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Nitrogen and me — How little did we, and do we know about "stikstof – azote – nitrogen"?

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ABSTRACT

This retrospective article reflects on the complex and evolving relationship between humans and nitrogen over several decades. Raised on a Flemish farm, the author's early experiences with nitrogen in agriculture – both its benefits and dangers – laid the foundation for a lifelong interest in this element. The article traverses a broad range of topics related to nitrogen, highlighting its critical role in various historical, agricultural, environmental, and industrial contexts. The narrative begins with a historical overview of nitrogen's role in agriculture and warfare. The development of industrial processes like the Haber and Ostwald methods transformed nitrogen into a key ingredient for both fertilizers and explosives. The dual nature of nitrogen – as a life-giver in agriculture and a destructive component in warfare and also in biodiversity – is an important theme. The article delves into the environmental impacts of nitrogen, particularly in the context of modern agriculture and industrialization. Issues like fertilization, water contamination, and the challenges of managing nitrogenous waste highlight the complex interplay between human activities and environmental health. Technological advancements are explored, including the development of bioaugmentation methods and the potential of genetic engineering in optimizing nitrogen fixation. Throughout the narrative, personal anecdotes are weaved with scientific information, offering a unique perspective on the historical and contemporary challenges of managing nitrogen. The discussion extends to the broader implications of nitrogen management in the context of sustainability, climate change, and global food security and its overall regulatory space. All these considerations call for a re-evaluation of our relationship with nitrogen, advocating for innovative solutions and systemic thinking to address the multifaceted challenges posed by this essential, yet often problematic element.

1. Preamble

I have come to an age where looking back is quite a challenge… there is so much that has evolved. These days, in Flanders and the Netherlands, we have major controversies at the eco-political level about nitrogen in agriculture, traffic, natural habitats, drinking water, wastewater, ambient air… Our governments resign because of policy matters with respect to nitrogen. What follows is a retrospective over almost 8 decades on how a beautiful swan has become a rather nasty but most intriguing duck.

2. In the beginning…

I was raised on a small farm in Flanders, the northern part of Belgium. We had a diversity of production animals, the soil was sandy and poor. My first recollection of what in Flemish is named 'stikstof' (the substance that makes you suffocate) was that it was abundantly present in all types of animal manures, and when applied to the crops, it made them grow luxuriously…provided rain was abundant. When that rain happened on a warm summer day, concomitant with a good amount thunder and lightning, there was nothing more enjoyable than a walk in the fields still draining the excess of water. The wisdom of the farmer was that the lightning brought nitrate to the soil and the summer soil responded by producing these wonderous relaxing gases, nowadays tentatively characterized as nitrous oxide also known as laughing gas. The happy feeling of the country side in full growth and fertility! Yet, the nitrogen coin had also a dark side. The well fertilized crop could be so top-heavy that a windstorm would flatten the field and made it a total loss. Moreover, in the fall season, the turnips could, at the onset of an early frost, in a matter of a few days accumulate so much nitrate from the overfertilized soil that the cows dropped dead because of nitrate blood poisoning. Industrial nitrogen in the form of magic blue granules, was much easier to handle than the stinky animal manure and more precise to dose, but my farther told me that a large heap of such granules, in the

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year 1942 at the site of the chemical fertilizer factory of Tessenderlo - when it had been hammered upon by a worker to make it lose and free flowing - had caused a massive explosion which destroyed half of the city. Clearly, as a youngster, I became acquainted with the fact that nitrogen had several ways of action.

In Flanders, the most urbanized region of northern Europe in the fourteenth and fifteenth centuries, the inhabitants succeeded already at those days to live the so-called Burgundian life style with plenty of food for the relative dense population. One of the elements was that farmers came to the cities to 'buy' the night soil (=fecal wastes) from the citizens. The night soil was then with great care 'tasted' on its salt content (salt as the proxy of 'non'-dilution) and thus accredited a proper price as fertilizer. These fecal liquors were -generally by boats - transported to the fields and optimally recycled to grow crops. In my high school time, I learned that the English philosopher Thomas Malthus in 1798 had perceived how critical the optimal usage and recycling of nutrients was for the healthy development of the demography in those days. By the start of the 19-th century, it became apparent that the world population had no bright prospects simply because the need of reactive nitrogen in the agriculture was mainly dependent on inputs by leguminous plants fixing nitrogen from air, and guano (excrements from marine birds) coming from South America. Clearly, sooner or later the pre-industrial society would face malnutrition due to lack of reactive nitrogen.

