Short-Term Effects of Mineral and Organic Fertilizer on Denitrifiers, Nitrous Oxide Emissions and Denitrification in Long-Term Amended Vineyard Soils

Enrico Tatti
Dipartimento di Biotecnologie Agrarie Sez. Microbiologia
Università degli Studi di Firenze
P.le delle Cascine 24
Firenze, Italy

and
Potato Research Centre
Agriculture Agri-Food Canada
Fredericton, NB
Canada, E3B 4Z7

Claudia Goyer*
Bernie J. Zebarth
Potato Research Centre
Agriculture Agri-Food Canada
Fredericton, NB
Canada, E3B 4Z7

David L. Burton
Dep. of Environmental Sciences
Nova Scotia Agricultural College
Truro, NS
Canada, B2N 5E3

Luciana Giovannetti
Carlo Viti
Dipartimento di Biotecnologie Agrarie Sez. Microbiologia
Università degli Studi di Firenze
P.zzale delle Cascine 24
Firenze, Italy

Short-term effects (i.e., 21 d) of mineral or organic fertilizer application on long-term (i.e., 8 yr of applications) amended soil on denitrifier community abundance, denitrification gene mRNA transcript numbers, denitrification rate, and emissions of N$_2$O were explored. Soil was collected from a vineyard in Italy receiving annual applications of either mineral fertilizer (conventional management system, CS) or municipal compost (organic management system, OS). Each soil was incubated using three treatments: no amendment, NH$_4$NO$_3$, or municipal compost. Microcosms set up with soil treated with compost showed higher nirS, nirK, and nosZ abundance in comparison to conventional fertilization. Short-term compost addition increased nirK gene abundance over time in OS and CS soils, whereas nirS and nosZ gene abundance increased after compost addition only in OS soil. In OS soil, nosZ gene mRNA transcript numbers were higher at all time-points for all treatments compared with CS soil. Furthermore, nosZ gene mRNA transcript number increased over time after compost addition for both soils, N$_2$O emissions were higher in both soils after NH$_4$NO$_3$ addition compared with no amendment and compost addition. Denitrification was higher in OS than CS soil following NH$_4$NO$_3$ treatment. Denitrification rates were much higher than N$_2$O rates in all cases suggesting most emissions occurred as N$_2$. Our study demonstrated that long-term urban-waste compost application clearly changed soil denitrifier communities and the response of denitrification and N$_2$O emissions to different short-term soil amendments.

**Abbreviations:** CS, conventional management system; OS, organic management system; WFPS, water-filled pore space.

Recent changes in household waste collection and waste management systems in the European Union countries has substantially increased the amount of compost generated from municipal solid wastes (Adhikari et al., 2010). Land application of compost to agricultural fields is desirable because it is a source of stabilized and complex C and N (Hargreaves et al., 2008). Moreover, repeated compost applications have been shown to preserve soil quality and to enhance soil organic matter pools, which play a crucial role in maintaining soil biological activity, soil aggregate stability, and mineral nutrition of crops (Garcia-Gil et al., 2000; Crecchio et al., 2001). Viticulture has been recently shown to cover an approximate area of 7.4 million hectares of plants worldwide (FAOSTAT, http://faostat.fao.org/site/569/default.aspx, accessed 2008), therefore the application of compost into productive vineyards is an interesting approach not only to reduce the amount of waste added to landfill sites but also to maintain soil quality and decrease the quantity of applied artificial fertilizers. However, the environmental implications of this practice (i.e., N$_2$O production) are not well understood.
N2O emissions, total denitrification, and denitrifier communities in a vineyard in Italy to different N amendment treatments (no addition, mineral fertilizer, municipal compost) with respect to N2O emissions, total denitrification, and denitrifier community abundance (nirS, nirK, and nosZ) and denitrification gene mRNA transcript number (nosZ) using soil microcosms. In addition, possible relationships between environmental and denitrification parameters, and denitrifier abundance and mRNA transcript numbers, were explored.

We hypothesized that long-term addition of compost led to a higher abundance of soil denitrifiers compared with the conventionally fertilized soil. Changes in fertilization practices in the two long-term management systems might affect denitrifiers and denitrification. Therefore, we assumed that mineral fertilizer amendments would result in a more important increase in N2O emissions, total denitrification rates, and denitrification gene expression in soil that has experienced long-term compost application compared to soil under long-term mineral fertilization due to the increased availability of C.

