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NITROGEN TRANSFORMATIONS AND THEIR CIRCUMSTANCES IN SOILS

T. Włodarczyk

Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin 27, Poland

A b s t r a c t. Nitrogen transformation and their circumstances in soils were reviewed. In this paper, the biological processes of nitrogen transformation e.g., ammonification, nitrification, assimilatory reduction of nitrate, dissimilatory reduction of nitrate, nitrogen fixation and the way in which the different interacting processes influence N transformation were outline. In this mini-review we have concentrated predominantly on papers concerned with N_2O production and reduction. Additionally were shown non-biological processes of nitrogen transformation, which are responsible for some N_2O emission.

K e y w o r d s: ammonification, nitrification, assimilatory and dissimilatory N reduction, N fixation, sink of N_2O, redox potential

INTRODUCTION

Together with carbon, oxygen and hydrogen, nitrogen is one of the four most common elements in living cells and an essential constituent of proteins and nucleic acids, the two groups of substances which can be said to support life. Yet the element is not particularly common on earth, with exception of the atmosphere, which contains almost 80% nitrogen. The estimated 11 000 to 14 000 teragrams (10^{12}) nitrogen is in living biomass (mainly terrestrial plants) is equivalent to about three parts per million of the atmospheric nitrogen. Other important nitrogen pools are soil organic matters, rocks (in fact the largest single pool) sediments, coal deposits, organic matter in ocean water, and nitrate in ocean water. The next most common gaseous form of nitrogen in the atmosphere after molecular nitrgen is dinitrogen oxide [162].

The N atom exists in different oxidation and physical states. Shifts between them are commonly mediated by soil organisms. The ease with which shifts occur

in the oxidation states results in formation of different inorganic forms that are readily lost from ecosystem. The NO_3^- form is readily soluble in water and thus subject to leaching and water transport. The NH_4^+-NH_3 forms are subject to volatilisation and fixation both by clays and by soil organic mater (SOM). Nitrogen shortages, therefore, often limit plant productivity. Also, both the gaseous and the soluble phases of this nutrient lead to environmental pollution [119].

The size of pools does not indicate anything about dynamics of annual global fluxes of nitrogen between the more important pools.

Table 1. Global pool size of nitrogen [from Paul and Clark [119]

Pool	g N
Lithosphere	1 x 1023
Atmosphere	3.9 x 1021
Coal	1 x 1017
Hydrosphere	2.3 x 1019
Soil organic N	1 x 1017
Soil fixed NH_4^+	2 x 1016
Biota N	3.5 x 1015
Microbial N	1.5 x 1015

NITROGEN IN PLANTS

Most plants and other living organisms need nitrogen in larger amounts than they need essential elements other than carbon, oxygen and hydrogen. Nitrogen is a major and essential constituent of living cells. The proteins are polymerised amino acids, and nucleic acids are also polymers containing nitrogen in their constituents. There is often a close relation between the amount of nitrogen available to roots and total plant biomass in the ecosystem, which can be traced back to the fundamental relation between available nitrogen and plant cytoplasm [162].

As nitrogen is constituent of chlorophyll and enzymes participating in photosynthesis, the chlorosis often observed in severely nitrogen – deficient plants is often taken as evidence that a direct relationship must exist between leaf nitrogen concentration and photosynthetic efficiency. Such a relationship has also been demonstrated in nitrogen – limited system on the single leaf level [48]. The amounts of nitrogen available at any given moment in terrestrial ecosystem are often limited. It is characteristic of nitrogen that only a small fraction of the total amount in terrestrial ecosystem occurs in inorganic form (mainly as ammonium or nitrate ions), the form in which nitrogen in normally

available to higher plants. There is a continuous decomposition of nitrogen – containing organic matter in the soil. Mineral nitrogen is released and then rapidly taken up again by roots and microorganisms and again transformed to organic form. Soil concentration of ammonium or nitrate ions are therefore not good expressions for the availability of nitrogen to roots, when different ecosystems are compared [174].

Even if physiological need for nitrogen is satisfied, plant roots continue to absorb ammonium and nitrate ions. Ammonium ions are rapidly metabolised, a process requiring comparatively little energy, as the redox state of nitrogen remains unchanged [117]. As the ammonium nitrogen is transferred to amino acid or amide nitrogen, cell metabolism must provide the organic acids necessary for this process. The acids in question are produced from carbohydrates in normal metabolic processes, as long as the cell has enough carbohydrates in storage. The most common intermediary products formed from ammonium ions ad organic acids are glutamine and asparagine, which serve both for translocation and for temporary storage of nitrogen in many plants [162].

Roots also easily take up nitrate ions, although at a higher energy cost than ammonium ions [117]. They are exchanged for bicarbonate or hydroxyl ions, which leads to a counteraction of the acidification caused by cation uptake. Upon entrance into the root two thins can happen: 1) rapid reduction of nitrogen and formation of amino acids or amides, as above, or 2) translocation of nitrate to other parts of the plant, including the leaves. The nitrate is not very toxic, and a reduction to amino nitrogen may take place in green organs, with a coupling to the photosynthesis, a pathway, which seems to require less energy than reduction in the dark [67,117,149]. The temporary accumulation of nitrate in the leaves or other organs and then reduction coupled to photosynthesis is a characteristic of certain plants, while others normally reduce all nitrate immediately upon entrance into the plant [162]. Determination of the enzyme nitrate reductase in plant leaves has become a useful indirect method to assess soil nitrification [76,59].

Plants can store excess nitrogen in two ways, either as organic compounds (glutamine, asparagine, nitrogen – rich amino acids such as arginine), or as inorganic nitrate nitrogen but long – term storage is usually in organic form (seeds, stems of deciduous trees during winter). Some species, e.g., grasses, use both organic and inorganic storage forms [162].

NITROGEN IN SOIL

As in plants, nitrogen in soil occurs both in organic and inorganic form. Organic nitrogen is in reduced form, some of it as amide nitrogen, relatively easily available to decomposer organisms unless protected mechanically or chemically. Another part of soil organic nitrogen occurs as a constituent of large and often resistant molecules with nitrogen in heterocyclic aromatic rings [162].

Inorganic nitrogen is usually fully reduced, ammonium, or fully oxidised, nitrate. Intermediary oxidation stages also exist but do not accumulate in measurable amounts, except for nitrite under special circumstances. There are transfers not only between the various soil nitrogen pools, but also between the soil pools and gaseous phase, where nitrogen compounds at different oxidation levels also occur (NH_3, N_2, N_2O, NO) [162].

Only a small part of nitrogen store in the soil is available to plant roots at any given moment. Most is in organic form, usually in large molecule insoluble in water. Organic nitrogen in natural ecosystems originates from dead organisms, plants, and microorganisms. Much of the nitrogen in fresh litter is still in protein form or in decomposition products of proteins, i.e., peptides and amino acids. These substances are attractive substrates for microorganisms, which often can use as a source of carbon as well as of nitrogen. Their residence time in the soil is short, unless association with less attractive substances in, e.g., cell walls protects them mechanically or chemically [162]. A bacterial cell synthesises over 1000 kinds of proteins. Proteins constitute the most abundant N-containing constituents of organisms and are readily attacked by many soil organisms via proteolitic enzymes that hydrolyse the peptide links [119].

The decomposition of litter does not mean that litter nitrogen immediately transferred to inorganic nitrogen or transformed into the limited number of low-molecular organic compounds in which it may be available to plant roots and mycorrhizal fungi. Microorganisms do the chemical degradation of the litter, and even if they may produce extracellular enzymes, most take of the nitrogen up themselves. The rate at which the microbial nitrogen is transferred to the available pool depends on the C/N ratio of the substrate and on the death rate the microorganisms [162]. Microorganisms are a major source for N mineralization in soil because of the much lower C:N ratios of bacteria and fungi relative to plant residue. Bacteria have C:N ratio as low as 3.5:1, fungi, of 10 to 15:1. The average soil population is found to have a C:N ratio of 4 to 7:1 [119]. Figure 1 shows nitrogen cycle in soil.

