Microbiology for a better future

**The human wellbeing-connectivity-planetary wellbeing nexus:
microbes to the rescue!**

Photo by Hannah Grapp: https://www.pexels.com/photo/boy-feeding-a-calf-9660871/

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Storyline

To pretend to be able to look in the future, is preposterous. Yet to evaluate the current problems and use our current knowledge to indicate certain potentials and identify opportunities for societal improvements is a testimony of wisdom. The field of microbiology and biotech has offered numerous new insights and plenty of new developments generate prospects for the future, one that is worth striving for by the next generation. In a few decades from now, the following suggestions may appear as naïve and completely dated; yet our world will have progressed and the generation that will have realized it, will receive full credit for it. What follow are some issues that we believe must occupy future efforts of microbiologists and the other natural and social scientists they collaborate with.

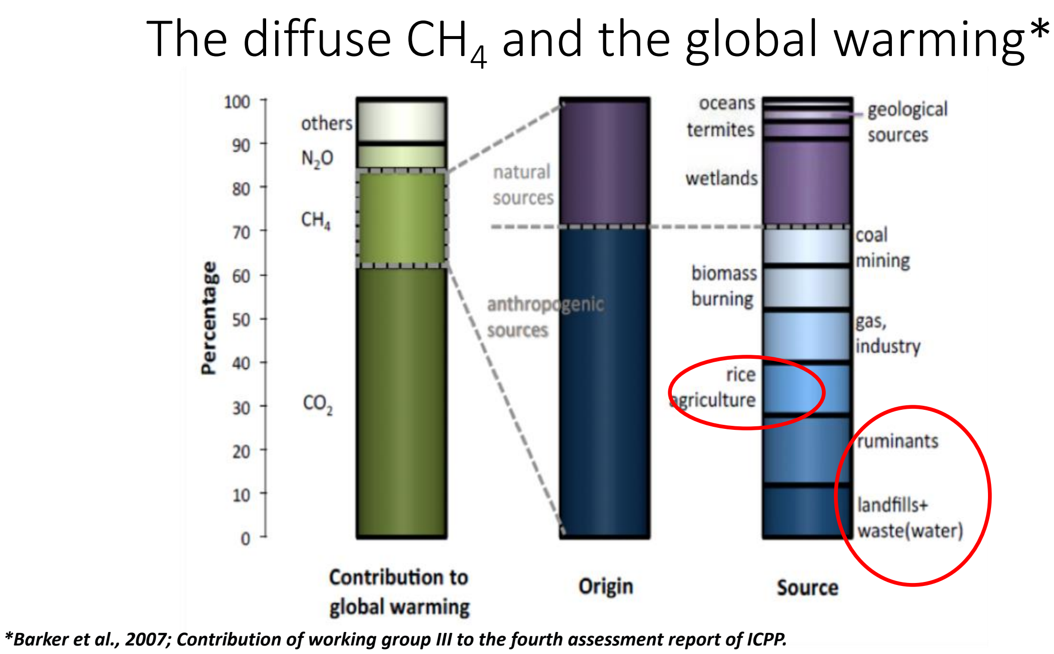
1. ***Climate change and the need to deal with unwanted microbial processes.*** Microbes are all around us, always have been and always will be on this planet. They are important drivers of essential biogeochemical processes worldwide. In doing so, microbes also contribute directly to the production of carbon dioxide (CO_2) and other, even more powerful greenhouse gases, such as methane and nitrous oxide (laughing gas).

For starters, the soil microbial ecosystem generates massive amounts of CO_2, actually 10 times the amount produced by all traffic. This originates from microbial degradation of organic matter in the soil: peat and humus are 'eaten' by the soil micro-organisms under aerobic conditions at a rate of a few percent per year. In addition, the lowering of groundwater levels may turn a soil more aerobic thereby increasing the rate of organic matter degradation and CO_2 emission. The combination of extensive organic matter breakdown and groundwater extraction for drinking water purposes will not only contribute to higher CO_2 emissions but also increases the risk of soil shrinkage, eventually resulting in the cracking of buildings on top of these sites and even sinking of entire cities (Gouda in The Netherlands, Jakarta in Indonesia).

