# Interactions Among Bacteria Metabolizing Inorganic Nitrogen Compounds

**J.G. Kuenen and L. A. Robertson**

Kluyver Laboratory of Biotechnology, Delft University of Technology, Delft -2628 BC, The Netherlands

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## Summary

It is essential that nitrogen compounds are kept where they are wanted, on cultivated fields, and not lost into the water table or the atmosphere. Potentially polluting nitrogenous output from domestic and industrial wastes must also be controlled, preferably by conversion to $N_2$. Among the many factors playing a role in the control of these processes, bacterial selection is crucial as its outcome may determine whether the products will be harmless ($N_2$) or harmful (e.g. $N_2O$, $NO_2^-$). Current knowledge of defined mixed cultures is limited, and modelling is thus difficult. This paper will review such experiments in the light of the N-cycle. N-assimilation (very general) and $N_2$ fixation (highly specialized) will not be discussed.

## 1. Introduction

As can be seen from the simplified nitrogen cycle shown in Figure 1, the processes that most obviously play a role in the conversion of inorganic and organic nitrogen to nitrogen gas are nitrification, denitrification and dissimilatory nitrate reduction. The bacteria that carry out these reactions must interact since they either produce or compete for their various electron donors or acceptors. Of course, it should be remembered that considering a single interaction represents a high degree of simplification, when compared to natural communities. Thus, in a complex mixture, bacteria may be competing for a substrate or electron acceptor, but inter-dependent because of the need for the removal of a toxic intermediate. An example of this type of relationship might be that between two organisms competing for a substrate under denitrifying conditions. If one organism can only reduce nitrate to nitrite (eventually becoming inhibited by the accumulating nitrite), and the other can denitrify, but only from nitrite because its lacks nitrate reductases, their relationship would be both mutualistic and competitive. A similar partnership where one bacterium produces $N_2O$ (a phenomenon known for nitrifiers and dissimilatory nitrate reducers as well as denitrifiers (1)), and another then uses the $N_2O$ as an electron acceptor might be considered symbiotic, rather than mutualistic, because $N_2O$ would normally diffuse into the atmosphere rather than accumulate to toxic levels. However, among the many

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potential forms of interaction, mutualism and competition appear to be the most prevalent in this type of study.

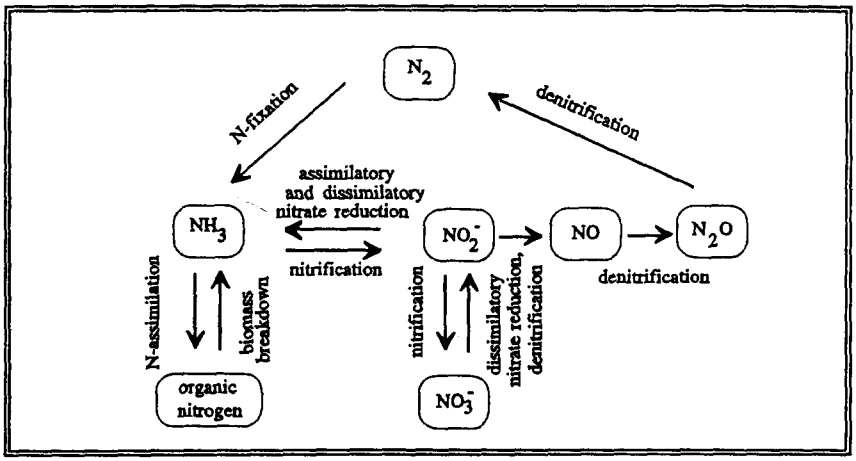


**Figure 1.** A simplified view of the nitrogen cycle.

## 2. Mutualism

Mutualism can be defined as a partnership in which all participants benefit in some way. The conventional nitrogen removal system using sequential nitrification (with autotrophic ammonia and nitrite oxidizers) and denitrification (with heterotrophic denitrifiers) has been challenged by a number of research groups working with mutualistic communities. For example, growth of the autotrophic nitrite oxidizer *Nitrobacter agilis* can be supported by nitrite from a heterotrophically nitrifying *Alcaligenes* species (2). The *Alcaligenes* sp. benefits because potentially toxic nitrite is removed. $NH_3$ conversion to nitrate and $N_2O$ without any of the traditional physiological types has been shown (3) using a consortium containing $C_1$ utilizers and *Pseudomonas* species (fig. 2). $NH_3$ was oxidized to hydroxylamine and nitrite or nitrate by the methanotroph (via its methane monooxygenase) and *Pseudomonas* Is-3. The remaining hydroxylamine was oxidized to nitrite, nitrate and $N_2O$ by *Pseudomonas* Is-2. *Methylobacillus* Is-1 consumed $CH_3OH$ produced by the $CH_4$ oxidizer, and produced the organic excretion products on which the pseudomonads grew.