3. Nitre heaps and gunpowder

In 1967, I could follow a summer course at the Pasteur Institute in Paris. I bumped into the history of 'La Salpêtrière'. Salpêtre is the French for 'salt stone = sel de pierre or maybe also sale pierre / dirty stone' which actually refers to potassium nitrate. Already in the Middle Ages, one had the knowledge to search for glazy salt deposits near zones where urine or manure was stored. Such stony deposits rich in potassium nitrate were upgraded to nitrate concentrate and the latter was used to produce gun powder (sulfur + charcoal + nitrate). Actually, under the French revolution and during the Napoleon period, nitrogen rich materials (manure, urine, blood,…) were requested and had to be delivered to the civil authorities. The latter had 'nitre heaps' installed. These heaps of manure mixed with chalky earth were watered with urine and manure water. Calcium nitrate crystallized on the surface and was scraped off and taken to a shed for processing. This was an industrial nitrification process to support warfare. In the center of Paris, one had installed such a 'stinky' industrial production site for the super important production of nitrate designated to produce gun powder. Much later, on that military site a top-level hospital was erected and still exists with that name. Note, in that hospital, princess Diana died after her tragic accident.

4. Nitrogen and war

The super urgent quest for easily accessible nitrates to rage more effectively war led in the 1910s and 1920s to the rise of the Haber process and the Ostwald process. This industrial process produces ammonia from methane (natural gas) and molecular nitrogen abundantly present in air. The ammonia from the Haber process is then partially converted into nitric acid. By accident, it was also discovered that lower quality nitrates, which were dumped outside the 'gunpowder' factory turned out to be… super fertilizers. Thus, the inventors of industrial nitrate made by serendipity a major contribution to open up agriculture for abundant feed and food production. Remarkably, as demonstrated in a most dramatic movie in the Ypres Museum of War, the same scientist Haber was confronted with the fact that during first world war, the German army had to fight the Allied Forces in Flanders and France. The Allied Forces sought protection in trenches and therefore the war appeared to be lasting forever. The much-praised Mr Haber, through which the German army had access to ample supplies of gun powder, came to the ethical dilemma of letting the war linger on, or use another chemical i.e. chlorine gas released in the wind to blow over the battlefields and trenches. The use of chlorine gas to sweep the fields was according to the historians, well pondered and judged to be 'morally correct' by Haber and the military staff. Incidentally, to counteract the chlorine gas, the poor soldiers had as a remedy to save their lives a piece of cloth with urine held before their noses to quench the chlorine gas which reacted with the urea to become nitrogen gas, a bit of chemistry later known in the drinking water production as "breakpoint chlorination".

5. The undesired encounter

I studied agricultural engineering at Ghent University. Yet, when I graduated in 1968, I could sense in the distance the coming of a new discipline: environmental engineering. The item that particularly intrigued me was the breakdown in the environment of those super magnificent chemicals which were applied in massive amounts all around the countryside, i.e. the "wonderful" pesticides!! They were proclaimed to disappear swiftly due to mysterious microorganisms naturally present and 'infallible'. In 1968, I could obtain a scholarship from Cornell University (Ithaca NY) and I hastened to get all the documents ready to go and study those phenomena of pesticide degradation in the lab of Martin Alexander, top soil microbiologist. Arriving there in the early fall, I learned that the biodegradation topic was given to a colleague doctoral student. Moreover, when hearing the topic I was assigned to, instead of pesticide biodegradation, all the colleagues tried to comfort me because I had apparently inherited the 'old cow in the ditch'. That topic was nitrification: the postulation at that period of time was that the process was probably in part due to autotrophs and in part to heterotrophs. Fortunately, a few months further on, we got a bacterium that could, when growing in an artificial medium rich in ammonium and acetate under highly aerated conditions, produce a series of metabolites i.e. free hydroxylamine, also bound hydroxylamine (hydroxamates-siderophores) and moreover also nitrite and nitrate and even nitrous oxide. Quite a novel set of products, albeit under artificial conditions. In those days, microbial taxonomy was a headache; the bacterium was tentatively identified as *Arthrobacter* (Verstraete and Alexander, 1973).

6. Manure all over

In 1971, I returned to Ghent University, and indeed the environmental movement had taken on. There had arisen a very remarkable new problem: too much manure and particularly too much reactive nitrogen for the available agricultural soils. Indeed, since the end of the Second World War, farmers had gotten ample access to industrial nitrogen fertilizer, and it was quite inexpensive. They preferred, by far, this commodity relative to manure. Concomitantly, due to the import of inexpensive grain from overseas, Flanders experienced an enormous growth in animal husbandry. Thus, the industrial nitrogen applied in the USA, Canada, and Brazil was transformed in Flanders to a top value food (meat, milk, eggs). That food was exported to other regions in the world, but the residual nitrogen remained and had to be disposed of on the available soils. Since the crops could only take limited levels of reactive nitrogen, and since farmers were tuned to industrial fertilizers, the problem of excess animal manure rapidly rose to major concerns.