Mitigating soil ecosystem CO_2 emissions will be of great significance to curb global warming: it is therefore often proposed that environmental management strategies should interfere with this microbial mineralization of organic matter in the soil. Yet, this is considered rather controversial as one should not forget that mineralization of organic matter is intrinsically related to the natural recycling of organic matter and nutrients and thus should be considered as 'nature at work'. On the contrary, humanity should tackle the core of the problem: the excessive drainage and pumping of groundwater causing the soil to become more aerobic thereby paving the way for microbial activity. Climate change and extended periods of droughts will obviously further aggravate the situation. While climate change can only be tackled at a global level, local policy changes can actually be highly effective in curbing soil CO_2 emissions and the unpleasant side-effect of soil shrinkage and sinking cities. ***Limiting groundwater extraction and groundwater replenishment in periods of rainfall are effective strategies to slow down the decrease of humus in agricultural soils.***

A more interventionist solution might be sought in a method to (bio)chemically crosslink soil organic matter and turn it into more complex molecules. This will result in a slower degradation of organic matter by the soil microorganisms. Slowing down the CO_2 production from agricultural soils is therefore considered beneficial as it will decrease the release of CO_2 to the biosphere. Yet, it would also come with an interesting side effect, improving soil fertility and increasing the water holding potential of the soil, thereby improving crop production.

Another evident issue in terms of global warming is methane production. Methane is a greenhouse gas with a 30 times higher global warming potential than CO_2 and is therefore considered a real nuisance in terms of climate change. Methane production by microorganisms from the enigmatic Archaea kingdom of life occurs naturally in waterlogged agricultural soils, in rice paddies, in waste treatment systems, in the human gut, and even more substantially in the rumen of cows, sheep and other ruminants.



Overview of different gases as they contribute to the global warming.

The solutions for mitigating methane emissions are obvious. We must avoid dumping waste in landfills and anaerobic lagoons. We should develop new high-yielding rice cultivars that allow more oxygen transport to the rootzone in order to obtain more microbial methane oxidation. Organic wastes must be digested in a contained reactor environment and the resulting methane must be captured and used in combined heat-and-power units to generate heat and electricity. This is already adopted policy in the EU.

For the rumen and the enteric system, we should dare to be ambitious, but the question is whether (micro)biology will allow us. Efforts to inhibit methane producing Archaea using natural dietary substances (e.g. rosemary constituents or oils) or chemical inhibitors (e.g. 3-nitro-oxypropanol pills) have so far failed to successfully deliver in animal production. Alternative and more specific strategies to abate methanogenic microbes by vaccination of ruminants in Australia have been unsuccessful so far. It seems that meat and milk production from ruminants is tidally interlocked with methane producing Archaea in the rumen. ***We may need to reconsider the human craving for animal protein, especially given the fact that production animals require feed crops that leave an additional ecological footprint.***

Nitrous oxide, N_2O, better known as laughing gas, is also a clear challenge for the microbiologist. Wherever nitrogen is cycled in the environment, the processes of nitrification and subsequent denitrification generates itself a 'waste' product: N_2O, a gas with 256 times the greenhouse potential compared to CO_2. Despite the fertilizing properties of nitrate in terms of crop production, nitrate is vulnerable to microbial reduction where N_2O will be generated. Clearly, in processes such as composting of organic wastes, sewage treatment, treatment of ammonia rich gases from animal housings, nitrogen metabolism is a nuisance because it generates considerable amounts of this laughing gas. Microbiologists have attempted to control nitrogen metabolism by specific chemicals (nitrapyrin), but the overall success of these specific biocides is thus far limited. Promising routes of exploration would be the upfront separation of ammonia as gas and to use microbial processes to reintegrate the ammonia by means of organotrophic microbial growth into microbial biomass. The latter can then serve as a starting point of producing slow-release organic fertilizers or feed or food as indicated in Fig 3.

