# Wetlands, swamps and bogs: life without air

## *Daddy, why are there so many bubbles coming out of the water? Is there something breathing underneath?*

Swamp in the Alps in Austria. Photo: Paul Bodelier

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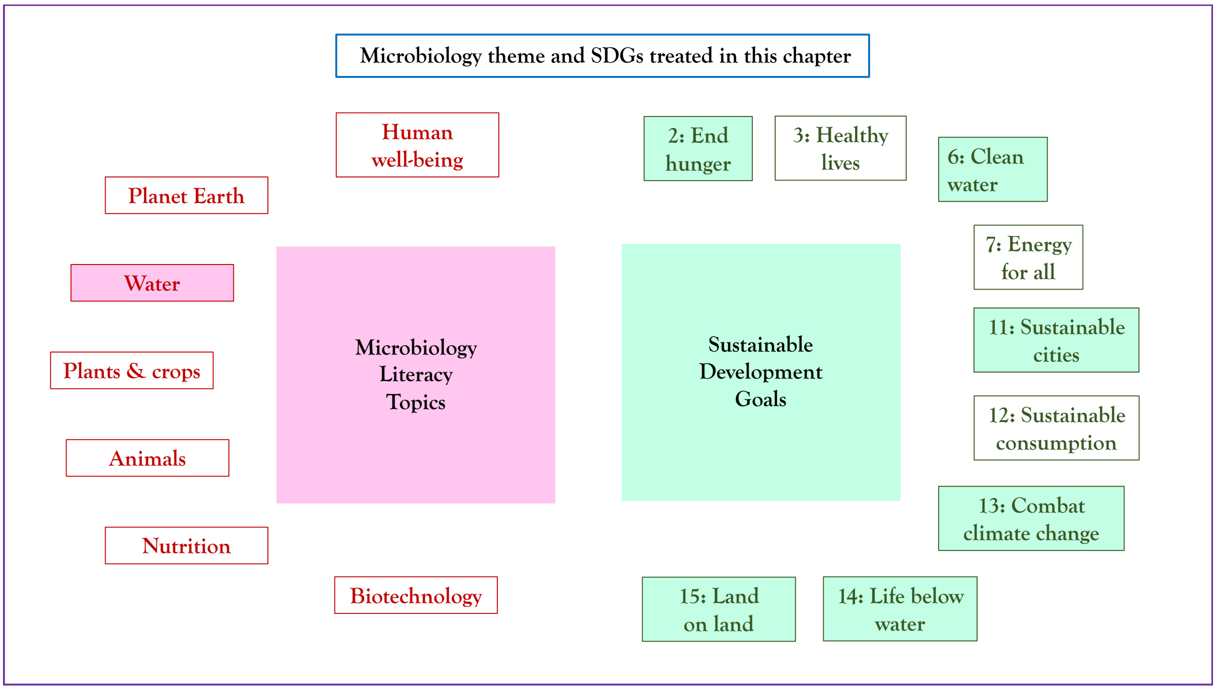
# Wetlands: Life without air

### *Storyline*

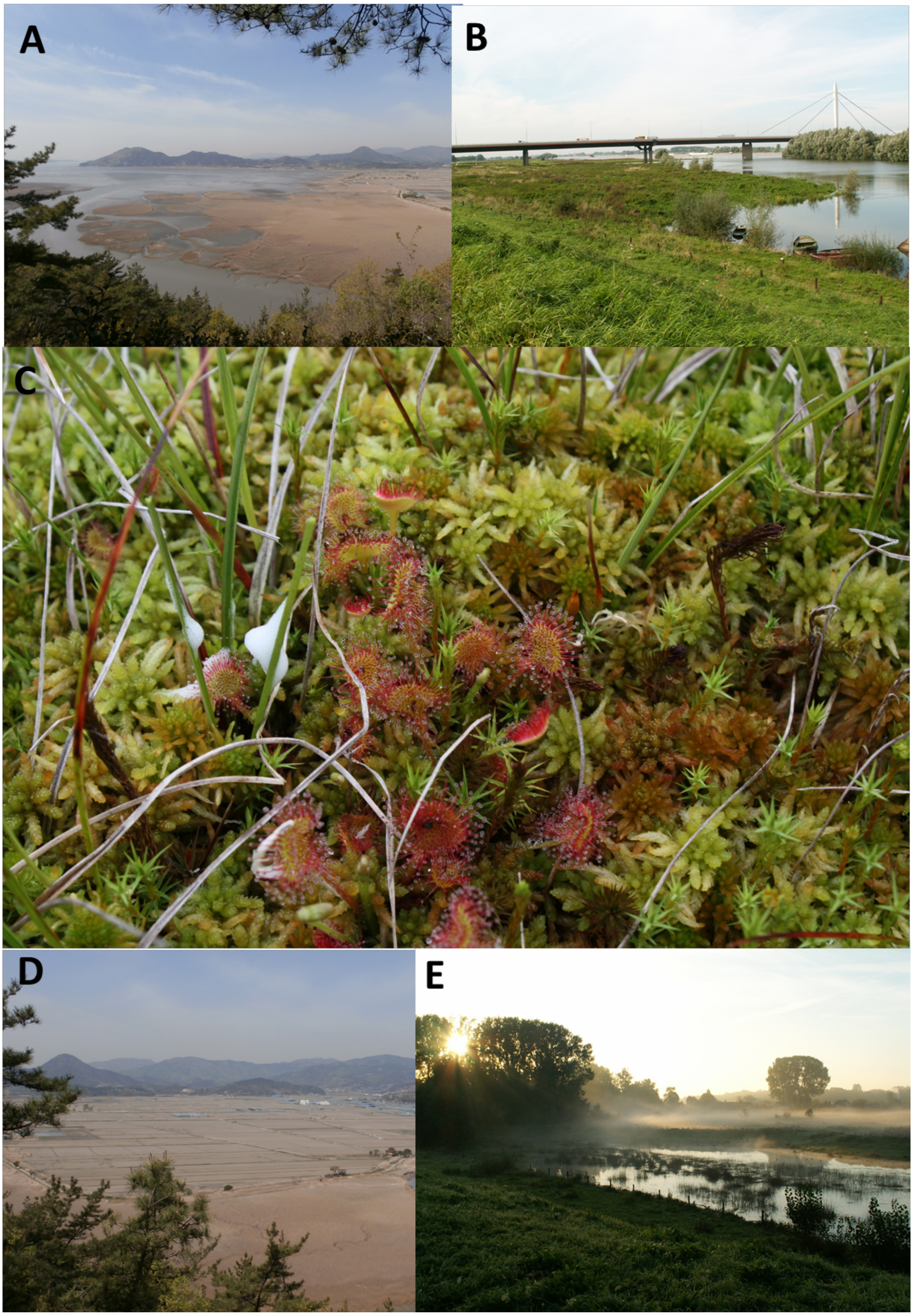
Although only 6% of the earth surface is covered by wetlands, a huge part of the global animal and plant populations depend on the ecosystem services wetlands provide. Wetlands, like swamps, bogs, peatlands, mangroves, floodplains, etc., are among the most productive ecosystems on the planet, harboring a huge diversity of plant and animal species and fulfilling vital functions in provision of food, water and a healthy climate. At the same time wetlands are among the most threatened ecosystems on the planet. Mainly due to human land use and climate change, already 87% of all wetlands have disappeared since the year 1700. Besides their live-saving functions and other positive aspects, swamps and peatlands also have this "spooky" image of inaccessible, dark misty areas where, once you go in, you never come out. If at all, you have to go well prepared, into the "twilight between wet and dry"! This also holds true for the microbes living in wetlands which have to be adapted to a life in the absence of, or with limited access to, air to be able to carry out their important, crucial but poorly known functions.

