Anaerobic respiration and denitrifying bacteria

# Breathing Without Oxygen

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## Microorganisms invisible to the naked eye, billions living in every gram of soil, can have an impact on such global processes as the level of greenhouse gasses in the atmosphere

Fourier (in 1827) and Arrhenius (in 1896) first hypothesized that our planet's life-sustaining temperature is attributable to atmospheric gasses which limit the radiation of energy back out into space. Indeed, if it were not for carbon dioxide, the climate on the Earth would be significantly harsher, with a mean temperature of -18 °C rather than +15 °C.

Although  $CO_2$  accounts for only 0.03% of our atmosphere, even this minimal level contributes to the "greenhouse effect." A correlation between increased  $CO_2$  concentration in the air and higher global temperature has been experimentally demonstrated. The same effect is now known to be evoked by other gases as well, such as nitrous oxide N<sub>2</sub>O, methane CH<sub>4</sub>, and freons. Even though the concentrations of those gasses are lower than that of  $CO_2$ , they do exert a significant environmental impact.

Mankind plays a significant role in driving the greenhouse effect, via industry, landfills, gas emissions from trash dumps, agricultural practices, excessive fertilization, deforestation, cattle husbandry, etc. A sharp rise in the use of nitrogen fertilizers seen in the 1970s, chiefly related to mounting world population numbers, has undoubtedly played a part.

Agriculture pollutes the atmosphere mainly through emissions of nitrous oxide (especially from the use of nitrogen fertilizers), methane, and ammonia. Soil may in fact



A sharp rise in the agricultural use of nitrogen fertilizers in the 1970s contributed to increased greenhouse gas emissions and water body eutrofication

be responsible for up to 56% of the overall quantity of  $N_2O$  released into the atmosphere – 40% of this figure coming from soils covered by natural vegetation (e.g. meadows and pasturage), 14% from fertilized cultivated soils.

Soil constitutes quite an extraordinary material. It is three-phase (containing solid, liquid, and gaseous components), and varies over time and space. It encompasses mineral particles of varying shape and size, which lump together with each other and with organic substances to form irregular, porous aggregates. Each gram of soil is inhabited by billions of microorganisms, whose life processes constitute an important part of every biogeochemical cycle. If soil were sterile, it could not perform its important function of supplying nutrients to plants.

Soil also possesses another special trait: it preserves a continuum of life under practically any physical and chemical conditions. If water inundation deprives aerobic microorganisms of the oxygen they need to survive, they are replaced by anaerobic organisms. Indeed, it is under such anaerobic conditions that the intensive production of "greenhouse gasses" occurs.

#### Electrons for the taking

Many microorganisms possess the extraordinary ability of anaerobic respiration. During cellular respiration, electrons move along what is called a transport chain, releasing energy. At the end of the chain there must be a "recipient" for the electrons (called an acceptor) - in the case of aerobic respiration this acceptor is oxygen, and electron attachment leads to the creation of water molecules. But if molecules other than oxygen receive the electrons, the respiration is then called anaerobic. One of the processes which occurs under reduced oxygen presence is called *denitrification*, in which bacteria utilize oxidized forms of nitrogen as their ultimate electron acceptors. The final product of this process is molecular nitrogen N<sub>2</sub> or nitrous oxide N<sub>2</sub>O - a greenhouse gas. The denitrification process is affected by three factors: the concentration of oxygen in the soil, the nitrate content, and the availability of organic carbon.

### 1,000 profiles

Our research has sought to identify how various doses of nitrogen fertilizers affect  $N_2O$  emissions, depending on soil type. Of 1,000 soil profiles collected at the Polish Mineral Soil Bank of the PAN Institute of Agrophysics, we choose to look at two brown soils (labeled B-1 and B-2) which distinctly differed solely in terms of their natural nitrate content. Soil samples were incubated while fully flooded with distilled water (as the control) or with nitrate solutions in concentrations of 25, 50, 100, 300, and 500 mg N-NO<sub>3</sub><sup>-</sup> per kg of dry soil mass. For soil B-1, nitrous oxide production increased as more nitrates were supplied to the soil, albeit up to a certain limit – with dosage



Soil is quite an extraordinary, three-phase material, every gram of which contains billions of microorganisms that significantly affect is properties

increases above 100 mg N-NO<sub>3</sub><sup>-</sup> no longer triggering any increase in the quantity of N<sub>2</sub>O emitted. Soil type B-1, therefore, can be classified as a soil inhabited by active denitrification microorganisms capable of producing N<sub>2</sub>O. In soils with properties similar to B-1, under conditions favorable to the growth of such bacteria (excessive moisture, a lack of oxygen), every dose of the nitrogen fertilizers currently used in agriculture could potentially contribute to the denitrification process, entailing increased emissions of the greenhouse gas N<sub>2</sub>O.

Soil B-2, in turn, evidenced a completely different reaction. Added nitrates had practically no impact on  $N_2O$  emission, which in all variations remained similar to the control soil level. The natural content of nitrate nitrogen in this soil was quite high. Thus, nitrates added to a soil of type B-2 do not pose a serious "greenhouse" threat to the environment. However, it must be borne in mind that nitrate nitrogen may pose a danger of a different kind: ground waters and water reservoirs, leading to their eutrophication.

Not all the greenhouse gas produced in soil is released into the atmosphere. In lower layers of the soil, or locally in sites of reduced oxygen concentration, nitrous oxide may become reincorporated into biochemical cycles. The soils we studied differed from one another in this respect as well: we observed such a phenomenon in soil B-1, but in B-2 sorption occurred only in the sample with natural nitrate content.

In conclusion, a seemingly small difference between the two brown soils studied proved to have a significant impact on the process of denitrification. Such differences in soil characteristics therefore need to be factored into estimates of  $N_2O$  emissions from soil and their overall environmental impact.

#### Further reading:

Gliński J., Stępniewski W. (1985). Soil Aeration and its Role for Plants. Boca Raton, Florida: CRC Press.

Włodarczyk T. (2002). Nitrogen transformation and their circumstances in soils. Acta Agrophysica, 63, 123–158.

Włodarczyk T., Stępniewski W., Brzezińska M., Stępniewska Z. (2004). Nitrate stability in loess soils under anaerobic conditions – laboratory studies. J Plant Nutr Soil Sci, 167, 693–700.