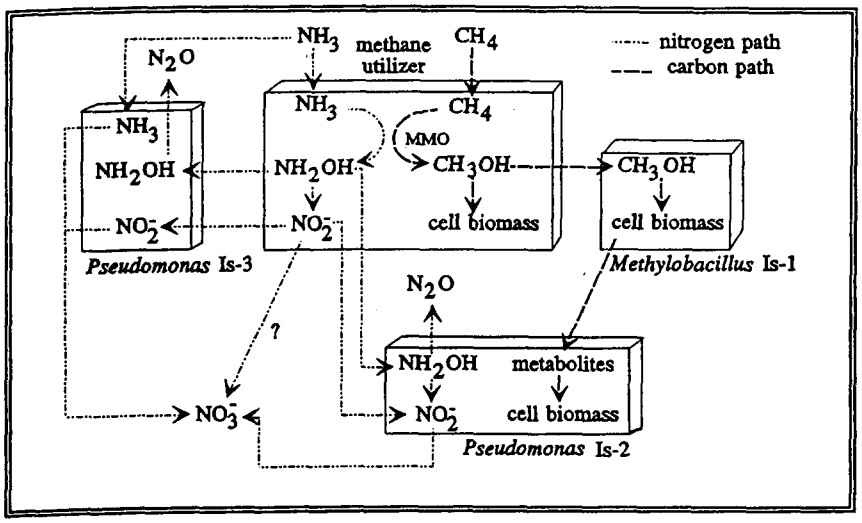


**Figure 2.** Interactions between 4 species growing on $CH_4$ and $NH_3$ (3).

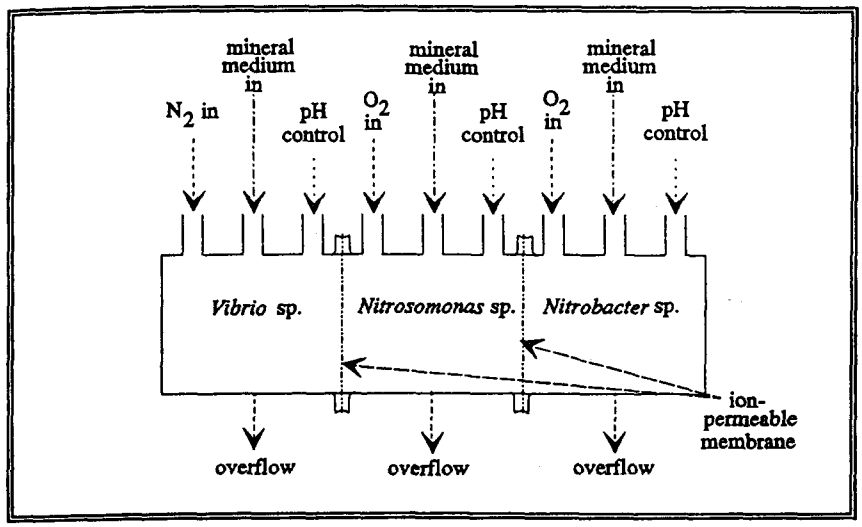


**Figure 3.** Layout of the 3-stage, bidirectional diffusion chemostat used to demonstrate a mini-N cycle (adapted from 4).

Mutualism, resulting in an almost closed nitrogen cycle, has also been demonstrated using cultures of a dissimilatory nitrate reducing *Vibrio* species, an autotrophic ammonia-oxidizing *Nitrosomonas* species, and an autotrophic nitrite oxidizing *Nitrobacter* species (4). The three species were grown axenically in

continuous cultures linked by membranes that allowed free exchange of medium components, but kept the bacteria separate (Figure 3). When anaerobically-grown under nitrogen limitation, the *Vibrio* sp. reduced the nitrate supplied in its medium to ammonia in sufficient quantities to support the aerobic growth of the *Nitrosomonas* sp. The *Nitrosomonas*, in turn, aerobically oxidized the ammonia to nitrite, thereby supporting the *Nitrobacter* sp. Ammonia oxidation by the *Nitrosomonas* sp. proved to be the rate-limiting step under these conditions. However, the picture changed if excess nitrate was supplied to the *Vibrio* sp. culture, when this culture produced more nitrite than ammonia. This stimulated *Nitrobacter* sp. growth, and the *Nitrosomonas* sp. population declined as the ammonia supply decreased.

### 3. Competition

Clear-cut competition experiments are only possible if the experimental system is well-defined, and all possible interactions have been considered. For this reason, continuous cultures where factors including pH, dissolved $O_2$ and substrate supply can be controlled are popular, and are often used (see, for example, (5)). The importance of considering all variables is emphasized by experiments designed to show the influence of dissolved $O_2$ and nitrate on competition for acetate between a "normal" denitrifier, *Paracoccus denitrificans*, and an aerobic, constitutive denitrifier, *Thiosphaera pantotropha* (6). Although theoretical curves derived using the Monod equation (7) indicated that at $O_2$ concentrations approaching air saturation, *Pa. denitrificans* would dominate co-cultures of the two species at high growth rates if ammonia was the sole nitrogen compound present, experiments designed to test this failed because *Tsa. pantotropha* formed biofilms on the culture vessel walls, and continued to dominate the culture. Subsequent experiments with axenic cultures suggested that *Pa. denitrificans* should have an advantage under continuously anoxic conditions, but that *Tsa. pantotropha* should adapt better if aerobic cultures were suddenly made anoxic, because its denitrifying enzymes are constitutive. *Pa. denitrificans* produced nitrite in quantities sufficient to make recovery after the return of aerobiosis difficult (6). Current experiments are being hampered by the loss of the actively aerobic denitrifying wild type, although the strains now available retain their constitutive denitrifying enzymes. Using dual $O_2/N_2O$ microelectrodes, it has not been possible to demonstrate *in situ* aerobic denitrifying activity, although other types of experiments with samples taken from soil, sediment and seawater suggest its existence (8,9,10,11).