At Cornell University, I learned about the possibility of setting up food chains, and it was considered proper and zootechnical fine to take, for instance, chicken manure - very rich in protein of which quite a fraction still has nutritive value – and supply this as feed to feedlots fattening cattle. This concept brought us to a startling invention. The fresh pig slurry of some 8–10 % dry matter was centrifuged to remove the coarse fiber particles. Subsequently the soluble matters were subjected to extended aeration and bioconversion and in a final step separated by acidification at a pH of 4 into a coagulate on the one hand (compare with cheese making) and a clear liquid on the other hand (Vanstaen et al., 1976). The remarkable fact was that indeed the organics aggregated in dense flocs, and just as nitrite/nitrate pickled protein in salami, they smelled fresh, could be stored and had a taste which even attracted choosy animals like sheep. The process had indeed some aspects in common with salami making: the proteins were set in contact with large concentrations of nitrite at acidic pH values. (Note: if the practice of producing salami where handfuls of nitrite are added to ground meat were invented today, the authorities would most probably never ever think of giving it a permission; the potential risks for the consumer would be considered enormous due the nitrosation reaction occurring in that matrix). Together with a team of veterinarians, feed tests were performed with the coagulum over a period of 2 generations of sheep. The zootechnical data were excellent and the thus generated 'gigot' was found by connoisseurs to be super delicious. This was a technology to convert fecal organics by aeration to single-cell protein. The single-cell protein made from fecal residues was preserved by nitrite treatment and was then further upgraded by the higher animal to a valuable food. The Belgian IWA branch granted this invention its prize of the year. The economics were not unattractive, and the process allowed the recycling of an important part of the nitrogen.… but the team had not at all given attention to communication with the consumer to an in-depth explanation of the process to the broader public. Within a matter of months, a journalist of a tabloid newspaper made a huge story about the fact that piggeries were now making big money from… the fecal matter, and this was the end of that 'upgrade' and recycling of nitrogen via that route.

7. Drinking water

The massive use of manure and industrial nitrogen on the fields in Flanders resulted in the enrichment of nitrate in groundwaters and surface waters. Flanders was getting top-quality drinking water from the Walloon region, but the language quarrels between the two regions in Belgium of those days resulted in those water supplies becoming politically labelled, and this forced Flanders to use its own surface waters to produce drinking water. The high nitrate levels gave rise to major debates, particularly about the 'blue baby syndrome'. It has been reported that infants, when they have milk prepared from powder and nitrate-rich water, can turn blue because infant hemoglobin reacts with the resorbed nitrite/nitrate. As a token of societal concern and protest, a minister of state got a tank load of manure dumped in his front garden. The possibility of building a fluid bed technology using methanol to denitrify the surface water field was explored at the site of drinking water production (Liessens et al., 1993). The process was technically sound, but costly. And a novel new remedy to scavenge much more mineral nitrogen from overfertilized soil was developed by the plant breeders. A series of cultivars of corn (maize) came into existence. The latter appeared to be unbelievably strong plants: they were shown to be able to grow successfully year after year on the same soil. Moreover, they were found to assimilate lots of mineral nitrogen to make plenty of plant biomass which, upon harvest can be chopped up and ensiled to become an excellent feed for dairy cows. In addition, the argument about nitrate as a dreadful poison was weakened strongly, certainly for adults, because one discovered that the sap of red beets, which can be extremely high in nitrate, is an excellent drink for athletes and invigorates - just as the famous blue pill - the blood recirculation and increases the stamina. The overall pressure for regulation of the over-use of nitrogen in agriculture remained minimal until the turn of the century.

