? High diversity and a right balance of the microorganisms and microbial functions makes it more difficult for a potential pathogen to find a niche to colonize and multiply in the gut, or any other environmental system. Reduced diversity or imbalance (dysbiosis), on the other hand, may facilitate potential pathogens to gain a foothold and multiply in the system – simply because there are fewer natural enemies. So far, several pathologies (including inflammatory bowel disease, colorectal cancer, diarrhea, diabetes and obesity) are directly associated with a dysbiosis of the gut microbiome. Fresh vegetables and fruits, like apples, not only provide us with important nutrients and fibers our gut microbiome needs to feed on, but also with microorganisms that can colonize our gut system at least transiently, and thus, maintain diversity. As an additional health benefit: several bacteria associated with fruits have been suggested to reduce food allergies.

**10.** ***From plant to gut and from gut to brain (or plant-gut axis & brain-gut axis)***. The plant-gut microbiome axis describes that microorganisms associated with plants eaten raw interact with our gut microbiome. The understanding of the brain-gut microbiome axis is that the gut microbiome has a profound influence on brain function, behavior and mental health, and that changes in the gut microbiome are associated with psychological issues, such as anxiety, depression and schizophrenia. From animal experiments, it has even been concluded that the transplantation of microorganisms to the gut microbiome can change human behavior and well-being. A diet rich in fresh vegetables and fruits, including their diverse microbiomes, can thus be beneficial in this regard. Meaning: *you are what you eat!!*

***11. Some microbes cause disease of apples, while others protect***. Apple trees are affected by several pests and pathogens, which can affect different parts of the tree, from root to fruit. While the word pest generally refers to insect infestations, pathogens also include microorganisms such as fungi, bacteria, and viruses, which can cause diseases in apple throughout its life cycle and even after harvest. Just like human diseases, apple diseases have descriptive names like Brown rot, Apple scab, Black pox, or Blue mold. Most apple diseases are caused by fungi and their impacts range from a reduction in the quality of the plant produce to a complete loss of yield. Management practices in the field can lower the risk for disease and toxin production. Enhanced diversity and evenness within the apple microbiome, achieved by managing the microbiome, e.g. by introducing counterparts of pathogens or other beneficial bacteria, can reduce diseases.

**12.** ***Apples can be spoiled by mycotoxin-producing fungi***. Several fungi that cause diseases of apples can produce mycotoxins, a by-product produced by the fungal pathogen while it consumes the plant tissue. Mycotoxins can cause acute severe illness but also long-term effects on health. In apple, the main mycotoxin, called Patulin, is produced by *Penicillium expansum* – the fungus that causes Blue mold. But no need to be alarmed; Patulin is not as dangerous as other mycotoxins, and is easily destroyed by the fermentation process, so is not found in apple beverages such as cider. As long as you don't eat a rotten apple, you should be fine.

And again, not all diseases are the same and, while it is not recommended to eat rotten or infected fruits, apples with russet-colored (brownish) patches that are rougher than healthy skin can be eaten and even taste well!

**13.** ***A few people are allergic to apples***. Sadly, some people, especially those who are allergic to birch pollen or peaches, can also be allergic to apples. This is because in apple there is a similar protein to the one that causes the allergic reaction to pollen. Interestingly, most people allergic to apples can eat old apple cultivars without problem. The effects behind that can be further investigated to find novel strategies for the treatment of other allergies, e.g. as treatment

for allergies against birch pollen. Moreover, the number of allergens in apples is influenced by the degree of maturity and storage conditions and, most probably, by the apple microbiome.

**14.** ***Don´t worry...*** Plant tissues are colonized by thousands of different microbial taxa and, despite the considerable quantity of vegetables and fruits consumed every day, food-borne outbreaks related to fresh produce consumption are rather exceptional. Moreover, we know that the majority of plant-associated microbiota are non-pathogenic to humans. New developments in detection and identification methods have shed light on the apple microbiome. From all we learnt so far, it is still important to say: *an apple a day keeps the doctor away*.