In soil and sediments, it has been observed that, among dissimilatory nitrate reducers, ammonifiers tend to be dominant when the level of organic carbon is high, and denitrifiers are more numerous when nitrate levels are high (12, 13). As might be expected, however, there appear to be exceptions to this trend. For example, chemostat-grown co-cultures of *Klebsiella* K312 (a dissimilatory nitrate reducer) and *Pseudomonas* P388 (a denitrifier), *Klebsiella* K312 dominated when glycerol was limiting, but *Pseudomonas* P388 had the advantage under nitrate limitation (14). However, subsequent experiments with

*Pseudomonas stutzeri* and *Citrobacter freundii* (15) have revealed that the C:N ratio is not the only factor controlling the outcome of competition between denitrifiers and dissimilatory nitrate reducers. If nitrate was the only limiting factor, *C. freundii* dominated. Double limitation (lactate and nitrate) gave a mixed culture at D=0.05 $h^{-1}$, but *C. freundii* washed out at D=0.1 $h^{-1}$. Supplementing the medium with glucose gave almost compiete domination by *C. freundii* at dilution rates at and below 0.12 $h^{-1}$, but at D=0.28 $h^{-1}$, 25% of the community was *Ps. stutzeri*. Different apparent $K_s$ values for nitrate with *Ps. stutzeri* were found on glucose and lactate (10 and 3 μM, respectively).

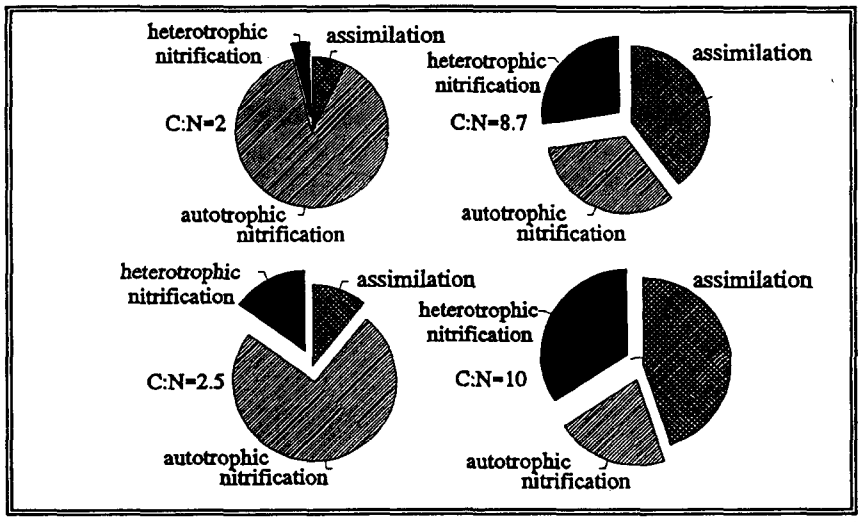


**Figure 4.** The fate of $NH_3$ supplied to mixed cultures of *N. europaea* and *Tsa. Pantotropha* at different C:N ratios in chemostat cultures (17).

The significance of heterotrophic nitrifiers in general, and in relation to autotrophic nitrifiers in particular, has long been a matter of debate. Many heterotrophic nitrifiers also denitrify (16), thus making it difficult to estimate nitrifying activity from product accumulation. N-balances for co-cultures of *Tsa. pantotropha* and *Nitrosomonas europaea* at different C:N ratios (Figure 4) revealed that heterotrophic nitrification became significant at a C:N ratio around 8.7 because of superior numbers of heterotrophs (17). Falling dissolved $O_2$ concentrations did not affect the composition of co-cultures until approximately 20 μM $O_2$ was reached, when *N.europaea* washed out.

## 4. Conclusions

If ammonia oxidation to nitrite is detected, this may be due to autotrophic nitrifying bacteria, heterotrophic activity, or both. Similarly, generation of $N_2O$ is not necessarily caused by denitrifiers.

The outcome of competition between autotrophic and heterotrophic nitrifiers, and denitrifiers and dissimilatory nitrate reducers, depends strongly on the C:N ratio. However, the influence of other parameters (e.g.type of C source, pH, $O_2$) requires further study.

There is no real evidence for aerobic denitrification outside the laboratory. Whether the significance of these bacteria is due to their constitutive denitrifying enzymes or some other factor remains to be seen. However, the apparent instability of this property in laboratory cultures of *Tsa. pantotropha* (11) will hinder further investigation.

Defined, mixed cultures of known organisms are required to reveal the principles of bacterial selection. However, due to the complexity of the situation, modelling is necessary in order to reveal whether the processes and interactions measured in the laboratory are valid in the wild.

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