8. Biological nitrogen fixation

In the 80 s, Ghent University was at the forefront of new developments in terms of genetically modified microorganisms and plants. The pioneering work of Jozef Deley, Walter Fiers, Jef Schell and Marc Van Montagu led to the creation of new perspectives in biotechnology. One of these was: no longer need to use industrial nitrogen fertilizers. The genes governing the fixation of biological nitrogen could be taken from nitrogen-fixing bacteria and implanted directly in higher plants. No more hunger in the world, even not in the very poor developing countries because nitrogen would be free of costs and come from now gratuitously from the air. A series of emotional debates followed: genetically modified organisms (GMOs) could be the beginning of 'Frankenstein food'. I have always believed that the technology of manipulating genes was a marvelous step forward and could be framed in proper risk management. Yet, there were two features that I brought forward in that debate. First, the fact that leguminous plants fix nitrogen in 'symbiosis' with the bacteria in their nodules is probably presented in a far too bucolic way. Indeed, the Rhizobium bacterium living free in the soil, is 'lured' by means of phytohormones into the leguminous plant. Once it penetrates the root hair, it is fully imprisoned, subsequently cut off from oxygen, and finally supplied with organic acidic metabolites by the plant. The only solution for the poor bacterium in total captivity is to convert dinitrogen to ammonium and thus maintain itself alive, until finally the plant dies off and it can return to the status of freedom. But aside from this mode of living, there is also the quantitative aspect. Chemically reducing dinitrogen to ammonia costs energy. Actually, the stoichiometric need of the Haber process is 0.5 kg of fossil methane (0.375 kg C) per kg of nitrogen fixed and converted to reactive nitrogen. These thermodynamics are not overruled in biology. Free-living nitrogen fixers need some 50 kg of carbohydrates to fix one kg of nitrogen. Under optimal conditions of a 'rhizobium suffocating in a nodule', still about 5 kg of carbohydrates are required to fix 1 kg of nitrogen (Matassa et al., 2023). Hence a nitrogen-fixing plant must deliver heavily to the bacterium to do the hard work of enzymatically at ambient temperature and pressure the conversion of dinitrogen into reactive nitrogen. In practice, the farmer has to make a choice: either have per ha of crop some 1000 kg or more of extra dry matter plant production due to the application of some 150 kg of industrial fertilizer nitrogen, or being sustainable and having the nitrogen fixed by the bacterium-plant association, but producing significantly less crop product. The Haber nitrogen fertilizer came up to now at such low prices that normally agriculture economically had no choice but to go for industrial fertilizer nitrogen. Yet, in the 2023 framework of the sustainable development goals, it still takes some1–2 L of fossil fuel equivalent to produce industrial fertilizer nitrogen and it is therefore worth to re-consider the potential of biological nitrogen fixation: can we continue to burn fossil fuel to fix nitrogen and produce reactive nitrogen from air? We should go for the production of Haber nitrogen by using no longer fossil but renewable energy, and moreover time has come to see to what extent we can recycle used nitrogen and restore biological nitrogen fixation in the framework of a more sustainable agriculture.

9. Bioaugmentation and biocontrol

In the 80 s, we learned about the problem of nitrite accumulation in hobby and professional aquaria. Indeed, the ammonium excreted by the fish is not healthy for aquatic animals. It is most often converted into nitrite; the latter thereby can accumulate to levels of several mg nitrogen per L of water, thus hampering the health of the precious fish. We looked into the various remedies used to alleviate this problem and found a bewildering array of bio-augmentation products, most of them without any demonstrable effect. Yet, they were sold to the hobby aquarist. A fed batch culturing procedure to grow at cubic meter scale the combination of ammonium and nitrite oxidizers was set up and a well-documented and effective bio-augmentation product for the hobby market was developed (Grommen et al., 2002). Ever since, this culture has been operated at a large scale, sold commercially and used with success in households and large-scale aquaria and also in fishponds. The remarkable aspect is that, so far, no major incidences of parasitism or predation on this ongoing dense nitrifying culture have been observed. This is apparently a very stable consortium which, over decades, has demonstrated to be effective in stimulating nitrification upon addition to all kinds of environments in which fish-derived ammonium tended to accumulate.

This raises a corollary. All along the past half of a century, there has been the quest to control nitrification, chemically and/or biologically. What a blessing it would be if one could in an elegant way, curb down the normally rapid conversion of ammonium to nitrate in soils. Plenty of chemicals have been developed to that extent, but their application is far from effective. The efficiency of urea- and ammonium-based fertilizers would drastically be improved and the leaching strongly decreased. Moreover, the losses of nitrate by denitrification and the concomitant formation of unwanted nitrous oxide would be decreased. The negative environmental aspect of nitrous oxide (laughing gas), i.e. its significant contribution (and indirectly of nitrification) to the formation of the greenhouse effect and hence of the rising of the planetary temperature must be fully emphasized. It is quite a mental setback and disappointment that the 'happy feeling' of the summer rain, which indeed is causal to stepped-up nitrous oxide release from fields (Miller et al., 2022), also has the nasty countereffect of contributing to the global temperature rise.