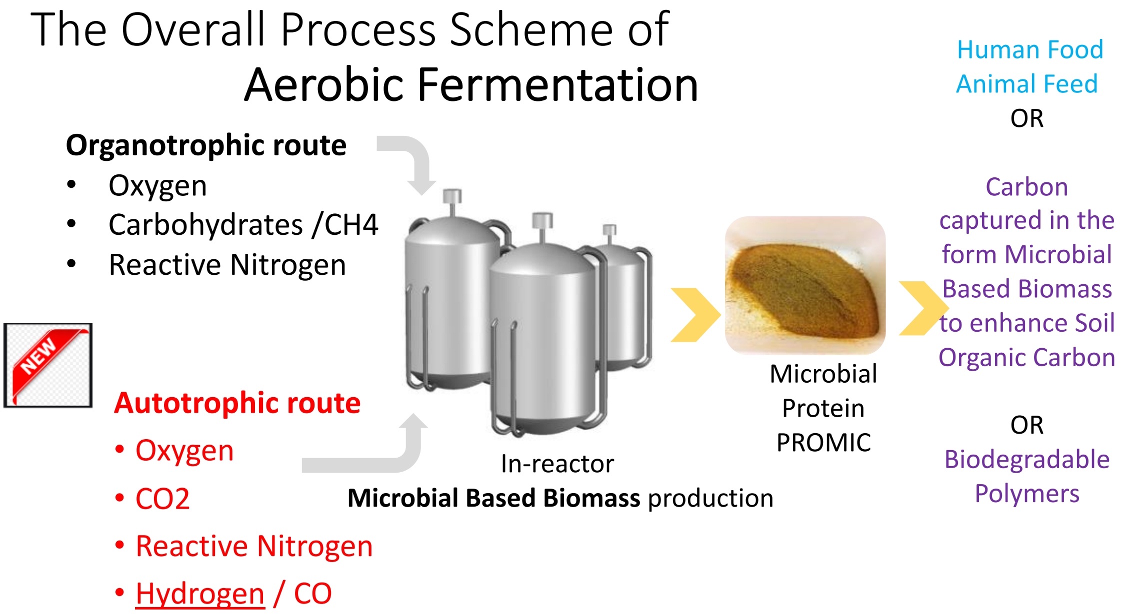
2. ***Food and the protein shift.*** The human body needs some 250 g of nutritious protein per day in order to function properly. This can be acquired through the consumption of a diversified plant-based diet, but care must be given to complement this with an additional source of vitamins, like vitamin B12. Indeed, we cannot make this vitamin ourselves and it is deficient in a plain vegetarian diet, so we rely primarily on meat as an important source of B12.

An innovative way to resolve meat reduction in/elimination from our diet is to substitute with microbial protein from algae, yeast, bacteria or fungi. At present, we already have microorganisms as regular constituents of our diet: lactic acid bacteria in fermented foods (yoghurt, sauerkraut, cheese, sour beers, etc), yeast in fermented beverages (wines, beers, etc.), and fungi (cheese, mushrooms). These types of protein have been labelled as single cell protein: indeed, the microbial cells are 'on their own', they do not make a tissue. This microbial biomass could consist of up to 80% of high-quality protein and also provide sufficient levels of vitamin B12. The idea behind single cell protein, and the growth of microorganisms with the purpose of using them as a source of protein-food, is not new. The well-known food Quorn is for instance composed of fungal cells which have been bound into a coherent texture. We already make use of yeast strains to ferment doughs in bakery products. Already in the 70's of the past century, a microbial biomass grown on fossil methane gas was industrially produced for feed and food purposes.

Unfortunately, the massive development of the soybean feed crop has significantly interfered with the development of single cell protein: the soybean protein produced in the USA and also in the vast plains of the Amazon in Brazil, has been inexpensive so far. Yet the feature of importance is the fact that all conventional protein leaves a very heavy footprint on our planet. To grow protein-rich crops, one needs water, plenty of water, but also further nutrient input through fertilization, the use of pesticides for crop protection and fossil fuels to plow and harvest. Soy is a plant that can fix nitrogen from air through the cooperation with bacteria. Yet, obliging the plant to make use of rootzone bacteria to get hold of atmospheric nitrogen comes with a cost: providing bacteria with sugars that are present in the root exudates and that concomitantly lower crop yield. That is the reason that even soybean production involves chemical fertilization most of the time.