### Apples: Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 1 No poverty.** Apple orchards provide income and food.
- **Goal 2 Zero Hunger.** Apples are superfood.
- **Goal 3 Good health and well-being.** Eating an apple is a short period in the lost paradise.
- **Goal 4 Quality education.** Learning from apples and bees is fantastic.
- **Goal 11 Sustainable cities and communities.** Instead of invasive/autochthonous trees plant apple trees.
- **Goal 12 Responsible consumption and production.** Take care where the apple comes from and how it is produced. Reduction of chemical fertilizers and pesticides.
- **Goal 13 Climate action.** Plant as much apple trees as possible. Each Kindergarten or school should have some apple trees!
- **Goal 15 Life on land.** Apple orchards, especially those which are extensively and organically managed, provide a healthy environment.
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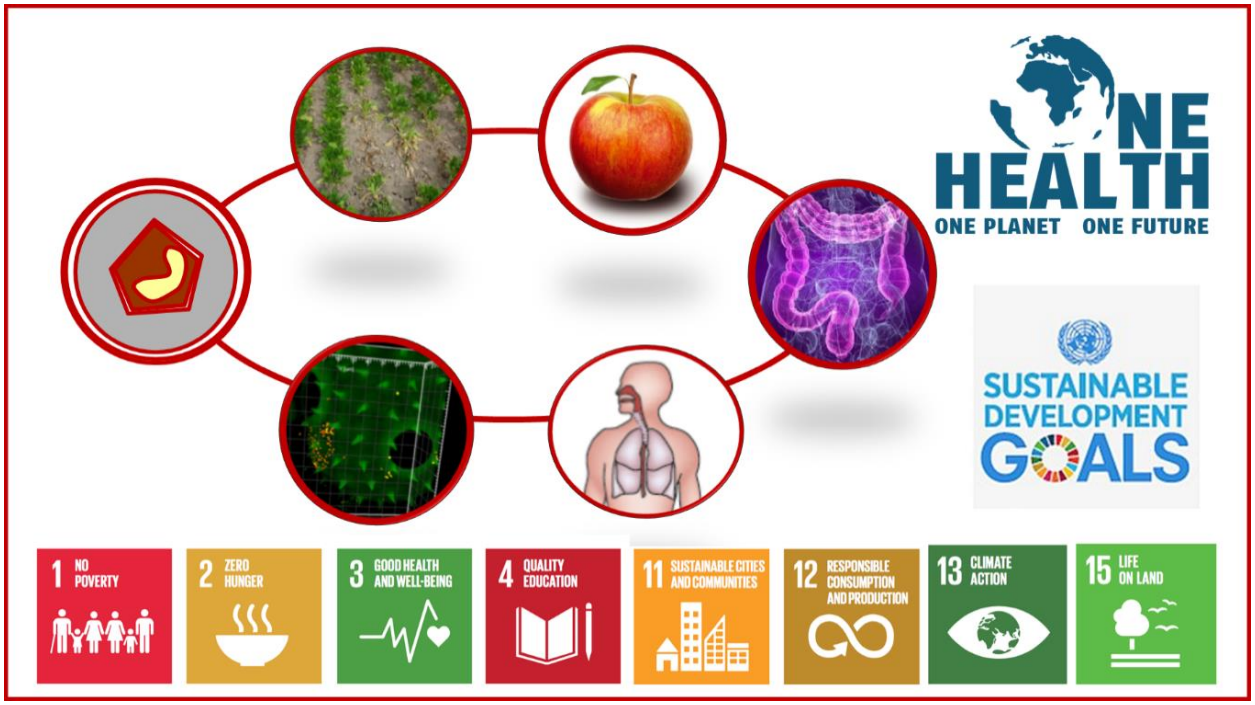

## Apples: Potential Implications for Decisions

1. ***Individual***
   a. **Eat fresh and local.** If possible, buy local apples.
   b. **Eat the whole apple fruit with peel and core** – as Adam and Eve. Then you get the whole microbial diversity for your gut microbiome.
   c. **Try to eat different cultivars.** They all have another taste and provide you with different microbes.
   d. **Small apples or apples with russet-colored (brownish) patches are healthy.** Avoid those with polished skin or those which are damaged by fungi. The latter can be dangerous for your health.
   e. **The microbes cannot be washed away.** Only a few of the millions of microbes are sitting on the skin; the majority lives inside. However, washing can clean them from pollution.

2. ***Community policies***
   a. Enable schools to provide facilities for pupil planting and care of apple trees
   b. Provide educational materials for young and old promoting healthy diets, including the consumption of raw vegetables and fruits, especially apples
   c. Plant non-commercial apple cultivars on common land to promote the consumption and appreciation of apple diversity
   d. Incentivise partnerships between local growers and sellers to market locally diverse apple varieties.

3. ***National policies***
   a. Encourage healthy diets, including the consumption of raw vegetables and fruits, especially apples, in the context of health policy
   b. Provide incentives to apple growers to increase the diversity of planted cultivars to extend beyond current commercial crops
   c. Incentivise the inclusion of apple trees in tree planting campaigns

## Pupil participation

1. ***Class discussion***
   a. Use the apple fruit as a vivid model to explain the importance of microorganisms for plant and human health.
   b. Discuss the health benefits of apple consumption, including flavonoids, pectin and vitamins, but also the microbiome.

2. ***Pupil stakeholder awareness***
   a. With regard to the issue of sustainability, how can different fruit farming practices as well as individual shopping behavior help to meet the SDGs?