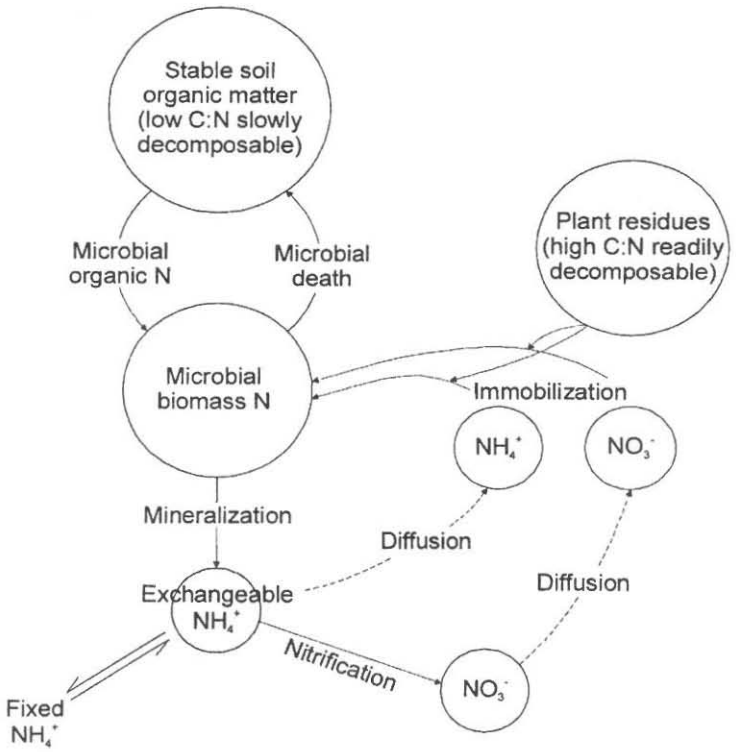


Fig. 1. A conceptual model of the soil nitrogen cycle. From Drury *et al.* [44]

As far as nitrogen is concerned, the end product of the decomposition process as such is ammonium ions. Ammonium ions in water solutions are in equilibrium with undissociated ammonia molecule, but the amounts of ammonia are negligible until pH rises above seven. In such cases some ammonia may well be emitted to the atmosphere. In dense vegetation, e.g., under a forest canopy, much of that ammonia may be reabsorbed by the foliage and thus retained within the ecosystem [162].

The normal case, however, is that most of the ammonium – liberated stays in the ecosystem, although rapidly removed from the soil solution along one of the following pathways: 1) uptake by plant roots (directly or via mycorrhizal hyphae), 2) uptake by microorganisms, 3) adsorption on the surface of soil colloids (in clay-rich soils partly followed by ammonium fixation in the lattice of certain clay mi-nerals), and 4) chemical binding to organic substances. Any ammonium ions left in the soil solution may leave the soil with percolating water, but this is seldom an important pathway in natural ecosystems [162].

Adsorption of ammonium ions to soil colloids is a removal from the pool of dissolved nutrients, but does not make them unavailable for plants; when roots or mycorrhizal hyphae deplete the soil solution of ammonium ions, such adsorbed ions go into solution again according to well – known chemical principles. However, ion transport by diffusion is a slow process. So unless there is a mass flow of soil water, toots and hyphae have to grow close to the sites of adsorption. The energy cost for uptake from a soil increases in comparison with that from a nutrient solution. Lattice-fixed ammonium ions can also be redissolved, but this is a slow, but this is a slow process of limited ecological importance under normal conditions and time perspectives (seasons, years, even decades) [162].

Chemical binding of ammonium nitrogen in high – molecular organic substances in the soil is very important and yet poorly understood process [162].

Humus is the term for the soil organic matter, which cannot macroscopically be recognised as plant, or animal remains [83]. The humus is very resistant to degradation, with half-lives varying from decades in some intensively cultivated organic soils to several thousand years for organic matter deep in mineral soils in certain soil types (as measured by radiocarbon dating). The chemical structure of humus is not well defined, even if fractions with different characteristic can be isolated by chemical methods (humic acids, fulvic acids). Much of nitrogen appears to occur in heterocyclic aromatic rings, which together with the size of the molecules may account for the resistance to enzyme degradation. Much of the carbon in the humus may originate from the lignin in plant cell walls, as terpenoid fragments can be obtained from both lignin and humus by chemical treatment. While many fungi and bacteria either lack lignin – degrading enzymes or produce them in small amounts, wood – degrading fungi of so – called white – rot type decompose lignin – rich plant residues relatively easily. Related soil living fungal species can decompose at least part of the soil humus [162].

The concentration of lignin and other high – molecular polyphenolic compounds appears to be one of the important controlling factors for the rate of organic matter decomposition in the forest soil [13].

The fluxes of N shown in Table 2 were obtained from number of independent estimates [119].

Table 2. Terrestrial fluxes of nitrogen. From Paul and Clark [119]

Tg^a N $year^{-1}$			Tg^a N $year^{-1}$
Soil N mineralised	3000	Plant utilisation	1200
Inputs		Losses	
Dinitrogen fixation	175	Denitrification	135
Fertiliser	85	NH_3 to atmpsphere	62
Lighting	20	Leaching	90
Anthropogenic	40	Runoff erosion	25
Total inputs	320	Total losses	312

NITROGEN TRANSFORMATIONS

Ammonification

The three biological forms of N proteins, microbial cell wall constituents such as chitin and peptidoglycans, and the nucleic acids. Protein is a basic constituent of all life forms. During decomposition, it is hydrolysed to peptides by proteinases and peptidases. The proteinases are classified as to whether they attack peptide linkages between specific amino acids. The reaction mechanism is the reverse of that used in formation of peptide bonds. The N group receives a proton (H^+), and C atom of the linkage receives an OH^- during the nucleophilic displacement reaction [119]. Most of the mineralization reactions are the result of the activity of extracellular degradative enzymes, released by soil microbes [28,90,41].

Mineralization of organic N refers to the degradation of proteins, amino sugars, and nucleic acids to NH_4^+, the mineral form. When deamination occurs, removal of NH_4^+ is most often carried out by enzymes as glutamate dehydrogenase, which requires the coenzyme nicotine adenine dinucleotide (NADH) as acceptor of the reducing equivalents [119].

The mineralization of N from decomposing materials with release of NH_4^+ by heterotrophic microbes is known as **ammonification**. Subsequently, a variety of processes affect the concentration NH_4^+ in the soil solution, including uptake by plants, immobilisation by microbes, and fixation in clay minerals [141].

Whether NH_4^+ is immobilised or accumulates in the soil depends on the microorgamisms requirement of N for growth. The C:N ratio of microorganisms is not constant. Fungi can have wide C:N ratios; their C contents are quite constant at approximately 45% C. With N contents of 3 to 10%, their C:N ratios range from 15:1 to 4.5:1. Bacteria have N in their cytoplasm and in the peptidoglycan of their cell walls: C:N ratios usually are in the range of 3:1 to 5:1 [119].

Nitrification

Nitrification is an aerobic process, performed both by autotrophs and heterotrophs in soils.

Autotrophic nitrification is defined as the biological oxidation of NH_4^+ to NO_2^- and NO_3^- in a two step reaction as presented in the following equations where *Nitrosomonas* performs the first energy yielding reaction:

$$NH_4^+ + 1.5\,O_2 \rightarrow NO_2^- + 2H^+ + H_2O + \text{energy} \quad (1)$$

and *Nitrobacter* the second energy yielding reaction:

$$NO_2^- + 0.5\,O_2 \rightarrow NO_3^- + \text{energy} \quad (2)$$

The chemoautotrophic nitrifiers are generally aerobes that derive their C largely from CO_2 or carbonates but NH_4^+ can originate from mineralization of soil organic material by other organisms or from fertiliser. All organisms in this family are capable of obtaining all their energy requirements for growth from oxidation of either ammonium or nitrite [10].

