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#### Authors

Adair, Karen  
Blazewicz, Steven J  
Hungate, Bruce A  
[et al.](#)

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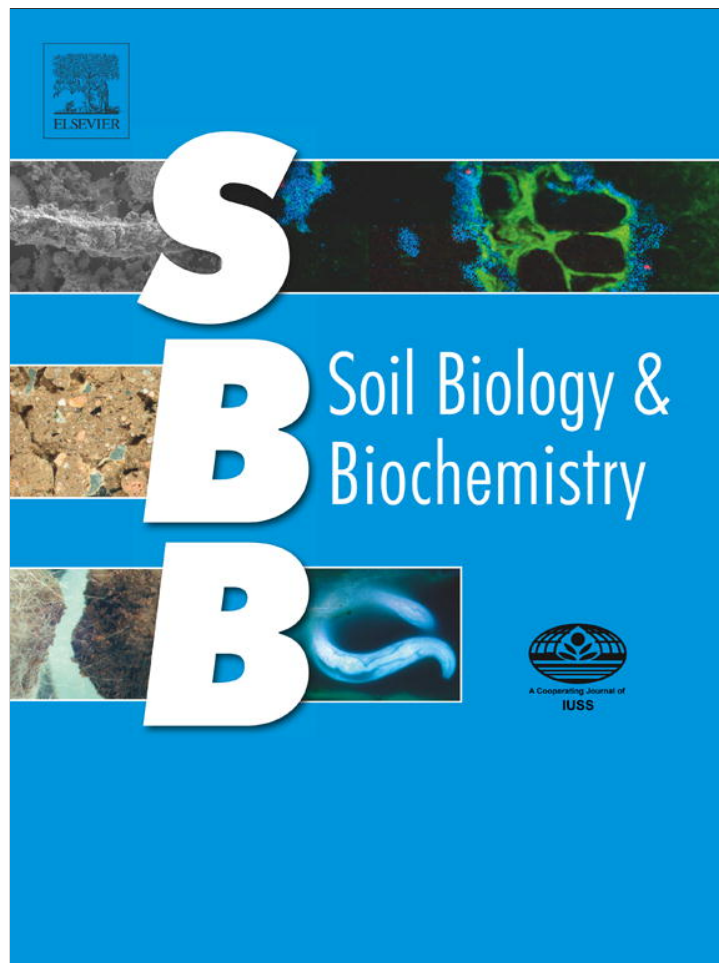
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## Short communication

A positive relationship between the abundance of ammonia oxidizing archaea and natural abundance  $\delta^{15}\text{N}$  of ecosystemsKaren Adair<sup>a,1</sup>, Steven J. Blazewicz<sup>a,2</sup>, Bruce A. Hungate<sup>a,b</sup>, Stephen C. Hart<sup>c,3</sup>, Paul Dijkstra<sup>a,b</sup>, Egbert Schwartz<sup>a,\*</sup><sup>a</sup> Department of Biological Sciences, Northern Arizona University, P.O. Box 5640, Flagstaff, AZ 86011, USA<sup>b</sup> Ecosystem Science & Society Center, Northern Arizona University, P.O. Box 5640, Flagstaff, AZ 86011, USA<sup>c</sup> School of Forestry, Northern Arizona University, P.O. Box 5018, Flagstaff, AZ 86011, USA

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## ABSTRACT

We present a significant relationship between the natural abundance isotopic composition of ecosystem pools and the abundance of a microbial gene. Natural abundance  $^{15}\text{N}$  of soils and soil DNA were analysed and compared with archaeal ammonia oxidizer abundance along an elevation gradient in northern Arizona and along a substrate age gradient in Hawai'i. There was a significant positive correlation between the abundance of archaeal *amoA* genes and natural abundance  $\delta^{15}\text{N}$  of total soil or DNA suggesting that ammonia oxidizing archaea play an important role in ecosystem N release.

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Nitrogen (N) release by ecosystems has important consequences for primary productivity, ground water pollution and greenhouse gas release from soil (Fenn et al., 1998). Soil N pools, in general, are enriched in  $^{15}\text{N}$  relative to the atmosphere (Högberg, 1990). During N mineralization, nitrification, and denitrification, the lighter isotope is preferentially released to form leachates or gaseous products so that ecosystem pools are enriched in  $^{15}\text{N}$  when large quantities of N are lost (Houlton et al., 2006; Templer et al., 2007). All three of these N transformations may occur concurrently in soils with high N availability, and there is often a positive relationship between nitrification and N availability in soil (Hart et al., 1994).

