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Review Paper

Nitrification and nitrifiers in acidic soils

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ABSTRACT

Nitrification, as a crucial step in nitrogen cycling and plant nutrition, is a biologically mediated process responsible for enormous losses of nitrogen fertilizer and a contributor to environmental pollution. The recent progress in our understanding of nitrification and nitrifiers, specifically in acidic soils, is discussed and reviewed. At one time it was assumed that rates of nitrification are relatively low in acidic soils. However, more recent studies have demonstrated nitrification down to pH 3.0 and that the rate of nitrification can equal, or even exceed, that found in neutral soils. Studies on acidic forest soils in Europe noted that they have a high potential for nitrate production. Furthermore, using the ^{15}N isotope-dilution technique it was shown that net nitrification measurements can markedly underestimate gross nitrification in these natural and highly organic systems. Using selective inhibitors it has been demonstrated that heterotrophic nitrifiers can contribute to nitrification. While heterotrophic nitrification can be performed by a wide range of bacteria and fungi, inhibitor studies point to fungi to be mainly responsible. Autotrophic ammonia-oxidizing bacteria (AOB), such as *Nitrosomonas* and *Nitrosospira*, have been known for some considerable time but have generally found to be inactive in acidic conditions. The discovery of ammonia monooxygenase in uncultured archaea that were functionally active at low pH pointed to an autotrophic microbial group (ammonia oxidizing archaea, AOA) that might be adapted to low substrate (ammonia) concentrations and responsible for nitrification in the wider range of acidic grassland and cultivated soils. Obligately acidophilic AOA have more recently been cultivated while stable isotope probing has been used to confirm the dominance of AOA over AOB in acidic soils. Detailed molecular studies using both 16*S* rRNA and *amo*A (ammonia monooxygenase sub-unit A) gene sequencing are continuing to expand our appreciation of the diversity of both AOB and AOA and how this varies over different pH ranges and in different ecosystems. Similar work is being directed towards nitrite oxidizing bacteria (NOB) but to date we do not fully know the role of pH in controlling NOB activity. Such understanding of nitrification and nitrifiers will help develop new effective nitrification inhibitors and aid the management of nitrogen cycling in acidic soils.

1. Introduction

Acidic soils (defined as pH < 5.5) occupy 30% of the world's ice-free land and mainly support forest, woodland and grassland, with a minor fraction used for arable crops (Vonuexkull and Mutert, 1995). Nitrification is a crucial step in nitrogen biogeochemical cycling and plant nutrition in soil-plant ecosystems. Nitrification in soil is generally considered to be a two-step process where ammonia is first oxidized to nitrite by ammonia oxidizers, and subsequently to nitrate by nitrite-oxidizing bacteria (NOB). However, the recent discovery of some species of *Nitrospira* capable of complete ammonia oxidation (comammox) in water systems (Daims et al., 2015; van Kessel et al., 2015) and detection in soil (Pjevac et al., 2017) indicates that this process could also be relevant. Nitrification can lead to nitrate leaching, losses of nitrogen-based fertilizers and the increased emission of the greenhouse gas nitrous oxide (Wrage et al., 2001). In particular, nitrification in acidic soils can lead to further acidification and aluminum toxicity (He et al., 2012). It was previously assumed that nitrification was relatively low in acidic soils since the availability of the substrate ammonia (NH_3) for the ammonia monoxygenase (AMO) enzyme of ammonia oxidizers would be limited and all isolated bacterial ammonia oxidizers did not grow in standard laboratory medium with a pH < 5.5 (De Boer and

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Kowalchuk, 2001). However, many recent studies have suggested that nitrification can occur at pH values as low as 3.0 (Norton and Stark, 2011) and that rates of nitrification based on mineralized organic nitrogen can be equal or even greater than that typically found in neutral pH soils (Booth et al., 2005).

There are probably three breakthrough discoveries that have promoted scientific interest and discussion on nitrification mechanisms in acidic soils. Firstly, in the 1980s, acidic forest soils in northwestern Europe were found to have a high potential for nitrate production (Vanbreemen and Vandijk, 1988) and subsequently resulted in increased public concern regarding the potentially damaging effects of nitrate leaching and pollution. Secondly, Stark and Hart (1997) used the ^{15}N isotope-dilution technique in intact soil cores to measure gross nitrification and microbial assimilation in a large number of acidic forest soils. They demonstrated that standard measurements of net nitrification could be significantly lower than gross nitrification rates determined using ^{15}N-based measurements and that microbial communities play an important role in promoting nitrate loss in acidic soil ecosystems. Thirdly, homologues of bacterial AMO-encoding genes were found in metagenomic fragments of uncultured archaea (Schleper et al., 2005; Venter et al., 2004) followed by the isolation of *Nitrosopumilus maritimus* (Könneke et al., 2005), confirming the potential for ammonia oxidation by organisms belonging to the (then-described) mesophilic crenarchaeota, and subsequently termed ammonia-oxidizing archaea (AOA). These organisms were found to be functionally important in soils and sediments (Leininger et al., 2006; Schleper and Nicol, 2010), especially in acidic soil ecosystems (Prosser and Nicol, 2008; Yao et al., 2011b). The discovery of obligately acidophilic AOA in these soils indicated that they possess specific adaptations allowing them to grow at low pH (Lehtovirta-Morley et al., 2011, 2014).