## The Microbiology and Societal Context

*The microbiology:* microbial cycling of nutrients; microbial adaptation to life without oxygen; microbial production and consumption of greenhouse gases; anaerobic respiration. *Sustainability issues:* clean water; provision of food and energy, economy and employment; sustainable cities by providing water purification and sanitation; combat climate change by sequestering carbon; life on land by providing shelter, irrigation, cooling and food.



*Wetlands: The microbiology*




Examples of wetlands. A is a tidal wetland near the Yellow Sea in South Korea. B is a riparian floodplain wetland along the river Waal in the Netherlands. C is an example of the plant species you can find in Peatlands, like this insect eating sundew. D are extended rice paddies in South Korea which emit lots of methane. E is simply a beautiful picture of a pool in a Dutch landscape. All photos Paul Bodelier.

1. ***What are wetlands?*** Wetlands are formally described as ecosystems of which the soils are frequently inundated or water saturated, and where the vegetation is predominantly hydrophytic - tolerating or adapted to life in water-saturated soil conditions. You find wetlands in the transition zones between land and water and, due to their large variation in environmental conditions, they are hotspots for biodiversity and ecological productivity. They play an important role in the environment as transformers in the global cycling of various chemical elements, including nutrients and pollutants.

We can distinguish several different types of wetlands, including: coastal wetlands, swamps, bogs, northern wetlands, and mangroves. They perform several key functions that are of importance to society: they store water and protect against both flooding and drought, improve water quality through retention and conversion of nutrients and contaminants; sequester carbon through primary production (the conversion of atmospheric carbon dioxide – $CO_2$ – to biomass by photosynthesis); produce fuel, fiber, food (domestic wetlands like rice paddies are estimated to feed half of the global population), facilitate tourism and, lastly, they harbor a huge biodiversity and a gene bank '*with known and unknown functions*'. Notwithstanding the importance of wetland functioning, the loss of wetlands and their species is even more rapid than for other ecosystems. The main driver of wetland loss is land-use change and associated drainage of wetlands.

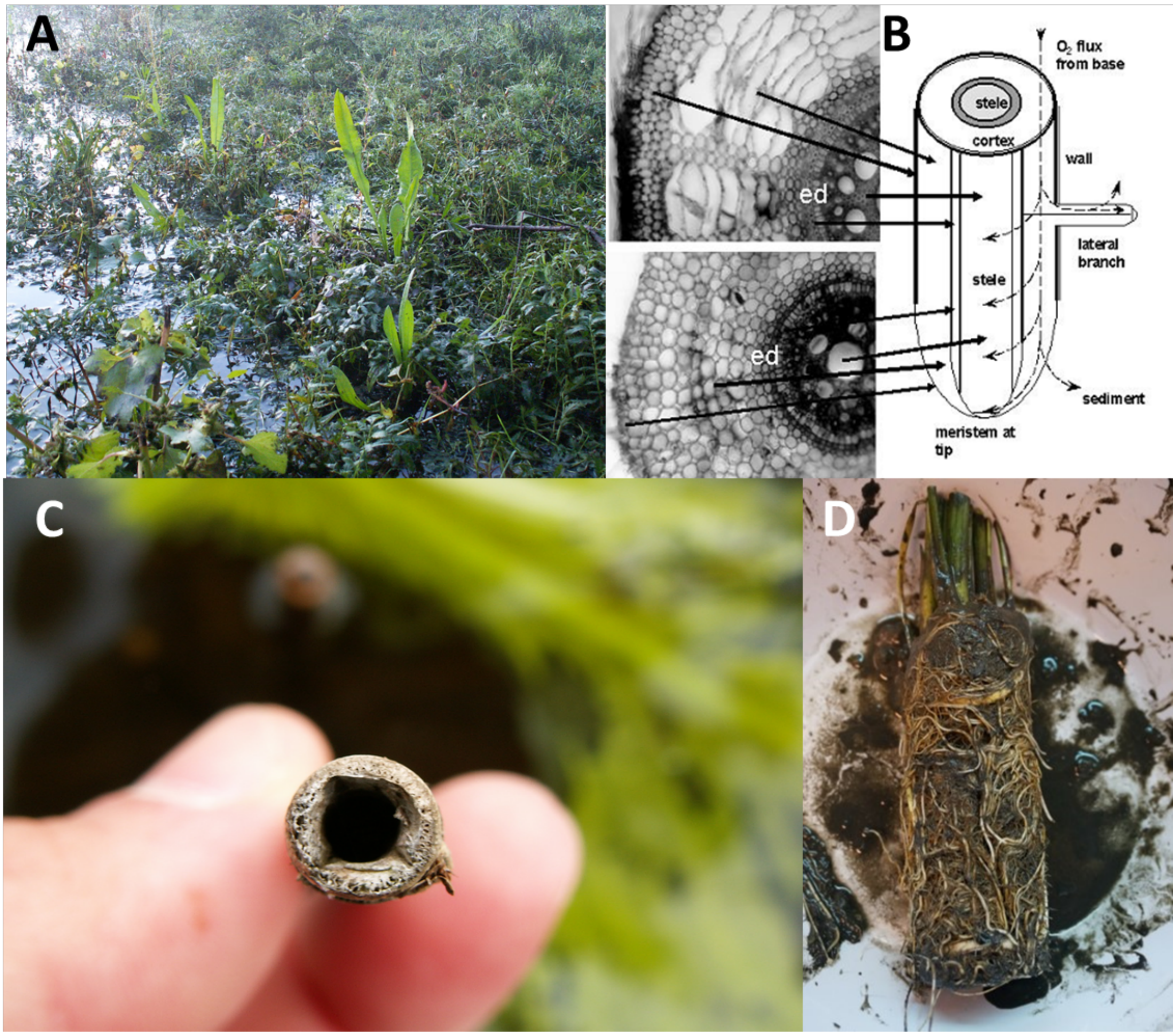
2. ***Characteristics of wetlands.*** Because of the high water content or even the complete submergence in water, wetland soils have very limited access to air. The solubility of oxygen in water is poor as is the diffussion of oxygen into water-saturated soils. Mainly due to oxygen consumption by soil microbes and plant roots, wetland soils turn "anoxic" (without oxygen) except for the upper few mm's where oxygen still can penetrate.