**MATERIALS AND METHODS**

**Soil Characteristics and Collection**

Soils were collected from a vineyard located near Marciano della Chiana, Arezzo, Italy (43°19′20″ N lat, 11°47′43″ E long) with mean annual air temperature of 14.1°C and mean annual precipitation of 40.5 mm. The experimental site was established in 2001 using field scale plots with two management systems including: (i) a CS using mineral fertilization corresponding to 50 kg N ha⁻¹ yr⁻¹, 30 kg P₂O₅ ha⁻¹ yr⁻¹, and 70 kg K₂O ha⁻¹ yr⁻¹ and (ii) an OS using 15 Mg ha⁻¹ yr⁻¹ dry weight compost generated from urban organic waste as previously reported (Tatti et al., 2012). The CS and OS soils are a silty clay loam Calcaric Cambisols with 169 g kg⁻¹ sand, 499 g kg⁻¹ silt and 332 g kg⁻¹ clay; the organic C concentration was 12.7 and 21.8 g kg⁻¹, and total N concentration was 1.1 and 1.7 g kg⁻¹, respectively (Tatti et al., 2012). The soil pH (1:2.5 soil/water ratio) was 8.1 (Thomas, 1996). Soil was collected at 0- to 15-cm depth from multiple locations within each management site along a 100-m transect to obtain spatially representative composite sample. The soil was then air-dried and shipped to Canada for microcosm experiments. Soils were passed through a 2-mm sieve and water added to achieve a gravimetric water content of 0.3 g g⁻¹ soil (approximately 50% water filled pore space, WFPS) then stored in the dark at room temperature for 10 d before use.

**Experimental Design**

The experiment used two soil management systems (CS or OS), three amendment treatments, and five incubation lengths (0, 2, 7, 14, and 21 d) replicated four times. The experimental unit was a 1-L glass canning jar of approximately 10 cm diameter (Bernardin, Toronto, ON). Amendment treatments included (i) a control with no amendment added (OS₀, CS₀); (ii) amendment with 87 mg N kg⁻¹ of dry soil as ammonium nitrate as a solution (OS₅₀, CS₅₀); and (iii) amendment with 40 g compost kg⁻¹ dry soil (OS₁₀₀, CS₁₀₀). Each treatment combination had a duplicate set of incubation jars to allow for either addition of 10% (v/v) acetylene (C₂H₂, to inhibit the reduction of N₂O to N₂) to the headspace to quantify total denitrification.
or no C₂H₂ addition to quantify N₂O emissions. The rate of compost addition was chosen to supply an equivalent quantity of mineral N as the mineral N treatments based on the compost physiochemical characteristics (Tatti et al., 2012). For each incubation jar, the required quantity of N amendment and water was added to approximately 290 g air-dried soil to achieve a water content equivalent to 70% WFPS, gently mixed to minimize loss of soil structure, and packed into the jar to a soil bulk density of 1 mg m⁻³. This resulted in approximately a 5-cm headspace. The jars were sealed with Parafilm (Pechiney Plastic Packaging, Chicago, IL) to reduce water evaporation and pierced with four holes to allow gas exchange. The jars were placed immediately in an incubation chamber at 25°C for 21 d. The weight of the jars was checked periodically and water added as required to maintain constant the chosen water content over time. Jars were destructively sampled at 0, 2, 7, 14, and 21 d.

For jars to be used for quantification of N₂O emissions (with no C₂H₂ addition), two soil cores (approximately 10 g total) were removed from the jar at the end of the incubation period, placed in two 15-mL tubes, flash-frozen using liquid nitrogen, and kept at −80°C for nucleic acid extraction. Jars were then sealed with a screw lid fitted with a rubber septum, and 10 mL of headspace gas were sampled at 0 and 3 h of incubation at 25°C. Gas samples were injected into previously evacuated Extetainer vials (Labco, UK) containing the desiccant magnesium perchlorate. After gas sampling was completed, the jars were opened and 25 g of soil was sampled to perform analytical measurements including NO₃⁻, NO₂⁻, and NH₄⁺ concentrations.

