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Evidence that invasive earthworms promote bacterial-mediated nitrous oxide emissions in forest ecosystems

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Earthworms are newcomers to South-Eastern Canada, as they were unable to survive the last glaciation period that ended about 11,000 years ago. Since their introduction by Europeans over recent centuries, these exotic earthworm species have substantially affected pedological processes and soil functions. For example, a recent study in the province of Quebec found that earthworms invading native sugar maple (*Acer saccharum* Marsh.) forests could potentially increase soil nitrous oxide (N_2O) emissions by increasing denitrification rates. However, the underlying microbial mechanisms driving the production of this greenhouse gas via denitrification remain unclear. This led us to conduct field and laboratory studies in order to explore whether earthworms preferentially promote bacterial and/or fungal denitrification pathways. We measured earthworm abundance and collected surface mineral soil samples from 38 sugar maple forests, half of which were earthworm-free. In each soil sample, we measured fungal, bacterial and total microbial biomass by substrate-induced respiration, we measured fungal, bacterial and total denitrification by acetylene inhibition, and we quantified the abundance bacterial (nirK, nirS and nosZ) and fungal (P450nor) denitrifying genes by qPCR. Earthworm abundance correlated positively with bacterial as well as fungal biomass, but did not affect the bacterial-to-fungal biomass ratio. Accordingly, bacterial-mediated and fungal-mediated denitrification rates both increased with the abundance of earthworms. However, earthworm abundance correlated positively with the specific bacterial denitrification rate (SBDR = (*bacterial-mediated denitrification rate*) ÷ (*bacterial biomass*)), but not with the specific fungal denitrification rate (SFDR = (*fungal-mediated denitrification rate*) ÷ (*fungal biomass*)). Moreover, qPCR analyses showed a positive correlation between earthworm abundance and the proportion of all bacterial denitrifying genes in the microbial population, but no such effect on fungal denitrifying genes. Taken collectively, our results suggest that earthworms may increase N_2O emissions in sugar maple forest soils by preferentially promoting the bacterial-mediated denitrification pathway.