

# Nitrification is a primary driver of nitrous oxide production in an agricultural soil

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In recent years, identification of the microbial sources responsible for soil N<sub>2</sub>O production has substantially advanced with the development of isotope enrichment techniques, selective inhibitors, and the discoveries of specific N-cycling functional genes. However, little information is available to effectively quantify the N<sub>2</sub>O sources (e.g. nitrification and denitrification) and the underlying microbial mechanism of N<sub>2</sub>O production is unclear as well. Here, a <sup>15</sup>N-tracing incubation experiment was conducted under controlled laboratory conditions [50%, 70% and 85% water-filled pore space (WFPS) at 25°C and 35°C] to determine nitrification-sourced N<sub>2</sub>O and denitrification-sourced N<sub>2</sub>O. 10 atom% <sup>15</sup>NH<sub>4</sub>Cl and K<sup>15</sup>NO<sub>3</sub> were applied to distinguish N<sub>2</sub>O production pathways, and QPCR was used to quantify the functional gene amoA to explore the relationship between N<sub>2</sub>O emission and ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) abundance. The soil used in the study with 0.52% total N, 5.2% organic C, 19% clay, 38% silt and 43% sand. The soil pH (H<sub>2</sub>O) was 4.5 and NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N were 13 and 93 mg kg<sup>-1</sup> soil, respectively. At 50%, 70% and 85% WFPS, nitrification contributed 87%, 80% and 53% of total N<sub>2</sub>O production, respectively, at 25°C, and 86%, 74% and 33% at 35°C. The percentage of nitrified N as N<sub>2</sub>O (PN<sub>2</sub>O) increased with temperature and moisture, except for 85% WFPS, when PN<sub>2</sub>O was lower at 35°C than at 25°C. AOA were the dominant ammonia oxidizers, but both AOA and AOB were related to N<sub>2</sub>O emitted from nitrification. AOA and AOB abundance was significantly influenced by soil moisture, more so than temperature, and decreased with increasing moisture content. These findings can be used to develop better models which may be added microbial variables for simulating N<sub>2</sub>O emission to inform soil management practices for improving N use efficiency.