For jars to be used for quantification of total denitrification, jars were sealed at the end of the incubation period with screw lids as described above. C₂H₂ was injected immediately, and 10 mL of headspace gas was sampled at 1 and 4 h of incubation at 25°C. The delay of gas sampling for the jars was to allow time for C₂H₂ diffusion into the soil. Gas samples were collected at time 0 and 3 h for N₂O emissions and time 1 and 4 h for total denitrification according to preliminary experiments that showed gas concentration increased linearly between 0 and 6 h (data not shown).

**Gaseous Fluxes and Soil Analytes Measurements**

Headspace gas was analyzed for N₂O and CO₂ concentrations using a Varian Star 3800 Gas Chromatograph (Varian, Walnut Creek, CA) fitted with an electron capture detector to measure N₂O, a thermal conductivity detector to measure CO₂, and a Combi-PAL Autosampler (CTC Analytics, Zwingen, Switzerland) as previously described (Henderson et al., 2010). Concentrations of NO₃⁻, NO₂⁻, and NH₄⁺ were analyzed colorimetrically following extraction with 0.5 M K₂SO₄ and then quantified as previously described (Henderson et al., 2010).

**Nucleic Acid Extraction**

DNA and RNA were co-extracted from 0.7 g of soil, divided using the method of Griffiths et al. (2000) as previously described (Henderson et al., 2010). Before extraction, 50 mg of sterilized skim milk was added to each tube to enhance extraction efficiency (Takada-Hoshino and Matsumoto, 2004). The RNA and DNA were quantified using the fluorescent dyes Ribogreen and Picogreen (Invitrogen, Burlington, ON, Canada), respectively. Average extracted DNA concentration was 1.64 (±0.103) and 1.05 (±0.86) µg g⁻¹ dry soil for OS and CS, respectively. Average extracted RNA concentration was 0.43 (±0.31) and 0.14 (±0.14) µg g⁻¹ dry soil for OS and CS, respectively. Genomic DNA was also extracted from 1 g of compost as described above.

**Quantitative PCR**

Gene copy number for nirS (Kandeler et al., 2006), nirK, and nosZ (Henry et al., 2006) was quantified via quantitative (qPCR) using an Applied Biosystems (Streetsville, ON, Canada) ABI PRISM 7000 thermal cycler and SYBR Green PCR Master Mix (Invitrogen) with PCR conditions as previously described (Dandie et al., 2008; Henderson et al., 2010; Dandie et al., 2011).

Primers targeting nirS or nirK bearing communities from soil previously failed to amplify cDNA in a qRT-PCR reaction even after extensive optimization of qRT-PCR conditions (Henderson et al., 2010). Primers targeting nosZ gene were suitable to quantify transcript abundance, as previously described (Henderson et al., 2010). Standard curves were used for absolute quantification of nirS, nirK, and nosZ gene number and transcripts as previously described (Dandie et al., 2008; Henderson et al., 2010; Dandie et al., 2011). Standard curve descriptors and detection level: nirS gene copy number: slope: −3.32 to −3.57, R² = 0.995–0.997, E = 97–101% y-intercept = 41.1–43.7. nirK gene copy number: slope: −3.34 to −3.59, R² = 0.997–0.999, E = 89–97% y-intercept = 33.7–34.4. nosZ gene copy number: slope: −3.42 to −3.77, R² = 0.999, E = 93–99% y-intercept = 37.8–38.8. nirS transcripts number: slope: −3.31 to −3.44, R² = 0.998–0.999, E = 83–95% y-intercept = 34.2–36.8. Successful amplification of the desired-size fragment was assessed by agarose gel visualization, and specificity of the amplification was checked by melt curve.

Soil DNA and RNA extracts were tested for the presence of co-extracted inhibitory substances as previously described (Henderson et al., 2010).