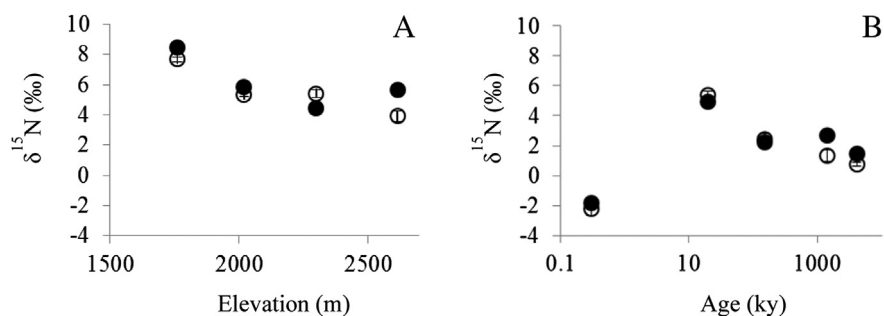
Ammonia oxidation is the rate limiting step in aerobic nitrification and is performed by two groups of organisms: ammonia oxidizing bacteria (AOB) in the  $\gamma$  and  $\beta$  subclass of Proteobacteria

and ammonia oxidizing archaea (AOA) or Thaumarchaea (Francis et al., 2005; Pester et al., 2011). The *amoA* gene, of which there is one gene copy per genome of ammonia oxidizing archaea characterized to date, can be targeted in quantitative PCR (qPCR) to measure ammonia oxidizer abundances (Adair and Schwartz, 2008). These analyses often show AOA are more abundant in non-fertilized soils than AOB (Leininger et al., 2006). For example, in nutrient limited arid soils from northern Arizona, USA, AOA were detected at levels that were consistently higher than those of AOB, often times by over two orders of magnitude (Adair and Schwartz, 2008). Similarly, along the Hawaiian Long Substrate Age Gradient only AOA were observed. We could not test if there was a positive correlation between AOB and ecosystem  $^{15}\text{N}$  enrichment because we could not detect AOB consistently in Hawaiian soils.

Previous studies have exploited natural abundance stable isotopic composition of nutrient pools (Högberg, 1990) to characterize N cycling, including nitrification, in ecosystems. In marine studies,  $\text{N}_2\text{O}$  produced by AOA had bulk  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values higher than observed for AOB but similar to that of the oceanic  $\text{N}_2\text{O}$  source to the atmosphere indicating AOA were an important source of  $\text{N}_2\text{O}$  released from oceans (Santoro et al., 2011). In studies of the central California current, natural abundance stable isotope ratios ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) in nitrate and nitrous oxide were used to calculate

\* Corresponding author. Tel.: +1 928 523 6168; fax: +1 928 523 7500.

E-mail address: [Egbert.Schwartz@nau.edu](mailto:Egbert.Schwartz@nau.edu) (E. Schwartz).<sup>1</sup> Present address: Bio-Protection Research Centre, PO Box 84, Lincoln University 7647, Canterbury, New Zealand.<sup>2</sup> Present address: United States Geological Survey, 345 Middlefield Rd., Menlo Park, MS 962, CA 94025, USA.<sup>3</sup> Present address: School of Natural Sciences & Sierra Nevada Research Institute, University of California, Merced, 5200 North Lake Rd., Merced, CA 95343, USA.



**Fig. 1.** Natural abundance  $\delta^{15}\text{N}$  of DNA (○) and total soil (●) along the elevation gradient in northern Arizona (A) and the Long Substrate Age Gradient in Hawai'i (B). Error bars represent standard errors of the mean. Where error bars are not apparent, the error bar is smaller than the data symbol.

nitrification rates (Santoro et al., 2010). However, these studies did not document a relationship between gene abundance and natural abundance stable isotope composition. We postulated that, in soils where Archaea are often the dominant ammonia oxidizers,  $^{15}\text{N}$  composition of soil total N pool would increase with higher ammonium oxidizing activity and greater AOA gene abundance, reflecting the role of ammonia oxidation in isotopic fractionation during ecosystem N loss.

We took three replicate soil samples from the top 10 cm of mineral soil from four sites along an elevation gradient in northern Arizona, USA, and five sites along the Long Substrate Age Gradient (LSAG) in Hawai'i, USA (Vitousek, 2004). The Arizonan gradient ranges from 1750 to 2640 m in elevation and each site supports a distinct plant community. The plant communities along the LSAG are similar to each other, but age of the soils ranges from 300 to 4.1 million years and there are large differences in the N cycle among the sites (Dijkstra et al., 2008). The sites in Arizona and Hawaii are described in more detail in Schwartz et al. (2007).

The samples were stored at  $-45\text{ }^{\circ}\text{C}$  until DNA was extracted from fifty grams of each replicate. The DNA was purified along a cesium chloride gradient produced through ultracentrifugation resulting in a very pure extract suitable for IRMS and qPCR analysis (Schwartz et al., 2007). The samples were spun at high g forces (267,000 rcf) so that AT rich genomes did not separate from GC rich genomes and the entire DNA band was subsequently harvested. Natural abundance  $\delta^{15}\text{N}$  of DNA and total soil were measured on a Carlo Erba NC 2100 Elemental Analyzer (CE Instruments, Milan, Italy) interfaced with a Thermo-Finnigan Delta Plus XL (Thermo-Electron Corp., Bremen, Germany) isotope ratio mass spectrometer at the Colorado Plateau Stable Isotope Laboratory (<http://www.isotope.nau.edu/>).

