Nitrous oxide production in soil: Microbial source partitioning to inform management options for mitigation

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Abstract
Terrestrial systems are major sources of atmospheric N_2O, which accounts for ~6% of the current greenhouse effect. Production of N_2O in soil is predominantly biological, and produced during several microbial processes, which may occur simultaneously within different micro-sites of the same soil. Here we explore the biogeochemical pathways in which these microbes can produce and reduce N_2O, consider the approaches available for determining the predominant N_2O-producing process under certain conditions, highlight any current uncertainties in microbial sources of N_2O to direct future research, and examine how understanding the N_2O source can aid us in managing terrestrial systems to lower emissions of this greenhouse gas. Such source partitioning of N_2O is inherently challenging, but is vital to close the N_2O budget and to better understand controls on the different processes, with a view to developing appropriate management practices for mitigation of N_2O.

Key Words
Nitrous oxide, stable isotopes, rhizosphere, denitrification, ammonia oxidation.

Introduction

Soil microbial N_2O production occurs via nitrification (ammonia oxidation) and nitrate dissimilation (denitrification and nitrate ammonification) pathways (Figure 1). The different processes involved in N_2O production respond differently to environmental parameters or imposed management, and the enzymatic systems of each process are regulated differently. This means that the down-regulation of N_2O production in one process as a result of management practice or change in environmental conditions may well lead to the up-regulation of N_2O production in another. Thus appropriate management for one process, may not be appropriate for another, and may well need to be flexible depending on the system, the prevailing environmental conditions, and the management options available. Unless the controls on enzyme regulation associated with these processes, and associated molar ratios of products are determined, then it will not be possible to target mitigation strategies. Here we give examples of stable isotope approaches that have provided advances in our understanding of the processes producing N_2O in soil, the conditions under which each process predominates, and suggest how this information can be used to develop more targeted management options to lower emissions.

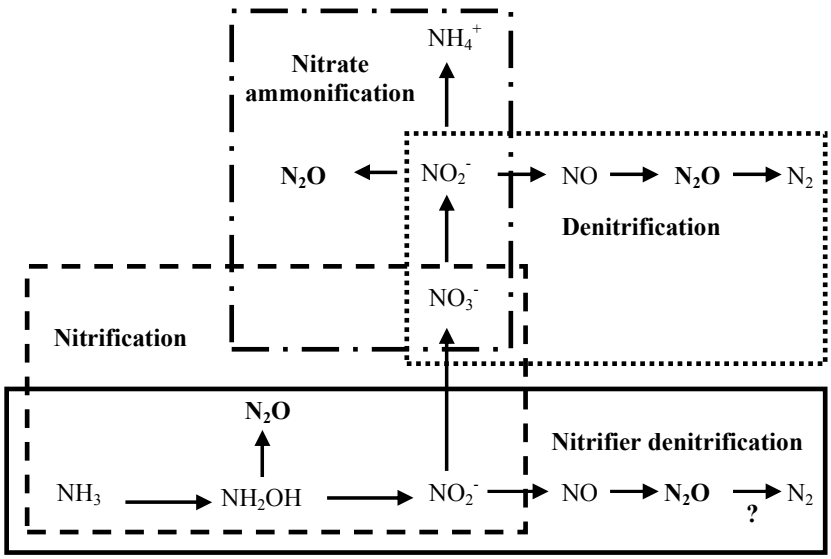

Figure 1. Microbial sources of N_2O in soil. Source Baggs (2008) showing N_2O production from all microbial processes, including nitrate ammonification.