The bacteria are classified based on whether they oxidise NH_4^+ to NO_2^- (*Nitroso-*) or NO_2^- to NO_3^- (*Nitro*). In most habitats they are closely associated and NO_2^- rarely accumulates [119].

The oxidation of NH_4^+ can be described as:

$$NH_4^+ + 2H + 2e^- + O_2 \xrightarrow{\text{ammonia mono-oxygenase}} NH_2OH + H_2O \quad (3)$$

The enzyme ammonia mono-oxygenase has broad specificity and also oxidises propene, benzene, cyclohexane phenol, methanol, and CH_4.

Hydroxylamine is oxidised to NO_2^- as follows:

$$NH_2 + OH + H_2O \xrightarrow{NH_2OH\text{oxidoreductase}} HONO + 4e + 4H^+ \quad (4)$$

$$2H^+ + \frac{1}{2}O_2 + 2e^- \xrightarrow{\text{terminal oxidase}} \quad (5)$$

The NO^-_2 oxidising bacteria catalyse the reaction:

$$HNO_2 + H_2O \xrightarrow{\text{nitrite dehydrogenase}} HNO_3^- + 2H^+ \quad (6)$$

Nitrification has been typically associated with chemoautotrophic bacteria, although it is now recognised that **heterotrophic nitrification** occurs in some soils too acid for known autotrophic nitrifiers, or lacking them for other reason and can be of significant especially in forest soils. It has been shown that nitrate formation may continue in presence of inhibitors known to stop autotrophic nitrification [85]. This indicates the occurrence of so – called heterotrophic nitrification, mediated by certain fungi [54] or by methylotroph bacteria [172]. It is clear that heterotrophic nitrifiers form nitrate at a much slower rate than autotrophic nitrifiers (with the same biomass). However, a slow rate may be compensated for by a high biomass [162].

Heterotrophic organisms use organic substances as both a carbon and an energy source. They can obtain part of energy from oxidation of NH_4^+ or organic nitrogen compounds. Fungi are apparently the most important of these. Different pathways have been postulated, but their role in fungal metabolism is largely unknown [79]:
inorganic:

$$NH_4^+ \rightarrow NH_2OH \rightarrow NOH \rightarrow NO_2^- \rightarrow NO_3^- \qquad (7)$$

organic:

$$RNH_2 \rightarrow RNHOH \rightarrow RNO \rightarrow RNO_2 \rightarrow NO_3^- \qquad (8)$$

The rate of nitrification in a soil is affected directly and indirectly by many factors, such as temperature, moisture, C/N ratio occurrence of inhibitors of the process itself, or of organic matter decomposition. Yet a prime prerequisite for nitrification is access to ammonium ions in the soil or, for some heterotrophic nitrifiers, easily available amino compounds. It was mentioned earlier that plant roots promptly absorb ammonium ions (as well as nitrate ions), while many microorganisms prefer the ammonium form. Some fungi cannot even use nitrate nitrogen. Concentration of ammonium ions high enough to support an active population of bacteria using oxidation of ammonium to nitrite as their sole source of energy (e.g., the genus *Nitrosomonas*) only occur when the competition for nitrogen is low or moderate, i.e., when ammonia influx to the soil compartment (by ammonification or as input from outside) temporarily or permanently exceeds biological uptake [162].

The best known nitrifiers are bacteria of the genera *Nitrosomonas*, which oxidise ammonium to nitrite, and *Nitrobacter*, which oxidise nitrite to nitrate. Both *Nitrosomonas* and *Nitrobacter* are favoured by alkaline to slightly acid soils and are unimportant in strongly acid environments. This does not necessarily exclude them from soils with an average acidity below pH 4.5 [54].

The heterogeneity of a soil means that there may be a large variation in many soil properties, including acidity, between microsites. pH is an important controlling factor, not only for the occurrence of nitrification, but also for any by – products that may be formed. As *Nitrobacter* seems to require somewhat higher pH than *Nitroso*monas, some accumulation of nitrite may occur under certain circumstances. Gaseous products may also be formed, at different rates under different conditions [162].

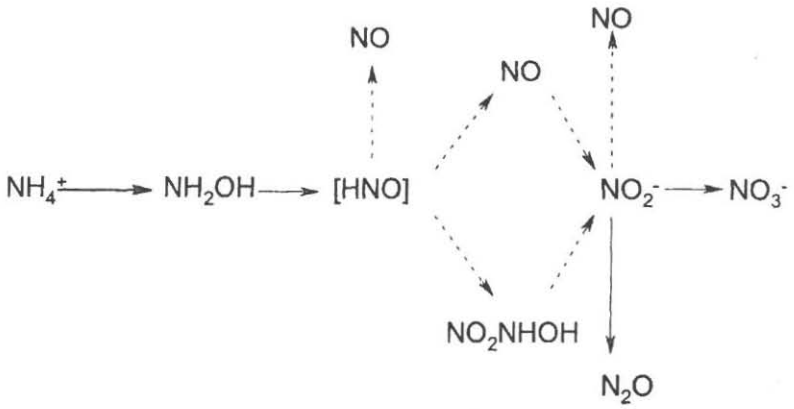
It remains to be stated that nitrification is an acidifying process. Under undisturbed conditions, when the nitrate formed is rapidly taken up by roots and reduced back to ammonium and other reduced forms, there is no net acidification [162].

Heterotrophic nitrification may dominate over autotrophic under certain conditions. A low pH is one factor that seems to strongly restrict autotrophic nitrification. Nitrification is probably heterotrophic in soils such as acid coniferous forest soils, where the microbial biomass is often dominated by fungi. The low nitrification potential per unit biomass observed for heterotrophic nitrifiers may be more than offset by the huge fungal biomass in these soils [79].

Nitrite accumulates only under conditions where Nitrobacter appears to be inhibited while Nitrosomonas is not. Typically these conditions are high pH (7.5) and very cold temperatures [20,153].

Although nitrification is understood to be an aerobic process there is strong evidence that it can also occur under anaerobic conditions. Nitrifying bacteria have been shown to produce NO and N_2O. This varies with O_2 concentration and usually does not go beyond 1% of NO_2^- added, but yields up to 10% of N in the medium have been reported. Nitrate reduction is now thought to be the major process involved in those gaseous emissions, with NH_4^+ oxidation providing the electrons for this denitrification process. This process is thought to possibly conserve O_2 for ammonia mono – oxygenase, keep NO_2^- from reaching toxic levels, and maintain optimum redox levels.

The intermediates in autotrophic nitrification showing the possible sites for gaseous losses during this process [119]:



A copper protein is responsible for the nitrite reduction which proceeds under aerobic and anaerobic conditions ("nitrifier – denitrification") with concomitant oxidation of ammonium [121,126]. This could be a process within nitrifiers to reduce accumulated nitrite levels which otherwise could cause intracellular toxicity [25].

Another route above mentioned for N_2O production via nitrification is the chemical reaction involving intermediates formed during ammonium (NH_4) oxidation to nitrite (NO_2^-). The reaction between hydroxylamine (NH_2OH) formed during nitrification in well aerated as well as anaerobic soils and nitrite has been proposed by a number of researchers [25,32,97]:

$$NH_2OH + NO_2^- \rightarrow N_2O + 2H_2O \qquad (9)$$

The first step in the process of nitrification is the synthesis of hydroxylamine, which is oxidised to produce HNO, this last intermediate being the precursor of HNO_2. However in anaerobiosis, the product of NH_2OH oxidation is N_2O, which presumably is produced by the nonenzymatic decomposition of HNO [71].