An exception are the immediate surroundings of wetland plant roots, which are adapted to life without air. Stems and roots of wetland plants contain so called "aerenchymatous tissues", which basically form a chimney inside the plants to transport air from above ground to below ground. In this way they can not only provide their roots with oxygen to breathe but also provide the microbes surrounding the roots in the "rhizosphere" with oxygen which leaks out of the roots. This creates many anoxic/oxic interfaces and gradients of oxygen availability within an anoxic environment.

In addition to plants, animals who make burrows, such as worms and insect larvae, also introduce oxygen by their burrowing activities, a process called bioturbation. Thus: microbes in wetlands have to be able to survive without oxygen and grasp the opportunity when a bit of oxygen becomes available.

3. ***How are microbes adapted to live in wetlands?*** Humans and all other animals, and plants, need oxygen to breathe. The oxygen is used as a so called "electron acceptor" in "redox" reactions, which yield the energy to perform all our activities and functions. In these reactions, electrons are transferred from one compound or molecule (which thereby becomes oxidized by loss of electrons) to another (which becomes reduced through gain of electrons) which generates energy. For humans this can be sugars from the diet transferring electrons to oxygen.

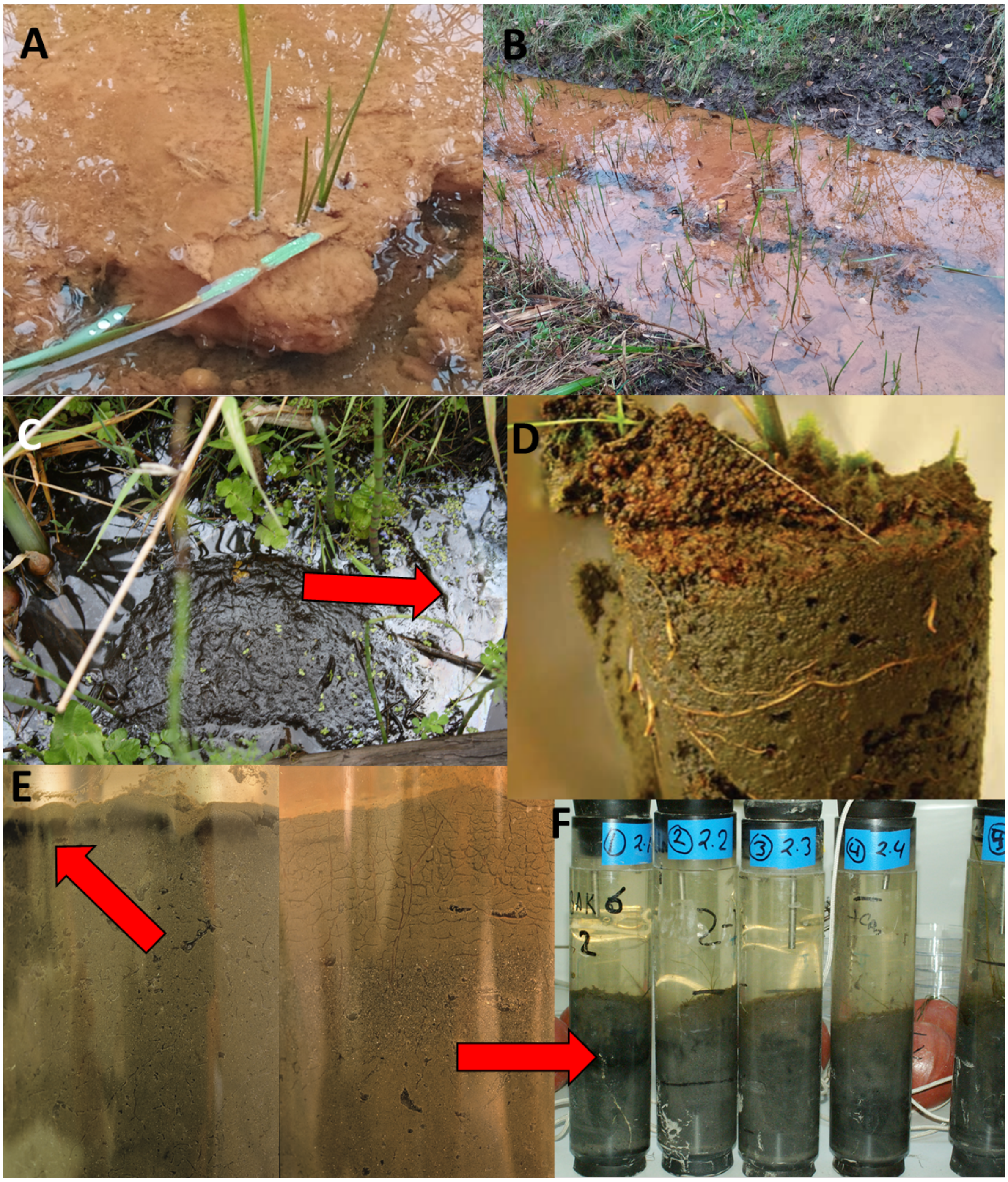
Microbes in wetlands have limited or no access at all to oxygen which they solve by using alternative compounds or molecules to which they transfer the electrons, a property which is a unique to microbes. Hence, they have the capacity to use "alternative" electron acceptors like nitrate ($NO_3^-$), ferric iron ($Fe^{3+}$), sulfate ($SO_4^{2-}$), manganese ($Mn^{2+}$), methane ($CH_4$), hydrogen ($H_2$) and carbon dioxide ($CO_2$). These compounds are changed in this process into a different chemical form like ferrous iron ($Fe^{2+}$) or sulfide ($S^{2-}$). The latter can give rise to hydrogen sulfide ($H_2S$) gas which gives the typical rotten egg smell when we wade through a ditch.




