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Nitrous oxide production by ammonia oxidisers: physiological diversity, niche differentiation and potential mitigation strategies

Running head – N_2O production by ammonia oxidisers

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Abstract

Oxidation of ammonia to nitrite by bacteria and archaea is responsible for global emissions of nitrous oxide directly and indirectly through provision of nitrite and, after further oxidation, nitrate to denitrifiers. Their contributions to increasing N_2O emissions are greatest in terrestrial environments, due to the dramatic and continuing increases in use of ammonia-based fertilisers, which have been driven by requirement for increased food production, but which also provide a source of energy for ammonia oxidisers, leading to an imbalance in the terrestrial nitrogen cycle. Direct N_2O production by ammonia oxidisers results from several metabolic processes, sometimes combined with abiotic reactions. Physiological characteristics, including mechanisms for N_2O production, vary within and between ammonia oxidising archaea (AOA) and bacteria (AOB) and comammox bacteria and N_2O yield of AOB is higher than in the last two groups. There is also strong evidence for niche differentiation between AOA and AOB with respect to environmental conditions in natural and engineered environments. In particular, AOA are favoured by low soil pH and AOA and AOB are respectively favoured by low rates of ammonium supply, equivalent to application of slow-release fertiliser, or high rates of supply, equivalent to addition of high concentrations of inorganic ammonium or urea. These differences between AOA and AOB provide the potential for better fertilisation strategies that could both increase fertiliser use efficiency and reduce N_2O emissions from agricultural soils. This article reviews research on the biochemistry, physiology and ecology of ammonia oxidisers and discusses the consequences for ammonia oxidiser communities subjected to different agricultural practices and the ways in which this knowledge, coupled with improved methods for characterising communities, might lead to improved fertiliser use efficiency and mitigation of N_2O emissions.

Introduction

Nitrous oxide (N_2O) is an important greenhouse gas produced by ammonia oxidisers (AO) and denitrifiers. It ranks third behind carbon dioxide (CO_2) and methane (CH_4) in terms of radiative forcing, with estimated global N_2O emission rates of 11 Tg y^{-1} (range 8.1 - 30.7 Tg y^{-1}) (IPCC, 2013). However, N_2O has a global warming potential that is 265- and 10-fold greater than those of CO_2 and CH_4, respectively, has a long atmospheric lifetime (109 - 125 years, Prather et al., 2015), contributed 17% to radiative forcing in 2005 (IPCC, 2013) and is predicted to be the primary contributor to ozone depletion in the stratosphere in the 21st Century (Ravishankara et al., 2009). Atmospheric N_2O levels are 20% greater than in pre-industrial times (MacFarling-Meure et al., 2006), largely through increases in reactive nitrogen, resulting from increased nitrogen fertilisation as manure, inorganic nitrogen and urea (Smil, 1999). N_2O levels are increasing by 0.73 ± 0.03 ppb y^{-1} (~0.3% y^{-1}) and are projected to increase significantly through this century, due to food and animal feed demands of an increasing global population (Tilman et al., 2001; Alexandratos & Bruinsma, 2012).

Nitrification, the sequential oxidation of ammonia (NH_3) to nitrite (NO_2^-) and nitrate (NO_3^-) in the presence of oxygen, can contribute indirectly to N_2O emissions, providing nitrogen oxides to denitrifying microorganisms for energy conservation under reduced oxygen conditions and anoxia (Kuypers et al., 2018). Denitrifiers are traditionally considered major producers of N_2O, but N_2O is also emitted by organisms that oxidise NH_3 to NO_2^-. This direct contribution to emissions is particularly important given projected increases in N fertiliser applications. Traditionally ammonia oxidation was thought to be performed by bacterial ammonia oxidisers (AOB), but marine ammonia oxidation is now known to be dominated by archaeal ammonia oxidisers (AOA) and both groups are important in other ecosystems. Recently discovered complete ammonia oxidisers (comammox bacteria) oxidise both NH_3 and NO_2^- within the same

cell (Daims et al., 2015; van Kessel et al., 2015), but little is currently known of their ecology. Cellular rates of N_2O production by AOA and AOB differ, leading to different contributions to emissions, and there is evidence for niche differentiation between AOA and AOB with respect to environmental factors and land use strategies. This article reviews evidence for differences in N_2O emissions by AO in culture and in natural ecosystems and assesses potential links between niche differentiation, N_2O emissions and agricultural systems and potential mitigation strategies.

The role of nitrifiers in biogeochemical cycling

In both terrestrial and aquatic ecosystems, the major source of bioavailable nitrogen is fixation of atmospheric dinitrogen (N_2) by free-living or plant-associated N_2-fixing bacteria, photosynthetic cyanobacteria and hydrogenotrophic methanogenic archaea (Boyd & Peters, 2013). Following consumption by higher trophic levels, excretion and death, mineralisation of organic N by microbes releases inorganic ammonium (NH_4^+), which can be assimilated by plants and microorganisms. Ammonium also provides an essential source of energy for obligate aerobic, lithotrophic microorganisms, the ammonia oxidisers. These organisms oxidise NH_3 to NO_2^-, which is then oxidised to NO_3^-, before dissimilatory reduction under anaerobic/microaerobic conditions. Facultative denitrifiers can return N_2 to the atmosphere (Kuypers et al., 2018). These nitrogen cycling processes are supplemented by a network of other biological and physicochemical processes.

In aerobic marine ecosystems, nitrification is highly efficient and results in concentrations of NH_4^+ in the nanomolar range (Horak et al., 2013), but can be limited under reduced oxygen concentration in oxygen minimum zones (OMZ; Ward, 2011) and within decaying particulate organic matter or polluted environments, where oxygen demand can be high. These conditions favour denitrification.

In natural terrestrial ecosystems, in which NH_4^+ is provided via mineralisation and NO_3^- is available, the balance between nitrification and denitrification is again controlled largely by oxygen concentration, which is itself controlled by soil moisture content. At high moisture content, the potential for aerobic nitrification will be low and denitrification will dominate. The situation reverses as soil moisture decreases, and oxygen concentration increases (Bateman & Baggs, 2005). These potential rates are moderated by other factors, e.g. rates of NH_3 supply from mineralisation or supply of organic carbon and nitrate for denitrification (Booth et al., 2005). This leads to variability in relative concentrations of NH_4^+ and NO_3^- and in relative activities of nitrifiers and denitrifiers at both 'bulk' and microscale levels. For example, even in 'aerobic' soils, denitrification may occur in microenvironments, where oxygen diffusion is limited, or in regions of high decomposition, where oxygen demand is high.

Despite spatial heterogeneity, and resultant complexity, soils that have not been subjected to modern agricultural developments have a 'closed' nitrogen cycle in which most NH_4^+ generated from mineralisation is assimilated by plants and soil microbes and <10% is oxidised to NO_3^- (Haynes, 1986). The closed nature of this cycle is facilitated further by the production of biological nitrification inhibitors (BNI) by some plants and NH_3 concentrations in such soils are relatively high and NO_3^- concentrations low (Subbarao et al., 2012).

This situation is reversed in managed, agricultural soils due to application of inorganic, NH_3- and urea-based fertilisers. Over the past 50 years, N fertiliser use increased in Western Europe until stabilisation in the 1990s, but continues to accelerate globally through increased usage elsewhere, with further increases projected to support future increases in world population (FAO, 2017; Lassaletta et al., 2014; Tilman et al., 2001). N entering terrestrial ecosystems produced via the Haber-Bosch process (170 Tg annually; FAO, 2017) exceeds that produced naturally and this energy-intensive process consumes >1% of the global energy demand (IFA-

UNEP, 1998). For AO, this represents an injection of energy and has created an imbalance in the nitrogen cycle, with high NO_3^- production due to high nitrification rates. These effects appear to have been exacerbated by crop breeding programmes developed on the assumption of high levels of inorganic nitrogen fertilisation, decreasing NH_3 competition between nitrifiers and plants and reducing the requirement for BNI, such that these are not produced by modern crop varieties (Subbarao et al., 2017). This has significantly reduced N fertiliser use efficiency to approximately 47% (Lassaletta et al., 2014), through leaching of anionic NO_3^-, in contrast to cationic NH_4^+ that is bound to negatively charged particles. It has also led to greatly elevated N_2O emissions.

Diversity of ammonia oxidising microorganisms

Three main groups of aerobic ammonia oxidising prokaryotes have been described: AOB, AOA and comammox bacteria. AOB belong to beta- and gammaproteobacteria classes, with two (*Nitrosomonas* and *Nitrosospira*) and one (*Nitrosococcus*) genera, respectively (Purkhold et al., 2000). These genera display different environmental distributions indicating distinct physiological characteristics. *Nitrosococcus* is mainly found in marine environments and salt lakes (Campbell et al., 2011), although one strain was recently enriched from an acidic soil (Hayatsu et al., 2017). *Nitrosomonas* has been found in similar environments but also in engineered systems, such as wastewater treatment systems (Mobarry et al., 1996; Schramm et al., 1996). *Nitrosospira* organisms dominate soil AOB but are found in a range of ecosystems and are genetically diverse, in terms of both 16S rRNA and *amoA* gene markers (Aigle et al., 2019; Purkhold et al., 2000, 2003).

AOA belong to the class *Nitrososphaeria* within the phylum Thaumarchaeota and known AOA diversity is represented by four order-level phylogenetic lineages: the *Nitrososphaerales*, *Nitrosopumilales*, *Ca.* Nitrosotaleales and the thermophilic *Ca*. Nitrosocaldales (Brochier-

Armanet et al., 2008; Kerou et al., 2016; Stieglmeier et al., 2014a). AOA are ubiquitous and mesophilic lineages and are genetically diverse, based on *amoA* and 16S rRNA gene phylogenies, with higher diversity described for terrestrial than marine lineages (Alves et al., 2018; Gubry-Rangin et al., 2011; Pester et al., 2011). Thermophilic AOA diversity is lower, possibly reflecting sampling bias (Alves et al., 2018; Gubry-Rangin et al., 2018).

All comammox bacteria belong to the genus *Nitrospira* of the class Nitrospira, which also include canonical nitrite oxidizing bacteria (Daims et al., 2016), and have been detected in a range of natural and engineered environments (Fowler et al., 2018; Palomo et al., 2018; Pjevac et al., 2017; Wang et al., 2019; Zheng et al., 2019), with the possible exception of oceanic environments. Available *amoA* gene sequences suggest two phylogenetic groups (clades A and B) of similar diversity with potential environmental specialisations. Currently cultivated representatives belong to clade A (Daims et al. 2015; van Kessel et al. 2015) and while both clades A and B are found in soil, conditions under which comammox bacteria are active in soil have only been demonstrated for clade B (Shi et al., 2018; Wang et al., 2019).

Mechanisms of ammonia oxidation

Ammonia oxidation to hydroxylamine

The first step in ammonia oxidation in all currently characterised aerobic, autotrophic AO is oxidation of NH_3 to hydroxylamine by NH_3 monooxygenase (AMO), a copper-based, broad-range, membrane-bound oxygenase. Although studied in detail in relatively few organisms (Arp and Stein, 2003; Kozlowski et al., 2016b), AMO has similar characteristics in AOA and AOB. It contains three major structural subunits (Tolar et al., 2017) and although the active enzyme complex has not been identified, comparison with the particulate methane monooxygenase suggests the β-subunit as the active site (Tolar et al., 2017). The structural gene for the α-subunit, *amoA*, has become the target gene for detection and distinction of AOA

and AOB in natural environments (e.g. Francis et al., 2003; Rotthauwe et al., 1997; Tourna et al., 2008) and, with 16S rRNA genes, for phylogenetic analysis (Alves et al., 2018; Gubry-Rangin et al. 2011; Pester et al., 2011; Purkhold et al., 2000; Stephen et al., 1996). Comparative genomic analysis of comammox *Nitrospira* indicates that their ammonia oxidation genomic repertoire is more similar to AOB than AOA, with homologues of *amoA* genes found in all autotrophic AO and of AOB-like hydroxylamine dehydrogenase and c-type cytochromes, which are responsible for transferring electrons to the quinone pool (Daims et al., 2015).