Approaches for distinguishing microbial sources of N_2O

Stable isotope enrichment

These studies have mostly focused on distinguishing between nitrification (ammonia oxidation or nitrifier denitrification) and denitrification following application of ^{15}N-labelled fertiliser. Application of $^{15}N\text{-}NH_4^+$ and/or $^{15}N\text{-}NO_3^-$ to soil and attribution of the $^{15}N\text{-}N_2O$ fluxes to nitrification or denitrification depending on the ^{15}N source applied negates the need for C_2H_2 inhibition, thereby overcoming the problems associated with application of C_2H_2 to soil (Baggs *et al.* 2003; Bateman and Baggs 2005). For example, using this approach Bateman and Baggs (2005) demonstrated denitrification to be the sole source of N_2O emissions in a silt loam soil at 70% water-filled pore space (WFPS), the predominance of nitrification between 35-60% WFPS, but, surprisingly, the dominance of denitrification in the 20% WFPS soil (Figure 2). Unfortunately, this approach is unable to distinguish denitrification from nitrate ammonification or nitrifier denitrification. A combined ^{15}N-, ^{18}O-enrichment approach has been proposed by Wrage *et al.* (2005) involving application of ^{18}O-labelled water to estimate N_2O production during nitrifier denitrification. Using this approach we have demonstrated the proportional contribution of ammonia oxidation but not nitrifier denitrification to vary with availability of different C amendments, and have also shown nitrifier denitrification to be proportionally a more significant N_2O source in soils amended with 5 g N/m^2 than those amended with 40 g N/m^2, where ammonia oxidation was more important (Figure 3). Unfortunately, quantification of nitrate ammonification N_2O by enrichment approach remains elusive.

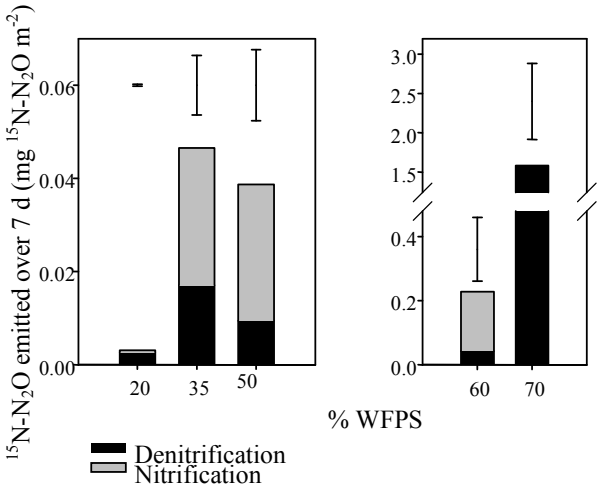

Figure 2. The contribution of nitrification and denitrification to $^{15}N\text{-}N_2O$ emissions after fertiliser application to a silt loam soil at different soil water-filled pore space (WFPS). Source: Baggs (2008).

Natural abundance approaches

These approaches have been applied to the determination of the microbial source of N_2O in a range of ecosystems and controlled environment experiments, and are most advantageous in natural or unfertilised systems (e.g. Webster and Hopkins, 1996; Wrage *et al.* 2004; Perez *et al.* 2006). Natural abundance approaches rely on the biological fractionation against ^{15}N and ^{18}O. Fractionation during nitrification is generally higher than for denitrification, so that N_2O produced during nitrification is more depleted (more negative δ) in ^{15}N and ^{18}O relative to substrates than that produced during denitrification (Wahlen and Yoshinari 1985; Yoshida 1988), but unfortunately the fractionation during nitrate ammonification has yet to be determined.

The isotopomer site preference of ^{15}N in N_2O has recently been proposed as a means to determine the microbial source of N_2O (Bol *et al.* 2003; Well *et al.* 2006). N_2O is a linear molecule, N-N-O, and the $^{14}N/^{15}N$ ratios of the central and outer N atom can naturally vary. Site preference (SP) is termed as the difference in $\delta^{15}N$ between the central and outer N atoms in N_2O, with different microbial processes and functional groups thought to exhibit distinct ^{15}N-SPs (Bol *et al.* 2003; Sutka *et al.* 2003; 2004). This technique is in its infancy and in our presentation we will critically assess the feasibility of this approach for estimating contribution of a wider range of processes than possible with ^{15}N-enrichment or $\delta^{15}N$, ^{18}O fractionation.