**Adaptations of wetland plants to survive in water saturated or flooded soils**. **A** shows leaves and stems of Rumex palustris, which are elongated upon flooding to stay above the water to make contact with air. **B** shows aerenchamatous tissue in roots and its location in the stems of rice plants. **C** shows the hollow stem of a reed (*Phragmites australis*) plant which serves to transport air into the soil. **D** dense root system of Reed sweet grass (*Glyceria maxima*) showing the many white aerenchymatous roots and also lots of blackish iron sulfide deposits which are a sign of anoxic conditions. All photos Paul Bodelier except for **B** which is adapted from Bodelier et al 2006, In: Wetlands and natural resource management. Volume 2: Wetlands: functioning, biodiversity conservation and restoration. Ecological Studies. Eds: Bobbink, R.; Beltman, B.; Verhoeven, J.T.A.; Whigman, D.F. Springer Verlag, Berlin.

If oxygen is present, many of these compounds can be used again as electron donors by for example iron-oxidizing bacteria which then turn $Fe^{2+}$ into the very well visible reddish iron oxide deposits in wetlands, or in the water of wetland ditches. Sulfide can react with iron and form iron sulfide which also can be visible as black deposits just underneath the surface of wetland sediment, and also around roots of wetland plants.

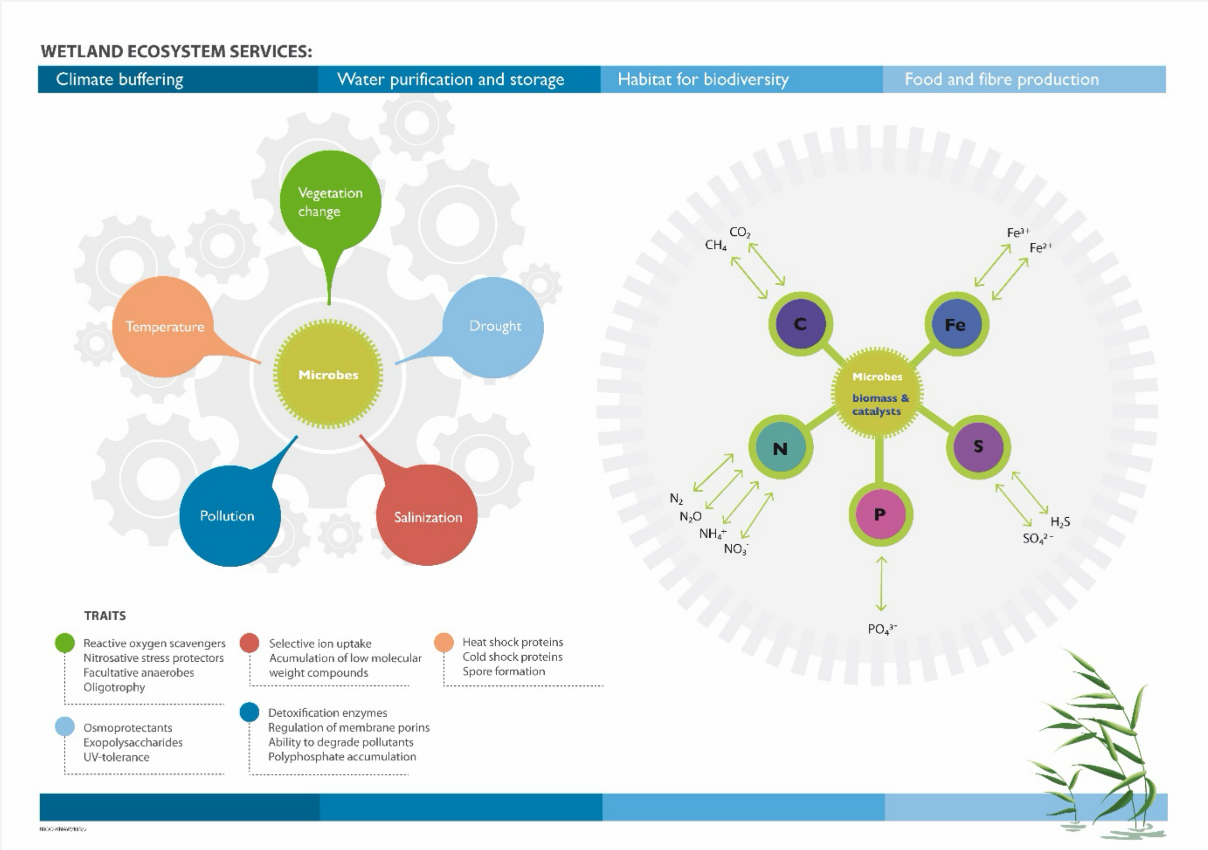
Methane gas, which is formed during decomposition of dead plants, can be used by so called "methanotrophs" which degrade methane using oxygen present around roots of wetland plants or in the upper surface centimetres of the soil where oxygen enters.