A potential difference between AO is substrate affinity. A report of high NH_3 affinity in *Nitrosopumilus maritimus* provided a convincing explanation for the dominance of AOA in marine environments, where NH_3 is present at nanomolar concentrations, and led to the suggestion that all AOA had higher affinity than AOB (Martens-Habbena et al., 2009). The generality of this finding was challenged by two studies (Hink et al., 2017a; Kits et al., 2017), which questioned the high affinity in *N. maritimus* and the clear distinction between AOA and AOB. The comammox strain, *Nitrospira inopinata*, also has high NH_3 affinity, leading to the view that comammox bacteria are oligotrophs with a competitive advantage in low NH_3 environments (Kits et al., 2017).

These studies raise a number of general points. Caution is required in generalising from results obtained from a small number (often one) laboratory cultures whose properties will differ from those of the ‘natural’ strain, through uncharacterised genetic and physiological changes during selective isolation. K_m values for whole cells, rather than individual enzymes, do not identify the processes (e.g. ammonia oxidation *vs.* ammonia uptake) that are limiting activity and affinity may be determined by non-enzymatic ‘characteristics’, e.g. cell surface area:volume ratio. In addition, affinity constants for activity (K_m) will differ from those for growth (K_s), which are more relevant for outcomes of competition (see Prosser, 2012). The relevance and

significance of substrate affinity for organisms in spatially complex and heterogeneous environments such as biofilms and soil, in which NH_4^+ exchanges with surfaces on which cells are attached, is also unclear.

Hydroxylamine oxidation

The conversion of hydroxylamine to NO_2^- is much less understood and leads to many of the difficulties in assessing contributions of different processes to N_2O production in natural environments. Until recently, it was commonly accepted that hydroxylamine is oxidised to NO_2^- by hydroxylamine dehydrogenase (previously referred to as hydroxylamine oxidoreductase) in AOB (Arp & Stein, 2003). This reaction generates four electrons, two of which fuel ammonia oxidation, while two enter the electron transport chain to generate ATP and reducing equivalents. This mechanism failed to explain fully a number of experimental observations and is now challenged by enzymatic studies that suggest that the direct product of the hydroxylamine dehydrogenase reaction is not NO_2^- but nitric oxide (NO) (Caranto & Lancaster, 2017). This reaction generates only three electrons and NO may then be converted abiotically to NO_2^-. Alternatively, generation of a fourth electron is possible, and likely, through enzymatic oxidation to NO_2^-. Caranto et al. (2016) suggested that under aerobic conditions NO could be rapidly oxidised to NO_2^- by NO_2^- reductase, encoded by *nirK*, as this enzyme can act reversibly. However, *nirK* expression is not high in AOB, expression is not coordinate with other ammonia oxidation genes, *nirK* is not found in genomes of all AOB and growth of *Nitrosomonas europaea* was unaffected by deletion of *nirK* (Cantera & Stein, 2007; Kozlowski *et al.*, 2014). Caranto et al. (2016) also proposed oxidation of NO by an as-yet uncharacterised NO oxidoreductase (NOO). A potential candidate is a red Cu protein, nitrosocyanin, encoded by *ncyA*, which is co-ordinately expressed with other ammonia

oxidation genes. Although this may fulfil this role in some AOB, *nycA* homologues are not present in all AOB genomes or in comammox bacteria (Kits et al., 2019).

Hydroxylamine oxidation in AOA is less well characterised. AOA (*N. maritimus*) can grow on hydroxylamine, producing NO_2^- (Vajrala et al., 2013), and ammonia oxidation is inhibited by the NO-quenching agent PTIO (2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide; Jung et al., 2014; Kozlowski et al., 2016b; Shen et al., 2013; Yan et al., 2012). There is currently no obvious candidate enzyme for hydroxylamine oxidation to NO in AOA and genomes contain no gene homologous to those encoding heme-based enzymes. The current model for hydroxylamine oxidation proposes hydroxylamine and NO as co-substrates for a currently unidentified, putative hydroxylamine dehydrogenase enzyme complex that produces two molecules of NO_2^-, generating 5 electrons, and that *nirK* (which is expressed highly in AOA) reduces one of these molecules to NO, for further oxidation (Kozlowski et al., 2016b). The absence of heme-based enzymes in AOA suggests that the enzyme may be copper-based.

Comammox bacteria contain hydroxylamine dehydrogenase and produce NO, but lack *ncyA* gene homologues. PTIO inhibition is similar to that in AOA, suggesting similar tight regulation of NO production, although a NO reductase has yet to be identified (Kits et al., 2019).

Mechanisms of nitrous oxide production during nitrification

Nitrifier denitrification

Classical heterotrophic denitrifiers rely on nitrifiers for NO_2^- or NO_3^- and are responsible for N_2O emissions under anaerobic conditions (coupled nitrification-denitrification). Ammonia oxidisers also produce N_2O directly through a number of partially understood mechanisms. Traditionally, the most important was considered to be nitrifier denitrification, the reduction of NO_2^- to N_2O via NO, mainly under reduced oxygen concentrations (Goreau et al., 1980;

Kozlowski et al., 2014; Poth and Focht, 1985; Shaw et al., 2006; Wrage-Mönnig et al., 2018; Zhu et al., 2013). There is currently no evidence of N_2O reductase gene homologues in AO genomes (e.g. Campbell et al., 2011; Chain et al., 2003; Norton et al., 2008; Spang et al., 2012; Tourna et al., 2011; Walker et al., 2010) and reports of reduction of N_2O to N_2 by AO are rare, although nitrosocyanin may have this function (Arciero et al., 2002; Beyer et al., 2009; Schmidt, 2009; Todt and Dörsch, 2016; Whittaker et al., 2000).

In denitrifiers, NO_2^- or NO_3^- acts as a terminal electron acceptor and is linked to growth and energy production from organic carbon (Kuypers et al., 2018). In AO, the most likely role of denitrification enzymes is the removal of excess electrons at high NH_3 concentration (Hink et al., 2017a) and possibly detoxification of any accumulated NO_2^- (Beaumont et al 2002, Stein and Arp 1998).

Nitrifier denitrification has been studied most in *N. europaea*, which possesses genes homologous to those of classical heterotrophic denitrifiers: *nirK*, encoding a Cu-containing nitrite reductase, and *norB*, encoding NO reductase (Chain et al., 2003; Kozlowski et al., 2014). While NorB is obligatory for nitrifier denitrification, NirK may be involved in oxidation of hydroxylamine rather than NO_2^- reduction, possibly involving an alternative nitrite reductase (Kozlowski et al., 2014). While studies have focused on *N. europaea*, there is evidence for genetic and physiological diversity within AOB, for example differences in gene content and responses to anoxia of oligotrophic and non-oligotrophic strains (Kozlowski et al., 2016a).

There is currently no evidence for nitrifier denitrification in AOA or comammox bacteria as both groups lack NO reductase genes (e.g. Daims et al., 2015; Kim et al., 2011; Kits et al., 2017; Spang et al., 2012; Tourna et al., 2011; Walker et al., 2010), although the neutrophilic soil AOA *Nitrosocosmicus oleophilus* may be capable of enzymatically denitrifying nitrite to N_2O at low pH (Jung et al., 2019). Furthermore, N_2O produced by both AOA and comammox

bacteria possesses a site preference (difference in $\delta^{15}N$ of the α and β N atoms of N_2O) which is distinct from that produced via nitrifier denitrification (Jung et al., 2014; Kits et al. 2019; Löscher et al., 2012; Santoro et al., 2011) and production is independent of oxygen availability (Hink et al., 2017b; Stieglmeier et al., 2014b).

N_2O production through hydroxylamine oxidation

Hooper and Terry (1979) provided evidence that NO and N_2O are also produced through incomplete oxidation of hydroxylamine. NO was considered to result from incomplete oxidation of hydroxylamine, with N_2O produced through the activity of NO reductase (Kozlowski et al., 2014; Pacheco et al., 2011). This mechanism is compatible with the revised, 2-intermediate model for ammonia oxidation (Caranto and Lancaster, 2017). Furthermore, there is evidence that cytochrome P460 converts hydroxylamine to N_2O with NO as an intermediate under anaerobic conditions enabling detoxification of hydroxylamine and NO and, in the presence of oxygen, NO would then abiotically dissociate to N_2O (Caranto et al., 2016).

Hydroxylamine oxidation in AOA differs from that in AOB with cycling of NO, which reacts with hydroxylamine to form NO_2^- (Kozlowski et al., 2016b), and there is currently no evidence for enzymatic N_2O production from either hydroxylamine or NO. However, N_2O may be produced through abiotic reactions linked to hydroxylamine oxidation (see below). Production mechanisms in *N. inopinata* are suggested to be similar to those of AOA, associated with abiotic conversion of hydroxylamine only, with no evidence for denitrification activity producing N_2O with decreasing oxygen availability (Kits et al. 2019).

Linked biotic and abiotic processes

A range of abiotic processes exists by which hydroxylamine, NO_2^- and NO can be converted to N_2O. The chemical reactions responsible, and their consequences for global emissions, have recently been reviewed (Heil et al., 2016; Zhu-Barker et al., 2015). They involve non-enzymatic conversions of products and intermediates of the nitrification process and therefore combine abiotic and biotic processes. NO_2^- may be reduced, by a range of reducing agents, and fixed by soil organic matter (SOM). The significance of abiotic processes is rarely considered, through difficulties in detection and measurement in natural environments, and measurement of NO_2^- concentration, rather than flux, but there is some evidence for NO_2^- accumulation in terrestrial and aquatic ecosystems (Shen et al., 2003; van Cleemput & Samater, 1995).

Chemical decomposition of hydroxylamine generates N_2O and small amounts of N_2 (Bremner et al., 1980). More importantly, hydroxylamine and nitrous acid (rather than NO_2^-) react to generate N_2O, particularly at low pH (Nelson, 1977). Detailed mechanisms are poorly understood and hydroxylamine can also be oxidised and, like NO_2^-, may form complexes with SOM (Heil et al., 2016). Abiotic production of N_2O from hydroxylamine has rarely been considered seriously because of lack of evidence for release of hydroxylamine from AO cells and low or undetectable levels in natural environments. Concentrations in marine environments are in the nM range, and highest when nitrification is active, and improved techniques have now led to its detection in soil at 0.3 – 35 µg N kg^{-1} dry soil (Liu et al., 2014). Low environmental concentrations may, in fact, reflect the high and diverse reactivity of hydroxylamine rather than low flux and Liu et al. (2017) demonstrated release of hydroxylamine from several AO in laboratory culture. Within AOB, production was greatest in *Nitrosospira multiformis* and *N. europaea*, but undetectable in *Nitrosomonas nitrosa* or *Nitrosomonas communis*, with low levels produced by the comammox strain *N. inopinata*.

Within AOA, hydroxylamine was produced by *Nitrososphaera gargensis* and *Nitrosotenuis uzonensis*, but not by *Nitrososphaera viennensis* or *Nitrosotalea devanaterra*. NO_2^- reduced chemical decomposition of hydroxylamine and proportions of NH_3 converted to N_2O were similar to those found previously in AOA and AOB.

Although AOB produce N_2O enzymatically, smaller contributions via abiotic reactions with hydroxylamine, NO or NO_2^-, and the observed influence of NO-quenching agents on AOA and comammox bacteria activity suggest leakage of NO (Jung et al., 2014; Kits et al., 2019; Kozlowski et al., 2016b; Shen et al., 2013; Yan et al., 2012). In the presence of oxygen, NO and hydroxylamine can be rapidly converted abiotically to N_2O (Kozlowski et al., 2016b; Stieglmeier et al., 2014b). Although it has implicitly been assumed that abiotic conversion is intracellular, direct evidence is lacking and it is possible that some or all abiotic production occurs after export or leakage of hydroxylamine from AO.

The situation is therefore complex. The two enzymatic pathways for N_2O production in AOB, from hydroxylamine and NO_2^-, are treated as distinct pathways but share NO as an intermediate. NO_2^-, NO and hydroxylamine are also intermediates in abiotic processes, which may require inorganic reducing agents, in solution or in solid form. Other factors can also influence production, e.g. storage compounds, including intracellular hydroxylamine, may be responsible for apparent anoxic production. In addition, physiological studies are generally performed in well-mixed systems in which environmental conditions are constant. In natural environments, conditions will be transient, potentially leading to metabolic imbalance that may result in accumulation of intermediates involved in N_2O production. It is difficult to assess the specific roles of abiotic reactions but there is sufficient evidence to merit further research into abiotic conversion of hydroxylamine and its relative contribution to emissions in comparison with nitrifier denitrification.