Nitrous oxides are well-documented gaseous products of litotrophic ammonia-oxidisers [73,92,161,166]. N_2O is produced when NO_2- is used as electron acceptor by ammonium oxidisers in O_2-limited environments. Poth [123] using *Nitrosomonas*, *Nitrosococcus* and *Nitrosolobus* species, showed the production of $^{15}N_2O$ and $^{15}N_2$ from $^{15}NO_2^-$ under oxygen stress. Poth [123] postulated during his work that the $^{15}NO_2^-$ was serving as an electron acceptor, so that any available oxygen could be used by the ammonia monooxygenase. It was suggested that it should be possible to grow autotrophic nitrifiers anaerobically, while denitrifying were

provided with hydroxylamine rather than ammonia. However, it has since been reported [171] that a mixed culture from a wastewater treatment system is capable of nitrification (and, by definition denitrification) under fully anaerobic conditions, implying that ammonia monooxidase may not be the sole ammonia-oxidising enzyme available to these bacteria. Bock *et al. [17]* showed that some nitrite-oxidising *Nitrobacter* species can grow anaerobically as heterotrophs, with nitrate serving in the presence of oxygen and an organic substrate and may simultaneously convert nitrite to gaseous products via denitrification.

N_2O production in soils at moisture contents below field capacity is generally attributed to nitrification [38,75,166]. Moreover, Tortosi and Hutchinsen [166] concluded those chemoautotrophic NH_4^+ oxidisers, rather than chemoautotrophic and heterotrophic NO_2^- oxidisers, are the predominant source of NO and N_2O production during nitrification in soil. In their study, the addition of nitrapyrin (an inhibitor of NH_4^+ oxidation) reduced gas production, while the addition of chlorate (an inhibitor of NO_2^- oxidation) spurred gas production. Moreover, the addition of glucose increased emission of NO and N_2O over the first few hours of incubation. Hence, gas production by mixotrophic growth of NH_4^+ oxidisers [160] cannot be discounted as a source of NO and N_2O. Hutchinson *et al.* [75] found that chemoautotrophic NH_4^+ oxidisers were the predominant source of gaseous N oxides at water contents % (ca. - 10 kPa) in a sandy loam. Furthermore, the addition of nitrapyrin eliminated the brief emission of N oxides that typically occurs upon wetting of dry soil.

Most heterotrophic nitrifiers appear to be also aerobic denitrifiers. Therefore, heterotrophic nitrification might be linked to the "nitrifier-denitrification". However, the contribution of nitrous oxide production through this pathway remains poorly understood. The situation is complex since it is very difficult to separate autotrophic and heterotrophic nitrification [132, 133]. During the batch culture experiments to discover *T. pantotropha* was denitrifying aerobically, nitrite was substituted for nitrate in a series of experiments, and it was observed that the nitrite concentration increased before eventually decreasing to 0. This phenomenon only occurred in the presence an organic substrate, ammonia, and oxygen, indicating that *T. pantotropha* is a heterotrophic nitrifier. In their words, *T. pantotropha* can catalyse the oxidation of ammonia to nitrite provided that an organic electron donor (in this case acetate) is available. Subsequent experiments revealed that the nitrifying enzymes of *T. pantotropha* were remarkably similar to those of autotrophic nitrifiers such as *Nirtosomonas europea* [130]. Nitrite only accumulated in

the presence of nitrite or an inhibitor of nitrite reductase, and it became clear that it was simultaneously reducing al or most of the nitrite to N_2 [86,131].

Kuenen and Robertson [86] found that a heterotrophic nitrifier could also denitrify, and accumulated little or no NO_3^- or NO_2^-. For such organisms nitrification rate cannot be estimated from the accumulation of NO_2^-. Thus, it seems possible that heterotrophic nitrifiers in significant amounts can also produce N_2O. However, this subject needs further investigation [61].

Episodes of N_2O production in response to C inputs may derive partly from mixotrophic or heterotrophic growth of nitrifiers. For example, Stuven et al. [160] proposed a scheme in which oxidation of organic matter during mixotrophic growth of *Nitrosomonas europaea* resulted in a release of hydroxylamine from cells and subsequent reduction of NO_2^- to NO and N_2O (chemodenitrification). Similarly, Abeliovich and Vonshak [1] demonstrated that NH_{4+} stimulated anaerobic reduction with pyruvate as an electron donor.

According to Groffman [63] two processes are responsible for N_2O formation from nitrification:

1. Ammonium oxidisers can use NO_{2-} as an alternative electron acceptor when O_2 is limiting and produce N_2O [124, 51]. This process is called nitrifier denitrification.
2. Intermediates between NH_4^+ and NO_2^-, or NO_2^- itself, can chemically decompose to N_2O, especially under acidic conditions (a type of chemodenitrification).

Nitrification is often considered to be the dominant source of N_2O in "aerobic" soils [23,139].

Assimilatory reduction of nitrate

Soil fixed nitrogen resources may be conserved through both assimilatory and dissimilatory nitrate reductive processes, or they are reduced by dissimilatory reduction. Assimilatory and dissimilatory nitrate reduction both involve the transfer of electrons to nitrogen compounds, but they differ in the ultimate fate of the reduced nitrogen atom.

In the absence of NH_4^+ and organic-N and under conditions where only NO_3^- is available, bacteria, fungi, yeast and algae have first to reduce the NO_3^- [55]. This process is less O_2 sensitive than denitrification and therefore would be expected to occur under aerobic conditions [121,101]. The aerobic assimilation of nitrate or assimilatory nitrate reduction is the process of NO_3^--N incorporation into biomass [101]. Some microorganisms reduce NO_3^- to NH_4^+. They use the N in

production of biomass (assimilatory reduction), but the process can also serve other purposes (dissimilatory reduction), e.g., as a source of energy or for detoxification of NO_2^-. N_2O can escape during these processes [34,78,140,165].

In nitrate assimilation, the first step is the reduction to nitrite, which is accomplished by the enzyme nitrate reductase. Subsequently, the nitrite is reduced to hydroxylamine by the enzyme nitrite reductase to finally be reduced to ammonia [120]. The net reaction is shown in following equation:

$$\begin{array}{c} NO_3^- \rightarrow NO_2^- \rightarrow [H_2N_2O_2] \rightarrow [NH_2OH] \rightarrow NH_3 \\ \downarrow \\ N_2O \end{array} \qquad (10)$$

where N_2O rather than N_2 may be produced as a by-product from the indicated intermediate (hyponitrite) [55]. The reaction shown is essentially the same as that which occurs during NO_3^- reduction to NH_4^+ and involves the same precursor of, N_2O again probably hyponitrite [55,101]. This pathway as a nitrous oxide source seems to be significant from studies on forest soils where fungal activity is important. Sextone [144] provided evidence that in an acidic organic coniferous forest soil the N_2O production due to fungal activity may be as much as 40% of the total. Furthermore fungal activity was also suggested by Robertson and Tiedje [129] as an alternate biological nitrous oxide source from forest soil. Finally, certain assimilatory nitrate-reducing yeast have been shown to be able to produce N_2O [80].

Some of the studied nitrate reductase shows the existence of an active form and an inactive form that depends on the oxydoreduction conditions of the environment [158]. Under reducing conditions, the enzyme is convert into the inactive form. The regulation of the synthesis of the enzyme varies in different species, being constitutive in several species and repressible in others. In *Rhizobium japonicum,* for instance, the assimilatory enzyme is induced in aerobiosis and in the presence of nitrate; meanwhile in anaerobiosis, a dissimilatory nitrate reductase is induced [36]. Both enzymes have different molecular weights and different sensitive to inhibitors [158].