**Data Analysis**

All statistical analyses were conducted using Systat Software 12 (Systat Software Inc., Chicago, IL). Data was tested for normality using the Shapiro–Wilk test and all non-normal data were log transformed. A mixed-model three-way analysis of variance was performed based on a randomized complete block design with management system, amendment, and incubation time as fixed effects. Treatment means in interactions were compared using Tukey adjusted least significant (LS) means and treatment means in simple main effects were compared by performing post hoc Tukey honestly significant difference (HSD) test. Regression analyses were performed to examine possible relationships between NO₃⁻, respiration (CO₂), N₂O, or total denitrification, and nirS, nirK, and nosZ gene numbers and nosZ gene transcripts at each time point. Treatment means and standard errors were calculated from untransformed data.
RESULTS

Soil NO$_3^-$, NO$_2^-$, NH$_4^+$ Concentrations

There were significant differences in NO$_3^-$ concentrations between management systems and among N amendment treatments ($P < 0.001$) (Fig. 1a). In the unamended soils, NO$_3^-$ concentrations were significantly higher ($P < 0.001$) for soil from the CS than the OS management system during the entire measurement period (average of 25.1 and 0.9 mg NO$_3^-$–N kg$^{-1}$ dry soil for the CS$_0$ and OS$_0$ treatments, respectively) (Fig. 1a). Addition of compost (i.e., OS$_C$ and CS$_C$ treatments) did not significantly increase soil NO$_3^-$ concentration compared with the unamended soil regardless of management system. Addition of mineral fertilizer resulted in the highest NO$_3^-$ concentrations for both management systems (average of 144 and 125 mg NO$_3^-$–N kg$^{-1}$ dry soil for the CS$_F$ and OS$_F$ treatments, respectively). Where mineral fertilizer was added, soil NO$_3^-$ concentrations differed significantly ($P < 0.001$) between management systems only at 21 d at 174 and 80 mg NO$_3^-$–N kg$^{-1}$ dry soil for the CS$_F$ and OS$_F$ treatments, respectively.

Soil NO$_2^-$ concentrations were very low in almost all cases (Fig. 1b). There was, however, a small transient increase in soil NO$_2^-$ concentration to 2.7 mg NO$_2^-$–N kg$^{-1}$ dry soil at 2 d for the CS$_F$ treatment.

Soil NH$_4^+$ concentrations were significantly different among amendment treatments ($P < 0.001$) (Fig. 1c). In unamended soils, soil NH$_4^+$ concentrations for both management systems were low (<1.6 mg NH$_4^+$–N kg$^{-1}$ dry soil) over the entire measurement period. Compost addition significantly increased NH$_4^+$ concentrations compared with unamended soils at time zero ($P < 0.001$), with average values of 41.6 and 23.1 mg NH$_4^+$–N kg$^{-1}$ dry soil for the CS$_C$ and OS$_C$ treatments, respectively. Soil NH$_4^+$ concentrations in compost amended soils decreased to an average of 9.2 NH$_4^+$–N kg$^{-1}$ dry soil at 2 d and remained low for the remainder of the incubation. Addition of mineral fertilizer significantly ($P < 0.001$) increased soil NH$_4^+$ concentrations compared with the other amendment treatments with average values of 71.6 mg NH$_4^+$–N kg$^{-1}$ dry soil at time zero and average values of 12.3 mg NH$_4^+$–N kg$^{-1}$ dry soil at Day 2 (Fig. 1c). Soil NH$_4^+$ concentrations did not differ among amendment treatments between 7 and 21 d.

Nitrous Oxide Emissions, Total Denitrification, and Respiration

In all cases, total denitrification rates (i.e., N$_2$O evolved in the presence of C$_2$H$_2$) were significantly higher than N$_2$O emissions (i.e., N$_2$O evolved in the absence of C$_2$H$_2$) ($P < 0.001$). The N$_2$O molar ratio (i.e., ratio of N$_2$O emissions to total denitrification) was always below 0.21 and mostly below 0.1 indicating that most gaseous N emissions from soils occurred as N$_2$ (data not shown).