3. ***Exercises***
   a. Visit an extensively managed or organic apple farm.
   b. Plant apple trees together, preferably old cultivars.
   c. Organize a blind tasting session with different apple cultivars.
   d. Think of all the different apple recipes and prepare some of them together (tip: raw apples are an important source for microorganisms).

## The Evidence Base, Further Reading and Teaching Aids

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## Glossary

**Allergies**: allergies are numerous conditions that can be caused by a hypersensitive reaction of the immune system to typically non-hazardous substances in the environment. Common allergies are food allergies, hay fever and allergic asthma and can be caused by e.g. certain food ingredients or pollen.

**Antagonistic**: in microbiology, antagonistic interactions refer to the activity of a microorganism to impede the growth of other microorganisms. In plant sciences and agriculture, antagonistic microorganisms are welcomed fellow players as their presence in the field can combat plant pathogens, and thus reduce the amount of pesticides required.

**Anti-aging**: aging happens when our cells lose the ability to effectively divide and replicate. If these so-called senescent cells are not cleared out by our immune system, they can be responsible for symptoms associated with aging and age-related diseases. Apple flavonoids, can help our immune system to clear out these senescent cells, thereby having an anti-aging effect.

**Anti-allergic:** anti-allergic refers to a reduction of allergic symptoms. It can be achieved by medication, but also by so-called allergen immunotherapy, in which patients are exposed to increasing amounts of the allergen. Especially the early exposure to allergens can have an anti-allergic, and thus, protective effect. For example, maternal apple consumption during pregnancy was observed to reduce the risk of their 5-year old children of developing allergic diseases.

**Anti-Alzheimer:** Alzheimer is a chronic disease, caused be progressive structural and functional loss of neurons in the brain. Adherence to a Mediterranian-type diet, which is largely based on fruits, vegetables and legumes, was found to reduce the risk of Alzheimer disease. Especially the flavonoids, frequent components in apple fruits, play an important role in disease reduction.

**Anti-cancer:** anti-cancer or carcinopreventive effects are assigned to substances that prevent the development of cancer or reacts to the effect of a carcinogen, which is the substance that promotes the formation of cancer. Consumption of apples was predicted to reduce the risk of bowel cancer by 50% due to the effects of apple flavonoids.

**Anti-melanogenic**: melanin is the pigment that gives color to skin and hair. Its primary effect is to protect the cells from UV radiation that causes damage to the DNA. However, melanin can also translate to various skin disorders, like hyperpigmentation, skin spots and melanoma, a type

of skin cancer. The anti-melanogenic effect of apples is provided by killing melanoma cells, thereby conferring an anti-cancer effect.

**Anti-oxidative**: antioxidants are compounds that supress oxidation, which is the process by which free radicals are produced that can damage cells of organisms.

**Apple scab**: apple scab is a frequent disease on various plant species from the plant family *Rosaceae* and is caused by a fungus called *Venturia inaequalis*. Infection with the fungus rarely leads to death of the plant but results in fruit and leaf deformation. Also an adverse effect on human health is highly unlikely.

**Archae**a: archaea are single-cell organisms and constitute one of the three domains of life: Bacteria, Archaea and Eukaryota (= 'higher organisms' including also fungi, plants and animals). While initially classified as bacteria, due to their shape and size, we know today that archaea are more closely related to Eukaryota due to specific cell functions. Archaea are omnipresent on earth, part of the microbiota of all organisms, and play important ecological functions in the environment. Until now, no pathogenic Archaeon has been identified.

**Black pox:** Black pox is an apple disease caused by the fungus *Helminthosporium papulosum*. It can affect apple bark, fruits and leaves, and appears as small to medium size black spots. It is less common in the Northern Hemisphere than in the Southern and is considered as less ecomonically important compared to other apple diseases.

**Blue mold:** Blue mold is the most economically important disease of apples after harvest. It is caused by the fungus *Pencillium expansum* and leads to soft and brown rot and greyish blue masses of spores on apple fruits. *Penicillium expansum* also produces the carcinogenic mycotoxin patulin.

**Brown rot:** Brown rot is a common and important disease on apple and other fruits caused by the fungus *Monilinia*. The disease appears as brownish rotten fruits in the field.

**Cultivar**: a cultivar describes a plant that is specifically selected and bred due to desirable characteristsics. It is the main classification for cultivated plants.

**Dysbiosis**: dysbiosis characterizes microbial imbalances on or inside the body. This appears when the normal microbiota of e.g. skin or gut becomes perturbed by usually dominating microbial species being underrepresented or usually less frequent ones becoming overrepresented. Dysbiosis is can be both a consequence and a cause of a disease. A diversified diet including vegetables and fruits can play an important role in preventing dysbiosis in the gut microbiome.