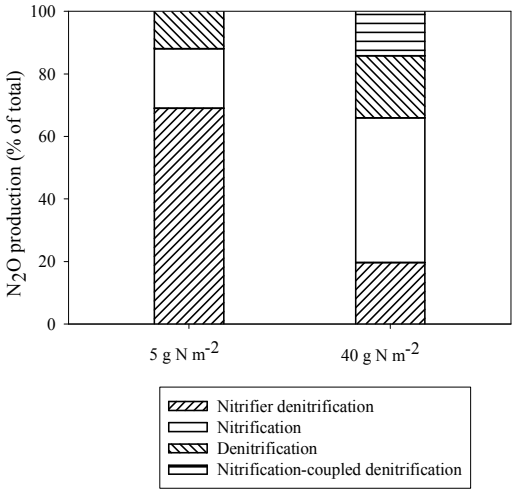

Figure 3. Proportional contributions of different microbial processes to ^{15}N-N_2O production following application of 5 and 40 g N/m^2 as NH_4NO_3 (10 atom % excess ^{15}N; 1 atom % excess ^{18}O in water) to soils.

Source partitioning across different scales

Source partitioning for N_2O has mostly been undertaken at plot or microcosm scales, these scales in part dictated by the technology available, but we currently do not have appropriate tools for verifying microbial sources at larger as well as smaller scales. Herrmann *et al.* (2007) have demonstrated the potential for detecting ^{15}N-enriched bacterial cells within a soil matrix using nano-scale secondary ion mass spectrometry (NanoSIMS) which can image and quantify isotopic enrichment down to a 50nm spatial resolution. In our presentation we suggest how such an approach could be coupled with stable isotope methods for source partitioning, offering the potential to understand controls on regulation at a scale relevant to the microbiology, and to determine the spatial location of different processes within a heterogeneous soil matrix. At the other end of the scale spectrum we need the tools to validate source partitioning at the catchment, landscape or even national scale and to unite the source partitioning with modeling approaches.

Opportunities for mitigation

Data on the environmental regulation of N_2O production during the different microbial processes is scarce, particularly for nitrifier denitrification and nitrate ammonification. Lowering application loads of inorganic N fertilizers has traditionally been considered as the most successful option for lowering net emission of N_2O. However, our source partitioning (Figure 3) adopting a ^{15}N-, ^{18}O-enrichment approach suggests that nitrifier denitrification may be increased under low N conditions and therefore lower N application may not necessarily be the most appropriate strategy in all systems. Other options lie in manipulating inputs into the plant rhizosphere, thereby changing the composition of plant-derived carbon flow or nitrogen uptake demand, or through crop spacing, tillage or integrated inorganic fertiliser, residue and soil organic matter (SOM) management, but their effects on N_2O-genic microbial processes are currently unknown. Richardson *et al.* (2009) argue that it is unlikely that it will ever be possible to develop practices that completely eliminate N_2O emission from agriculture. They propose that a more reliable approach to mitigating N_2O emissions lies in the understanding the regulation of the denitrifier N_2O reductase, which will inform the development of management options to lower net N_2O emissions by enhancing its reduction to N_2, rather than to trying to eliminate denitrification. In our presentation we explore how this may be possible in the rhizosphere environment.

Conclusion

Our ability to determine the microbial source of N_2O in terrestrial systems, and to quantify the contribution from each source, is improving with the advent and development of new techniques outlined here. This offers us exciting opportunities for targeted management options to optimise mitigation potential, but we still have some way to go before this can become a reality. To be able to determine the microbial source of N_2O with any degree of accuracy in natural, unfertilised, or fragile ecosystems we need to refine methodologies, or combine established and new methodologies, moving us away from reliance on application of ^{15}N-labelled substrates, which may artificially favour one process over others. Any mitigation approach should be grounded in predictions of future emissions from different management scenarios under a continuing changing climate. To achieve this, models require further development to encompass all microbial sources of

N_2O. This will be facilitated by advances in techniques to unite source partitioning with upscaling of emissions from the microplot. It is also essential to ensure that any management option imposed to lower emissions has no adverse effect on the diversity and functioning of the microbial community.

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