

## Nitrification and ammonia oxidising microbes in soil

Jim Prosser

Soil is home to an enormous number and diversity of microbes that are essential for creation and maintenance of soil fertility and consequent crop growth. Our inability to see microbes reduces appreciation of their significance but living cells within one hectare of soil have similar mass to ~100 sheep. One crucially important group of soil microbes are the ammonia oxidisers, which gain energy by oxidising ammonia to nitrite, which is then oxidised to nitrate in the process of nitrification.

Ammonia oxidisers in agricultural soils have considerable economic and environmental impact globally for two reasons (**Figure 1**):

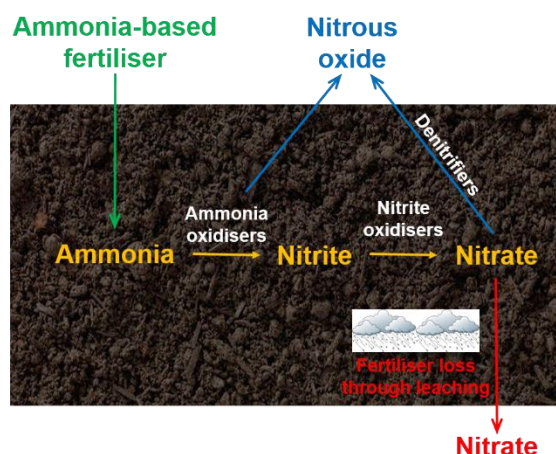
1. Nitrate, unlike ammonia, is rapidly leached from soil and nitrification leads to loss of around 50% of added fertiliser. This represents a significant economic loss, as manufacture of ammonia fertiliser consumes 3 – 5% of global energy and costs up to \$100 billion per year.
2. Ammonia oxidisers are also the major direct and indirect causes of emissions of the greenhouse gas nitrous oxide from terrestrial environments.

Although study of lab strains of ammonia oxidisers gave information on their metabolism and potential soil activity, we always worried that these 'lab rats' or 'domesticated' strains were unrepresentative of natural communities.

### Molecular ecology of ammonia oxidisers

We were right to be worried. In the early 1990s, we began using molecular techniques to characterise soil ammonia oxidiser communities. This was achieved by sequencing ammonia oxidiser genes in DNA extracted directly from soil, eliminating the need for lab culture. Analysis of

these sequences showed that bacterial ammonia oxidisers were orders of magnitude more diverse than lab strains, uncovered completely new groups and enabled experimental studies of the influence of soil characteristics and of different fertiliser strategies on community composition. Molecular techniques continue to improve, through advances in DNA sequencing technology, and we have now also developed methods to assess activity of different groups in soil without 'domesticating' them.



**Figure 1.** Soil Nitrification. Ammonia, supplied as ammonia-based fertiliser, is oxidised to nitrite by ammonia oxidising bacteria or archaea. Nitrite is then oxidised to nitrate by nitrite oxidising bacteria. Ammonia is retained in the soil, through adsorption of positively charged ammonium ions to soil particles, and can be taken up by plants. In contrast, nitrate is negatively charged and is leached from soil following rainfall. This leads to considerable loss of added ammonia-based fertiliser and also to nitrate pollution of groundwater. In addition, nitrous oxide is a side product of ammonia oxidation and nitrous oxide is also produced by denitrifiers, which are microbes that reduce nitrate to nitrous oxide and nitrogen gas. Ammonia oxidation therefore results in direct and indirect emissions of the greenhouse gas nitrous oxide.

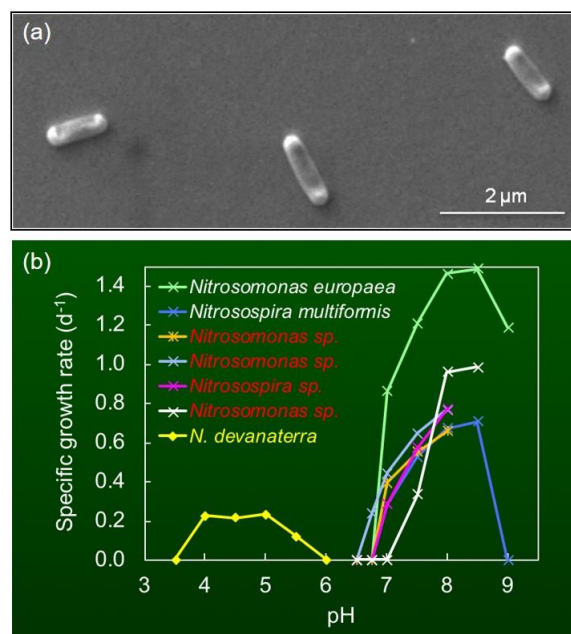
A further revolution was the discovery that archaea could also oxidise ammonia. Bacteria and Archaea represent the two major domains of life and we, and all animals, plants and eukaryotic

microbes (fungi, protozoa and algae) probably evolved from archaea. Traditionally, archaea were thought to exist only in 'extreme' environments, with low pH or oxygen concentration, or high salt or temperature. Molecular techniques showed them to be present in soil, many are ammonia oxidisers and they are frequently more abundant and active than bacterial ammonia oxidisers.

### Why does this matter?

Although bacterial and archaeal ammonia oxidisers share many characteristics, there is evidence for two major differences. Some archaeal ammonia oxidisers are obligate acidophiles, i.e. they only grow at low pH (**Figure 2**). Approximately 30% of soils are acidic and many exhibit high rates of ammonia oxidation and fertiliser loss. However, bacterial ammonia oxidisers do not grow below pH 7. A combination of molecular techniques, laboratory soil systems and traditional isolation techniques led to discovery of *Nitrosotalea devanattera*, an obligate archaeal acidophile, and demonstration of its growth in acid soils. This currently provides the best of explanation for ammonia oxidation in, and fertiliser loss from these important soils.

Bacterial, but not archaeal ammonia oxidisers can reduce nitrite to nitrous oxide. As a consequence, emissions of nitrous oxide during ammonia oxidation are much greater in soil dominated by bacterial rather than archaeal ammonia oxidisers. There is also evidence that different fertiliser strategies favour archaeal over bacterial ammonia oxidisers. It should therefore be possible for farmers to reduce fertiliser losses and nitrous oxide emissions by modifying fertiliser strategies. This therefore provides an important example of the ways in which greater understanding of the activity and diversity of soil microbes can impact on the economic and environmental costs of agriculture.



**Figure 2.** (a) A scanning microscope image of *Nitrosotalea devanattera*, an ammonia oxidising archaeon isolated from an acidic agricultural soil in which it is active. (b) The influence of pH on growth of laboratory cultures of several bacterial ammonia oxidisers, including some isolated from acidic soils, and *Nitrosotalea devanattera*, demonstrating very different responses of this archaeal ammonia oxidiser to pH, which enables it to dominate ammonia oxidation in acid soils.

### AUTHOR PROFILE

**Jim Prosser** is Professor in Environmental Microbiology, School of Biological Sciences, Aberdeen University. His research focuses on the community ecology of soil microbes, particularly those involved in nitrification, which is central to the global nitrogen cycle. He has demonstrated the influence of pH and ammonia supply on nitrifiers and the consequences for fertiliser loss and nitrous oxide emissions. He is a Fellow of the Royal Society and the Royal Society of Edinburgh, received an OBE in 2012 and is a Director of NCIMB Ltd.