Overall, to feed the human population with high quality protein, either from land or from the sea (fish protein), the costs on the planet have become very high: they represent some 3-4% of the total energy budget of the planet. Moreover, to achieve top crop productivity, the industrial nitrogen fertilizer is dosed at 5-10 times the level needed and leaching or runoff of fertilizer excesses damage the planet by phenomena of acidification, decreases in species diversity, groundwater deterioration, the creation of oxygen minimum zones in aquatic systems, and generation of nitrous oxides which destroy the ozone layer.

There is another route with more optimal use of the industrial nitrogen possible. One can grow plants, which mainly produce carbohydrates such as sugar cane, potatoes, cassava, etc. These crops do not need much nitrogen fertilizer and do not have such a negative impact on the environment (less loss of nitrogen fertilizer via leaching, runoff; less formation of unwanted nitrogen oxide species in the soil). Then the sugars and starch formed by these crops can be brought into a fermenter and upgraded to excellent food protein by means of common bacteria/yeast and mineral nitrogen produced by the chemical industry.



Growing nutritious single cell protein in a reactor thus avoiding the heavy impact of reactive nitrogen on the environment

Even more striking is the fact that one can produce green energy (photovoltaic panels, windmills) and use it to split water into hydrogen gas and oxygen gas. The energy of the sun is thus preserved in two gases, which when one allows them to re-unite provides ample energy for bacteria to grow. The best suited organisms are the so-called 'knallgas', i.e. hydrogen oxidizing bacteria. These natural organisms use the energy released by the reaction between hydrogen and oxygen which they carefully bring about, to fix – just as plants do directly with sunlight – CO_2 from the air. They thus also upgrade the minerals one provides to the reactor and produce a nice nutritious protein perfectly suited for human consumption.

Winston Churchill already wondered about the inefficient way humanity acquired protein: "We shall escape the absurdity of growing a whole chicken in order to eat the breast or wing, by growing these parts separately under a suitable medium."

The figure above depicts how the long route of protein production via the crop and the production animal can be replaced by a passage of hardly a few days in a reactor system. The microbial protein grown in a simple reactor can subsequently be formulated into a form of veggie-meat. When society shifts to protein from plants and microorganisms, a substantial amount of the current agricultural area will be freed up for many other applications, to the benefit of all its creatures.

3. ***Pandemics and cows as company.*** Some decades ago, a medical doctor in Bavaria (Germany) came to a remarkable observation. In Bavaria, there were many dairy farms built in a quite special configuration: the house of the farmer had a direct connection and passage to the dairy house where the milking cows were kept and milking (often still by hand) was performed. As a result, those farms were characterized by the farmer constantly bringing the microbiota from the cows directly in their living quarters and hence the children were constantly and directly exposed to the micro-organisms of the cows (see image of title page). In the same villages, there were also houses of non-farmers of which the children do not have such exposure. The key feature was that the children growing up in constant exposure to the multitude of farm microorganisms were much less prone to infection and had a much better immunological resistance against diseases, as monitored over several decades by the clever medical doctor.

Later on, other studies corroborated the fact that 'farm dust'-loaded with micro-organisms can be sufficient to step up the overall immunology of people. This brings us to a surprising conclusion: our body, more in particular our immune system, is trained by getting exposed to a diversity of microorganisms from our direct surroundings. It appears infancy offers a unique window in time to experience such advantage by distinguishing endogenous microorganisms we tolerate from unwanted microorganisms we need to keep at bay. The current coronavirus pandemic teaches us that the best way to stop the virus is to get a vaccine. The first vaccine was a kind of puss from a cow with the pox disease (*vacca* is Latin for cow) and it was found to establish resistance in the human body against the horrible human pox disease.

