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# Use of biological indicators of soil health to estimate reactive nitrogen dynamics in long-term organic vegetable and pasture systems

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ABSTRACT

Diverse crop rotations, cover crops and the possibility of integrating livestock make organic systems potentially more sustainable than other agroecosystems. Lower reactive nitrogen (N) in organic systems minimizes the potential for N losses. However, addition of organic manures and residues containing mineralizable N and carbon (C) have the potential to enhance nitrous oxide ($N_2O$) emissions. We conducted a 39 d laboratory incubation to assess key microbiological drivers controlling nitrification and denitrification in long-term organic agroecosystems during simulated freeze-thaw cycles. Soils were collected from two annual organic vegetable systems receiving 1) mixed-compost, or 2) broiler litter and 3) an organic perennial pasture system cropped to vegetables every third year. Soil microcosms amended with $^{15}N$ labelled sugar beet residue or unamended were maintained at 40, 60 and 80% of water filled pore space (WFPS). Significant $N_2O$ was emitted (4287–6138 μg $kg^{-1}$soil) via denitrification from amended soil microcosms at 3 °C and 80% WFPS. Archaeal (AOA) and bacterial (AOB) nitrifier *amoA* gene copies were affected by temperature and reactive N species during freeze-thaws. Long-term organic vegetable cropping systems receiving mixed-compost additions had the potential to accumulate C and immobilize excess reactive soil N (particularly nitrates) thereby improving soil health and reducing $N_2O$ emissions.



## 1. Introduction

Almost 70% of global nitrous oxide ($N_2O$) emissions originate from fertilization of agronomic systems and are produced by the microbial transformations, nitrification and denitrification (Syakila and Kroeze, 2011). The final product of nitrification is nitrate ($NO_3^-$), a substrate required for heterotrophic denitrifiers (Zhu et al., 2013). Ammonia oxidation, the first and rate limiting step of nitrification is catalyzed by the ammonia monooxygenase enzyme the production of which is controlled in part by the *amoA* gene. In soils, this process is mediated by nitrifiers, ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) (Hastings et al., 2000; Venter, 2004). Nitrous oxide is an obligate intermediary of denitrification whereas ammonia oxidation produces $N_2O$ as a byproduct of nitrification in both AOA (Stieglmeier et al., 2014) and AOB (Lipschultz et al., 1981). However, there is a body of literature that suggests AOA contribute to $N_2O$ emission via nitrification particularly in soils that with limited N (Jung et al., 2011).

The primary group of microorganisms controlling denitrification in agronomic soils are heterotrophic denitrifiers. This anaerobic form of microbial respiration results in the reduction of $NO_3^-$ to nitrogen gas ($N_2$) via intermediates that include $N_2O$ that is further reduced to $N_2$ via the enzyme nitrous oxide reductase encoded in the *nosZ* gene (Braker and Conrad, 2011). Not all denitrifiers possess the complete set of enzymes required to transform nitrate to $N_2$ (Dandie et al., 2008) and the *nosZ* gene is inhibited by small quantities of oxygen ($O_2$). An additional factor contributing to the partial conversion of $NO_3^-$ to $N_2$ resulting in the buildup of $N_2O$ is the proportion of $NO_3$, organic carbon (C) and $O_2$ (Firestone and

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Davidson, 1989). Addition of C substrate in the form of residues and manure can enhance microbial respiration thereby further reducing $O_2$ concentrations in the soil and creating anaerobic microsites that foster $N_2O$ production. Soil moisture also regulates $O_2$ concentrations affecting aerobic nitrifiers and anaerobic denitrifiers (Firestone and Davidson, 1989; Avrahami and Bohannan, 2009). Nitrification can account for 55–95% of $N_2O$ emissions when water filled pore space (WFPS) is between 40 and 60% (Linn and Doran, 1984) while denitrification is the primary source of $N_2O$ in soils at > 70% WFPS (Saggar et al., 2013).

Climate and management practices are major drivers influencing mineralization of C and N, nitrification and denitrification that in turn reduce or enhance $N_2O$ production (Livesley et al., 2009; Johnson et al., 2007). Organic agroecosystems, by definition, mimic natural ecosystems "relying on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects" such as synthetic N fertilizers, a major contributor to $N_2O$ production (The International Federation of Organic Agriculture Movements, http://www.ifoam.bio/en/organic-landmarks/definition-organic-agriculture). Diverse crop rotations, cover crops and the possibility of integrating livestock make organic systems potentially more sustainable than many other agroecosystems. Specifically, adaption of the above management practices has the potential to provide ecological and environmental services that include the reduction of greenhouse gas emissions (GHG) and improved soil health (National Organic Program, 2015). Soil health is "the capacity of a soil to function within ecosystems and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant, animal, and human health (Doran and Parkin, 1994)" and is assessed using physical, chemical, and biological properties of soil related to soil function. Because organic growers rely on the mineralization of organic sources to provide nutrients to crops, these systems typically, foster low reactive N and have the potential to minimize $N_2O$ emissions due to coupling of N and C (Ghorbani et al., 2010). Therefore, there is a critical need to quantify and identify keystone microorganisms that control nitrification and denitrification in these systems. Addition of C substrates from residues or animal amendments can facilitate heterotrophic denitrification if there is sufficient $NO_3^-$ in a soil system (Mitchell et al., 2013).