**Signs of microbial activity in wetlands**. **A and B** depict iron oxides formed in a wetland ditch due to activity of iron-breathing (oxidising) bacteria. **C** the arrow points to a thin film of iron-oxidizing bacteria on the water surface of a reed stand. **D** shows red iron oxides around roots of *Glyceria maxima* as removed from an experimental microcosm. **E** depicts the iron sulfide deposits (red arrow in left picture) which indicates the transition zone between oxic and anoxic conditions in the surface of wetland soil. F: In the right panel worms have been burrowing in the top layer of the soil, thereby introducing air, preventing the formation of iron sulfide due to inactivity of sulfate- and iron-reducing bacteria.

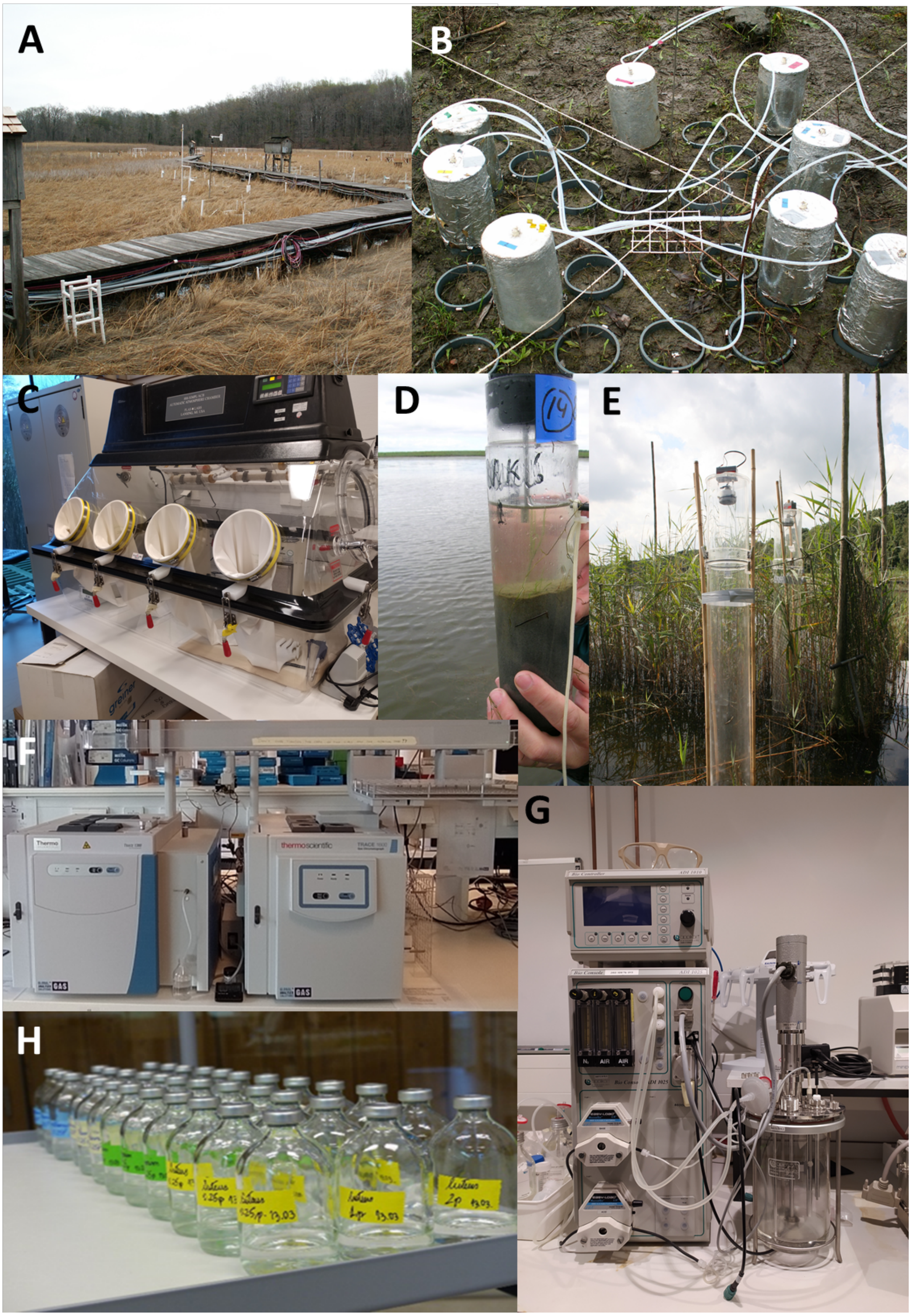
Energy can also be gained by the transfer of electrons from one carbon-containing compounds (so called organic compounds) to another, which we call "fermentation". Many wetland microbes can do this by which they produce many compounds that are responsible for the nasty smells coming from wetlands, like butyrate which smells like old cheese, and trimethylamine which smells like fish.

It is obvious that wetland microbes live in a highly dynamic environment where they have to survive periods without food, but also periods of exposure to compounds which are toxic to them. Many do not like oxygen and need special enzymes to deal with this. There are also many microbes who can only live with oxygen and need some other way of surviving, which may be using so called “reserve materials”, or relying on hydrogen gas in a way we still do not know. But wetlands can also run dry, leading also to higher salt concentrations, or can be loaded with pollutants from municipal discharge, all conditions microbes need to face and adapt to.




**Scheme of wetland functions, threats to microbes and main elemental conversions.** Microbes in wetlands have to be adapted to stresses (climate change, drought, salinization, pollution, temperature) for which they have developed a number of traits to keep on carrying out the most important redox conversions in the carbon, nitrogen, phosphorus, iron and sulfur cycles. Scheme from NIOO-KNAW.

4. ***How to study microbes in wetlands.*** Wetlands are not so easily accessible and special equipment or facilities are required to enter wetlands and also to disturb the soils/sediments as little as possible. Boardwalks can be installed by which equipment can be reached. But, also wading boots, wet/dry suits or even boats can be necessary. To measure microbial activity in the field (on site), chambers are often used to measure gasses that are formed and emitted from wetlands soils, like methane that is emitted from rice paddies for example, or from river floodplains. These gasses can be stored in small vials that are brought to the lab where they are identified and quantified using instruments like a gas chromatograph.



