Nitrous oxide is a critical greenhouse gas that continues to increase in the atmosphere, yet the microbial pathways and processes that control its production are still largely uncharacterized. Aside from heterotrophic denitrifiers, ammonia-oxidizing bacteria and Thaumarchaea are implicated as major producers of N₂O. Our comparative studies of ammonia-oxidizing bacteria and Thaumarchaea revealed several interesting insights to N₂O production including essential and non-essential enzymology, metabolic intermediates, and interactions between biotic and abiotic processes. This work places NO as a key molecule that ammonia-oxidizers produce and use in a surprising variety of physiological contexts to ultimately produce N₂O.