Dissimilatory reduction of nitrate

Dissimilatory reduction is the process through which some microorganisms use the energy generated by the electron transport from an organic or inorganic source to nitrate or to a more reduced nitrogen oxide. This metabolic reduction

uses cytochromes mostly as electron donors and occurs with a liberation of dinitrogen as the final product. However, some bacteria lack N_2O reductase, and so produce this gas as a terminal product, or lack nitrite reductase, yielding nitrite as an end product [77].

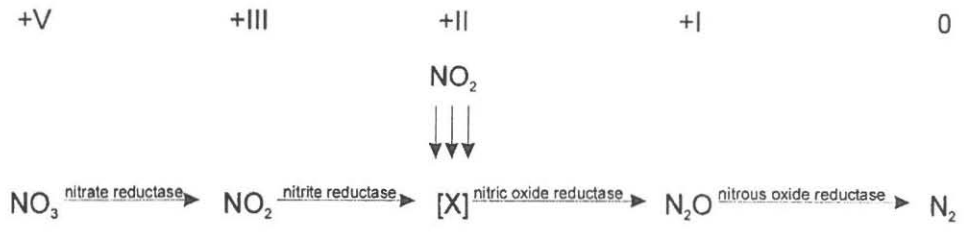
When the dissimilative reduction produces the gaseous dinitrogen or nitrous oxide compounds, the process is termed **denitrification**. However, since reduction, through the metabolic pathway of cytochromes, in some case results in the production of ammonia or nitrite, some authors prefer the more general name of **nitrate respiration** for the process. In other cases, the metabolic pathways do not involve membrane-bound enzymes, cytochromes, or electron transport phosphorylations, and the main product is ammonia. This process is called **fermentative nitrate reduction**. [47].

In contrast to assimilatory reduction (nitrogenous compound is incorporated into cellular biomass) for dissimilatory nitrate reduction, the nitrogenous compounds accept electrons in support of cellular respiration. The final products, dinitrogen, nitrous oxide, or ammonium are released from the cell and accumulate in the environment in concentrations far beyond that necessary for biomass synthesis. Three commonly evaluated microbial processes are classed under the tittle of dissimilatory nitrate reduction. These processes can be distinguished by their respective products: a) nitrite, b) ammonium, and c) nitrous oxide and dinitrogen denitrification.

Biological denitrification is the last step in the N-cycle, where N is returned to the atmospheric pool of N_2. It is an anaerobic process [61].

Biological denitrification is a respiratory process in which N-oxides (electron acceptors) are enzymatically reduced under anaerobic conditions to nitrous oxide and dinitrogen for ATP production by organisms that normally use O_2 for respiration. Most denitrifying organisms are heterotrophic. However, heterotrophic denitrification is the most important processes as a source for N_2O. Nitrous oxide is well – documented gaseous products of the heterotrophic denitrifiers [2,12,105].

The process of denitrification (including rhizobial denitrification) can be presented as follows [51]:



Nitric oxide (NO) is believed to be either a true intermediate or rapid exchange with an unidentified intermediate [X].

Anaerobic conditions and the presence of readily oxidisable carbonaceous substrates are necessary for denitrification. Denitrifiers gain carbon for cell growth from the concomitant oxidation of organic molecules [120].

Many microorganisms can use NO_3^- as their primary electron acceptor for obtaining energy from organic compounds when low O_2 availability restricts their metabolism [61]:

$$5(CH_2O) + 4NO_3^- + 4H^+ \rightarrow 5CO_2 + 7H_2O + 2N_2 + \text{energy} \qquad (11)$$

Some microorganisms can obtain energy by using NO_3^- for oxidation of inorganic compounds, e.g., S^{2-}, Fe^{2+} (autotrophic denitrification). This occurs where NO_3^- diffuses into zone rich in FeS, e.g., sediments in shallow waters [60].

The majority of soil bacteria seem able to denitrify [167,168]. The complete reduction of nitrate proceeds via nitrite, nitric oxide, and nitrous oxide, but not all denitrifiers can carry out the complete reduction from nitrate to N_2. Denitrifying bacteria exhibit a variety of incomplete reduction pathways. The enzymes most commonly missing are nitrate reductase or nitrous oxide reductase; some bacteria produce only N_2, while others give a mixture of N_2O and N_2, and some only N_2O [133,159].

Nitrate reductase of the dissimilatory reduction is a molybdo-iron sulphide protein, but different from the assimilatory enzyme [136,137]. Nitrate reductase has been found to be a membrane – bound enzyme except in *Spirillum iteronii* where is found as a soluble enzyme [58].

Nitrite reductase is the key enzyme that drives the NO_2^- ion toward the synthesis of the gases and NO in contrast with the more economic pathway of ammonia synthesis.

Nitrous oxide reductase is possibly a Cu protein and closes up the recycle of nitrogen by releasing dinitrogen back to the atmosphere [82]. Thus, the function of this enzyme is essential and prevents N_2O from being released into the atmosphere, avoiding the photochemical production of NO; this gas is supposed to be responsible for destroying the atmospheric ozone [40].

Some denitrifiers lack the ability to catalyse the last step from N_2O to N_2 [165].

There has been some doubt if NO is a true intermediate or by product [4] in the process, but a bacterial nitric oxide reductase has recently been characterised: *Pseudomonas stutzeri* loses the ability to denitrify if the genes for this enzyme are blocked [21].

That N_2O is an obligatory intermediate in denitrification is widely accepted [121,178].

N_2O is reduced to N_2 by the labile enzyme nitrous oxide reductase [159]. The reduction can also be carried out by the even more labile enzyme nitrogenase (the enzyme that reduce N_2 to NH_3).

Apart from free living denitrifiers such as *Pseudomonas ssp.*, *Rhizobium ssp.* which live in a symbiotic relationship with leguminous plants have the ability to denitrify. This later process is referred to as rhizobial denitrification [111].

The denitrification process may be performed by N_2-fixers, specifically by *Azospirillum*, and by *Rhodopseudomonas* [3,30]. These species are capable of using nitrate as an electron acceptor, an alternative to oxygen, for generating ATP for nitrogenase activity. Studies with stable isotopes showed that *Rhodopseudomonas* spheroides, strain IL-106, did not directly assimilate nitrate into cell nitrogen, but rather denitrified nitrate to dinitrogen gas which was reutilized via nitrogenase as a source of ammonia for its assimilation [109]. *Rhizobium japomicum* and cowpea strains exhibit substantial rates of denitrification as either free-living or bacteroid cells. *R. triflii, R. leguminosarum* and *R. hedysarum* were able to use nitrate as an electron acceptor, liberating N_2O gas. This liberation was inhibited in the absence of nitrate by aerobiosis or when rich media were used. Similar studies were carried out with nodulated plants, with the aforementioned fast-growing rhizobia, showing that *Rhizobium* in an active denitrifier in symbiosis as well as in the free-living state [30].

Denitrification is usually thought as a bacterial process, but Shoun *et al.* [146] reported that many fungi are capable of evolving N_2O under anaerobic conditions.

Some researchers have suggested that soil microbial population dynamics may be more important factor than soil physical and soil chemical factors in explaining the characteristics of nitrous oxide production from soil [2,61,125,142].

The influence of aeration on N_2O emission is complex and dependent on interacting factors. N_2O production and emission is usually greatest when the average soil conditions are such that both aerobic and anaerobic sites are abundant. This has been found in several laboratory studies [53].

Soil is heterogeneous and commonly has both aerobic and anaerobic sites. The oxygen status in soil, which is inversely, proportional to the amount of moisture held there, appears in many studies to be one of the key factors influencing nitrous oxide production. As the free oxygen in soil is depleted, a number of predictable changes in microbial activity occur. When the soil oxygen tension has been reduced to less than 1 percent (v/v), the microbial population appears to shift from

being predominantly aerobic to anaerobic. With the development of reducing atmosphere, growth yields decline because the energy yielded per mole of fixed carbon oxidised anaerobically is far less that produced from aerobic respiration. The inverse relationship between the rate of denitrification and O_2 concentration has been demonstrated in many studies [14,29,53].