Assessment of global rates and contributions of abiotic processes is technically difficult and requires development and improvement of approaches for application of stable isotope tracing methods, including analysis of ^{15}N site preferences of N_2O for the different processes. Estimates for the formation of N_2O in sterile soils were suggested to be 31 - 75% of total N_2O production in non-sterile agricultural soils (Venterea et al., 2007). However, there are difficulties in studying sterilised soil including incomplete sterilisation, alteration of soil properties and an absence of biotic production of hydroxylamine or NO_2^-, which are precursors for abiotic production (Lotrario et al., 1995; McNamara et al., 2003; Nowak et al., 1987).

Distinction of archaeal and bacterial N_2O production

Biochemical and physiological studies of individual organisms provide a basis for the assessment of differences between AO and have been used in developing techniques to distinguish growth and activity of different groups in natural communities, e.g. differential inhibitors or isotopic methods. ^{15}N- and ^{18}O-enriched substrates can distinguish N_2O production from nitrification and denitrification processes in the environment (see Ostrom and Ostrom, 2017; Wrage- Mönnig et al., 2018), although their ability to distinguish autotrophic and heterotrophic processes has been questioned (Bakken and Frostegård, 2017). Importantly, however, these techniques cannot differentiate emissions associated with AOA, AOB and comammox bacteria activities, which have common substrates and products. Attempts to distinguish these activities involve correlation of nitrification activity and N_2O production with growth or transcriptional activity of different AO, usually via quantification of temporal changes in group-specific *amoA* genes or transcripts, respectively. There are significant limitations to this approach, but their value is increased when used in conjunction with inhibitors targeting specific groups in short term assays. Alkynes can inhibit monooxygenases through irreversible covalent binding of the active site. Acetylene is a potent inhibitor of AMO

of all AO, but 1-octyne has recently been established as a specific inhibitor of AOB (Taylor et al., 2013; Hink et al., 2017b). The NO-scavenger PTIO has been used as a specific inhibitor of AOA (Yan et al., 2012; Shen et al., 2013; Kozlowski et al., 2016b), but also inhibits comammox bacteria (Kits et al., 2019), and simvastatin can specifically inhibit AOA (Zhao et al., in revision).

N_2O yield

N_2O production associated with nitrification is quantified as the ratio of N_2O to NO_x^- (NO_2^- and/or NO_3^-) produced or NH_3 consumed, and is termed N_2O yield. Yields vary significantly within AO through differences in the physiology, and comparison with yields in environmental samples provides information on likely sources of production. N_2O yield of AOB cultures ranges from 0.1 to 8% (Anderson et al., 1993; Hink et al., 2017a; Jiang & Bakken, 1999; Shaw et al., 2006), due to differences in available NH_4^+ and oxygen concentrations that affect enzymatic reactions. Higher yields are observed with increasing NH_4^+ concentration, potentially due to a lower reaction rate of hydroxylamine dehydrogenase than AMO, leading to accumulation of hydroxylamine, which is subsequently transformed abiotically to N_2O (Hink et al., 2017a). An alternative explanation is redox balancing, in which electrons generated by hydroxylamine dehydrogenase are shuttled to denitrification enzymes when they exceed the capacity of terminal oxidases (Hink et al., 2017a). N_2O yields from AOA cultures are below or in the lower range of those observed for AOB i.e. 0.04 - 0.3%, and NH_4^+ and oxygen concentration has no or little effect on yield (Jung et al., 2011; Hink et al., 2017a; 2014; Kim et al., 2012; Löscher et al., 2012; Santoro et al., 2011; Stieglmeier et al., 2014b; Qin et al., 2017). This is consistent with the assumption that N_2O is produced only from abiotic reactions of intermediate compounds (Kozlowski et al., 2016b), although enzymatic production has been reported in *Nitrosocosmicus oleophilus* (Jung et al., 2019). Comammox bacteria and AOA lack

homologues of AOB NO reductase, suggesting low yields of N_2O, and abiotic production with a yield of 0.07% has been reported in *N. inopinata* (Kits et al., 2019).

Niche specialisation and differentiation in ammonia oxidisers

AOA and AOB belong to different domains of life and differ considerably in cellular structure and fundamental aspects of metabolism and physiology. This suggests the potential for niche differentiation associated with different physiologies and has fuelled the search for associations between environmental characteristics and AOA and AOB abundance and community composition. Comammox studies are in their infancy but all current evidence indicates that they are oligotrophs (Kits et al., 2017).

Marine AO communities are dominated by AOA (Beman et al., 2008), AOA are often absent in wastewater treatment plants (Mussmann et al., 2011) and investigation of niche differentiation between AO has focussed on terrestrial environments, with two factors attracting particular attention: pH and NH_4^+. In laboratory culture, many AO can grow at pH ≥6.5 (Hatzenpichler et al., 2008; Lehtovirta-Morley et al., 2016; Tourna et al., 2011), but obligate autotrophic growth in acidic liquid batch culture is restricted to acidophilic AOA belonging to the *Nitrosotalea* group, which grow in the pH range 4 - 6 (Jung et al., 2014; Lehtovirta-Morley et al., 2011, 2014). This is reflected in their global presence and activity in acidic soils (Gubry-Rangin et al., 2011), which contain AOA clades (particularly *Nitrososphaera* clade C11) that are currently not represented in culture, restricting physiological studies (Gubry-Rangin et al., 2018). AOA dominate ammonia oxidation in acidic soils, which comprise 30% of soils globally, some of which are heavily fertilised arable soils (von Uexküll and Mutert, 1995). Betaproteobacterial AOB activity has been observed at pH as low as 5.5 in biofilms (Allison & Prosser, 1993), but AOA and AOB activities are found in soils down to pH 4.5. AOB activity may be linked to an uncharacterised *Nitrosospira* clade

active in acidic soils (Aigle et al., 2019) and growth of ureolytic AO at low pH is also possible, if NH_3 is supplied as urea, as ureolytic activity is not pH-dependent (Burton et al., 2001). Evidence for this mechanism in soil is lacking and the role of AOB and *Nitrososphaera* clade C11 in acid soils, and potential metabolisms, require further study.

There is also evidence for niche differentiation associated with NH_3 supply. AOB growth is favoured in soils fertilised by single additions of high levels of inorganic NH_3, while AOA grow preferentially when NH_3 is produced through mineralisation of organic N (Hink et al., 2017b, 2018; Verhamme et al., 2011). AOA and AOB relative activities may therefore be influenced by different N fertilisation strategies, with subsequent effects on N_2O yields.

Terrestrial ecosystems

Terrestrial environments contribute 56 - 70% of N_2O emissions globally with agricultural systems contributing ~40% of that derived from soils (Davidson, 2009; Syakila & Kroeze, 2011). As described above, AO activity contributes directly to N_2O emissions through both biotic and linked abiotic processes. However, coupled with the activity of NOB, they also contribute indirectly to N_2O emissions by producing NO_3^- for heterotrophic denitrifiers and subsequent reduction of NO to N_2O as part of denitrification. The application of AO inhibitors has therefore been considered as a method to inhibit N_2O emissions directly or indirectly (Ruser & Schulz, 2015). Although NH_3 can be lost through volatilisation, reduction of ammonia oxidation is generally considered beneficial for plant uptake and decreased fertilisation loss, and niche differentiation of AOA and AOB suggests that controlling the activities of each group may also have substantial impacts on reducing N_2O emissions. This leads to the hypothesis that inorganic fertilisation will benefit AOB, and high N_2O yield and emissions, while slow release of ammonia from native organic N, organic fertiliser or slow-release fertiliser will favour AOA and lower emissions (Figure 1).

This hypothesis has been tested most critically in microcosms in which AOA and AOB N_2O production is distinguished using differential inhibitors or comparison of isotopic signatures with those obtained in AOA or AOB cultures. The inhibitor approach was first used by Hink et al. (2017b) in soil microcosms in which N_2O production was dominated by AO and N_2O emissions by AOA and AOB were discriminated by differential inhibition of AOB using 1-octyne. Inorganic N fertilisation stimulated N_2O production, which was reduced in the presence of 1-octyne, and estimated N_2O yields associated with AOA and AOB activity were comparable to those obtained in pure cultures, i.e. the N_2O yield of AOA was approximately half that of AOB. AOA-associated N_2O emission was also determined in unfertilised soils, in which NH_3 is supplied through mineralisation of organic N, and N_2O yields were again similar to those reported for AOA cultures. These results therefore indicate that the relative contribution to ammonia oxidation and N_2O generation by AOB may be greatly reduced by controlling the rate of NH_4^+ supply.

To test the potential for reduction in N_2O through use of different fertiliser strategies, a similar approach compared single addition of urea-N at high concentration and continuous low production from a slow-release urea-N fertiliser (polymethylene urea) or organic N mineralisation (Hink et al., 2018). When fertiliser was supplied at high concentration, AOB dominated activity and N_2O production, with high yield (Figure 2).

Inhibition of AOB activity by 1-octyne reduced N_2O production and yield but AOA activity increased, demonstrating the ability of AOA to grow at high NH_4^+ concentration in the absence of AOB. Low NH_4^+ supply, through slow release, led to dominance of activity by AOA and low N_2O yield.

AOB-dominated nitrification and high AOB-associated N_2O production have also been observed after N fertilisation of alluvial (pH 8) and red (pH 6) soils (Wang et al. 2016).

Fertilisation did not result in AOA growth and, in fact, led to a decrease in AOA abundance in the red soil. AOA did not grow in the alluvial soil but grew in control and 1-octyne-treated, unfertilised red soils, but not following acetylene inhibition. AOA growth was not associated with detectable N_2O production. In fertilised soils, proportions of emissions associated with AOB and AOA were similar (70.5 - 78.1% and 18.7 - 19.7%, respectively) to those observed by Hink et al. (2017b). Meinhardt et al. (2018) complemented this approach by additional use of PTIO, to inhibit AOA, and isotopic analysis, to distinguish N_2O arising from hydroxylamine oxidation and nitrifier denitrification in an alkaline switch-grass soil. Nitrification, AOA and AOB growth and activity and N_2O production were low in control soil microcosms. Inorganic N fertilisation favoured AOB growth and increased N_2O production, and isotopic analysis indicated that N_2O production was associated with AOB activity, while production in unfertilised soil was similar to that from AOA cultures. These results were consistent with field data, in which AOB abundance correlated with N_2O emissions.

While NO_2^- concentration in soil is typically low, accumulation of NO_2^- and its influence on N_2O emissions has also been investigated in microcosms. Venterea et al. (2015) and Breuillin-Sessoms et al. (2017) reported NO_2^- accumulation and associated N_2O production in bovine urine- and urea-fertilised soil. Increased N_2O production after fertilisation was thought to be due to nitrifier denitrification of accumulated NO_2^-. Interestingly, Giguere et al. (2017) reported NO_2^- accumulation in three non-cropped soils that was not associated with increased N fertilisation. This led to increased N_2O production in soil in which ammonia oxidation was dominated by AOA or shared between AOB and AOA and production was stimulated by addition of NO_2^-. While this can be explained by nitrifier denitrification by AOB, some of the activity was associated with AOA. The mechanism for this is unclear, but could be through abiotic reaction between NO_2^- and leaked hydroxylamine or currently uncharacterized AOA

nitrifier denitrification. Regardless of the mechanism, the authors highlight the potential for nitrite accumulation to influence N_2O yields in soil studies.

Duan et al. (2019) used 1-octyne to distinguish AOA- and AOB-associated N_2O emissions in urea-amended microcosms using several Chinese soils cultivated with vegetables in greenhouses and previously treated with different levels of urea fertiliser. AOA dominated N_2O production in microcosms containing previously unfertilised soil and AOA and AOB contributed to production in soil with a history of intermediate fertilisation. AOA abundance increased with historically high urea fertilisation levels and dominated nitrification and N_2O production in the two most heavily fertilised soils. The authors also reported a correlation between N_2O production and accumulated NO_2^-, suggesting differential inhibition of NOB at high NH_4^+. These, and other studies of complex soil ecosystems, indicate that the potential for localised depletion of oxygen and nitrate accumulation must also be considered, through ammonia oxidation following high levels of fertilisation, that will provide conditions favourable for N_2O production by heterotrophic nitrifiers.