Clearly, civilization has profited enormously from the domestication of animals in general and of that of the cow in particular. The time has come when we may have to look at domesticated animals in a different way: they can be considered our companions – even sparring partners – who constantly challenge us a with a diversity of microbial agents that keep our immune system alert. Environmental microbes have thus far mostly been considered a threat; we must learn to live in harmony with all the creatures around us, including bacteria, fungi, viruses, and particularly associate with the hot spots of cooperative microbes that we pick up from our direct environment: not only from specific animals, but also from organically grown crops, soils and surface waters. ***This continuous immune training by our surrounding ecosystem is microbiology at microbe-human interactions at their best.***

4. ***Pollution and biodegradation.*** All over the world, the phenomenon of plastic trash piling up is well known. It clearly demonstrates that the beautiful invention of a molecule that is non-biodegradable by microorganisms, gives rise to tremendous problems. Indeed, the various types of plastic molecules are incredibly well-designed: they have functionalities which we all appreciate (plasticity, water and air tightness, transparency, ...) and moreover they are so long and have special bonds so that they do not disintegrate. The microbial world has no way to decompose them. This is in sharp contrast with all-natural materials, such as wood, hair, nails, teeth... Over

time, environmental microbes will always find a way to decompose provided the conditions for growth (temperature, moisture, oxygen, acidity …) are right. This is simply wonderful: if this was not the case, we would not be able to go in our garden without stumbling on one or the other remainder of former plant or animal life; nature would be one horrendous graveyard.

Yet, when it comes to some molecules invented by men (xenobiotics), we have been able to surpass the capacity of degradation present in nature. It is naïve to assume that micro-organisms eventually will evolve to degrade xenobiotics. Certainly, for some detergents, pesticides, pharmaceuticals, solvents … microbiology has 'cracked the code' to decompose. However, others are of such composition that to untangle them, it takes so much 'investment' by the decomposer that these molecules are left alone. Hence, what is the solution for the future? We do need plastics, pharmaceuticals… but we must from now on take on board the wisdom that biology is not 'infallible'.

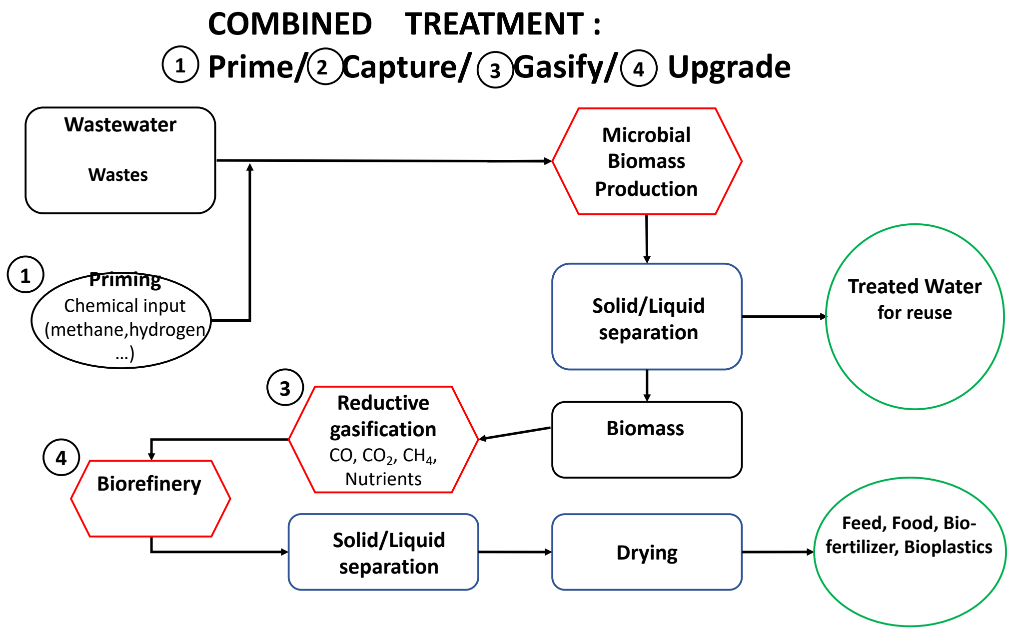
A striking example of such insight took place at the end of the millennium when Nobel prize winners Molina, Crutzen and Rowland informed two rather conservative politicians about the disturbing facts on halogenated hydrocarbons, the so-called CFCs (chlorofluorocarbons). These compounds were endangering life on our planet by destroying the ozone layer that protects us from the scorching UV irradiation that causes massive skin cancer. Convinced by this insight, former US president Ronald Reagan and British PM Margaret Thatcher were able to push forward a worldwide ban on CFCs as refrigerants and propellants through the famous Montreal protocol from 1989, which is nowadays still considered the very first universally ratified and most successful international treaty.