Similar results were obtained by Parkin and Tiedje [113]. Denitrification rates in their soil cores remained low, less than 2% of anaerobic rate, as low as O_2 concentration in the gas was greater than 3%. At lower O_2 concentrations the rates increased, and rapidly approached anaerobic rates when the O_2 concentration decreased below 0.5%.

The inverse relationship between denitrification rate and O_2 concentration is more pronounced at high (34.5°C), rather than at low (19°C), temperature [54].

Non-denitrifying fungi and bacteria can produce N_2O during the process of dissimilatory reduction of NO_3^- to NH_4^+. This pathway, which is regulated by oxygen and unaffected by ammonium, can be a contributing source of N_2O from systems which suffer prolonged anaerobic periods, e.g. in sediments and rice paddy fields [165]. According to Bleakley and Tiedje [16] this pathway of N_2O production is of minor importance. However, with the high activity of these microorganisms coupled with an appreciable NO_2^- accumulation in soil, this pathway may be more important than is generally acknowledged [165].

In aerobic soils denitrification can occur in anaerobic microsites such as in the centre of aggregates [71,114] or in areas of localised high oxygen consumption ("hot spots") which can be associated with the breakdown of particulate organic material [114]. Furthermore some groups of denitrifiers are able to use simultaneously both oxygen and nitrate or nitrite as electron acceptor. Therefore, denitrification by those organisms can occur under aerobic conditions. **Aerobic denitrification** can occur in the presence of significant amounts of oxygen. Those denitrifiers are able to simultaneously utilise oxygen and nitrate or nitrite, even when the dissolved oxygen concentration approaches air saturation. An explanation for the usage of both acceptors might be the presence a rate-limiting step in the transfer of electrons from its substrate to oxygen. The provision of a second electron acceptor, in this case nitrate, would allow it to use an additional branch in the electron transport chain [132,133,178].

In anaerobic respirometry experiments, it was observed that aerobically grown *Thiobacillus pantotropha* began to denitrify immediately when it was supplied with substrate and nitrate. Similarly grown cultures of the other strains required 2 to 4 h to induce their denitrifying enzymes [127]. Oxygen and nitrate electrodes

were used to monitor the activity of these cultures, and simultaneous nitrate and oxygen removal in *T. pantotropha* suspension was clearly observed [128]. Oxygen and nitrate electrodes were used to monitor the activity of these cultures, and simultaneous nitrate and oxygen removal in *T. pantotropha* suspension was clearly observed [128]. When grown in batch cultures with acetate as the substrate, *T. pantotropha* cultures provided with both oxygen (at a dissolved oxygen concentration of 80% air saturation) and nitrate grew more rapidly than similar cultures which had only one electron acceptor [127].

Mention must be made of another condition, which can favour low O_2 levels, and hence N_2O production within soils. This is the presence of anaerobic microsites, particularly within heavy textured clay soils, where gaseous diffusion is slowed or restricted. Nitrous oxide emissions are often high from these soils, especially those with a large proportion of anaerobic microsites [27,96]. Such microsites exist where root or soil respiration rates exceed the capacity of the soil to allow adequate gaseous diffusion to or from the microsites. The role of O_2 diffusion in soil for denitrification was described in the model of K.A. Smith [152]. This model calculates concentrations in soil and describes how O_2 diffuses down the profile and into aggregates, and the fraction of the soil volume that is anaerobic. The diffusion of O_2 into aggregates rather than down the soil profile appears to be the main rate-determining step for denitrification in this model. Diffusion of NO_3^- from aerobic to anaerobic sites with subsequent reduction in the later may also occur. In aerobic soil, denitrification and autotrophic nitrification, each with its associated N_2O production may occur simultaneously at spatially distinct microsites [20]. Highest N_2O fluxes are expected under microaerophilic conditions in soil where N_2O reduction to N_2 during denitrification is inhibited by O_2 gas and where nitrifiers are sufficiently limited in O_2 gas supply to also form N_2O [81].

After a heavy rainfall, with the presence of nitrate and suitable carbon sources, significant losses of fixed nitrogen from soil can result from the induction of denitrifiers.

In soils and wastewater, even if well aerated, anaerobic energy – conserving processes can occur inside aggregates and sewage flocculates in the sequence NO_3^-, MnO_2 and Fe_2O_3 respiration followed by SO_4^{2-} and CO_2 reduction [112].

Soil water content is a major factor determining the rate of denitrification [65, 106]. Highest emission are often correlated with very wet soil conditions [5,11, 42,64,93,94,102,104,115,156,176]. Such findings reflect the fact that denitrification is an anaerobic process. Increasing denitrification rate with increasing soil water content seems most marked above about 60% WFPS [water-filled pore space] [7,70,91,110,164].

Several workers observed highest nitrous oxide fluxes from soil during fluctuating moisture conditions compared to either continuously well-aerated or continuos anaerobic conditions [49,151,155].

Denitrification may cease if the soil remains wet for some time, and higher denitrification rates are observed where soils are going through wetting/drying cycles than where soil water content is constantly high [103]. Groffman and Tiedje [62] showed that the rate of denitrification did not depend on water content in a simple manner. They dried intact soil cores and found that denitrification rates decreased markedly when water content declined from flooding to field capacity. With further drying the decline was less rapid. However, when water content was increased from dry conditions, the sharpest increase in rate of denitrification occurred at low water content. Others also found that denitrification rates depend on history of the sample [57,88].

During fluctuating soil moisture conditions, drying and rewetting cycles may enhance the availability of soil organic matter and this will also favour denitrification. Drying causes shrinkage and disruption of soil aggregates and exposes organic matter not previously accessible to microbial attack. In addition, death of part of the microbial biomass during drying releases additional available carbon. As a result, upon rewetting there is a characteristic flush of soil microbial activity [118].

Microbial processes in soils are the most important sources of N_2O [61]. Nitrous oxide is produced during denitrification and nitrification. It is an intermediate of the denitrification and a by-product of nitrification.

The amount of nitrous oxide emitted via denitrification is related to the factors, which influence the enzyme production for the several steps in the denitrification sequence. Low pH, high nitrate concentration, low moisture and low availability of oxidisable organic material all tend to increase the nitrous oxide fraction in the denitrification products [6]. At saturated moisture conditions or under strictly anaerobic conditions (e.g. poorly drained soils and in sediments) N_2-production is favoured as the principal gaseous product [37,103]. With an increase in aeration to an air-filled porosity of about 10%, denitrification and hence the overall gas production (N_2 plus N_2O) declines but the mole fraction of N_2O trends to increase [89].

Many studies showed that the reduction of N_2O to N_2 is more prone to inhibition by O_2 than reduction of NO_3^- to N_2O, thus the N_2O/N_2 ratio decreases with decreasing O_2 concentration. Thus, the presence of O_2 reduces the activity and delays the synthesis of nitrous oxide reductase relative to nitrate reductase and nitrite reductase, so that the N_2O/N_2 ratio increases with increasing O_2 concentration [14,19,46,50,53,95,150,154,165]. The N_2O/N_2 ratio usually decreases with in-

creasing soil water content and tends to be high when the denitrification rate is low [8,104,134,135,143,164,176].