In addition, an increasing number of microcosm and field studies have generated data on correlations between N_2O and AOA or AOB *amoA* gene or transcript abundance. These studies have generally been carried out to assess the effects of fertiliser on production rates and AO abundances, rather than to test the above hypotheses, and suffer from the fundamental limitations of correlation-based studies and use of gene abundance as a measure of activity. They frequently report AOB growth only, following fertilisation, and therefore find correlations between N_2O production and AOB, rather than AOA abundance, e.g. studying fertilised paddy soil subjected to wetting and drying (Abid et al., 2018), biochar-treated wheat-maize soil (Liu et al., 2019), arable soil (Song et al., 2018) and forest soil (Martins et al., 2017). Several studies, however, reported a greater role for AOA in nitrification and correlation

between AOA and N_2O, e.g. in tropical rainforest soil (Soper et al., 2018) and Tibetan alpine soil (Peng et al. 2018).

Directed microcosm studies, using inhibitor and isotopic approaches to distinguish AOA and AOB activities, therefore support proposed links between niche specialisation and the consequences for N_2O production and yield. Care must be taken in interpreting results, inhibitor specificity may require more rigorous testing and additional inhibitors may be required to differentiate comammox bacteria. PTIO inhibits *N. inopinata* and *N. maritimus* (Martens-Habbena et al., 2015; Kits et al., 2019) and is therefore not specific for AOA activity, and both PTIO and 1-octyne can inhibit both AOA and AOB at sufficiently high concentrations (Shen et al., 2013; Taylor et al., 2013). They must therefore be used with care with complex natural communities. In addition, greater activity of AOA when AOB are inhibited may lead to overestimation of N_2O production by AOA when both groups are present and potentially active. Studies based only on AO abundances produce a range of effects with results that are difficult to interpret because of a number of limitations, including lack of control, inability to distinguish effects of different factors and difficulty in relating abundance to activity.

Aquatic ecosystems

The major source of nitrogen in marine and freshwater ecosystems is biological nitrogen fixation. AO therefore obtain NH_3 through mineralisation of organic N and N_2O is produced by both ammonia oxidisers and traditional denitrifiers, the latter also providing a sink for N_2O. Production is greatest in regions of equatorial and coastal upwelling (transporting N_2O produced in deep sediments), OMZ and areas of high productivity, with hot-spots in the Arabian Sea and East tropical South Pacific (ETSP) (Nevison et al., 1995). Marine systems are estimated to contribute ~3.8 Tg N_2O y^{-1}, equivalent to 21% of global emissions, although the range of estimates is large (1.8 – 9.4 Tg N^{-1}) (IPCC, 2013). This high range is due to

uncertainties in methodology, reliance on assumed correlations between oxygen utilisation and N_2O production and lack of information for parametrisation of simulation models, in addition to uncertainties regarding biophysical processes, such as transfer functions from surface waters to atmosphere, wind dispersal and hydrogeography.

AOA dominate AO communities in the open ocean and are the major contributors to marine ammonia oxidation and AO-associated N_2O production (Santoro et al., 2011), although a minority of studies report similar abundances for AOA and AOB. AOA abundance is low in surface waters, except in the Arctic (Müller et al., 2018), through photoinhibition and low NH_3 flux, but increases between ~100 - 1000 m, before decreasing at greater depths. AOA communities are dominated by two phylogenetic groups within the *Nitrosopumilales* (Santoro et al., 2019). The water column A (WCA) or high NH_4^+ (HAC) group is found in polar regions, contains the cultivated AOA *Nitrosopelagicus brevis* and dominates when total AOA abundance is high. The water column B (WCB) or low NH_4^+ (LAC) group dominates at depths >300 m and currently has no cultivated representative. Correlations of gene and transcript abundances and nitrification rates with environmental conditions suggest that WCA has a broad environmental niche, but WCB genes correlate with lower temperature, higher nutrients and low chlorophyll (Smith et al., 2014). The basis of niche differentiation between these two groups, however, remains uncertain.

A role for ammonia oxidation and N_2O production by AOA is based on correlations between gene and transcript abundances and N_2O production rates and isotopic methods. For example, Löscher et al. (2012) reported N_2O production in the East Tropical North Atlantic (ETNA) (oxygen concentration >40 µmol l^{-1}) and the OMZ of ETSP waters. In the ETSP, N_2O emissions correlated with both archaeal *amoA* gene abundance and those involved in denitrification, suggesting a mixed origin for N_2O, while ETNA production correlated with

archaeal *amoA* gene abundance only. AOA outnumbered AOB by 1 - 2 orders of magnitude. N_2O production was also inhibited when sea water was incubated with the inhibitor N1-guanyl-1,7-diaminoheptane (GC7), which inhibits synthesis of hypusine, required for protein synthesis in archaea. Peng et al. (2015) used on-board incubations of ETNP (East tropical North Pacific) water to assess depth-related nitrification potential, *amoA* gene abundance, N_2O production and selective inhibition of AOB and AOA by ATU and PTIO, respectively. AOA outnumbered AOB by approximately one order of magnitude and estimated K_m values were similar to those of *N. maritimus*, confirming the potential for activity in the OMZ. Horak et al. (2013) also measured a K_m of 98 nmol l^{-1} in incubations of Puget Sound sea water where, again, AOA outnumbered AOB by 1 – 2 orders of magnitude. In contrast, Ji et al. (2015) measured rates and gene abundances in ETSP water and suggested nitrification and associated N_2O production were greatest between the euphotic zone and the OMZ, but that denitrification dominate production in the OMZ. Effects of climate change have also been investigated by Rees et al. (2016), who found that acidification (pH reduction by 0.06 – 0.4) of polar and subpolar Atlantic Ocean waters did not affect AOA community composition but reduced N_2O production in proportion to the reduction in NH_3 availability (vs. NH_4^+) due to the pH reduction.

Many simulation models estimate N_2O production rate from measurements of oxygen concentration, assuming a linear negative relationship. Trimmer et al. (2016) tested this assumption by determining relationships between AOA *amoA* and *nirK* gene abundances (AOB were below the detection limit), oxygen concentrations and N_2O production in an ETNP oxycline. Correlations between oxygen and molecular data were similar to those from low-oxygen regions of the Baltic Sea (Berg et al., 2015), supporting AOA activity at low oxygen concentration. N_2O production increased exponentially as oxygen concentration decreased from 30 – 1 µmol O_2 l^{-1} and there was no evidence for denitrification or nitrifier denitrification.

This confirmed early work of Goreau et al. (1980) and the improved information on dynamics improved modelling of N_2O emissions in OMZs. This led to estimates of 17 µmol N_2O m^{-2} d^{-1}, which agree with previous estimates, and predictions that ETNP and global OMZ production generates 2.1 Tg N y^{-1} and 5.1 Tg N y^{-1} as N_2O, respectively.

Lakes and coastal regions

AOB appear to dominate ammonia oxidation in freshwater environments and Wenk et al. (2016) used isotope measurements to distinguish different sources of N_2O production in two basins (North and South) of Lake Lugano. Production was higher in the holomictic lake and was associated with denitrification and bacterial (rather than archaeal) ammonia oxidation in the redox transition zone. N_2O production in the meromictic North Basin was lower and was due to nitrifier denitrification. Frame et al. (2017) compared N_2O production in Lake Lugano and Namibian coastal waters. In the former, AOB outnumbered AOA and isotope methods indicated AOA or AOB production by abiotic conversion of hydroxylamine and insignificant nitrifier denitrification. In Namibian seawater, AOA outnumbered AOB but the mechanism of production was not clear. Angell et al. (2018) correlated abundance of functional genes associated with N_2O production with process data in salt marshes subjected to different levels of N fertilisation. N fertilisation increased rates of production and consumption of N_2O, increased the contribution of denitrification, had no effect on AOA composition but changed AOB community composition, which contributed more than AOA to N_2O production.

Wastewater treatment systems

Wastewater treatment encompasses a wide range of aerobic and anaerobic processes in which N_2O is generated by ammonia oxidisers and/or denitrifiers, contributing to 1.3% of global anthropogenic emissions (Kampschreur et al., 2009). AO will generate N_2O in aerobic processes but their contributions will be influenced by spatial heterogeneity, with anoxic

conditions within flocs of activated sludge processes and biofilms formed in trickling filters. The impact of these conditions and of pH are discussed in recent reviews (Blum et al., 2018; Sabba et al., 2018; Todt & Dörsch, 2016). AOB are generally considered to dominate AO communities but Yin et al. (2018) found that AOB dominated 13 of 23 wastewater treatment plants, including 3 in which AOA were not detectable. AOA outnumbered AOB by ~1 – 2 orders of magnitude in 10 plants but, if cellular ammonia oxidation and N_2O rates are lower for AOA, AOA will not necessarily dominate activity. These findings do, however, suggest that AOA should not be considered insignificant, but it is currently not clear which conditions influence relative abundances of AOA and AOB. Mitigation of N_2O emissions may therefore be possible, but requires investigation of niche specialisation and sources of emissions.

Potential strategies for mitigation of N_2O emissions

N_2O emissions in open oceans are from natural, rather than anthropogenic sources, and mitigation in coastal regions and freshwater environments is achieved by reducing N run-off. Soils are the main source of N_2O globally and advances in the understanding of the ecology of the organisms involved presents an opportunity to influence agricultural practices and decrease global emissions.

Ammonia oxidation directly and indirectly leads to all microbially-mediated N_2O production. Ammonia oxidation can reduce volatilisation of NH_3 in alkaline soils but reduction of AO activity in non-alkaline agricultural soils will not only improve fertiliser use efficiency, by increasing the residence time of NH_4^+ in soil and the opportunity for plant uptake, but will also decrease AO-associated N_2O emissions. This can be achieved by use of synthetic nitrification inhibitors, whose benefits and limitations have been reviewed elsewhere (Coskun et al., 2017). BNIs associated with arable crops may provide a more efficient approach to nitrification inhibition (Subbarao et al., 2012) and Byrnes et al. (2017) demonstrated the use of forage

grasses with high BNI activity in decreasing N_2O emissions in urine bovine pasture. Both synthetic inhibitors and BNIs have been developed against AOB and, while some also inhibit AOA, others have not been tested. Future studies should therefore assess inhibition of a range of both AOB and AOA, given increasing appreciation of diversity within these groups.

Increased understanding of niche differentiation within AO, and within AOA and AOB, and significant improvements in the ability to quantify these organisms, adds a further dimension and presents new opportunities. For example, fertiliser use efficiency will be greater in soils dominated by AOA or AOB, following application of inorganic or slow-release fertilisers, respectively, given their different NH_3 supply preferences. Ammonia oxidation by either group will result in N_2O emissions, but management strategies that favour AOA would have an additive effect in reducing N_2O emissions, as N_2O yield from AOB activity is double that of AOA (Hink et al., 2017b, 2018). In contrast, liming of acidic soil is likely to increase AOB, rather than AOA, increasing N_2O emissions associated with AOB activity. This highlights the need to consider the impact on relevant and important microbial communities, in addition to benefits for plant productivity and is consistent with calls for greater consideration of sustainability in agricultural practice (Rockström et al., 2017).

Realisation of this potential requires a broader range of microcosm studies with a greater range of soil types and conditions and fertilisation strategies to confirm the relatively limited data currently available. Importantly, it also requires well-designed field experiments to translate laboratory findings to agricultural soils and to assess impacts of mitigation strategies on microbial communities, plant productivity, fertiliser use efficiency and N_2O emissions under field conditions. To date, field studies have focused largely on inorganic N fertilisers and few have considered diversity within AO and the consequences for N_2O emissions.

