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Review paper

# Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems

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ABSTRACT

The understanding of nitrogen (N) cycling in forest ecosystems has undergone a major shift in the past decade as molecular methods are being used to link microorganisms to key processes in soil. The analysis of the abundance and community structure of functional genes involved in the biogeochemical cycling of N in forest soils offers an approach to directly link microbial groups to soil characteristics and ecosystem processes. The majority of N entering ecosystems is biologically-derived from fixation of atmospheric $N_2$. Molecular studies of N-fixation use the nitrogenase reductase (*nifH*) marker gene, and can be used to link N-fixation to other N- and C-cycling processes. Inorganic N entering soil via N-fixation, fertilization and deposition can have several fates, depending on the soil environment and the microbial community. The loss of N from forests stands subject to fertilization and atmospheric deposition is of increasing interest as the outputs of nitrate ($NO_3^-$) and nitrous oxide ($N_2O$) are implicated in ground water pollution and climate change, respectively. Ammonia-oxidizing bacteria (AOB) and archaea (AOA) oxidize ammonia ($NH_3$) to $NO_3^-$ as the first step of nitrification and are studied using the ammonium monooxygenase (*amoA*) marker. The abundance and community structure of ammonia-oxidizers is largely dependent on pH and availability of reactive N forms, and can change rapidly following N addition or after fire. These organisms can also release $N_2O$ during nitrifier denitrification or through linked nitrification–denitrification. In some forest soils, $N_2O$ emissions are correlated with genes in the denitrification pathway (*napA*, *narG*, *nirK*, *nirS*, *nosZ*) making these genes useful indicators of greenhouse gas (GHG) flux potential. A review of this topic is timely as there is currently much concern regarding the effect of N fertilization and deposition on North American and European forests due to the potential alteration of dissimilative N-cycling processes and the potential for increased $N_2O$ emissions in forest stands.



## 1. Introduction

The microbial cycling of nutrients affects many ecological properties of forests including tree growth, productivity, soil carbon (C) sequestration and greenhouse gas (GHG) emissions. Nitrogen (N) availability is often the limiting factor in terrestrial ecosystem productivity (Vitousek and Howarth, 1991; LeBauer and Tresender, 2008), including forest soils in western North America (Hooper and Johnson, 1999). The limit on N availability in forest soils is a result of the lack of inputs, rapid immobilization and removal by leaching and gaseous emission (Vitousek et al., 1997, 2002). However, anthropogenic N inputs to terrestrial ecosystems through fertilization and atmospheric deposition can remove these limitations, increasing reactive N availability and N loss from the soil. Nitrogen fertilization is used in forests to increase aboveground biomass production and shorten rotation times, and can enhance belowground C sequestration (Brockley and Simpson, 2004; Grayston, 2007; Van Miegroet and Jandl, 2007). Alterations to the net addition of N in forests soils are likely to have reverberating effects on the function of the soil community, including rates of decomposition (Janssens et al., 2010), N mineralization (Wallenstien et al., 2006a,b,c) and the abundance and activity of nitrifying and denitrying microorganisms (Wallenstien et al., 2006a,b,c; Hallin et al., 2009). Quantification and characterization of microbial functional genes in the N-fixation, nitrification and denitrification pathways can help create informative models of N cycling process rates, reactive N availability and $N_2O$ emissions from soil, providing predictions and mitigation strategies for GHG emissions (Bothe et al., 2000; Richardson et al., 2009; Morales et al., 2010).

The cycling of N in soil can be subdivided into (i) decomposition processes, (ii) assimilative processes and (iii) dissimilative processes (Fig. 1). Decompostion processes include high

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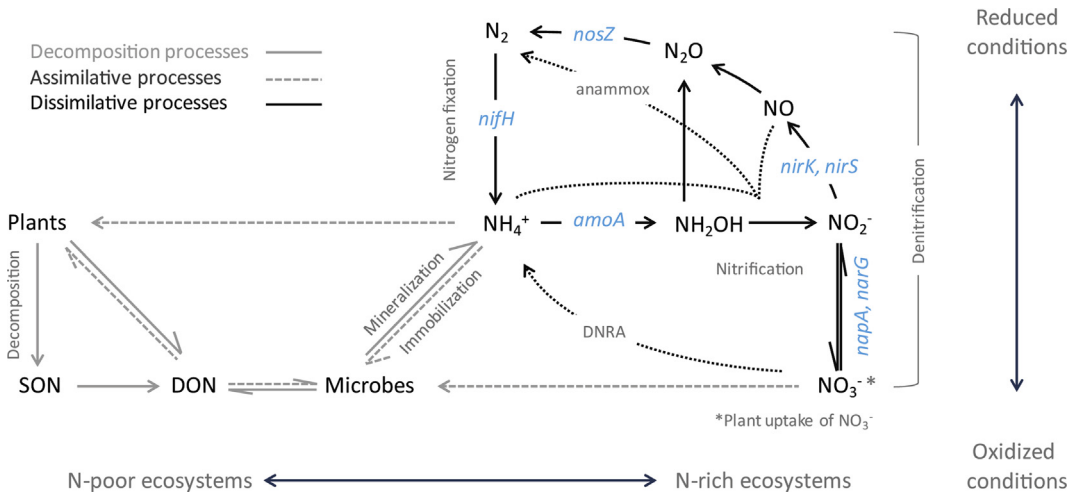