At low soil water content, N_2O emission is low because microbial activity is low and the O_2 supply is ample so that nitrification goes all the way to NO_3^-, and denitrification rates are low. With increasing water content mineralization rate increases and nitrification increasingly produces N_2O. Also denitrification becomes significant with a high N_2O/N_2 ratio as O_2 diffusion becomes impeded. At high soil water content gas diffusion is severely hindered, denitrification proceeds increasingly towards N_2 and N_2O emission declines. Thus, soil water content where both denitrification and nitrification can proceed will generally give the maximum emission of N_2O. The range of soil water content is normally 45 to 75% WFPS [61]. Though Klemedtsson *et al.* [81] and Hansen *et al.* [68] have indicated a higher level. The maximum N_2O emission for denitrifies or nitrifies is normally close to FC (field capacity) [38,81,116,143]. Most authors find a strong and positive correlation between N_2O emission and soil water content when either denitrification [71, 39] or nitrification [39,74,81] is the main N_2O generating process.

The relationship between soil moisture content and N_2O emission rate is also often seen in field studies as an association between corresponding values N_2O emission and water content obtained over a period of time, e.g season or year and over a wide range of water content levels [45,52,116,148]. This relationship is illustrated by Mosier *et al.* [100] who found N_2O emission from a native shortgrass steppe during a summer sampling period to be positively correlated with soil water content in the upper 5 cm. Emission were some 10-fold higher at 18 vol-% (36% WFPS) than at 10 vol-% (20% WFPS). Conrad *et al.* [35] made similar observations at water contents of 10 to 20 weight-%. Maximal N_2O fluxes from soils are reported shortly after irrigation or rainfall [31,35,69,68].

Davidson *et al.* [39] studied N_2O emission in a dry tropical forest. Emissions were higher in the wet season than in the dry season, but addition of water to dry soil caused rapid formation of NH_4^+ from mineralization and large pulses of N_2O emission.

Waterlogged conditions are mostly undesirable in agriculture, except for paddy rice. These fields usually emit only small amounts of N_2O while flooded [26].

Mosier and Hutchinsen [99] reported that an irrigated field of maize lost 59% of the seasons loss of N_2O during the week following the first irrigation, when restricted O_2 diffusion favoured denitrification.

The high rates of denitrification that occur when soils pass through wetting/drying cycles also show up as high N_2O emissions [38,118]. When a soil is wetted sufficiently by rain or irrigation water to cause anoxic conditions and to initiate denitrification, N_2O will be produced more rapidly than it is reduced. If the soil dries within 24 to 72 h, insufficient time will have elapsed for the development of nitrous oxide reductase, thereby preventing N_2O reduction to N_2 [31].

Firestone and Tiedje [49] showed that after the onset of anaerobiosis essentially three time periods could be distinguished based upon the response of the native microbial population. In the period from 16 to 33 h following anaerobiosis, 40 to 90% of the gaseous denitrification product is evolved as N_2O. Initially NO_3^--reductase production is stimulated and enzyme is produced more rapidly than N_2O-reductase. Thus, N_2O accumulates and can be released into the atmosphere. The moisture conditions which seem to favour N_2O production are, therefore, alternating wetting and drying cycles during which both autotrophic nitrification and denitrification are active but where there is not enough time for substantial levels of N_2O-reducatse to form. The large pulses of N_2O, which typically follow rainfall or irrigation may exceed, background levels by up to 3 orders of magnitude especially after long periods of dryness [35,145].

N_2O formation, accumulation, and subsequent emission from the soil depend both on its production and its reduction to N_2. The production of N_2O depends on the process rate of denitrification and nitrification and on the relative N_2O production, which is the percentage of the reduction (denitrification: $N_2O*100/[N_2O+N_2]$) or the oxidised (nitrification: $N_2O*100/[NO_2^-+N_2O]$) substrate being transformed into N_2O [9]. Firestone and Davidson [51] suggest that the process rate is the most important factor determining the N_2O production.

Changes in soil **redox potential** are related to changes in oxygen levels. If organic matter is added to soil, oxygen is depleted and the potential drops - at time quite precipitously. This is a microbial reaction, because inhibitors of microbial activity prevent both oxygen depletion and the development of reducing conditions. The occurrence of a variety of microbial processes is related to specific redox potential. Some of these are as follows:

Aerobic carbon oxidation – 0.2 V

Denitrification – 0.15 to 0.2 V

Methanogenesis – 0.2 to -0.1 V

Sulphur reduction – 0.2 to -0.1 V.

Masscheleyn *et al.* [95] reported on N_2O emission from rice paddy soils at various redox potentials, ranging from +500 to -250 mV. Two maximums for N_2O

evolution were found, at +400 mV when nitrification was the source, and at 0 mV when N_2O was produced by denitrification.

Kralova *et al.* [84] got similar results in a study on denitrification in a soil suspension amended with NO_3^-. The maximum amount of N_2O was evolved at a redox value of 0 mV, while denitrification rates and N_2 emission continued to increase with lower redox levels.

Smith C.J. and Patrick [155] showed that alternate anaerobic-aerobic cycling increased N_2O evolution by a factor of 10 to 20 relative to constant aerobic conditions for soil suspensions amended with NH_4^+. No N_2O evolved during constant anaerobic conditions. The redox potential fluctuated during cycling, but was always lower than the redox potential for constant aerobic, and much higher therefor-constant anaerobic conditions.

Włodarczyk [177] studied nitrous oxide emission from Eutric Cambisol observed the highest N_2O evolution at 250 mV.

Total denitrification fluxes (N_2O plus N_2) are directly proportional to soil NO_3^- concentrations when the other important component, a readily metabolizable organic substrate, is also present and non rate – limiting. When a lack of metabolizable organic matter limits potential denitrification, N_2 plus N_2O fluxes do not increase with increasing NO_3^- concentration [139].

Freney *et al.* [56] found that emissions increased by 1 to 2 orders of magnitude following heavy irrigation of a field cropped with sunflower and fertilised with urea. Most of the urea had been converted to NO_3^- at the time of the emission measurements.

High emissions associated with rainfall/irrigation are favoured when fertiliser is applied simultaneously with, or soon before, the event [74,99,175].

Complete reduction of $2NO_3^-$ to N_2 generates $2OH^-$, which may cause environmental pH to rise [157].

It is well established that an increase in soil or sediment NO_3^- concentration leads to an increase in the N_2O:N_2 ratio in the product gases. This is attributed to the inhibition of N_2O reductase by NO_3^- [15,49,163,178] and, as noted earlier, this effect is further enhanced at low pH.

Nitrification and denitrification are the main microbial processes producing N_2O and NO. Other biochemical oxidation or reduction reactions like N_2 – fixation and dissimilatory nitrate reduction may yield some traces of N_2O and NO as well. Abiotic production may occur through chemodenitrification [169].

Sinks N_2O

Soil can remove atmospheric N_2O under conditions favourable for N_2O reduction [88,138,147]. This is probably only a minor sink on the global scale, but elimination of N_2O in the stratosphere is so slow that even a small soil sink can contribute significantly to reduction of the atmospheric residence time of N_2O [33].

Silvola *et al.* [147] also observed occasional uptake of N_2O in field studies on Finnish peat soils. Soil absorption of N_2O is illustrated by result of Ryden [138] for fertilised grassland. He observed that the unfertilised control invariably removed atmospheric N_2O when the water content exceeded 20 weight - %. However wet field conditions suitable for extensive N_2O reduction, are also the conditions that will restrict N_2O movement from the air into soil. This suggests that there is little removal of atmospheric N_2O by reduction in the soil to N_2, but the topic cannot be regarded as settled.

Dowdell *et al.* [43] reported that the N_2O content of rainwater was about 0.3 $\mu g\ N_2O\text{-}N\ l^{-1}$. A rainfall of 1000-mm $year^{-1}$ will therefore return only about 3 $g\ N_2O\text{-}N\ ha^{-1}\ year^{-1}$ to the soil.

Lensi and Chalamet [87] reported that plants could take up and remove N_2O from air. Grundmann *et al.* [66] reported that $^{15}N_2O$ are taken up by maize leaves and metabolised as a source of N.