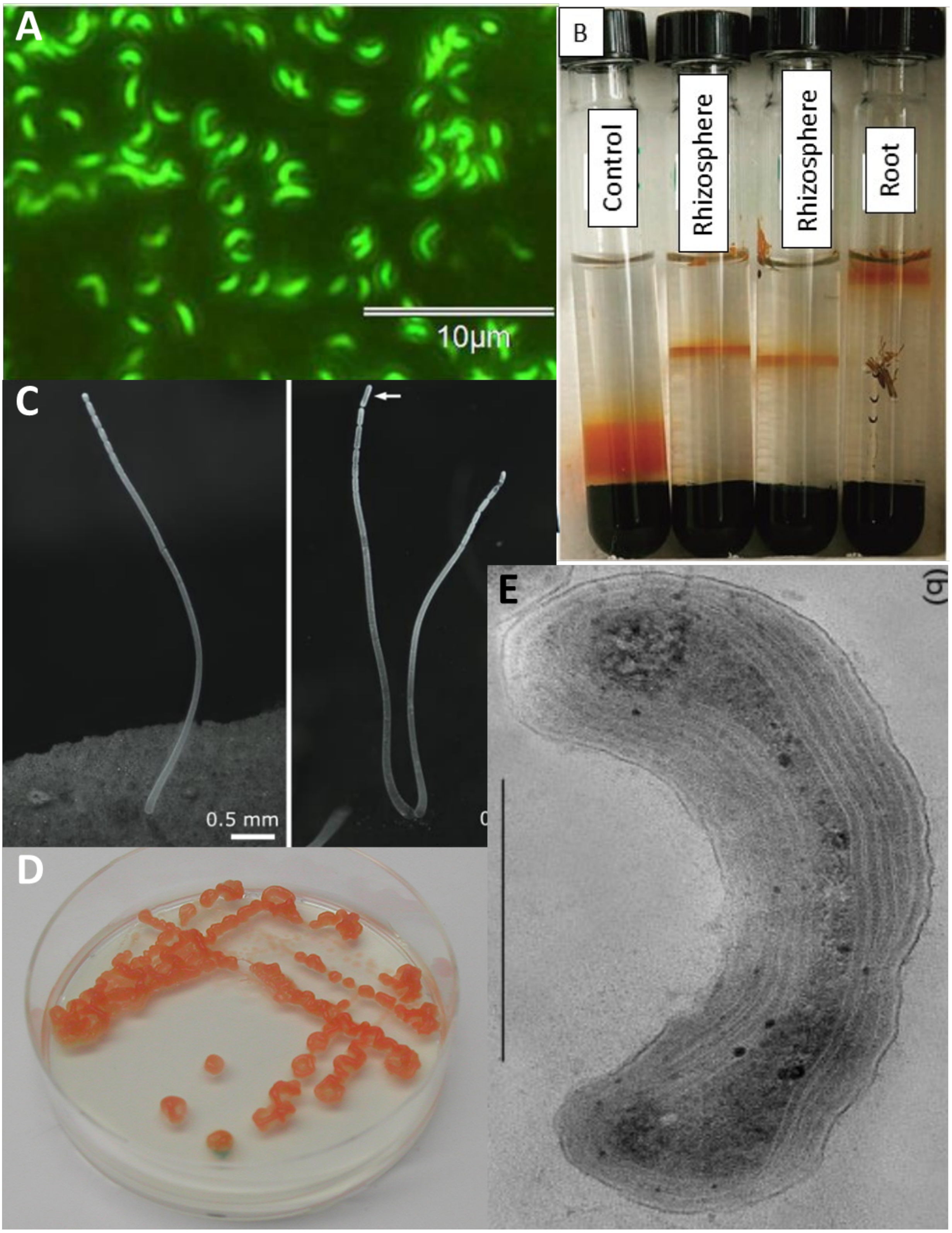

**Equipment and methods to study microbes in wetlands. A** shows a boardwalk system with cables and lines to access wetlands without disturbances and without getting stuck in the swamp. This picture shows a tidal wetland in the Chesapeake Bay area in Maryland (US) and equipment to measure gas emissions from these wetlands. **B** depicts portable gas-chambers to measure emission and/or uptake of major greenhouse gasses (methane, nitrous oxide, carbon dioxide) in a river floodplain in the Netherlands. **C.** To study and also to grow anaerobic microbes, we need an anaerobic chamber like this which allows us to keep and manipulate oxygen sensitive microbes in an oxygen-free environment. **D** depicts a sediment core which is used to collect intact wetland soils and sediment for transportation to the laboratory. **E** shows large transparent chambers to measure emission of greenhouse gasses, produced mainly by microbes, from large emergent wetland plants like reeds. **F.** To identify and to measure consumption or production of gasses by microbes we use so called “gas chromatographs”. **G** shows a reactor (also called a chemostat) which is used to study the behavior of wetland microbes under controlled conditions. **H** depicts incubation bottles used to grow microbes which breathe gasses like methane and study their activity. All photos Paul Bodelier

A closer look at wetland microbes in the lab is usually done by pushing a plastic or metal tube into the soil or sediment, which is subsequently extracted from the soil/sediment and transported to get the microbes as intact as possible to the lab. Their activities, like production or consumption of various compounds, can be measured, or microbes can be extracted from the soil and brought into a suitable growth medium to cultivate and eventually isolate them. Sometimes we also want to study the behavior of these microbes under controlled conditions which can be done in so called reactors or chemostats. In the case of oxygen-sensitive microbes, special chambers can be used which are free of oxygen.

5. ***Role of wetland microbes in climate.*** Wetlands and their microbes play important roles in the climate of our planet. Most important is the role of wetlands to store and to sequester carbon. The high productivity (biomass production) of wetland vegetation also results in high amounts of $CO_2$ that are fixed through photosynthesis. The high versatility of microbes to live with, without, and under low amounts of oxygen, and under highly dynamic conditions, contributes to this function of wetlands, providing plants with nutrients made available by high recycling rates.