Total denitrification rates responded significantly to amendment treatment, time, and management system ($P < 0.001$) (Fig. 2a). In unamended soils, total denitrification rates were significantly higher for the OS than the CS management at 7 d (16.1 and 1.1 µg N$_2$O–N kg$^{-1}$ dry soil h$^{-1}$ for OS$_0$ and CS$_0$ treatments, respectively) and 14 d (15 and 5.7 µg N$_2$O–N kg$^{-1}$ dry soil h$^{-1}$ for OS$_0$ and CS$_0$ treatments, respectively). For compost amended soils, there was no significant difference in total denitrification between management systems with average value over time of 5.2 µg N$_2$O–N kg$^{-1}$ dry soil h$^{-1}$. Moreover, compost application did not increase denitrification rates in both OS and CS compared with unamended soils at any sampling date. Addition of mineral N significantly increased denitrification rates in both OS and CS compared with unamended soils at 2, 7, and 14 d. At 7 d, total denitrification rates significantly decreased compared with Day 2.
in both CS F and OS F treatments ($P < 0.001$). After 14 and 21 d, total denitrification rates were not significantly different among amendment treatments for the CS soil, whereas denitrification rate for the OS F treatment was still higher than for the OS0 and OSC treatments. The OS F treatment had higher denitrification rates (average of 156 μg N₂O-N kg⁻¹ dry soil h⁻¹) than the CS F treatment (average of 68 μg N₂O-N kg⁻¹ dry soil h⁻¹) (Fig. 2a).

The rate of N₂O emission responded significantly to amendment treatments ($P < 0.001$) but not management systems (Fig. 2b). Unamended and compost amended treatments were not significantly different and were stable over time with an average of 0.035 μg N₂O-N kg⁻¹ dry soil h⁻¹. Addition of mineral N significantly increased N₂O emission rates ($P < 0.001$) compared with the unamended and compost amended soils at Day 2 (average of 1.6 μg N₂O-N kg⁻¹ dry soil h⁻¹) and at Day 7 (average of 0.37 μg N₂O-N kg⁻¹ dry soil h⁻¹) but not at Day 21 (Fig. 2b).

Soil respiration (CO₂ concentration) was measured in this study because it reflects microbial activity and is a measure of carbon availability. Carbon dioxide concentrations did not differ between soils incubated with or without C₂H₂ ($P = 0.217$), suggesting that acetylene was not a significant C source for the soil microorganisms (data not shown). Respiration rates responded significantly to amendment treatment and management system ($P < 0.001$) (Fig. 2c). In unamended soils, CO₂ emissions were significantly higher ($P < 0.001$) for the OS than the CS management system for the entire measurement period (average of 0.44 and 0.14 mg CO₂–C kg⁻¹ dry soil h⁻¹ for the OS0 and CS0 treatments, respectively). Respiration was stable over time after addition of NH₄NO₃, indicating that addition of mineral N did not affect respiration (Fig. 2c). Compost amendment significantly increased respiration rates in both CS and OS soils compared with other amendment treatments ($P < 0.001$), and respiration for the OSC treatment was significantly higher than for the CS C treatment at 2, 7, and 14 d (Fig. 2c).

**Denitrifier Abundance and nosZ Transcript Numbers**

The nirS gene abundance was about 10 times higher ($P < 0.001$) in OS than CS soil when averaged over time and amendment treatments (Fig. 3a). In unamended soil, nirS gene copy number was significantly lower ($P < 0.001$) in the CS0 treatment compared with the OS0 at each sampling point (Fig. 3a). Compost and mineral N treatments had significant effects on nirS gene copy numbers in both management systems ($P < 0.001$). In soils from the CS management, addition of mineral N significantly increased ($P < 0.001$) nirS gene copy numbers compared with unamended soils after 2, 7, and 21 d with average values of $1.8 \times 10^7$ and $1 \times 10^7$ copies g⁻¹ dry soil for the CS F and CS0 treatments, respectively (Fig. 3a). In comparison, addition of mineral N in soils from the OS management significantly increased nirS gene copy numbers compared with unamended soils only at 21 d (Fig. 3a). Compost addition significantly increased ($P < 0.001$) nirS gene copy numbers in soils from both management systems compared with unamended soils. In soils from the CS management, nirS gene abundance in the CS C treatment (average of $1.6 \times 10^8$ copies g⁻¹ dry soil) was significantly higher ($P < 0.001$) than for the CS F and CS0 treatments at each sampling point (Fig. 3a). In soils from the OS management, compost addition increased nirS gene copy numbers from $7.2 \times 10^8$ copies g⁻¹ dry soil at 2 d to $1.7 \times 10^9$ copies g⁻¹ dry soil at 21 d (Fig. 3a).