10. The diplomat and visionaire

Thermodynamics have been a constant intriguing denominator in the exploration of the microbial world. One day, I stumbled over a remarkable paper (Broda, 1977). E. Broda, a career diplomate (stationed at the border of the then called Iron Curtain) who happened to be also microbiologist, had carefully examined the concept of Ernest Gale of 1985 that stated that, if there is energy to be gained from a compound, a microorganisms "will figure" out how to extract it and create a niche for itself. This concept had also the connotation of 'microbial infallibility'. Thus Broda postulated that two kinds of lithotrophs are missing in nature. The first one is oxidizing ammonium with nitrite/nitrate and obtains energy from this conversion. The second one is probably a phototrophic bacterium growing with ammonium as energy and hydrogen source. I remember a Biotechnology workshop in Ghent in the 90′s and I discussed these concepts with Gijs Kuenen who came to visit us. We explored the route of the so-called Oxygen Limited Nitrification and Denitrification (OLAND) (Verstraete and Vlaeminck, 2011). By the turn of the millennium, the team of Gijs succeeded to fully characterize the Anammox organism (Kuenen, 2008). This special nitrogen transformation has revolutionized the world of biology. It has also found major application in water technology, but it is sub-perfect in view of the nitrous oxide it produces. The other Broda postulate, which actually could be as simple as a bacterium which has a nitrogenase working in reverse, thus converting the ammonia to dinitrogen gas and hydrogen gas and getting energy from the further catabolism of this hydrogen has not been described yet! It would be a most useful biotech tool because it would allow to eliminate the excess reactive nitrogen present in wastes to neutral dinitrogen and…. useful hydrogen gas. The feammox reaction indicates that this concept of non-oxidative conversion of ammonia could be operational in biology (Le et al., 2021). Let us hope that one or other inspired scientist encounters that moment of serendipity and will bring the potential to recycle effectively waste ammonium nitrogen in the form of useful hydrogen to practice in the near future.

11. Aquaculture

By the turn of the millennium, we experienced a major development of aquaculture. Yoram Avnimelech at the Technion (Kochba et al., 1994) and Patrick Sorgeloos at Ghent University (Verschuere et al., 2000) were super driving forces. Yet aquaculture is a rather wasteful way of producing food. The ponds and cages are fed with high-value protein feeds (often made from fish caught at the high sea) and have a low conversion efficiency whereby some 80 % of the nitrogen is excreted as waste nitrogen. Hence, together with Avnimelech (2006), we developed the concept of dosing into such waters enriched with fecal fish nitrogen, a proper amount of carbohydrates (starch). Subsequently, one gets assimilation of the nitrogen into microbial biomass. By imposing proper process conditions, the microbes can grow in flocs which can then be grazed upon by the higher aquatic biota and thus give rise to a doubling of the overall efficiency of the nitrogen in terms of aqua-products harvested. This bioflocs technology is now used worldwide and of great value in terms of making aquaculture more sustainable (De Schryver et al., 2008). It should be noted that bioflocs technology is in fact nothing else but recycling of fecal matter towards food; and this does not - as far as we know - raises cultural or religious distrust. It is a remarkable constatation and a putative positive stepping stone for the cyclic economy, which is very often hampered by hygienic considerations which do not integrate the current insights in microbial ecology.

12. Relicts for future generations

Rather by accident, I had the privilege to serve a ten-year term as a member of the Technical Commission of Soil Protection in the Netherlands. The many aspects of soil and groundwater pollutants and the methods to abate them passed the various administrative and technical considerations. In 2009, during a site visit organized by this commission, I could experience how the mindset in terms of valuable nutrients had changed, particularly with regard to nitrogen. The case relates the region between the Maas and the Rijn in the Netherlands. Shortly after the second world war, -a war where famine had occurred in the Netherlands-, the Dutch government had supported the start-up of small dairy farms in that Maas -Rijn region. Soils were poor and the farmers had, often by manual labor, turned over the top soil and enriched it with organic matter and dung to make it a humus and organic nitrogen rich fertile agricultural land. Yet, 50 years later, this region around Maasduinen was subject to massive industrial excavation of top-quality sand for various industrial applications. Lo and behold, the fertile top soils were now a major problem; their nutrients risked to bring eutrophication. The solution for the 'too rich in organic nitrogen soils' which was selected by the Dutch government came as a shock: in 2010, the rich top soils were skimmed, loaded on trucks and transported to be buried 25 m deep in the sites where sand had been taken out. The valuable nitrogen produced by hard farmer labor during the postwar period had become of such concern, that it had to be stored forever isolated under a layer of clay at the bottom of the water storage basin which was created in that sand grove. Maybe, in the far future, this 'postwar formed organic nitrogen' can be re-discovered as a relic from what will probably be labelled the 'nitrogen-obsessed' time period in history.