**Fertilize**: fertilization describes the process of applying any natural or synthetic compound to soil or plants to provide nutrients that are important for growth and productivity of plants.

**Fiber**: dietary fiber is part of plant-based food that cannot be entirely broken down by human enzymes in the gut system but can be by gut bacteria. Fibers can act by changing how other nutrients in the gut are absorbed and can be fermented by the gut microbiome to produce short-chain fatty acids conferring health benefits, like decreased rates of colon cancer, coronary heart disease and type 2 diabetes.

**Flavonoids**: flavonoids are naturally-occurring compounds in fruits and vegetables; so far, more than 800 different flavonoids have been described. Their main function for plant health is to protect against UV radiation, pathogens and herbivores. As dietary supplemets for humans, they are considered to promote health and prevent especially cardiovascular diseases and cancer. Apples are the most important source of flavonoids for people in the US and Europe.

**Immune system**: the immune system is the defensive system that protects against diseases, caused by various agents like bacteria, fungi, viruses and parasitic worms. To function properly, the immune system must distinguish between pathogens and harmless microorganisms, and must also recognize and neutralize rapidly evolving pathogens. Our immune system even creates memory which, after initial recognition and response to a specific pathogen, can lead to an enhanced response at a later time point, and thus prevent a disease. This is also the basis function of vacciniation. Especially during childhood, our immunological memory can be trained in a natural way.

**Minerals**: minerals are essential compounds for human health. Vegetables and fruits represent the main source of the 22 minerals known to be required for proper organ function. Apples contain several minerals like potassium, phosphorus and calcium, which are mandatory e.g. for muscle development and assisting nerve functions (potassium), bone and teeth stregthening (calcium) and kidney function, cell growth and heart muscle function (phosphorus).

**Nutrients**: a nutrient is any substance that is required by any organism to grow, reproduce and survive.

**Organic**: here, refering to organic agriculture, describes agricultural management that is based on the use of fertilizers of organic origin and biological pest control, while the application of synthetic substances are prohibited or strictly limited. Organic agriculture is defined as "an integrated farming system that strives for sustainability, the enhancement of soil fertility and biological diversity while, with rare exceptions, prohibiting synthetic pesticides, antibiotics, synthetic fertilizers, genetically modified organisms, and growth hormones".

**Patulin**: patulin is a toxic compound produced by fungi, in particular by *Penicillium* species, which are important pathogens of apple fruits. The amount of patulin in apples is also used to measure the fruit quality. Regarding adverse health effects for humans, there are only few reported cases, although a carcinogenic effect is assumed.

**Pesticide**: pesticides, also known as plant protection products, are chemical or biological substances that are meant to control pests. The target pests can be insects, fungi, nematodes, bacteria, weeds or animals.

**Pests**: a pest is any microorganism, plant, insect or animal that is considered as harmful to humans or human concerns. The term pest, however, is closely related to human modification of the total environment and the intolerance to other creatures utilizing the same space. Many important pests of our crops only emerge as a result of intensive agriculture and monocultures.

**Pollen**: pollen is a powdery substance that is formed by seed plants and represents the male microgametophytes, which is the sexual phase in a plant´s life cycle. When the pollen lands on a compatible female structure (pistill) it forms a pollen tube by which the sperm is transferrred to

female ovule. Pollen, especially when produced in large quantities and dispersed by air, can cause human allergies.

**Pollination:** pollination is the process of transferring pollen from the male to the female part of a plant to enable fertilisation. Pollination can be dependent of diverse agents like, animals, birds, insects, water or wind.

**Pollinators**: a pollinator can be any animal that transfers, actively or passively, the pollen from the male part of the plant to the female part, thus enabling fertilization.

**Plant genotype**: the genotype represents the entirety of genes of an organism that can be inhereted to the next generation. It forms the genetic basis for the development of morphological and physiological characteristics of an individual, which are in turn referred to as the phenotype (= resulting from gene expression).

**Sexual attractants**: sexual attractants, also known as sex phormones, are pheromones released by an organism to attract the opposite sex of the same species, to encourage mating or to perform any other function related to sexual reproduction. Sex phormones have found application in monitoring and control of pests; e.g. pheromone traps are used to determine whether the quantity of pests present demand further action, or to disturb mating of males and females of a pest species.

**Symbiotic**: symbiosis describes the close and long-term interaction of two species, which is advantageous for both partners. The term was introduced by Anton de Bary based on his work on lichens. A symbiosis can be either obligatory for both partners, which means that they entirely depend on each other, or facultative, when the two species can also survive independently.

**Vitamins**: vitamins are organic compounds that represent micronutrients essential for the proper functioning of the metabolism. As all essential nutrients, vitamins can be either not, or only in insufficient quantities, synthesized by the organism itself and must therefore be taken up through the diet.