Conclusion

Research over the last 25 years has dramatically increased our understanding of AO diversity and the importance of AO community composition in determining rates of ammonia oxidation, the influence of environmental factors and important consequences for N_2O emissions. This provides the basis for better quantitative information required for parameterisation of biogeochemical models to improve predictions and assess impacts of different management strategies. Physiological studies should consider these requirements but care should also be taken in generalising and extrapolating findings from single isolates, single genomes and single studies and information is required to determine the degree to which isolates represent active members of natural communities. Ecological studies require greater focus on structured experiments that identify the combinations of factors that determine the activity of different AO groups based on niche specialisation, with less emphasis on descriptive studies. Research would also benefit from development of better techniques to distinguish the many different biotic and abiotic processes contributing to N_2O production and to distinguish activity of different functional microbial groups *in situ*. A critical requirement, however, is for studies that test the predictions of physiological and microcosm experiments ultimately to inform mitigation strategies under field conditions.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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References

Abid, A. A., Gu, C., Zhang, Q., Wang, J., Di, H. (2018). Nitrous oxide fluxes and nitrifier and denitrifier communities as affected by dry-wet cycles in long term fertilised paddy soils. *Applied Soil Ecology*, *125*, 81-87.

Aigle, A., Prosser, J. I., Gubry-Rangin, C. (2019). The application of high-throughput sequencing technology to analysis of *amoA* phylogeny and environmental niche specialisation of terrestrial bacterial ammonia-oxidisers. *Environmental Microbiome*, *14*(3), 1-10.

Alexandratos, N., J. Bruinsma (2012). World agriculture towards 2030/2050: The 2012 revision. ESA Working paper No. 12-03 (Food and Agriculture Organization of the United Nations, Rome).

Allison, S. M., Prosser, J. I. (1993). Ammonia oxidation at low pH by attached populations of nitrifying bacteria. *Soil Biology and Biochemistry*, *25*(7), 935-941.

Alves, R. J. E., Minh, B. Q., Urich, T., von Haeseler, A., Schleper, C. (2018). Unifying the global phylogeny and environmental distribution of ammonia-oxidising archaea based on *amoA* genes. *Nature Communications*, 9(1), 1517.

Anderson, I. C., Poth, M., Homstead, J., Burdige, D. (1993). A comparison of NO and N_2O production by the autotrophic nitrifier Nitrosomonas europaea and the heterotrophic nitrifier Alcaligenes faecalis. *Applied Environmental Microbiology*, *59*(11), 3525-3533.

Angell, J. H., Peng, X., Ji, Q., Craick, I., Jayakumar, A., Kearns, P. J., ... Bowen, J. L. (2018). Community composition of nitrous oxide-related genes in salt marsh sediments exposed to nitrogen enrichment. *Frontiers in Microbiology*, *9*, 170.

Arciero, D. M., Pierce, B. S., Hendrich, M. P., Hooper, A. B. (2002). Nitrosocyanin, a red cupredoxin-like protein from *Nitrosomonas europaea*. *Biochemistry*, *41*(6), 1703-1709.

Arp, D. J., Stein, L. Y. (2003). Metabolism of inorganic N compounds by ammonia-oxidizing bacteria. *Critical Reviews in Biochemistry and Molecular Biology*, *38*(6), 471-495.

Bakken, L. R., Frostegård, Å. (2017). Sources and sinks for N_2O, can microbiologists help to mitigate N_2O emissions? *Environmental Microbiology* 19: 4801-4805.

Bateman, E. J., Baggs, E. M. (2005). Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils*, *41*(6), 379-388.

Beaumont, H. J., Hommes, N. G., Sayavedra-Soto, L. A., Arp, D. J., Arciero, D. M., Hooper, A. B., ... Van Spanning, R. J. (2002). Nitrite reductase of *Nitrosomonas europaea* is not essential for production of gaseous nitrogen oxides and confers tolerance to nitrite. *Journal of Bacteriology*, *184*(9), 2557-2560.

Beman, J. M., Popp, B. N., Francis, C. A. (2008). Molecular and biogeochemical evidence for ammonia oxidation by marine Crenarchaeota in the Gulf of California. *The ISME Journal*, *2*(4), 429.

Berg, C., Vandieken, V., Thamdrup, B., Jürgens, K. (2015). Significance of archaeal nitrification in hypoxic waters of the Baltic Sea. *The ISME Journal*, *9*(6), 1319.

Beyer, S., Gilch, S., Meyer, O., Schmidt, I. (2009). Transcription of genes coding for metabolic key functions in *Nitrosomonas europaea* during aerobic and anaerobic growth. *Journal of Molecular Microbiology and Biotechnology*, *16*(3-4), 187-197.

Blum, J. M., Su, Q., Ma, Y., Valverde-Pérez, B., Domingo-Félez, C., Jensen, M. M., Smets, B. F. (2018). The pH dependency of N-converting enzymatic processes, pathways and microbes: effect on net N_2O production. *Environmental microbiology*, *20*(5), 1623-1640.

Booth, M. S., Stark, J. M., Rastetter, E. (2005). Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecological Monographs*, *75*(2), 139-157.

Boyd, E., Peters, J. W. (2013). New insights into the evolutionary history of biological nitrogen fixation. *Frontiers in Microbiology*, *4*, 201.

Bremner, J. M., Blackmer, A. M., Waring, S. A. (1980). Formation of nitrous oxide and dinitrogen by chemical decomposition of hydroxylamine in soils. *Soil Biology and Biochemistry*, *12*(3), 263-269.

Breuillin-Sessoms, F., Venterea, R. T., Sadowsky, M. J., Coulter, J. A., Clough, T. J., Wang, P. (2017). Nitrification gene ratio and free ammonia explain nitrite and nitrous oxide production in urea-amended soils. *Soil Biology and Biochemistry*, *111*, 143-153.

Brochier-Armanet, C., Boussau, B., Gribaldo, S., Forterre, P. (2008). Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. *Nature Reviews Microbiology*, *6*(3), 245.

Burton, S. A. Q., Prosser, J. I. (2001). Autotrophic ammonia oxidation at low pH through urea hydrolysis. *Applied and Environmental Microbiology*, *67* (7), 2952-2957.

Byrnes, R. C., Nùñez, J., Arenas, L., Rao, I., Trujillo, C., Alvarez, C., ... Chirinda, N. (2017). Biological nitrification inhibition by Brachiaria grasses mitigates soil nitrous oxide emissions from bovine urine patches. *Soil Biology and Biochemistry*, *107*, 156-163.

Campbell, M. A., Chain, P. S., Dang, H., El Sheikh, A. F., Norton, J. M., Ward, N. L., ... Klotz, M. G. (2011). *Nitrosococcus watsonii* sp. nov., a new species of marine obligate ammonia-oxidizing bacteria that is not omnipresent in the world's oceans: calls to validate the names ‘Nitrosococcus halophilus’ and ‘Nitrosomonas mobilis’. *FEMS Microbiology Ecology*, *76*(1), 39-48.

Cantera, J. J. L., Stein, L. Y. (2007). Molecular diversity of nitrite reductase genes (*nirK*) in nitrifying bacteria. *Environmental Microbiology*, *9*(3), 765-776.

Caranto, J. D., Lancaster, K. M. (2017). Nitric oxide is an obligate bacterial nitrification intermediate produced by hydroxylamine oxidoreductase. *Proceedings of the National Academy of Sciences*, *114*(31), 8217-8222.

Caranto, J. D., Vilbert, A. C., Lancaster, K. M. (2016). *Nitrosomonas europaea* cytochrome P460 is a direct link between nitrification and nitrous oxide emission. *Proceedings of the National Academy of Sciences*, *113*(51), 14704-14709.

Chain, P., Lamerdin, J., Larimer, F., Regala, W., Lao, V., Land, M., ... Sayavedra-Soto, L. (2003). Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph *Nitrosomonas europaea*. *Journal of Bacteriology*, *185*(9), 2759-2773.

Coskun, D., Britto, D. T., Shi, W. M., Kronzucker, H. J. (2017). Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. *Nature Plants*, *3*,17074. https://doi.org/10.1038/nplants.2017.74

Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., ... Kirkegaard, R. H. (2015). Complete nitrification by *Nitrospira* bacteria. *Nature*, *528*(7583), 504.

Daims, H., Lücker, S., Wagner, M. (2016). A new perspective on microbes formerly known as nitrite-oxidizing bacteria. *Trends in Microbiology*, *24*(9), 699-712.

Davidson, E. A. (2009). The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. *Nature Geoscience*, *2*(9), 659.

Duan, P., Fan, C., Zhang, Q., Xiong, Z. (2019). Overdose fertilization induced ammonia-oxidizing archaea producing nitrous oxide in intensive vegetable fields. *Science of the Total Environment*, *650*, 1787-1794.

FAO (2017). World fertilizer trends and outlook to 2020. Food and Agriculture Organization United Nations, Rome, Italy.

Fowler, S. J., Palomo, A., Dechesne, A., Mines, P. D., Smets, B. F. (2018). Comammox *Nitrospira* are abundant ammonia oxidizers in diverse groundwater-fed rapid sand filter communities. *Environmental Microbiology*, *20*(3), 1002-1015.

Frame, C. H., Lau, E., Nolan IV, E. J., Goepfert, T. J., Lehmann, M. F. (2017). Acidification enhances hybrid N_2O production associated with aquatic ammonia-oxidizing microorganisms. *Frontiers in Microbiology*, *7*, 2104.

Francis, C. A., O'Mullan, G. D., Ward, B. B. (2003). Diversity of ammonia monooxygenase (*amoA*) genes across environmental gradients in Chesapeake Bay sediments. *Geobiology*, *1*(2), 129-140.

Giguere, A. T., Taylor, A. E., Suwa, Y., Myrold, D. D., Bottomley, P. J. (2017). Uncoupling of ammonia oxidation from nitrite oxidation: impact upon nitrous oxide production in non-cropped Oregon soils. *Soil Biology and Biochemistry*, *104*, 30-38.

Goreau, T. J., Kaplan, W. A., Wofsy, S. C., McElroy, M. B., Valois, F. W., Watson, S. W. (1980). Production of NO_2^- and N_2O by nitrifying bacteria at reduced concentrations of oxygen. *Applied and Environmental Microbiology*, *40*(3), 526-532.

Gubry-Rangin, C., Hai, B., Quince, C., Engel, M., Thomson, B. C., James, P., ... Nicol, G. W. (2011). Niche specialization of terrestrial archaeal ammonia oxidizers. *Proceedings of the National Academy of Sciences*, *108*(52), 21206-21211.

Gubry-Rangin, C., Williams, W., Prosser, J. I. (2018). Approaches to understanding the ecology and evolution of understudied terrestrial archaeal ammonia-oxidisers. *Emerging Topics in Life Sciences*, *2*(4), 619-628.

Hatzenpichler, R., Lebedeva, E. V., Spieck, E., Stoecker, K., Richter, A., Daims, H., Wagner, M. (2008). A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. *Proceedings of the National Academy of Sciences*, *105*(6), 2134-2139.

Hayatsu, M., Tago, K., Uchiyama, I., Toyoda, A., Wang, Y., Shimomura, Y., ... Akiyama, H. (2017). An acid-tolerant ammonia-oxidizing γ-proteobacterium from soil. *The ISME Journal*, *11*(5), 1130.

Haynes, R. J. (1986). Mineral Nitrogen in the Plant–Soil System (pp. 127–165). London: Academic Press Inc.

Heil, J., Vereecken, H., Brüggemann, N. (2016). A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. *European Journal of Soil Science*, *67*(1), 23-39.

Hink, L., Gubry-Rangin, C., Nicol, G. W., Prosser, J. I. (2018). The consequences of niche and physiological differentiation of archaeal and bacterial ammonia oxidisers for nitrous oxide emissions. *The ISME Journal*, *12*(4), 1084.

Hink, L., Lycus, P., Gubry-Rangin, C., Frostegård, Å., Nicol, G. W., Prosser, J. I., Bakken, L. R. (2017a). Kinetics of NH_3-oxidation, NO-turnover, N_2O-production and electron flow during oxygen depletion in model bacterial and archaeal ammonia oxidisers. *Environmental Microbiology*, *19*(12), 4882-4896.

Hink, L., Nicol, G. W., Prosser, J. I. (2017b). Archaea produce lower yields of N_2O than bacteria during aerobic ammonia oxidation in soil. *Environmental Microbiology*, *19*(12), 4829-4837.