13. Ammonia is in the air

A special note relates to the practice of scrubbing the ventilation gases of stables to remove the odor and also the ammonia they tend to contain. This technology is well established in the lower countries and results in the fact that quasi all the ammonium can be absorbed from the ventilated gases. There are different types of washers (Van der Heyden et al., 2016): some work with mineral acids (sulfuric) and thus produce a kind of ammonium sulfate liquor which can be used as fertilizer. Yet, the concentration of nitrogen of the proposed reuse product is a few percent and the contents of such liquors are variable; the crop growers have little interest for such recovery products. Another approach is to let the ammonium nitrogen become nitrified. In that case, the recirculating liquor becomes loaded particularly with nitrite (up to several grams per liter) and also nitrate. Two aspects deserve special mentioning. In many of these farm-installed washers, the pH of the liquor in which the nitrifiers thrive, gradually decreased over the months and years to values below 5 and even 2.5 and the nitrification keeps going on. These full-scale systems demonstrated that nitrifying bacteria could also adapt and evolve towards acidic pH values (Picone et al., 2021). Yet the textbooks mention that nitrification has a lower limit of approximately

6.0, and therefore in several countries the authorities constantly have accused the farmers operating the biological washers of false information and practice. Secondly, the nitrite in these waters could be effectively removed by adding sulfamic acid and thus converted to dinitrogen gas. Also for this approach, fully documented in terms of mass balance and potential side products, there has been no willingness of the regulatory instances to examine further practical application. Innovation is, in many respects, bouncing back on regulations and mindsets cast in concrete. Education is the remedy we must keep promoting.

14. 'Flemish fries', single-cell protein and positive mindsets

Anaerobic digestion is a marvelous microbial technology: it allows us to distill the energy present in the multitude of organic molecules in the form of biogas. The latter is far from well-defined nor devoid of contaminants such as trace organics or sulfur compounds but since the recovery product is 'taken by fire ', no questions are asked. Fire apparently has for the human being always been the mechanism of cleansing by preference, both for material and spiritual 'commodities'. Yet, anaerobic digestion leaves behind a digestate which is often rich in nitrogen. For industrial waters from the potato industry - Flanders is top producer and exporter of fries worldwide - the effluents of the UASB reactors constitute a real nitrogen problem. Nitrification and denitrification is a possibility, but the organic carbon to denitrify is starch which could otherwise be converted to biogas. For these industries, we tried to revitalize the bioflocs concept. These waters have an organic carbon to nitrogen ratio which, upon direct aeration of the matrix, allows to harvest both the organic carbon, the nitrogen and part of the phosphorous in the form of young single cells rich in protein. The latter can be centrifuged and upon drying, qualifies as a nice proteinaceous feed formulation for chickens, pigs and fish. This approach of assimilating the nitrogen into a new form of feed, although technically feasible and also in term of sustainability positive (Owsianiak et al., 2022), has thus far not seen applications, particularly due to regulatory restrictions. These harvested proteins are by EU law labelled as 'novel proteins' and therefore have to be subjected to rigorous standardization and continuous control at the level of molecular biology. In this respect, the strive for strict and very detailed regulation by the EU authorities needs to be monitored with care. It risks to quench innovation and to be restrictive to the establishment of a substantial flow of circular economy products and services. The long-term mismatch in the EU between innovator-entrepreneur and regulator-consumer over the past decades about genetic manipulation is currently re-emerging in the configuration of endless considerations about dangers of resource recovery products. This mismatch is counterproductive. Open minded explorations on how to reconcile scientific progress and public perception and well-being are warranted. We must hope for long-term conceptual 'sand boxes' in which new technologies are evaluated and tested with the tri-partite of the innovator, the investor and the regulator-consumer. We are in need of 'intelligent EU regulatory spaces' which integrate the new scientific insights with the future needs.

15. Ammonia and nitrous oxide emission

Since the end of the second world war, the term nitrogen was generally positive all the way to the end of the 20th century. Nitrification also was considered positive: it removes the potentially toxic ammonia in waters and soils, it has a significant role in providing the rapidly assimilable nitrate to various plants, and moreover it has a yet not fully understood role in generating various types of 'carriers of iron', i.e. siderophores which – as a kind of EDTA molecules - make iron bioaccessible to higher forms of life (Bossier et al., 1988). But rather suddenly, over the past 3 years nitrogen became in Flanders and the Netherlands a highly loaded word. It has become of central political importance, very much in the negative direction. The Flemish and Dutch parliaments have debated for weeks over issues of emission and deposition. Critical deposition values and rates have been narrowed down and mathematical models determine the all or not allowance to operate an animal stable resp an industrial installation. Politicians have broken their careers over nitrogen and its environmental impact. Farmer unions have upset the country by massive protests against the proposed restrictions on nitrogen emissions via groundwater and air. Major industries have in the past 2 years seen their authorizations to operate withdrawn. Cars on the highway have in the Netherlands speed limits of 100 km per hour, in order to curb emissions of nitrogen oxides. Remarkably, only in Flanders and the Netherlands is nitrogen such an issue; Denmark, France, Germany and Italy, having similar situations, have not such focus on nitrogen. Our decades of dealing with various aspects of environmental nitrogen suddenly came to stand under highly fierce spotlights.