The bright scientists who were able to put their scientific insight on the political agenda should receive major credit from humanity. We must learn from it: no longer should we embrace xenobiotics that are non-biodegradable and which accumulate at infinity on our planet. This is one of the objectives of the REACH legislation within the European Union and should be adopted worldwide to protect this and the generations to come.

Plastics and other xenobiotic chemicals must be engineered to be at least slowly consumable by natural organisms and all countries should agree to abandon the use of totally unbiodegradable materials.

5. ***Circular economy: the biorefinery.*** The concept that we must make our economy circular is great. In the past, old iron scrap was collected and melted again. Old clothes were collected to make paper. Fecal matter in the cities was collected and sold to farmers who carried the night soil out of the city dwellings to fertilize their fields. In Flemish history, you can find city regulations prohibiting to add water to make larger volumes of the precious good. The value of the night soil was 'tasted'; its salt level assured that it was rich in nutrients and thus worthy to be transported and thus capable to produce the next crop. These are just a couple of old-school methods that unknowingly embraced the concept of circularity.

What can be the role of microbiology in the circular economy of tomorrow? Microbial biotechnology can upgrade molecules starting from low concentrations and it can bring forward catalysis under a broad range of conditions. Chemical processes and catalysts on the other hand, can operate under much more extreme conditions of temperature and pressure and concentrations levels, but to bring forward the synthesis to valuable components, they require quite strict conditions of input. Hence, the future is to combine harsh physics and chemistry with agile biochemistry. We can use the biology to capture what is present in wastes and then subject it to full disintegration by the powerful physical and chemical technology. In other words, we can rely on the microorganisms to trap nutrients in their cells. We can subsequently use thermal gasification of this 'unclean' biomass to gases such as carbon monoxide, ammonia, methane, carbon monoxide and phosphorous. The latter are then 'cleansed by the high temperature' and used as an input for biotechnological processes. In this phase, the bio-catalytic processes are able to refine this complex mix of gases and synthesize valuable microbial products.