Hooper, A. B., Terry, K. R. (1979). Hydroxylamine oxidoreductase of *Nitrosomonas*: production of nitric oxide from hydroxylamine. *Biochimica et Biophysica Acta - Enzymology*, *571*(1), 12-20.

Horak, R. E., Qin, W., Schauer, A. J., Armbrust, E. V., Ingalls, A. E., Moffett, J. W., ... Devol, A. H. (2013). Ammonia oxidation kinetics and temperature sensitivity of a natural marine community dominated by Archaea. *The ISME Journal*, *7*(10), 2023.

IFA/UNEP (1998). The Fertiliser Industry. World Food Supplies and the Environment. International Fertiliser Industry Association & United Nations Environment Programme, Paris, France.

IPCC (2013). Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (1535 pp). UK & USA: Cambridge University Press.

Ji, Q., Babbin, A. R., Jayakumar, A., Oleynik, S., Ward, B. B. (2015). Nitrous oxide production by nitrification and denitrification in the Eastern Tropical South Pacific oxygen minimum zone. *Geophysical Research Letters*, *42*(24), 10-755.

Jiang, Q. Q., Bakken, L. R. (1999). Nitrous oxide production and methane oxidation by different ammonia-oxidizing bacteria. *Appl. Environ. Microbiol.*, *65*(6), 2679-2684.

Jung, M. Y., Gwak, J. H., Rohe, L., Giesemann, A., Kim, J. G., Well, R., ... Rhee, S. K. (2019). Indications for enzymatic denitrification to N_2O at low pH in an ammonia-oxidizing archaeon. *The ISME Journal*.

Jung, M. Y., Park, S. J., Min, D., Kim, J. S., Rijpstra, W. I. C., Damsté, J. S. S., ... Rhee, S. K. (2011). Enrichment and characterization of an autotrophic ammonia-oxidizing archaeon of mesophilic crenarchaeal group I. 1a from an agricultural soil. *Applied and Environmental Microbiology*, *77*(24), 8635-8647.

Jung, M. Y., Well, R., Min, D., Giesemann, A., Park, S. J., Kim, J. G., ... Rhee, S. K. (2014). Isotopic signatures of N_2O produced by ammonia-oxidizing archaea from soils. *The ISME Journal*, *8*(5), 1115.

Kampschreur, M. J., Poldermans, R., Kleerebezem, R., Van Der Star, W. R. L., Haarhuis, R., Abma, W. R., ... Van Loosdrecht, M. C. M. (2009). Emission of nitrous oxide and nitric oxide

from a full-scale single-stage nitritation-anammox reactor. *Water Science and Technology*, *60*(12), 3211-3217.

Kerou, M., Alves, R. J. E., Schleper, C. (2016). Genus Nitrososphaera. In: W. B. Whitman (Ed.), Bergey's Manual of Systematics of Archaea and Bacteria (pp. 1–8). USA: John Wiley & Sons, Inc.

Kim, B. K., Jung, M. Y., Yu, D. S., Park, S. J., Oh, T. K., Rhee, S. K., Kim, J. F. (2011). Genome sequence of an ammonia-oxidizing soil archaeon, "*Candidatus* Nitrosoarchaeum koreensis" MY1.

Kim, J. G., Jung, M. Y., Park, S. J., Rijpstra, W. I. C., Sinninghe Damsté, J. S., Madsen, E. L., … Rhee, S. K. (2012). Cultivation of a highly enriched ammonia-oxidizing archaeon of thaumarchaeotal group I.1b from an agricultural soil. *Environmental Microbiology*, *14*(6), 1528-1543.

Kits, K. D., Jung, M. Y., Vierheilig, J., Pjevac, P., Sedlacek, C. J., Liu, S., ... Brüggemann, N. (2019). Low yield and abiotic origin of N_2O formed by the complete nitrifier Nitrospira inopinata. *Nature Communications*, *10*(1), 1836.

Kits, K. D., Sedlacek, C. J., Lebedeva, E. V., Han, P., Bulaev, A., Pjevac, P., ... Daims, H. (2017). Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. *Nature*, *549*(7671), 269.

Kozlowski, J. A., Kits, K. D., Stein, L. Y. (2016a). Comparison of nitrogen oxide metabolism among diverse ammonia-oxidizing bacteria. *Frontiers in Microbiology*, *7*, 1090.

Kozlowski, J. A., Price, J., Stein, L. Y. (2014). Revision of N_2O-producing pathways in the ammonia-oxidizing bacterium *Nitrosomonas europaea* ATCC 19718. *Applied and Environmental Microbiology*, *80*(16), 4930-4935.

Kozlowski, J. A., Stieglmeier, M., Schleper, C., Klotz, M. G., Stein, L. Y. (2016b). Pathways and key intermediates required for obligate aerobic ammonia-dependent chemolithotrophy in bacteria and Thaumarchaeota. *The ISME Journal*, *10*(8), 1836.

Kuypers, M. M., Marchant, H. K., Kartal, B. (2018). The microbial nitrogen-cycling network. *Nature Reviews Microbiology*, *16*(5), 263.

Lassaletta, L., Billen, G., Grizzetti, B., Anglade, J., Garnier, J. (2014). 50-year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. *Environmental Research Letters*, *9*(10), 105011.

Lehtovirta-Morley, L. E., Ge, C., Ross, J., Yao, H., Nicol, G. W., Prosser, J. I. (2014). Characterisation of terrestrial acidophilic archaeal ammonia oxidisers and their inhibition and stimulation by organic compounds. *FEMS Microbiology Ecology*, *89*(3), 542-552.

Lehtovirta-Morley, L. E., Sayavedra-Soto, L. A., Gallois, N., Schouten, S., Stein, L. Y., Prosser, J. I., Nicol, G. W. (2016). Identifying potential mechanisms enabling acidophily in the ammonia-oxidizing archaeon "*Candidatus* Nitrosotalea devanaterra". *Applied and Environmental Microbiology*, *82*(9), 2608-2619.

Lehtovirta-Morley, L. E., Stoecker, K., Vilcinskas, A., Prosser, J. I., Nicol, G. W. (2011). Cultivation of an obligate acidophilic ammonia oxidizer from a nitrifying acid soil. *Proceedings of the National Academy of Sciences*, *108*(38), 15892-15897.

Liu, Q., Liu, B., Zhang, Y., Hu, T., Lin, Z., Liu, G., ... Ambus, P. (2019). Biochar application as a tool to decrease soil nitrogen losses (NH_3 volatilization, N_2O emissions, and N leaching) from croplands: Options and mitigation strength in a global perspective. *Global Change Biology*, *25*(6), 2077-2093.

Liu, S., Han, P., Hink, L., Prosser. J. I., Wagner, M., Brüggemann, N. (2017). Abiotic conversion of extracellular NH_2OH contributes significantly to N_2O emission during ammonia oxidation. *Environmental Science and Technology 51*, 13122-13132.

Liu, S., Vereecken, H, Brüggemann, N. (2014). A highly sensitive method for the determination of hydroxylamine in soils. *Geoderma 232-234*, 117−122.

Löscher, C. R., Kock, A., Könneke, M., LaRoche, J., Bange, H. W., Schmitz, R. A. (2012). Production of oceanic nitrous oxide by ammonia-oxidizing archaea. *Biogeosciences*, *9*(7), 2419-2429.

Lotrario, J. B., Stuart, B. J., Lam, T., Arands, R. R., O'Connor, O. A., Kosson, D. S. (1995). Effects of sterilization methods on the physical characteristics of soil: implications for sorption isotherm analyses. *Bulletin of Environmental Contamination and Toxicology*, *54*(5), 668-675.

MacFarling Meure, C., Etheridge, D., Trudinger, C., Steele, P., Langenfelds, R., Van Ommen, T., ... Elkins, J. (2006). Law Dome CO_2, CH_4 and N_2O ice core records extended to 2000 years BP. *Geophysical Research Letters*, *33*(14).

Martens-Habbena, W., Berube, P. M., Urakawa, H., José, R., Stahl, D. A. (2009). Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature*, *461*(7266), 976.

Martins, C. S., Nazaries, L., Delgado-Baquerizo, M., Macdonald, C. A., Anderson, I. C., Hobbie, S. E., ... Singh, B. K. (2017). Identifying environmental drivers of greenhouse gas emissions under warming and reduced rainfall in boreal–temperate forests. *Functional Ecology*, *31*(12), 2356-2368.

McNamara, N. P., Black, H. I. J., Beresford, N. A., Parekh, N. R. (2003). Effects of acute gamma irradiation on chemical, physical and biological properties of soils. *Applied Soil Ecology*, *24*(2), 117-132.

Meinhardt, K. A., Stopnisek, N., Pannu, M. W., Strand, S. E., Fransen, S. C., Casciotti, K. L., Stahl, D. A. (2018). Ammonia-oxidizing bacteria are the primary N_2O producers in an ammonia-oxidizing archaea dominated alkaline agricultural soil. *Environmental Microbiology*, *20*(6), 2195-2206.

Mobarry, B. K., Wagner, M., Urbain, V., Rittmann, B. E., Stahl, D. A. (1996). Phylogenetic probes for analyzing abundance and spatial organization of nitrifying bacteria. *Applied and Environmental Microbiology*, *62*(6), 2156-2162.

Müller, O., Wilson, B., Paulsen, M. L., Rumińska, A., Armo, H. R., Bratbak, G., Øvreås, L. (2018). Spatiotemporal dynamics of ammonia-oxidizing thaumarchaeota in distinct arctic water masses. *Frontiers in Microbiology*, *9*, 24.

Mußmann, M., Brito, I., Pitcher, A., Damsté, J. S. S., Hatzenpichler, R., Richter, A., ... Wagner, M. (2011). Thaumarchaeotes abundant in refinery nitrifying sludges express *amoA* but are not obligate autotrophic ammonia oxidizers. *Proceedings of the National Academy of Sciences*, *108*(40), 16771-16776.

Nelson, D. W. (1977). Transformations of hydroxylamine in soils. *Proceedings of the Indiana Academy of Science 87*, 409-413.

Nevison, C. D., Weiss, R. F., Erickson III, D. J. (1995). Global oceanic emissions of nitrous oxide. *Journal of Geophysical Research: Oceans*, *100*(C8), 15809-15820.

Norton, J. M., Klotz, M. G., Stein, L. Y., Arp, D. J., Bottomley, P. J., Chain, P. S., ... Starkenburg, S. R. (2008). Complete genome sequence of *Nitrosospira multiformis*, an ammonia-oxidizing bacterium from the soil environment. *Applied and Environmental Microbiology*, *74*(11), 3559-3572.

Nowak, A., Wronkowska, H. (1987). On the efficiency of soil sterilization in autoclave. *Zentralblatt für Mikrobiologie*, *142*(7), 521-525.

Ostrom, N. E., Ostrom, P. H. (2017). Mining the isotopic complexity of nitrous oxide: a review of challenges and opportunities. *Biogeochemistry*, *132*(3), 359-372.

Pacheco, A. A., McGarry, J., Kostera, J., Corona, A. (2011). Techniques for investigating hydroxylamine disproportionation by hydroxylamine oxidoreductases. *Methods in Enzymology*, *486*(11), 447-463.

Palomo, A., Pedersen, A. G., Fowler, S. J., Dechesne, A., Sicheritz-Pontén, T., Smets, B. F. (2018). Comparative genomics sheds light on niche differentiation and the evolutionary history of comammox *Nitrospira*. *The ISME Journal*, *12*(7), 1779.

Peng, X., Fuchsman, C. A., Jayakumar, A., Oleynik, S., Martens-Habbena, W., Devol, A. H., Ward, B. B. (2015). Ammonia and nitrite oxidation in the Eastern Tropical North Pacific. *Global Biogeochemical Cycles*, *29*(12), 2034-2049.

Peng, Y., Wang, G., Li, F., Zhou, G., Yang, G., Fang, K., ... Yang, Y. (2018). Soil temperature dynamics modulate N_2O flux response to multiple nitrogen additions in an alpine steppe. *Journal of Geophysical Research: Biogeosciences*, *123*(10), 3308-3319.

Pester, M., Schleper, C., Wagner, M. (2011). The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. *Current Opinion in Microbiology*, *14*(3), 300-306.