The facts are quite straightforward. In 1992, the EU installed the Natura 2000 directive. Stretching over 18 % of the EU's land area and more than 8 % of its marine territory, Natura 2000 is the largest coordinated network of protected areas in the world. It offers a haven to Europe's most valuable and threatened species and the habitats they live in. This directive requires that the EU members, in those designated zones, should protect the high quality of the nature and moreover should allow no deterioration of nature in those zones. For decades, this law was disregarded and neglected. Animal husbandry continued to expand and the impacts of overfertilization were not properly administered. However, in the past few years, it was postulated that the emission and subsequent deposition of nitrogen, particularly as ammonium by animal husbandry and in the form of nitrogen oxides by various combustive activities, was directly causal to the decrease of plant biodiversity in nature reserves in Europe and beyond. A most particular consideration is that deposition of nitrogen (as ammonium or as nitrogen oxides) in those areas must stay below a particular 'Critical Deposition Value' (CDV). The Critical Deposition Values vary mostly between 5 and 25 kg nitrogen per ha per year. In most cases, the range is between 10 and 20 kg of nitrogen per ha per year, with a range of 5 to 10 kg of nitrogen per ha per year for sensitive marshes and dunes. Those proposed values of the CDV are not cast in concrete and research is ongoing to better delineate the quantities relative to the specific ecological sites (Guthrie et al., 2018). Also, different countries have different levels of allowed deviations with respect to the CDV (ranging from 500 g to 0,07 g nitrogen per ha per year). Yet, environmental regulators do impose such low amounts of nitrogen per ha per year as cut-off and as reason to close farms and industries. Although the Critical Deposition Values, and their boundaries of statistical confidence, still need to be demonstrated in a rigorous cause-effect statistical experiments, they are now strong stepping stones in legal enforcement of nitrogen emissions. In addition, there is also the point is that there might be negative effects on human health (fine dust formation as a result of nitrogen species present in the air). Finally, there is also a link between the emission of nitrogen oxides (NO, NO_2 by traffic combustion engines) and N_20 (by nitrification-denitrification processes) and the growing concern of global warming. Yet, the key feature of attention is the decrease in biodiversity. The latter issues, all in relation to nitrogen, became in a matter of 2–3 years super important arguments voiced by a group of clever and motivated nature activists. They confronted the judges in Netherlands and Flanders with the EU Natura 2000 directive and the CDV values which had been, in the meantime, become established as facts. In the courts, the judges have acted according to the law and thus forced the politicians in Flanders and the Netherlands to come to terms. The observation that other countries in the EU do not act yet is of secondary importance; it is crucial that the issues of nitrogen and quality improvement of nature have to be faced and clarified. The ongoing searches for better insights have caused many misunderstandings and turmoil. The fairness of the various approaches is the subject of discussion and plenty of fine-tuning.

Let us focus on one segment i.e. the treatment of nitrogen present in wastewater. At present, due to the low concentrations of about 50 mg reduced (Kjeldahl) nitrogen per L wastewater, the line of treatment is evident: nitrify the ammonium and concomitantly remove the nitrate by denitrification. Yet, there is a critical remark to make: during such oxidative /reductive conversion of the ammonium, a side product is formed, i.e. nitrous oxide. By optimally tuning of the process system, the production and emission of this greenhouse gas 'par excellence' from sewage treatment installations, can be kept low but overall the amount of nitrous oxide thus formed and released in the environment constitutes a significant percent in the production on greenhouse gas. Of all reactive N entering in a conventional activated sludge plant, about 1 % is converted to N_2O! The latter, due to its strong greenhouse gas effect, constitutes, as calculated for Switzerland, some 1 % of all greenhouse gas produced by that country (Gruber et al., 2021).This may seem small, but the whole of the mobility sector is responsible for some 20 % of all greenhouse gases and very much deals with the matter; hence the water cleaning sector should also address its contribution via N_2O with care.