Since the turn of the century there has been a continual increase in the number of studies examining nitrification and nitrifiers in acidic soils. The majority of studies have focused on the distinct ecological niches of ammonia oxidizing bacteria and archaea, their relative importance to autotrophic nitrification and their environmental drivers (Erguder et al., 2009; Hu et al., 2014; Yao et al., 2013; Zhalnina et al., 2012). Some studies report on the methodology for the measurement of nitrification, the functioning of isolated microorganisms and the mechanisms responsible for nitrification.

2. Methodology of measuring nitrification and nitrifiers

2.1. Detection of nitrification rates

Accurate determination of nitrification rates is essential to understanding nitrogen-cycling processes. Nitrification rates have been measured using a wide variety of methods, such as the laboratory incubation of soils (e.g. in potential nitrification assays), the use of ^{15}N tracers to determine changes in pools of ammonium and nitrate, and the use of inhibitors to differentiate the relative contribution of different microbial groups to ammonia oxidation (Hart et al., 1994b; Liu et al., 2015a; Stark and Hart, 1997).

Generally, nitrification rates can be described by three terms: net, gross, and potential nitrification. The laboratory incubation method, which is the simplest and most frequently used, measures net nitrification where nitrate accumulation is measured at the end of an incubation period. Net nitrification is calculated by subtracting initial NO_3^- concentration from final NO_3^- concentration (Hart et al., 1994a). Soil pH and ammonium supply are the two main environmental factors that influence nitrification (Hanan et al., 2016; Kemmitt et al., 2006); however, substrate addition generally has a lower impact on net nitrification compared to pH (Ste-Marie and Paré, 1999; Yao et al., 2011a).

Nitrification potential is a method that was first developed as an approach to estimate the biomass of nitrifiers in soil. It aims to determine the maximum capacity of nitrifiers to transform ammonium into nitrate under assumed optimal conditions (or to nitrite when chlorate is used to inhibit NOB). The shaken soil-slurry method (Hart et al., 1994b) is a widely used approach to determine nitrification potential where buffered liquid medium (pH 7.2) is typically used, assuming that all ammonia oxidizers grow optimally in a pH-neutral environment (Belser, 1979; De Boer et al., 1996), even including those that have been isolated from acidic soils (Allison and Prosser, 1993; De Boer et al., 1991; Jiang and Bakken, 1999). However, this approach requires a number of assumptions including that, irrespective of taxonomic diversity, all ammonia oxidizers present in a soil sample grow at similar rates, have similar affinities for ammonia and are inhibited by (or tolerant to) similar concentrations of ammonia. However, current knowledge of ammonia oxidizer physiology demonstrates that there is considerable diversity between different populations. For example, some species of AOA are inhibited at the ammonia concentrations typically used in potential assays (Martens-Habbena et al., 2009) and AOB often outcompete AOA for added inorganic ammonia (Hink et al., 2016), subsequently underestimating or ignoring their contribution to activity in soil. Some recent studies have indicated that the neutral buffer solution can decrease the activities of nitrifiers in acidic soils and in fact ammonia oxidation in these soils can be dominated by organisms that are obligate acidophiles when isolated from soil. The traditional method of using a neutral pH medium may not therefore reflect the maximum nitrification capacity (Xue et al., 2009; Yao et al., 2011a). Xue et al. (2009) evaluated two different methods (with and without neutral buffer solution) to measure nitrification potentials in Chinese tea plantation soils and suggested that the shaken slurry method without phosphate buffer was the better choice for analyzing nitrification potential in these highly acidic soils.

Changes in nitrate pool concentrations do not always reflect actual gross nitrification rates, since nitrate can be transformed by microbial immobilization and denitrification (Norton and Stark, 2011). Therefore, in conditions where a stoichiometric conversion of ammonia to nitrate is not observed, the ^{15}N isotope dilution technique is a more appropriate approach to evaluate gross nitrification. Davidson et al. (1992) used this technique to investigate gross N transformation processes in acidic forest soils, with gross nitrification rates being much greater than observed net nitrification rates. Stark and Hart (1997) also demonstrated that > 50% of nitrification-derived nitrate can be immobilized and low nitrate concentrations in studied coniferous forest soils were not due to the suppression of nitrification but a tight coupling of nitrification and microbial nitrate assimilation.

With the use of selective inhibitors and stable isotope labeling techniques, it is possible to distinguish between autotrophic and heterotrophic nitrification, which are driven by chemolithotrophic and chemo-organotrophic microorganisms, respectively. Acetylene is the most commonly used specific inhibitor of autotrophic ammonia oxidation (Hynes and Knowles, 1982). Low concentrations of acetylene (often 1–10 Pa) are typically used, with acetylene covalently, and irreversibly, binding to AMO. Liu et al. (2015a) used the acetylene inhibition technique to determine the relative importance of heterotrophic and autotrophic processes and concluded that heterotrophic nitrification was the main nitrate production pathway in highly organic acidic soils. Other inhibitors such as dicyandiamide (DCD) and nitrapyrin (2-chloro-6-(trichloromethyl) pyridine) have been used in both laboratory studies and in agriculture and may inhibit ammonia oxidation by chelating copper (Subbarao et al., 2006). Fisk et al. (2015), for example, examining the inhibition potential of nitrapyrin, found that nitrapyrin increased soil ammonium retention and decreased autotrophic nitrification in agricultural soils.

The use of the combined $^{15}NH_4$ and $^{15}NO_3$ dilution method has been suggested as an effective way of distinguishing heterotrophic and autotrophic pathways (Barraclough and Puri, 1995). Dilution of added $^{15}NO_3$ indicates nitrification by combined heterotrophic and autotrophic pathways with dilution of added $^{15}NH_4$ in a parallel experiment indicating gross mineralization. Simulation modelling over time then