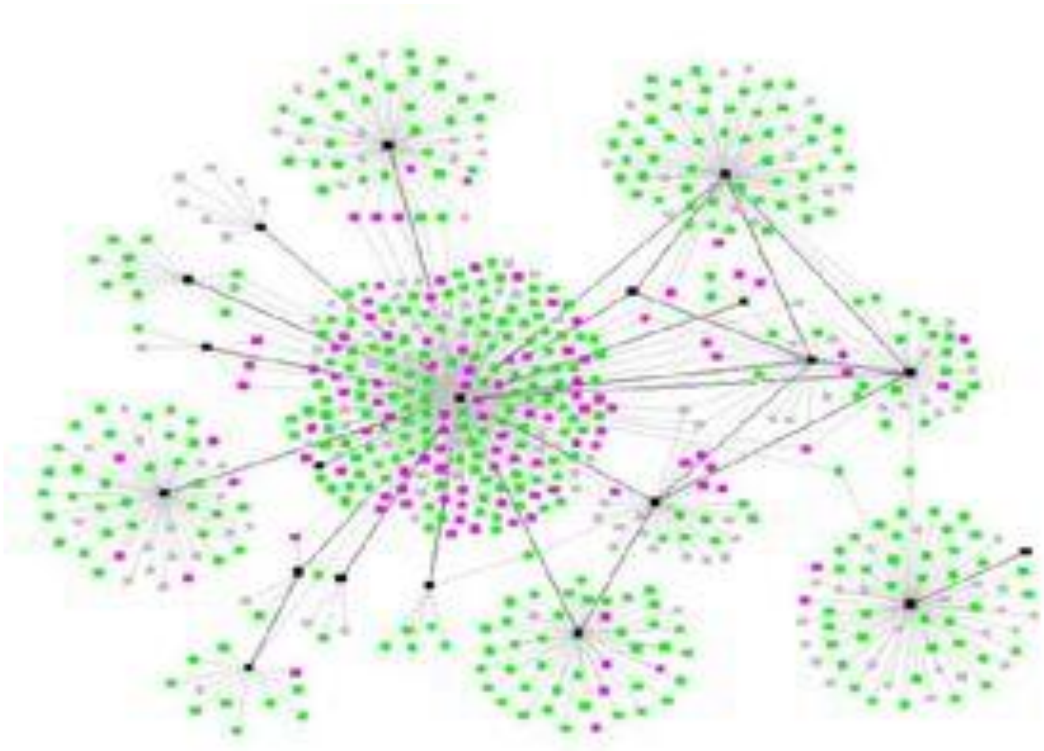
By providing them with chemical energy, micro-organisms capture the nutritive components of wastes, which subsequently are converted by physical processes to unit molecules. In the subsequent biorefinery, these units are upgraded to valuable commodities.

6. ***Well-being and the Gaia homeostasis.*** Nature has evolved by the principle of survival of the fittest. Yet the more we look into ecosystems and the way they function, the more we learn about positive interactions such as cooperation and even symbiosis. In the microbial world, the thousands of different species co-exist and clearly compete with one another but they also form value chains: one type of organism starts a process, and a series of species follow on, each one

bringing about a part of the process in which they particularly specialize and excel. Moreover, various vitamins and growth factors are often shared and there is an intensive communication between species of the same and of different types.

A simple gram of soil receiving a dead leaf, or a dead insect body witnesses the very complicated, yet orderly process of gradual degradation with optimal use of energy and nutrients. Another example comes from the gastrointestinal tract where each (well-balanced and health supporting) meal that enters the gut results in the microbial ecosystem bringing about a series of processes that deliver important services to our body, such as nutrients, energy, but also wall protection and even immunological support. This endogenous microbiota is tolerated by the human host and this results in a stable relationship, termed homeostasis.

Yet, a genetic predisposition, our craving for the wrong (fiber-poor and sugar- and fat-rich) diet, our exposure to environmental pollutants or our fetish for a hygienic sterilized world, sometimes causes turmoil in this human-microbiota relationship: dysbiosis, characterized by an unbalanced microbiota where one or more microbial groups or species proliferate and contribute to or aggravate disease.



We all connect to one another in small hubs, and the hubs link with one another. This interconnectivity helps to maintain stability, in the microbial and the human world. In the future, we might learn to better interact via a clever understanding of the microbial ecology and the way it helps to bring about stability.

The fact that millions of individual species apparently can live alongside each other and maintain a kind of "well-being" altogether, is most intriguing. Microbial ecology is a research domain where one learns how very large numbers of very different organisms can strive for co-existence under continuously challenging and variable conditions. These microbial-ecological mechanisms are of universal value to us. Microbial ecology helps us understand how the complexity of interactions lends resilience to microbial ecosystems and how that yields stability for crucial biogeochemical processes on our planet or how that can even support our health. Let us cherish the gifts that microbial ecosystems have offered us and let us discover and embrace the functional potential that still lies hidden in their genetic codes.

The microbial world is a world to learn from. We human beings, and all other life forms on this planet, are humble colonizers of this microbial world. After all, microbes hold the status of the oldest life form: they should be appreciated and honoured for this.