Pjevac, P., Schauberger, C., Poghosyan, L., Herbold, C. W., Van Kessel, M. A., Daebeler, A., ... Daims, H. (2017). *AmoA*-targeted polymerase chain reaction primers for the specific detection and quantification of comammox *Nitrospira* in the environment. *Frontiers in Microbiology*, *8*, 1508.

Poth, M., Focht, D. D. (1985). ^{15}N kinetic analysis of N_2O production by *Nitrosomonas europaea*: an examination of nitrifier denitrification. *Applied and Environmental Microbiology*, *49*(5), 1134-1141.

Prather, M. J., Hsu, J., DeLuca, N. M., Jackman, C. H., Oman, L. D., Douglass, A. R., ... Isaksen, I. S. (2015). Measuring and modeling the lifetime of nitrous oxide including its variability. *Journal of Geophysical Research: Atmospheres*, *120*(11), 5693-5705.

Prosser, J. I. (2012). Ecosystem processes and interactions in a morass of diversity. *FEMS Microbiology Ecology*, *81*, 507-519.

Purkhold, U., Pommerening-Röser, A., Juretschko, S., Schmid, M. C., Koops, H. P., Wagner, M. (2000). Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: implications for molecular diversity surveys. *Applied and Environmental Microbiology*, *66*(12), 5368-5382.

Purkhold, U., Wagner, M., Timmermann, G., Pommerening-Röser, A., Koops, H. P. (2003). 16S rRNA and *amoA*-based phylogeny of 12 novel betaproteobacterial ammonia-oxidizing isolates: extension of the dataset and proposal of a new lineage within the nitrosomonads. *International Journal of Systematic and Evolutionary Microbiology*, *53*(5), 1485-1494.

Qin, W., Meinhardt, K. A., Moffett, J. W., Devol, A. H., Virginia Armbrust, E., Ingalls, A. E., Stahl, D. A. (2017). Influence of oxygen availability on the activities of ammonia-oxidizing archaea. *Environmental Microbiology Reports*, *9*(3), 250-256.

Ravishankara, A. R., Daniel, J. S., Portmann, R. W. (2009). Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. *Science*, *326*(5949), 123-125.

Rees, A. P., Brown, I. J., Jayakumar, A., Ward, B. B. (2016). The inhibition of N_2O production by ocean acidification in cold temperate and polar waters. *Deep Sea Research Part II: Topical Studies in Oceanography*, *127*, 93-101.

Rockström, J., Williams, J., Daily, G., Noble, A., Matthews, N., Gordon, L., … Smith, S. (2017). Sustainable intensification of agriculture for human prosperity and global sustainability. *Ambio*, *46*(1), 4-17.

Rotthauwe, J. H., Witzel, K. P., Liesack, W. (1997). The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Applied and Environmental Microbiology*, *63*(12), 4704-4712.

Ruser, R., Schulz, R. (2015). The effect of nitrification inhibitors on the nitrous oxide (N_2O) release from agricultural soils - a review. *Journal of Plant Nutrition and Soil Science*, *178*(2), 171-188.

Sabba, F., Terada, A., Wells, G., Smets, B. F., Nerenberg, R. (2018). Nitrous oxide emissions from biofilm processes for wastewater treatment. *Applied Microbiology and Biotechnology*, *102*(22), 9815-9829.

Santoro, A. E., Buchwald, C., McIlvin, M. R., Casciotti, K. L. (2011). Isotopic signature of N_2O produced by marine ammonia-oxidizing archaea. *Science*, *333*(6047), 1282-1285.

Santoro, A. E., Richter, R. A., Dupont, C. L. (2019). Planktonic marine archaea. *Annual Review of Marine Science*, *11*, 131-158.

Schmidt, I. (2009). Chemoorganoheterotrophic growth of *Nitrosomonas europaea* and *Nitrosomonas eutropha*. *Current Microbiology*, *59*(2), 130.

Schramm, A., Larsen, L. H., Revsbech, N. P., Ramsing, N. B., Amann, R., Schleifer, K. H. (1996). Structure and function of a nitrifying biofilm as determined by in situ hybridization and the use of microelectrodes. *Applied and Environmental Microbiology*, *62*(12), 4641-4647.

Shaw, L. J., Nicol, G. W., Smith, Z., Fear, J., Prosser, J. I., Baggs, E. M. (2006). *Nitrosospira* spp. can produce nitrous oxide via a nitrifier denitrification pathway. *Environmental Microbiology*, *8*(2), 214-222.

Shen, Q. R., Ran, W., Cao, Z. H. (2003). Mechanisms of nitrite accumulation occurring in soil nitrification. *Chemosphere*, *50*(6), 747-753.

Shen, T., Stieglmeier, M., Dai, J., Urich, T., Schleper, C. (2013). Responses of the terrestrial ammonia-oxidizing archaeon *Ca*. Nitrososphaera viennensis and the ammonia-oxidizing bacterium *Nitrosospira multiformis* to nitrification inhibitors. *FEMS Microbiology Letters*, *344*(2), 121-129.

Shi, X., Hu, H. W., Wang, J., He, J. Z., Zheng, C., Wan, X., Huang, Z. (2018). Niche separation of comammox *Nitrospira* and canonical ammonia oxidizers in an acidic subtropical forest soil under long-term nitrogen deposition. *Soil Biology and Biochemistry*, *126*, 114-122.

Smil, V. (1999). Nitrogen in crop production: An account of global flows. *Global Biogeochemical Cycles*, *13*(2), 647-662.

Smith, J. M., Casciotti, K. L., Chavez, F. P., Francis, C. A. (2014). Differential contributions of archaeal ammonia oxidizer ecotypes to nitrification in coastal surface waters. *The ISME Journal*, *8*(8), 1704.

Song, A., Liang, Y., Zeng, X., Yin, H., Xu, D., Wang, B., ... Fan, F. (2018). Substrate-driven microbial response: A novel mechanism contributes significantly to temperature sensitivity of N_2O emissions in upland arable soil. *Soil Biology and Biochemistry*, *118*, 18-26.

Soper, F. M., Sullivan, B. W., Nasto, M. K., Osborne, B. B., Bru, D., Balzotti, C. S., ... Porder, S. (2018). Remotely sensed canopy nitrogen correlates with nitrous oxide emissions in a lowland tropical rainforest. *Ecology*, *99*(9), 2080-2089.

Spang, A., Poehlein, A., Offre, P., Zumbrägel, S., Haider, S., Rychlik, N., ... Böhm, C. (2012). The genome of the ammonia-oxidizing *Candidatus* Nitrososphaera gargensis: insights into metabolic versatility and environmental adaptations. *Environmental Microbiology*, *14*(12), 3122-3145.

Stein, L. Y., Arp, D. J. (1998). Loss of ammonia monooxygenase activity in *Nitrosomonas europa*ea upon exposure to nitrite. *Applied and Environmental Microbiology*, *64*(10), 4098-4102.

Stephen, J. R., McCaig, A. E., Smith, Z., Prosser, J. I, Embley, T. M. (1996). Molecular diversity of soil and marine 16S rRNA gene sequences related to beta-subgroup ammonia-oxidizing bacteria. *Applied and Environmental Microbiology*, *62*(11), 4147-4154.

Stieglmeier, M., Alves, R. J., Schleper, C. (2014a). The phylum thaumarchaeota. In: E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt & F. Thompson (Eds.), The Prokaryotes: Other Major Lineages of Bacteria and the Archaea (pp. 347-362). Berlin, Heidelberg, Germany: Springer.

Stieglmeier, M., Mooshammer, M., Kitzler, B., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., Schleper, C. (2014b). Aerobic nitrous oxide production through N-nitrosating hybrid formation in ammonia-oxidizing archaea. *The ISME Journal*, *8*(5), 1135.

Subbarao, G. V., Arango, J., Masahiro, K., Hooper, A. M., Yoshihashi, T., ….. Iwanaga, M. (2017). Genetic mitigation strategies to tackle agricultural GHG emissions: The case for biological nitrification inhibition technology. *Plant Science*, *262*, 165-168.

Subbarao, G. V., Sahrawat, K. L., Nakahara, K., Ishikawa, T., Kishii, M., Rao, I. M., ... Bonnett, D. (2012). Biological Nitrification inhibition - A novel strategy to regulate nitrification in agricultural systems. In: D. Sparks (Ed.), Advances in Agronomy (Vol. 114, pp. 249-302). USA: Academic Press.

Syakila, A., Kroeze, C. (2011). The global nitrous oxide budget revisited. *Greenhouse Gas Measurement and Management*, *1*(1), 17-26.

Taylor, A. E., Vajrala, N., Giguere, A. T., Gitelman, A. I., Arp, D. J., Myrold, D. D., … Bottomley, P. J. (2013). Use of aliphatic n-alkynes to discriminate soil nitrification activities

of ammonia-oxidizing thaumarchaea and bacteria. *Applied and Environmental Microbiology*, *79*(21), 6544-6551.

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., … Swackhamer, D. (2001). Forecasting agriculturally driven global environmental change. *Science*, *292*(5515), 281-284.

Todt, D., Dörsch, P. (2016). Mechanism leading to N_2O production in wastewater treating biofilm systems. *Reviews in Environmental Science and Bio/Technology*, *15*(3), 355-378.

Tolar, B. B., Herrmann, J., Bargar, J. R., van den Bedem, H., Wakatsuki, S., Francis, C. A. (2017). Integrated structural biology and molecular ecology of N-cycling enzymes from ammonia-oxidizing archaea. *Environmental Microbiology Reports*, *9*(5), 484-491.

Tourna, M., Freitag, T. E., Nicol, G. W., Prosser, J. I. (2008). Growth, activity and temperature responses of ammonia oxidizing archaea and bacteria in soil microcosms. *Environmental Microbiology*, *10*, 1357-1364.

Tourna, M., Stieglmeier, M., Spang, A., Könneke, M., Schintlmeister, A., Urich, T., ... Schleper, C. (2011). *Nitrososphaera viennensis*, an ammonia oxidizing archaeon from soil. *Proceedings of the National Academy of Sciences*, *108*(20), 8420-8425.

Trimmer, M., Chronopoulou, P. M., Maanoja, S. T., Upstill-Goddard, R. C., Kitidis, V., Purdy, K. J. (2016). Nitrous oxide as a function of oxygen and archaeal gene abundance in the North Pacific. *Nature Communications*, *7*, 13451.

Vajrala, N., Martens-Habbena, W., Sayavedra-Soto, L. A., Schauer, A., Bottomley, P. J., Stahl, D. A., Arp, D. J. (2013). Hydroxylamine as an intermediate in ammonia oxidation by globally abundant marine archaea. *Proceedings of the National Academy of Sciences*, *110*(3), 1006-1011.

Van Cleemput, O., Samater, A. H. (1995). Nitrite in soils: accumulation and role in the formation of gaseous N compounds. *Fertiliser Research*, *45*(1), 81-89.

Van Kessel, M. A., Speth, D. R., Albertsen, M., Nielsen, P. H., den Camp, H. J. O., Kartal, B., ... Lücker, S. (2015). Complete nitrification by a single microorganism. *Nature*, *528*(7583), 555.

Venterea, R. T. (2007). Nitrite-driven nitrous oxide production under aerobic soil conditions: kinetics and biochemical controls. *Global Change Biology*, *13*(8), 1798-1809.

Venterea, R. T., Clough, T. J., Coulter, J. A., Breuillin-Sessoms, F., Wang, P., Sadowsky, M. J. (2015). Ammonium sorption and ammonia inhibition of nitrite-oxidizing bacteria explain contrasting soil N 2 O production. *Scientific Reports*, *5*, 12153.

Verhamme, D. T., Prosser, J. I., Nicol, G. W. (2011). Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. *The ISME Journal*, *5*(6), 1067.

von Uexküll, H. R., Mutert, E. (1995). Global extent, development and economic impact of acid soils. *Plant and Soil*, *171*, 1-15.

Walker, C. B., De La Torre, J. R., Klotz, M. G., Urakawa, H., Pinel, N., Arp, D. J., ... Hemp, J. (2010). *Nitrosopumilus maritimus* genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. *Proceedings of the National Academy of Sciences*, *107*(19), 8818-8823.