Włodarczyk [177] studied nitrous oxide emission from Eutric Cambisol found that the range of reduction of N_2O under investigated conditions was from 10 to 100% of emitted gas depending on kind of soil and time incubation. The boundary value of redox potential for emission of nitrous oxide was 250 mV and for sink of N_2O was about 200 mV (Fig. 2).

Non-biological processes

Chemodenitrification is a non-biological process. NO_2^- can react with organic compounds (e.g., amines) to form N_2, NO_2^- and N_2O [22]. Chemodenitrification is a term usually employed to describe the chemical decomposition (dismutation) of nitrous acid, HNO_2, in soil but is also used more generally to denote chemical reactions involving NO_2^-. As a N_2O producing process it gains importance whenever NO_2^- accumulates in soil, e.g., in soils with an alkaline pH where the nitrification of NO_2^- to NO_3^- is inhibited and also under acidic pH conditions when HNO_2 can form more readily [101].

Under acidic conditions (pH <4.9) and a redox potential of 0 to 200 mV HNO_2 dismutates chemically according to the following equations [20,139,156]:

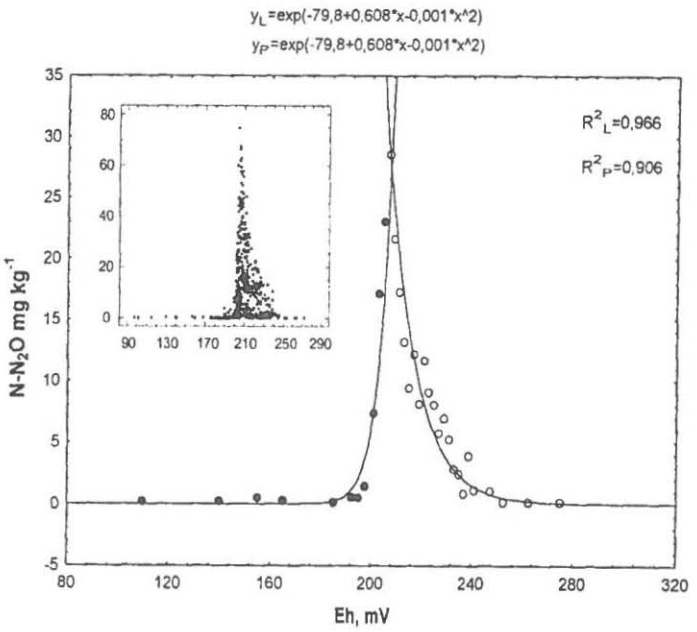


Fig. 2. Equilibrium content of N_2O in the phase of emission (P right side of figure) and absorption (L left side of figure) in the headspace of gas as a function of Eh values (y = mean values for the determined ranges of x value). Insertion shows single data from all soils and entire time of incubation. From Włodarczyk [177]

$$3HNO_2 \rightarrow 2NO + HNO_3 \rightarrow H_2O \quad (12)$$

or

$$2HNO_3 \rightarrow NO + NO_2 + H_2O \quad (13)$$

The NO and NO_2^- produced during these processes can be further reduced chemically by organic constituents to N_2 and N_2O [107,108]. It has been found that the prevailing gaseous products under these conditions are N_2 and NO_2^- as well as small amounts of N_2O [108] and NO [18]. With increasing pH, the HNO_2 level and N_2O production through HNO_3 dismutation declines. Under neutral or alkaline pH conditions biological processes are mainly responsible for N_2O and N_2 production [18,169]. However, in NH_4^+ and NO_2^- amended soils under alkaline conditions small amounts of N_2O may be produced chemically because NO_2^- oxidation may be inhibited [24,32,169,153].

Nitrous oxide may also be produced by the reaction between nitrous acid and oximes formed during organic matter decomposition [122]:

$$R_2C = NOH + HNO_2 \rightarrow R_2C = O + N_2O + H_2O \quad (14)$$

The next way is the chemical reaction between HNO_2 and phenconstituents in soil and with compounds containing free amino groups (the **"Van Slyke"** reaction) may be responsible for some N_2O emission from soils [98].

Nitrogen fixation

Diverse groups of prokaryotes contain the enzyme nitrogenase responsible for the fixation of N_2. These diazotrophs include organotrophs, phototrophic sulphur bacteria, and cyanobacteria (Blue-green algae). Substrates range from poliphenols to H_2 and CH_4. The aerobic, free-living, N_2-fixing bacteria that utilise organic, often recalcitrant substrates as a source of energy include *Azotobacter*, found in neutral and alkaline soils. Members of the same family, *Beijerinckia* and *Derxia* have a broader pH range and are more often found in acids soils, especially in the tropics [119].

Azotobacter, Beijerinckia, and *Rhizobium* require aerobic conditions for the production of the extensive energy required for N_2 fixation. However, in these organisms as in all other diazotrophs, the activity of nitrogenase is inhibited by O_2. Special mechanisms for protection of nitrogenase include the association of the N2-fixing complex with membranes within the cell, slime production, and clump formation. Another feature of aerobic N2-fixing bacteria is the high level of respiration within the cells. This in *Azotobacter* helps protect the enzyme from O_2 by maintaining low O_2 concentrations [119].

Facultative microaerophilic organisms such as *Klesiella, Azospirillum,* and *Bacillus* produce energy in the form of ATP by oxidative pathways in an environment where nitrogenase does not need to be as well protected from O_2. Anaerobic diazotrophs such as *Clostridium* and the sulphate reducers, *Desulfovibrio* and *Desulfotomaculum,* also use organic compounds as electron donors. The fermentative pathways of these organisms lead to the build-up of organic intermediates and results in low amounts of energy being available for N_2 fixation. However, certain environmental conditions with high substrate availability combined with anaerobic conditions, such as waterlogging, result in extensive N_2 fixation. The amount of N_2 fixed by free – living diazotrophs such as *Azotobacter*, and *Pseudomonas* is generally only a few kilograms per hectare [119].

Nitrogen fixation in the legumes is attributed to a group of bacteria consisting of a number of genera collectively known as rhizobia. Nitrogen fixation in the

legu- mes is within a bacteriod in the rhizobia. Oxygen is controlled by haemoglobin and is low in legume nodules [119].

Free-living bacteria are usually less effective than the symbiotic ones, but in many ecosystems they contribute more nitrogen than that added with wet and dry deposition in unpolluted areas. Nitrogen fertilisation depresses fixation of both symbiotic and free – living bacteria, so it might be assumed that emission of NO_x and NH_4, if intensive might also affect fixation. Other factors of importance for the rate of fixation are soil pH (most nitrogen fixers prefer relatively high pH, even if some may by active down to pH = 4.5 or lower), and the supply of nutrients other than nitrogen [162].

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PRZEMIANY AZOTU W GLEBIE I ICH UWARUNKOWANIA

T. Włodarczyk

Instytut Agrofizyki im. B. Dobrzańskiego PAN, Doświadczalna 4, 20-290 Lublin 27

S t r e s z c z e n i e. Przedstawiono przemiany azotu glebowego ze szczególnym podkreśleniem warunków w jakich procesy te zachodzą. Omówiono następujące procesy biologicznej przemiany azotu: amonifikację, nitryfikację, redukcję asymilacyjną azotanów, redukcję dysymilacyjną azotanów, wiązanie azotu cząsteczkowego oraz warunki i drogi ich wzajemnych interakcji w procesach transformacji N. W tym mini-przeglądzie skoncentrowano się głównie na procesach produkcji i redukcji N_2O. Dodatkowo praca zawiera opis reakcji chemicznych (przemian N bez udziału drobnoustrojów), w efekcie których powstaje podtlenek azotu.

S ł o w a k l u c z o w e: amonifikacja, nitryfikacja , asymilacyjna i dysymilacyjna redukcja N, wiązanie N, redukcja N_2O, potencjał oksydoredukcyjny.