NO laughing matter: the toxic gases of the nitrogen cycle

Nitric oxide (NO) is one of the most versatile and important molecules in living organisms but as **David J. Richardson**, **Andrew J. Thomson** and **Nicholas J. Watmough** describe, it is also a potent cytotoxin. Converting it to nitrous oxide renders it relatively harmless, but the resultant greenhouse gas causes different problems.

As humans we are dependent on oxygen for our respiration. We are obligate aerobes. This is not so for many species of bacteria. Faced with a shortage of oxygen in their environment many bacterial species are able to switch to using nitrate (NO_3^-), rather than oxygen to support respiration. One of these energy yielding processes, known as denitrification, converts water-soluble nitrates to gaseous products, nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2). This denitrification process can take place extensively in agricultural soils where nitrogen-rich fertilizers added to stimulate plant growth can also stimulate bacterial nitrogen cycling (Fig 1).

Destroying a cytotoxin, producing an environmental toxin

NO is one of the most versatile and important molecules in living organisms. In higher animals and plants it is an important signalling molecule, for example it is the effector responsible for stimulating the dilation of blood vessels. However, it is also a potent cytotoxin and specialized cells called macrophages produce NO as part of a generalized response to invasion by pathogenic bacteria. Such bacteria have evolved a number of enzymic systems to defend themselves against this 'gas attack'. Soil bacteria which can denitrify also need to protect themselves from the autotoxic effects of NO produced through their own metabolism. They have an enzyme, nitric oxide reductase (NOR) that has evolved to keep endogenous NO levels low by converting it to the relatively benign N_2O which can be released into the atmosphere. From the perspective of bacterial metabolism, the job of detoxifying cytoxic NO is done when it is converted to N_2O, but from an environmental perspective an envirotoxin, a greenhouse gas, has been produced. When discussing greenhouse gas emissions, the public are acutely aware of the problems posed by carbon dioxide and possibly methane. However, emissions of N_2O, perhaps best known as the dental anaesthetic 'laughing gas' should also cause concern.

N_2O was first discovered by the British chemist Joseph Priestley in 1793 and its effects on the human senses were famously explored by a number of notable scientists and poets of the time, such as Sir Humphrey Davy (President of the Royal Society 1820–1827) (Fig. 2) and Robert Southey (Poet Laureate, 1813) who both wrote about it:

► Fig. 1. The nitrogen cycle. *Background: Thinkstock Images / Jupiter Images*

Yet are my eyes with sparkling lustre fill'd
Yet is my mouth replete with murmuring sound
Yet are my limbs with inward transports fill'd
And clad with new-born mightiness around.

Sir Humphrey Davy

I am sure the air in heaven must be this wonder
working gas of delight

Robert Southey

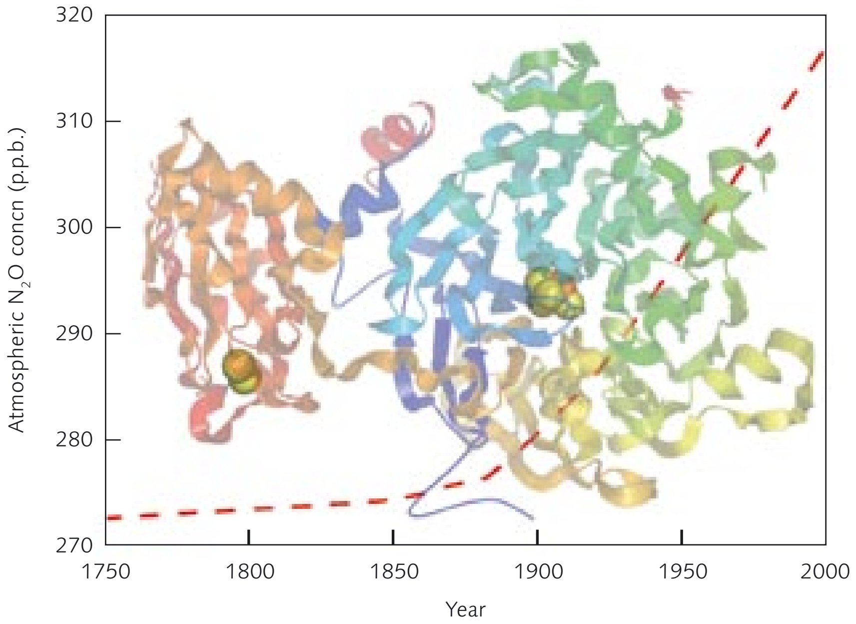
When Joseph Priestley discovered N_2O, its atmospheric levels had been steady for millennia. However, throughout the 20th century (Fig. 3), and continuing into the 21st century, N_2O in the environment has increased by 50 parts per billion. The levels of this atmospheric loading are rising by 0.25 % each year, with most commentators linking the increase to intensive use of fertilizer to improve farmland productivity in the 20th century (Fig. 3). Although its atmospheric levels are only a fraction of that of CO_2, N_2O has a 300-fold greater global warming potential. Thus when expressed in terms of CO_2 equivalents, it represents around 9 % of total global emissions of greenhouse gases. With an atmospheric lifetime of some 150 years, the N_2O produced today will potentially influence the climate experienced by our great-great grandchildren. This is most definitely not a laughing matter and so it is important to predict the impact of N_2O emissions on environmental change and devise strategies to mitigate these releases now.