To minimize the production of nitrous oxide in sewage treatment, in many countries sugars are dosed into the sewage treatment plant. Such dosage of quality commodities (feed/food stuffs) to sewage to avoid nitrous oxide, is confrontational. Clearly, there is an un-easy feeling about the ongoing route of wastewater treatment. If the farmers, the traffic, the industry have to curb down on greenhouse gas emissions, so should the industry of waste and wastewater treatment also, particularly since this branch is focused on dealing with environmental sustainability. The issues of nitrogen recovery from domestic sewage are very complex. Items such as urine separation, stripping of ammonium from digestate, re-use of recovered ammonium all deserve to be explored. Yet, none of these routes is elegant or evident because one should not forget that recovery products are mistrusted by the re-user and in general only rewarded by some 10 % of their factual value. In that context, there is sign of hope. The current attention on ammonium as an energy carrier, i. e. a molecule which binds hydrogen at sites of production and delivers hydrogen at sites of use, is quite special. Indeed in that context., one can strip 'waste' ammonium, clean it so that it can thermally or electrochemically be cracked to nitrogen gas and hydrogen gas (Lucentini et al., 2021). The hydrogen gas can subsequently be used – not so much for synthesis due to its waste origin – but as a source of energy, for instance generating kWh-electric by means of an adequate fuel cell system. This perspective is quite powerful and would be a blessing in the overall context of climate change abatement and the circular economy. And who knows, maybe there might, along with the Broda postulate, even be a biological process bringing the removal of ammonium without any danger of generating nitrous oxide.

16. What do we not know and needs to be explored asap

There were times that mankind used the very valuable amounts of reactive nitrogen present in urine, feces, wastes to produce gunpowder, thereby risking food shortage and famine. Today, we dose carbonaceous resources and even sugar to wastewater systems to convert nitrogenous end products back to dinitrogen. We continue to use fossil fuel based industrial fertilizers at a rate of 232 million ton N per year of which there is a surplus use of 119 million ton N per year (Rockström et al., 2023). According to the latter authors, the current way we handle the nitrogen balance of the biosphere certainly strongly surpasses (about factor 2) what they call the Safe and Just Earth System Boundaries. All this nitrogen inputs (representing some 400 million tons of oil equivalent per year) need to find a proper path of resource recovery. Mankind has to become aware that for this massive input of reactive nitrogen, a proper channel towards dinitrogen has to be assured. Preferably the path of nitrification and denitrification should be abandoned. For this huge amount of nitrogen in the reactive form, an adequate value chain should be engineered in the near future.

There are plenty of unknowns to be addressed and the challenges are enormous

- Do we, in view of the many negatives of nitrification, have to try to minimize the occurrence of this process in our industrial world? Should we - maybe at the exception of being at the basis of generating siderophores – maintain it for anything else? Should we not fully try to have better tools to control by chemical, biotechnological or ecological means the nitrifying organisms, particularly in our agricultural soils and waste treatment systems? The late Canadian soil scientist Eldor Paul has said (but not put in print) that nitrification is a top level "environmental disease". In the current status of the planet, it indeed looks that we must label nitrification as 'disaster process'. As a sign of hope, agronomists have discovered that the core germplasm of some tropical grasses is endowed with the capacity for Biological Nitrification Inhibition (BNI) (Villegas et al., 2020). Hence, there could a potential to produce by genetic or technical means BNI active substances thus suppressing nitrification in situations where it is deleterious.
- There is clearly a need to search for novel biology - natural or genetically engineered - which does not follow the routes which produce the very worrying nitrous oxide. Is such a new route realistic to hope for? Can possibly the conversion of ammonium by MnO_2 be engineered in a way that the end product is only dinitrogen (Liu et al., 2022; Zhao et al., 2022)?
- Can we not find ways to capture the biologically produced nitrous oxide (e.g. in sewage treatment systems) and use the thus captured gas as a fuel (indeed nitrous gas is used as a booster for engines). Similarly, is there not the possibility to develop another 'value chain' for 'used ammonia' which does not go via nitrification and denitrification but via cracking of the ammonia to reusable hydrogen and nitrogen gas.
- How to give a renaissance to biological nitrogen fixation and minimize use of fossil fuel based industrial nitrogen fertilizer without risking imbalances in food supply and food prices?
- Are the cause-effect relationships about deposition of nitrogenous products on the biodiversity and on the formation of fine dust appropriate? Are the models calculating the depositions and predicting the effects of nitrogen depositions in the environment sufficiently adequate and predictive?
- Should the major attention about nitrogen not be first given to the overall impact on the greenhouse gases and the concomitant rise of the temperature of our planet?

Obviously, we must educate ourselves and our political representatives in a new way which encompasses systemic thinking in terms of science in general and in relation to nitrogen chemistry, biology, ecology and technology in particular so that this planet can deal in a more coherent and long-lasting way with that powerful and most intriguing element called 'stikstof'. Not panic but better documented insight is needed. Let's cooperate, see how to make progress day by day and frame the latter in a proper intelligent regulatory space.

CRediT authorship contribution statement

W. Verstraete: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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