Wang, Q., Zhang, L. M., Shen, J. P., Du, S., Han, L. L., He, J. Z. (2016). Nitrogen fertiliser-induced changes in N_2O emissions are attributed more to ammonia-oxidising bacteria rather than archaea as revealed using 1-octyne and acetylene inhibitors in two arable soils. *Biology and Fertility of Soils*, *52*(8), 1163-1171.

Wang, Z., Cao, Y., Zhu-Barker, X., Nicol, G. W., Wright, A. L., Jia, Z., Jiang, X. (2019). Comammox *Nitrospira* clade B contributes to nitrification in soil. *Soil Biology and Biochemistry*, *135*, 392-395.

Ward, B. B. (2011). Nitrification in the Ocean. In: B. B. Ward, M. G. Klotz & D. J. Arp DJ. (Eds.), Nitrification (pp. 325-345). USA: American Society for Microbiology Press.

Wenk, C. B., Frame, C. H., Koba, K., Casciotti, K. L., Veronesi, M., Niemann, H., ... Zopfi, J. (2016). Differential N_2O dynamics in two oxygen-deficient lake basins revealed by stable isotope and isotopomer distributions. *Limnology and Oceanography*, *61*(5), 1735-1749.

Whittaker, M., Bergmann, D., Arciero, D., Hooper, A. B. (2000). Electron transfer during the oxidation of ammonia by the chemolithotrophic bacterium *Nitrosomonas europaea*. *Biochimica et Biophysica Acta - Bioenergetics*, *1459*(2-3), 346-355.

Wrage-Mönnig, N., Horn, M. A., Well, R., Müller, C., Velthof, G., Oenema, O. (2018). The role of nitrifier denitrification in the production of nitrous oxide revisited. *Soil Biology and Biochemistry*, *123*, A3-A16.

Yan, J., Haaijer, S. C., Op den Camp, H. J., van Niftrik, L., Stahl, D. A., Könneke, M., ... Jetten, M. S. (2012). Mimicking the oxygen minimum zones: stimulating interaction of aerobic archaeal and anaerobic bacterial ammonia oxidizers in a laboratory-scale model system. *Environmental Microbiology*, *14*(12), 3146-3158.

Yin, Z., Bi, X., Xu, C. (2018). Ammonia-oxidizing archaea (AOA) play with ammonia-oxidizing bacteria (AOB) in nitrogen removal from wastewater. *Archaea*, *2018*.

Zhao, J., Bello, M. O., Meng, Y., Prosser, J. I., Gubry-Rangin, C. Selective inhibition of ammonia oxidising archaea by simvastatin stimulates the growth of ammonia oxidising bacteria. *Soil Biology and Biochemistry* (in revision).

Zheng, M., Wang, M., Zhao, Z., Zhou, N., He, S., Liu, S., ... Wang, X. (2019). Transcriptional activity and diversity of comammox bacteria as a previously overlooked ammonia oxidizing prokaryote in full-scale wastewater treatment plants. *Science of the Total Environment*, *656*, 717-722.

Zhu-Barker, X., Cavazos, A. R., Ostrom, N. E., Horwath, W. R., Glass, J. B. (2015). The importance of abiotic reactions for nitrous oxide production. *Biogeochemistry*, *126*(3), 251-267.

Zhu, X., Burger, M., Doane, T. A., Horwath, W. R. (2013). Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. *Proceedings of the National Academy of Sciences*, *110*(16), 6328-6333.

Figure legends

Figure 1. A schematic representation of changes in AO communities and directly AO-associated N_2O emissions during incubation of soil after addition of single application of high inorganic NH_4^+-based fertiliser or with slow release of NH_4^+ from soil organic nitrogen or a slow release fertiliser. The initial AO community, prior to incubation, is dominated by AOA, which are generally assumed to be smaller than AOB.

Figure 2. Changes in (a) NH_4^+ and (b) NO_3^- concentrations, (c) AOA and AOB *amoA gene* abundances and (d) N_2O emissions from soil microcosms incubated after addition of water (green triangle, blue cross) or under conditions of high NH_4^+ supply (0 – 10 days) or low NH_4^+ supply (10 – 25 days) (black circle, red square). Ammonium was supplied as a slow-release, urea-based fertiliser that contained residual free urea. (Urea was converted rapidly to NH_4^+.) This generated high initial concentrations of available NH_4^+, which was oxidised within 10 days. Thereafter, NH_4^+ was generated by mineralisation of native organic nitrogen or of the slow-release fertiliser. In addition, microcosms were incubated with (black circle, green triangle) or without (red square, blue cross) 1-octyne, a specific inhibitor of AOB.

AO growth in the absence of fertiliser (green triangle, blue cross) was due to mineralisation of native organic nitrogen. Under these conditions, AOB growth was not detectable, AOA grew slowly and N_2O emissions were low and unaffected by the 1-octyne. Initial high inorganic NH_4^+ after fertiliser addition (1 – 10 days) resulted in greater growth of AOB, increases in the AOB:AOA ratio and substantial N_2O emissions that were significantly reduced, almost to those of control microcosms, in the presence of 1-octyne. During slow release of NH_4^+ (10 – 25 days), growth was dominated by AOA, N_2O emissions were significantly reduced and

emissions were much less affected by the AOB inhibitor, 1-octyne. Note, however, that AOA growth was stimulated to some extent when AOB were inhibited. (See Hink et al. 2018 for experimental details and further discussion of results.)

Figure 1

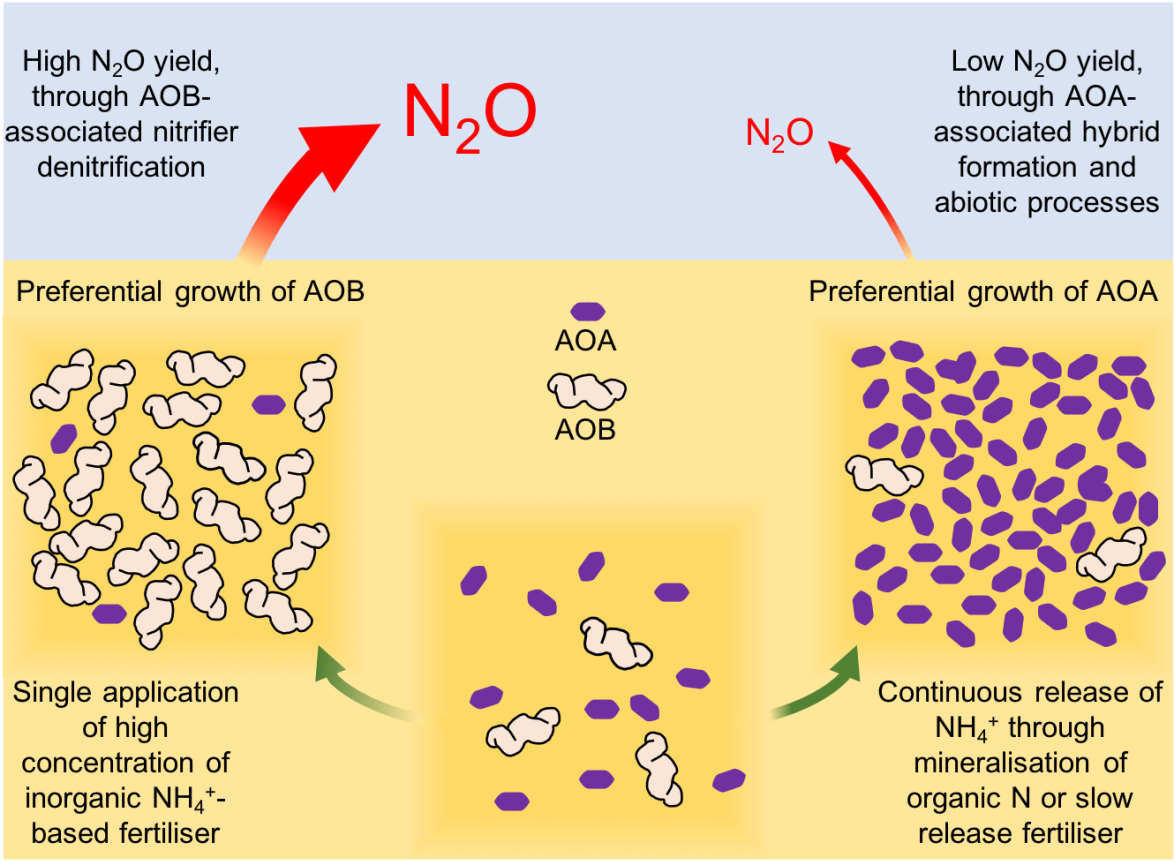
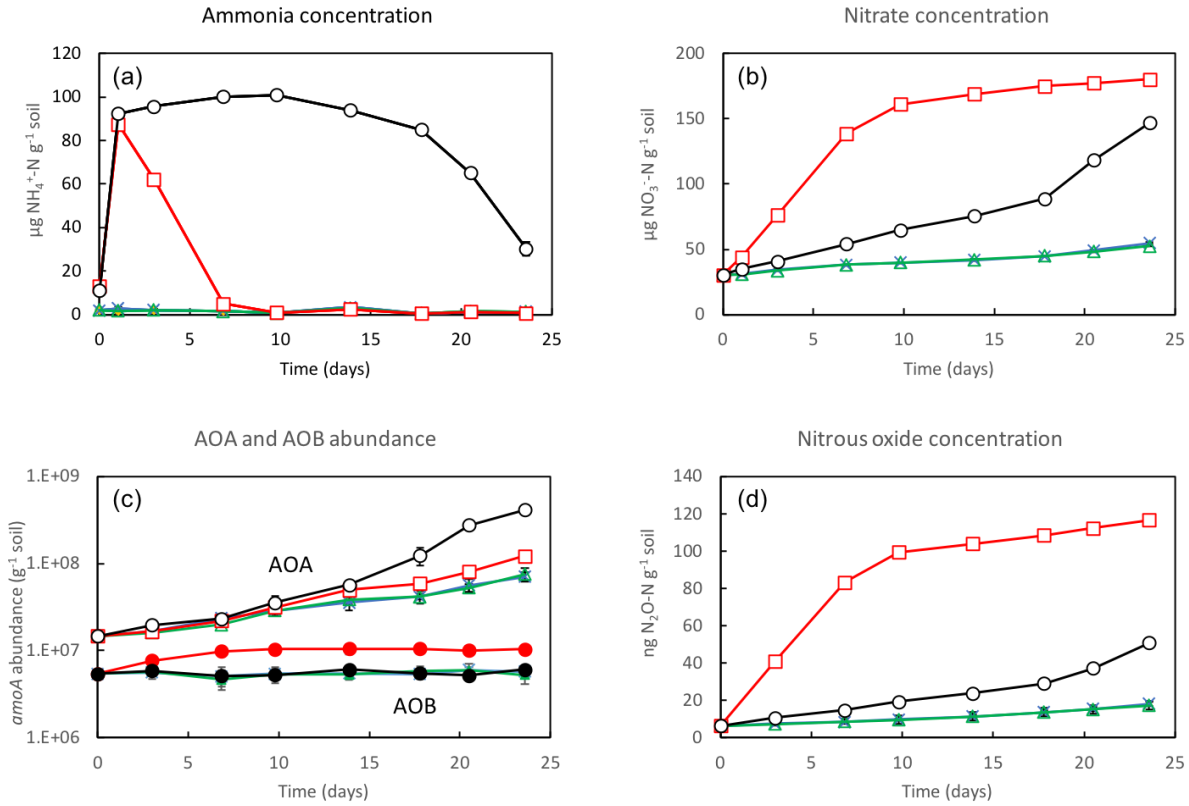

High N_2O yield, through AOB-associated nitrifier denitrification
N_2O
N_2O
Low N_2O yield, through AOA-associated hybrid formation and abiotic processes
Preferential growth of AOB
AOA
AOB
Preferential growth of AOA
Single application of high concentration of inorganic NH_4^+-based fertiliser
Continuous release of NH_4^+ through mineralisation of organic N or slow release fertiliser

Figure 2


Ammonia concentration
(a)
µg NH4+-N g-1 soil
Time (days)
Nitrate concentration
(b)
µg NO3--N g-1 soil
Time (days)
AOA and AOB abundance
(c)
AOA
AOB
amoA abundance (g-1 soil)
Time (days)
Nitrous oxide concentration
(d)
ng N2O-N g-1 soil
Time (days)