Understanding the denitrification enzymes that make and break N_2O

The pathways by which denitrifying bacteria produce NO from nitrate are now well understood from a molecular level, with the structures of enzymes that convert nitrate to nitrite (nitrate reductases) and nitrite to nitric oxide (nitrite reductases) being known. These enzymes are metalloproteins that depend on transition metals such as molybdenum, iron and copper for activity.

The molecular structure of the membrane-associated enzyme complex (NOR) that synthesizes N_2O in bacteria is not yet known. It is, however, a close relative of the enzyme in the mitochondria of human cells that allows us to respire oxygen. The chemical reaction takes place at a special dinuclear metal centre in the heart of the enzyme that binds two iron ions. In order to produce one molecule of N_2O, bacterial NOR requires not only two molecules of NO, but also two electrons and two protons (hydrogen ions) whose arrival at the enzyme's active site (which consists of two iron atoms) must be carefully co-ordinated. The enzyme that breaks down N_2O is a copper-containing enzyme (nitrous oxide reductase; N_2OR). It is the major enzyme on the planet responsible for catalysing the two-electron reduction of N_2O to N_2. Without it, the atmospheric levels of N_2O would be much greater than they currently are.

◀ Fig. 2. Sir Humphrey Davey and colleagues at the Royal Institution inhaling gases such as nitrous oxide as part of the science of pneumatics. Coloured etching by J. Gillray, 1802. *Wellcome Library, London*



◀ Fig. 3. Atmospheric N_2O accumulation since 1750. *Data adapted from the 4th Assessment of the Intergovernmental Panel on Climate Change.* Background: Molecular model of nitrous oxide reductase (N_2OR) from *Paracoccus denitrificans* drawn using the co-ordinates 1FWX.

The molecular structure of N_2OR is known. It exists as a functional homodimer (one monomer is shown as the background to Fig. 3), that contains 12 atoms of copper with each subunit having two different types of copper clusters. The dinuclear cluster, known as CuA, serves to pass electrons to the active site which is known as CuZ, that breaks the N–O bond to make N_2 which is not a greenhouse gas. The correct assembly of CuZ, a tetranuclear copper sulfide centre, unique in biology, utilizes a dedicated biosynthetic pathway that is limited by the bioavailability of copper in the environment.

How can we mitigate N_2O release?

The largest source of anthropogenic N_2O emissions is agricultural soils because of the application of nitrogenous fertilizers to soils that began in the early 1900s and continues to increase today. This intensive use of fertilizers provides an interesting paradox for policy makers in that some strategies based on biofuel production designed to mitigate the effects of CO_2 release from fossil fuel actually lead to increases in global warming potential because of the increased requirement for artificial fertilizers. Since the UK signed up to the Kyoto Protocol, many non-biological sources of N_2O emissions have been reduced, but emissions from biological sources are less easy to manage. Efforts to improve the prediction and management of agricultural N_2O emissions will benefit from a better understanding of the factors that influence the net production of N_2O by bacteria. It is imperative that the outcomes of this research are translated into policy and practices through the development of appropriate management techniques for a range of soil systems that both mitigate emissions and improve existing agricultural and waste-treatment practices.

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The authors are all members of a new Nitrous Oxide Focus Group (www.nitrousoxide.org) that brings together scientists from a range of disciplines with various stakeholders with the aim of sharing knowledge on nitrous oxide and exploring strategies for mitigating release. University of East Anglia, Norwich NR4 7TJ, UK (e d.richardson@uea.ac.uk; a.thomson@uea.ac.uk; n.watmough@uea.ac.uk)