An even more important aspect of the role of wetland microbes in climate is the production of greenhouse gasses. Due to an absence or low availability of oxygen, methane and also nitrous oxide can be formed by microbes which, through the “chimneys” of wetland plants, can escape to the atmosphere. In this way wetlands contribute up to 40% to the warming of our planet. But, on the bright side, there are also microbes that can degrade these gasses even without oxygen. For example, the amount of methane which is produced in rice paddies is way higher than what is released into the atmosphere because methane-consuming microbes degrade it and thereby help save our planet.

6. ***Role of wetland microbes in clean water.*** Wetlands are the transition zone between land and water and form a living filter for all kinds of nutrients, but also pollutants. When wetlands are degraded or removed, or when too much polluted water is passing through, this filtering activity no longer functions normally and ditches, lakes and even marine waters can become overloaded with nutrients and or pollutants, with drastic consequences for these essential ecosystems. Everybody will be familiar with green lakes or swamps, dominated by algae, where plants and many other living creatures are absent. The combined action of microbes and plants in wetlands prevent this from happening by retaining the nutrients in the soil, in plants or in microbes. This cleaning principle of wetland is even used in so-called “constructed wetlands”. These are wetland sites specially created to accept polluted waters, like sewage or mine drainage waters, in order to clean them up before discharging into natural surface waters.




**A** shows a microscopic image of an iron-breathing bacterium called *Ferricurvibacter nieuwersluisiensis*, isolated form a Dutch wetland. **B** depicts so called gradient tubes, with a gradient of ferrous iron (high at the bottom) and oxygen (high at the top) to grow ferrous iron-breathing bacteria, the growth of which is visible as the orange band in the tube. Tubes labelled "rhizosphere" and "root" have been inoculated with environmental samples which leads to oxygen consumption by ferrous iron-breathing bacteria, and therefore a higher position of the bands in the tubes compared to the control without bacteria. **C.** This gigantic bacterium, *Thiomagarita magnifica*, which can be up to 9cm long and is visible by eye, lives in mangrove wetlands. It can breathe without air inside the soil/sediment and with air in the water layer. Photo Volland et al 2022, with permission. **D** shows an agar plate with beautiful pigmented colonies of *Methylomonas* LL1, which is a bacterium isolated from a riparian wetland and which can breathe methane. **E** depicts an electron microscope image of *Methylocystis bryophila*, which is a methane-breathing microbe isolated from an acidic peatland. Photo from Belova et al 2013, with permission.

## Relevance for Sustainable Development Goals and Grand Challenges

Wetlands and their microbes relate to the following UN Sustainable Development Goals.

- **Goal 2: Zero hunger.** Rice paddies are wetlands that provide food for a huge part of the world's population. Wetlands in general provide fish and other food. Microbes are responsible for cycling of elements in wetland soils and water and thereby contribute substantially to the production of the food wetlands provide.
- **Goal 6: Clean water and sanitation.** Wetlands have a very important function to filter pollutants coming from land before run-off water and also groundwater flows reach surface waters. Constructed wetlands can also be used to clean sewage water, especially in local remote communities. Microbes convert these nutrients and pollutants to be taken up by wetland plants.
- **Goal 11: Sustainable cities and communities.** Sustainable cities and communities can only live by having access to clean water and also by cleaning their sewage and discharge.
- **Goal 13: Climate action.** Wetlands contribute both to positive and negative feedbacks to climate change by fixing carbon but also by emitting greenhouse gasses like methane. It is of high importance to manage wetlands in a sustainable way to promote their climate mitigating action. Also, here, microbes with all their biogeochemical functions are of utmost importance.
- **Goal 14: Life below water.** Wetlands and their filtering capacity are a prerequisite for clean water and hence, everything living in it. Also, in surface waters like lakes, there are microbes performing important functions keeping the water clean and suitable for plants, animals and microbes.
- **Goal 15: Life on land.** Considering the high productivity of wetlands, they also provide food and shelter for all other life on land. Wetlands also have a number of physical functions like cooling but also providing water to irrigate land.

## Pupil participation

***Class discussions/*group exercises**

1. Think about what will happen with wetland microbes after drainage/drying up?
2. What can happen in wetlands if temperature goes up?
3. What can happen to the planet when there are no wetlands anymore?
4. How do wetland microbes influence human life?

## The Evidence Base, Further Reading and Teaching Aids

***Information on wetlands, including information for teachers and students:***

https://microbewiki.kenyon.edu/index.php/Wetlands
https://www.wetlands.org/
https://www.epa.gov/wetlands/wetlands-education-students-and-teachers
https://www.sws.org/education-outreach/
https://www.epa.gov/wetlands/why-are-wetlands-important
https://education.nationalgeographic.org/resource/wetland
https://www.epa.gov/wetlands/constructed-wetlands

***Further reading***

Mitsch, William & Gosselink, James. (2015). Wetlands, 5th edition. John Wiley & Sons, Inc. ISBN: 978-1-118-67682-0. Interactions between oxygen-releasing roots and microbial processes in flooded soils and sediments (2003). Bodelier, P.L.E. *In*: Root ecology. Ecological Studies, vol. 168. *Eds:* De Kroon, H.; De Visser, E.J.W. Springer Verlag, Berlin.

Ecological aspects of microbes and microbial communities inhabiting the rhizosphere of wetland plants (2006). Bodelier, P.L.E.; Frenzel, P.; Drake, H.L.; Hurek, T.; Küsel, K.; Lovell, C.; Megonigal, P.; Reinhold-Hurek, B.; Sorrell, B. *In*: Wetlands and natural resource management. Volume 2: Wetlands: functioning, biodiversity conservation and restoration. Ecological Studies. *Eds*: Bobbink, R.; Beltman, B.; Verhoeven, J.T.A.; Whigman, D.F. Springer Verlag, Berlin. Microbiology of wetlands. 2013**.** Bodelier P.L.E., Dedysh, S.N. Frontiers Media SA. **ISBN:** 978-2-88919-144-4

Steenbergh, A. K., Veraart, A. J., Ho, A., & Bodelier, P. L. E. (2017). Microbial Ecosystem Functions in Wetlands Under Disturbance. In K. Tate (Ed.), *Microbial Biomass: A Paradigm Shift in Terrestrial Biochemistry.* World Scientific Publishing.