**Fig. 1.** The nitrogen (N) cycle in forest soil. The cycling of N in soil can be subdivided into (i) decomposition processes, (ii) assimilative processes and (iii) dissimilative processes. Anammox, anaerobic ammonia oxidation; DNRA, dissimilatory nitrate reduction to ammonium.

molecular-weight soil organic N (SON) released during decomposition of plant litter, which can be further degraded to low molecular weight dissolved organic N (DON) (Jones et al., 2004). Assimilative processes include the uptake and utilization of DON, $NH_4^+$, or $NO_3^-$ by plants and microorganisms for growth and replication. Dissimilative processes, which are the focus of this review, include the oxidiation of $NH_3$ for the generation of reducing equivalents ($NADPH^+$) or the use of oxidized N products as electron acceptors during facultatively anaerobic respiration by denitrifying microorganisms. Dissimilative process rates are likely to be highest in N-rich ecosystems. Denitrification proceeds stepwise as soil redox potential decreases. Two additional dissimilative processes that will not be examined in this review are dissimilatory nitrate reduction to ammonium (DNRA) and the anaerobic oxidation of ammonium (anammox). DNRA has been measured in tropical forest soil (Silver et al., 2001) and in paddy soil (Yin et al., 2002), though DNRA is not expected to be a major source of $NO_3^-$ loss in non-flooded soil (Silver et al., 2001). The anammox bacteria are able to combine both oxidized and reduced inorganic N compounds to produce $N_2$ (Strous et al., 2006). Common in marine environments (Kuenen, 2008), anammox bacterial 16S rRNA has been detected in flooded terrestrial environments (Humbert et al., 2010, 2012; Zhu et al., 2011). Long et al. (2013) have used the hydrazine oxidase (*hzo*) gene as a functional marker for the quantification of anammox bacteria in fertilized agricultural soil, though the role of these organisms in $N_2$ loss from non-flooded soil has yet to be resolved. Nitrification and denitrification are linked to the loss of N from forest soil through the leaching of nitrate and the emission of NO, $N_2O$ and $N_2$.

Soils are the source of about 70% of the $N_2O$ emitted to the atmosphere (Conrad, 1996). Forest soil $N_2O$ emissions are substantially less than those from industrial or agricultural sources, but are increasing due to fertilization (Grayston, 2007; Smethurst, 2010) and atmospheric deposition (Gundersen et al., 2012). At about 314 ppb, the concentration of $N_2O$ in the atmosphere is minute, although the gas has a global warming potential (GWP) 296 times that of $CO_2$ over a 100- year period (IPCC, 2007). $N_2O$ is also an important ozone-depleting molecule (Ravishankara et al., 2009). Forest soil can either be a source or sink of $N_2O$ depending on the activity and structure of the nitrifier and denitrifier communities (Matson et al., 1992; Chapuis-Lardy et al., 2007; Dalal and Allen, 2008; Jassal et al., 2010).

Studies of N cycling processes and $N_2O$ emissions in forest soils following fertilization demonstrate inconclusive or contradictory results (Grayston, 2007). For example, $N_2O$ emissions can be negligible (Pang and Cho, 1984; Wallenstein et al., 2006a,b; Basiliko et al., 2009; Gundersen et al., 2012) or pronounced (Brumme and Beese, 1992; Sitaula et al., 1995), and in notable cases can account for up to 5% loss of applied N following fertilization (Jassal et al., 2008). Temperate forest soils can also act as sinks for $N_2O$, in both very wet (Chapuis-Lardy et al., 2007; Gundersen et al., 2012) and aerated soil (Chapuis-Lardy et al., 2007; Goldberg and Gebauer, 2009). In order to explain apparently contradictory responses to forest fertilization these studies could benefit from assessing the microbial community responsible for N transformations by targeting process-specific functional genes. We aim to demonstrate in this review how some of these uncertainties can be resolved with an understanding of microbial functional gene dynamics.

The N-fixing, nitrifier and denitrifier communities will be the focus of this review due to their importance for N availability and loss in forest ecosystems and their ability to be studied using microbial functional genes. We pay particular attention to dissimilatory processes that drive $N_2O$ emissions from forest soil. This review will describe recent advances in the use of molecular methods to relate functional gene diversity and abundance to activity of microorganisms primarily to dissimilative N cycling processes in forest soil ecosystems, with a focus on forest stand fertilization and $N_2O$ emissions. Although several excellent reviews of the molecular biology of N-cycling microorganisms in soil exist (e.g., Bothe et al., 2000; Wallenstien et al., 2006c; Hayatsu et al., 2008), there is a lack of synthesis of the role of microbial functional genes in elucidating the key players in the N cycle in forest soil. This review will focus on temperate and boreal forest ecosystems of North America and Europe where the majority of research on functional gene communities has been undertaken. Studies that link soil characteristics and N cycling dynamics to functional gene abundance and diversity can be used to identify key factors to assess the functioning of forest ecosystems, incorporate microbial dynamics into biogeochemical models, improve soil management and mitigate N loss from forest soil.

## 2. Molecular analysis of microorganisms in forest soil

One gram of soil can contain up to about $10^9$ microbial cells (Gans et al., 2005; Roesch et al., 2007). Approximations of the