Most studies focus on soil temperatures at or above 25 °C, a temperature at which the production of reactive nitrogen species in soil is optimal (Cui et al., 2016). However, the community structure and activities of soil nitrifiers and denitrifiers vary within the range of 4–37 °C (Braker et al., 2010). Denitrifiers are more tolerant of subzero temperatures relative to most bacteria including nitrifiers (Avrahami and Conrad, 2003; Sharma et al., 2006; Knowles, 1982). As a consequence, the addition of organic C through incorporation of manures or plant residues in late fall is expected to stimulate $N_2O$ emissions when temperatures range from 5 to 10 °C (Singurindy et al., 2009). Wertz et al. (2013) reported significant shifts in nitrifier and denitrifier communities and increased $N_2O$ production in the presence of $NO_3^-$ and C at soil temperatures of −1 °C. Seasonal $N_2O$ emissions in winter can be equivalent to or greater than the $N_2O$ emitted during the growing season (Christensen and Tiedje, 1990; van Bochove et al., 2000; Wagner-Riddle et al., 1997). An increase in $N_2O$ emissions due to the release of additional C and N substrates made available by winter freeze-thaws has also been reported (Phillips, 2008; Singurindy et al., 2009). The temperature during a freeze-thaw cycle (amplitude), its duration, and soil moisture content at the time of a freeze regulates the release of C and N influencing the potential for mineralization, nitrification and denitrification (Zhao et al., 2010). The balance among water content, soluble C and nitrate concentrations [$(C_{H2O})/NO_3^-$] can be used to predict the effect of a soil management(s) on $N_2O$ production originating from heterotrophic denitrification in soil (Benckiser et al., 2015). Yet, our knowledge of the effects of freeze-thaw events on nitrifier and denitrifier communities and their contribution to $N_2O$ emissions is incomplete.

Inventories of GHG emissions and knowledge with respect to how nutrients cycle in organic agroecosystems is limited, their biogeochemical cycling complex, and the range of existing climatic conditions and managements diverse (Franzluebbers, 2005; Johnson et al., 2007). Therefore, it is of pivotal importance to study how these complex biogeochemical processes occur via nitrification/denitrification pathways and the influence best management practices have on the potential to mitigate $N_2O$ production during freeze-thaws. Our research provides a unique opportunity to study the interaction of multiple abiotic factors (temperature, moisture, C availability, $NH_4^+$-N, $NO_3^-$-N) on the abundance of genetic markers associated with nitrifiers and denitrifiers and to relate the production of $N_2O$ to organic management practices (disturbance, amendment type). The objectives of this study are 1) to assess the microbiological drivers controlling the fluxes of key N cycle processes (nitrification, denitrification) in long-term annual and perennial vegetable systems, 2) evaluate interactions among nitrifier (*amoA*) and denitrifier (*nosZ*) gene copies, potential biological indicators of soil health, and reactive forms of N (ammonium, nitrate and nitrous oxide) during simulated freeze-thaws and 3) investigate the potential for niche separation of nitrifier and denitrifier communities on the basis of moisture, temperature and substrate affinity.

## 2. Materials and methods

### 2.1. Site description and soil sampling

The Long-term Organic Vegetable Systems Experiment was established at Washington State University Puyallup Research and Extension Center, USA (47° 11′24″ N, 122° 19′48″ W; elevation 13 m) in 2003 as a USDA certified organic research site (see Supplementary Methods). The plots (6.1 × 15.2 m) were in a randomized complete block with four field replicates for each treatment (see Supplementary Methods). Ten soil cores were collected to a 10 cm depth in November of 2013 from each of the three organic systems in all four field plot replicates: 1) annual fall-seeded cover crop – vegetable rotation, mixed-compost amendment; 2) annual fall-seeded cover crop – vegetable rotation, broiler litter amendment; 3) 2-year pasture –1-year vegetable rotation with push probes (2.5 cm in diameter) and composited. The treatments provided contrasts in organic C additions and disturbance (annual vs. perennial). The seasonal N supplied from amendments was comparable. However, the mixed-compost supplied 2–5 times higher C than the broiler litter (Table S1). Cover crop biomass and N content were not statistically different between treatments. The pasture rotation treatment has received no amendments (in the form of mixed-compost or broiler litter) since 2005 (Table S2). Field moist soils were passed through 2-mm sieve and pre-incubated for one week. Soil moisture content was recorded gravimetrically. Baseline inorganic ($NH_4^+$ and $NO_3^-$)-N was extracted from 10 g (field moist soil) with 100 mL of 2 M KCl solution.

### 2.2. Microcosm experiments

Two separate but simultaneous 39 d laboratory incubations were conducted to measure: 1) $N_2O$ and $CO_2$ emissions, and 2) soil inorganic ($NH_4^+ + NO_3^-$)-N and gene copies of nitrifiers (*amoA* genes) and denitrifiers (*nosZ* gene) in soils. Temperatures