Bodelier, P. L. E., Pérez, G., Veraart, A. J., & Krause, S. (2019). Methanotroph Ecology, Environmental distribution and functioning. In E. Y. Lee [Ed.], *Methanotrophs: Microbiology Fundamentals and Biotechnological Applications* (Microbiology Monographs MICROMONO; Vol. 32). Heidelberg: Springer. https://doi.org/10.1007/978-3-030-23261-0_1

Bodelier, P.L.E. (2011). Interactions between nitrogenous fertilizers and methane cycling in wetland and upland soils. Current Opinion in Environmental Sustainability, 3: 379-388.

P.L.E. Bodelier and A.K. Steenbergh (2014). Interactions between methane and the nitrogen cycle in light of climate change. Current Opinion in Environmental Sustainability, 9-10:26-36.
Bodelier, P.L.E. 2015, Bypassing the methane cycle. Nature 535:523-524

## Glossary

**Ecosystem services:** The many benefits humans and other organisms obtain from healthy functioning ecosystems like, food, clean water, shelter, healthy climate, etc.
**Mangrove:** a mangrove is a shrub or tree that grows in coastal saline or brackish water and can often be found as main coastal vegetation in tropical countries, where it also functions as coastline protection against storms and floods.
**Aerenchyma:** Spongy tissue that creates spaces or air channels in the leaves, stems and roots of some wetland plants, which allows exchange of gases between the shoot and the root. The channels of air-filled cavities provide a pathway for the exchange of gases such as oxygen, carbon dioxide and ethylene between the plant above the water and the submerged tissues.
**Rhizosphere:** The rhizosphere is the narrow region of soil or substrate that is directly influenced by root secretions or by root uptake and associated soil microorganisms. In wetlands, the excretion of oxygen by plant roots is crucial for microbial activity and survival.
**Iron sulfide:** Also called ferrous sulfide is a black, water insoluble mineral formed from ferrous iron ($Fe^{2+}$) and sulfide ($S^{2-}$) which are both products of microbial activity under anoxic conditions.

**Iron oxide:** Chemical compounds that are formed of iron and oxygen, of which the most well-known one is rust. In wetlands this "rust" can be formed by iron-oxidizing bacteria using ferrous iron ($Fe^{2+}$) and oxygen.
**Hydrogen sulfide:** A compound with chemical formula $H_2S$ which is toxic and smells like rotten eggs. It is a gas that can be formed in wetland soils under anoxic conditions.
**Microcosm:** The word comes from the Greek "mikros kosmos" with means little world. In science it is used to indicate experimental systems that are smaller and simpler than the real world but resemble the real situation as much as possible.
**Greenhouse gasses:** Gasses that trap heat in our atmosphere. The balance between the rates at which they enter the atmosphere, and their rates of decay, determine the average temperature of our planet surface and how appropriate this is for the various life forms of the biosphere. The large amount of these gasses released into the atmosphere by human activity has disturbed this balance and caused global warming. The most important greenhouse gasses are $CO_2$ (carbon dioxide), $CH_4$ (methane) and $N_2O$ (nitrous oxide).
**Aerobic vs anaerobic microbes:** Microbes that use oxygen to generate energy have an aerobic metabolism, while microbes that use other electron acceptors have an anaerobic metabolism. There are also microbes that can do both, depending on the conditions, which are called facultative aerobic or anaerobic microbes.
**Gas chromatograph:** Gas chromatographs are laboratory machines used to separate, identify and quantify compounds that can be vaporized and which occur in mixtures. GC are often used to separate gasses in air like oxygen, carbon dioxide, methane, hydrogen and nitrous oxide.
**Chemostat:** A chemostat is a bioreactor to which fresh medium is continuously added, while culture liquid containing left over nutrients, metabolic end products and microorganisms is continuously removed at the same rate to keep the culture volume constant. By changing the rate with which medium is added to the bioreactor the specific growth rate of the microorganism can be easily controlled and is completely determined by the environmental conditions. In this way the behavior and response of microbes to changes in mostly abiotic conditions can be assessed.
**Electron acceptor:** An electron acceptor is a chemical compound that accepts electrons transferred to it from another compound. It is an oxidizing agent that, by virtue of its accepting electrons, is itself reduced in the process. Electron acceptors are sometimes mistakenly called electron receptors. In contrast to humans, microbes can use other compounds besides oxygen as electron acceptors.
**Electron donor:** An electron donor is a compound that donates electrons to another compound. It is a reducing agent that, by virtue of its donating electrons, is itself oxidized in the process.
**Redox reactions:** "Redox" is a combination of the words "reduction" and "oxidation". The processes of oxidation and reduction occur simultaneously and cannot occur independently. In redox processes, the reductant transfers electrons to the oxidant, a process that yields energy. Thus, in the reaction, the reductant or *reducing agent* loses electrons and is oxidized, and the oxidant or *oxidizing agent* gains electrons and is reduced. The pair of an oxidizing and reducing agent that is involved in a particular reaction is called a *redox pair*. A *redox couple* is a reducing species and its corresponding
oxidising form, e,g, Fe2+/Fe3+. The oxidation and the reduction alone are each called a *half-reaction* because two half-reactions always occur together to form a whole reaction. Elemental cycling in wetlands depends on redox reactions catalyzed by microbes.
**Biogeochemical functions:** Biogeochemical functions are chemical or biological activities by which chemical substance cycle (turned over or moves through) through the biotic and the abiotic compartments of Earth. The biotic compartment is the biosphere and the abiotic compartments are the atmosphere (air) , hydrosphere (water) and lithosphere (soil/rocks).

**Organic compounds:** An organic compound is a molecule which at least contains 1 carbon atom. $CH_4$ (methane) is the simplest organic molecule while DNA, the basic molecules of life, are highly complex organic compounds.
**Butyrate:** Butyric acid is a so-called organic acid which is named after the Greek word for butter. It can cause a typical unpleasant smell and is usually formed in wetland soils by fermentation processes.
**Trimethylamine:** TMA is a compound that can cause a strong fish-odor and is also formed during fermentative metabolism. In wetlands it can be used by so called methanogenic microbes to form methane gas.