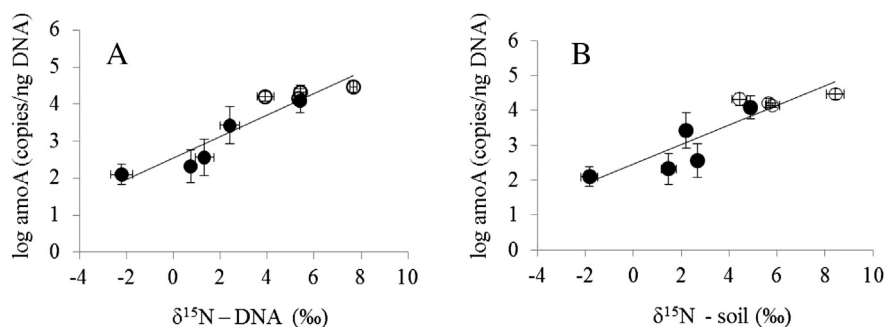
The abundance of archaeal *amoA* genes was quantified in soil DNA extractions through qPCR as described in Adair and Schwartz

(2008). The relationship between the abundance of *amoA* copies and  $\delta^{15}\text{N}$  of DNA or total soil N was analysed through linear regression performed with JMP software (SAS Institute, Cary, NC).

In Arizona,  $\delta^{15}\text{N}$  of soil and soil DNA declined with increasing elevation (Fig. 1A), while  $\delta^{15}\text{N}$  of Hawaiian soil DNA increased along the younger sites but declined with substrate age (Fig. 1B). Combining data from Arizonan and Hawaiian sites, there was a significant positive correlation ( $R^2 = 0.886$ ,  $p < 0.001$ ) between the number of archaeal *amoA* genes per  $\mu\text{g}$  DNA and  $\delta^{15}\text{N}$  of soil DNA (Fig. 2A). There was also a significant positive correlation ( $R^2 = 0.793$ ,  $p < 0.001$ ) between the abundance of archaeal *amoA* genes and  $\delta^{15}\text{N}$  of soil (Fig. 2B).

The exponential equation that describes the correlation between AOA abundance and  $\delta^{15}\text{N}$  of DNA or soil from the humid tropics to temperate deserts may indicate a general relationship between ammonia oxidizer abundance and ecosystem  $^{15}\text{N}$  enrichment. It is likely that soils with high ammonia volatilization rates (Frank et al., 2004) will deviate from this relationship because not all ammonium is utilized by ammonia oxidizing organisms; under these conditions, even though the soil is enriched in  $^{15}\text{N}$ , ammonia oxidizer abundance may remain low. Similarly, the correlation in soils with limited oxygen availability may also not be described by the exponential relationship because ammonia oxidizing archaea may be obligate aerobic organisms (Könneke et al., 2005), so that their abundance will remain low in N rich, but anaerobic, environments. However, high densities of Thaumarchaea populations have been observed in oxygen limited marine environments (Stewart et al., 2012).

Evidence that AOA control nitrification rates in soil is mounting (Nicol and Schleper, 2006; Gubry-Rangin et al., 2010; Yao et al., 2011; Prosser and Nicol, 2012; Levicnik-Höfferle et al., 2012). There are now reports in the literature that show positive correlations between ammonia oxidation, nitrification rates, nitrification



**Fig. 2.** Relationship between the abundance of archaeal *amoA* genes and  $\delta^{15}\text{N}$  of DNA (A) or  $\delta^{15}\text{N}$  of total soil (B) in sites along the Arizonan elevation gradient (○) and Hawaiian Long Substrate Age Gradient (●). The relationship was best fit with the exponential equation  $\text{amoA gene copies} = 349.79 e^{0.6714(\delta^{15}\text{N DNA})}$ .

potentials and AOA abundance (Gubry-Rangin et al., 2010; Yao et al., 2011; Levcnik-Höfferle et al., 2012). Demonstration that isolates in pure culture oxidize ammonia and generate nitrite remains the most direct evidence (Könneke et al., 2005), but to date few AOA have been isolated from soil (Tourna et al., 2011), although several enrichment cultures have been reported (Jung et al., 2011; Kim et al., 2012). The strong correlation between natural abundance  $\delta^{15}\text{N}$  of soil pools and abundance of AOA, as demonstrated in this study, is one more piece of evidence suggesting that AOA do indeed play an important role in the soil N cycle, particularly in unfertilized systems and soils with low ammonium availability (Schleper and Nicol, 2010). Correlation does not establish causality, but combined with findings in previous studies that nitrification impacts N retention in soil and that N retention impacts  $^{15}\text{N}$  enrichment in ecosystems (Fenn et al., 1998; Högberg, 1990; Houlton et al., 2006; Timpler et al., 2007), control by AOA over nitrification in these soils is the most plausible explanation for the observed correlation between ecosystem  $^{15}\text{N}$  enrichment and AOA gene abundance.

The lack of reports linking natural abundance isotope patterns to microbial population abundances is surprising, since the activity of microorganisms affect isotopic ratios of ecosystem pools. Coupling specific microbial groups to natural abundance isotope patterns in ecosystem pools may provide a powerful tool for elucidating the functions of these organisms in soil.

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