The nirK gene abundance was significantly higher ($P < 0.005$) in soils from the OS system than the CS system, with average values of $4.1 \times 10^8$ and $1.9 \times 10^8$ copies g⁻¹ dry soil, respectively. The nirK gene copy numbers did not change significantly over time for
nirK gene copy numbers increased significantly in soils amended with compost or mineral N from time zero to Day 2 (Fig. 3b). Compost addition significantly increased \( (P < 0.001) \) nirK gene abundance, with about a 70-fold and 10-fold compared increase at 21 d compared with time zero for the OSC and CS C treatments, respectively \( (P < 0.001) \) (Fig. 3b). In contrast, addition of mineral N did not have a significant effect on nirK gene copy numbers compared with unamended soils in both management systems after Day 2 (Fig. 3b).

The nosZ gene copy numbers were higher in soils from the OS management (average of \( 3.1 \times 10^8 \) copy g\(^{-1}\) dry soil over treatment and time) compared with the CS management (average of \( 1.6 \times 10^8 \) over time and treatments) (Fig. 4a). There were no significant differences in nosZ gene abundances among amendments of OS or CS soils from Day 0 to 7. Addition of mineral N had no significant effect on nosZ gene copy numbers for soils from the CS management and had inconsistent effects in soils from the OS management; nosZ gene copy numbers were significantly higher for the OS\(_0\) than the OS\(_F\) at 14 d, whereas the reverse was true at 21 d. Addition of compost in CS and OS soils increased nosZ abundance compared with unamended and mineral N amended soils at 14 and 21 d. The nosZ gene copy numbers was higher in the OS\(_C\) treatment than the CS\(_C\) treatment at 21 d with \( 9.2 \times 10^8 \) and \( 3.9 \times 10^8 \) copy g\(^{-1}\) dry soil, respectively (Fig. 4a).

The abundance of denitrifiers communities was quantified in compost to evaluate the number of gene copies that would be added to soil. qPCR analysis using primers for nirK and nirS on DNA directly extracted from compost resulted in \( 7 \times 10^6 \) and \( 5 \times 10^6 \) gene copy numbers g\(^{-1}\) dry compost, respectively. Thus, addition of around 10 g of dry compost to 290 g of soil resulted in addition of \( 2.2 \times 10^6 \) and \( 1.4 \times 10^6 \) of nirK and nirS gene copy numbers g\(^{-1}\) dry soil, respectively. Therefore compost addition contributed at time zero for an average of 2.5 and 2% in nirS and nirK copy number, respectively. No amplification using nosZ primers was achieved in compost extracted DNA.
Gene transcript number could only be measured for the \textit{nosZ} gene. \textit{nosZ} gene transcript numbers were significantly higher ($P < 0.001$) in soils from the OS management ($5.6 \times 10^6$ transcripts g$^{-1}$ dry soil) than from the CS management ($2.9 \times 10^6$ transcripts g$^{-1}$ dry soil). mRNA transcript numbers of \textit{nosZ} did not change significantly over time in soils from either management system (Fig. 4b). Similarly to \textit{nosZ} gene copies, the \textit{nosZ} gene mRNA transcript numbers increased numerically from time zero to 2 d, but the changes were not significant (Fig. 4b). Compost addition resulted in a significant ($P < 0.005$) increase in \textit{nosZ} mRNA transcript number in both soils on some sampling dates. The \textit{nosZ} transcript numbers in the OSC treatment were significantly higher ($P < 0.001$) at 21 d compared with the OS$_F$ and OS$_0$ treatments ($4.9 \times 10^6$, $3.5 \times 10^6$, and $2.3 \times 10^6$ transcripts g$^{-1}$ dry soil, respectively) (Fig. 4b). \textit{nosZ} mRNA transcript numbers in the CSC treatment were significantly higher than for the CS$_F$ and CS$_0$ treatments at 7 d (Fig. 4b). Conventional management system and OS soils had an RNA/DNA ratio of 0.03 and 0.015, respectively, when averaged over N fertilizer treatments and time (data not shown).

Regression analysis was used to identify possible relationships between analytical measurements, respiration, N$_2$O emission rates, denitrification rates, and the abundance and \textit{nosZ} mRNA transcript numbers of denitrifier communities. There were significant positive relationships ($P < 0.001$) between respiration rates and \textit{nirK}, \textit{nirS}, \textit{nosZ} gene abundance, and \textit{nosZ} gene mRNA transcript number (Fig. 5). No significant relationships were found between denitrification gene abundance and \textit{nosZ} mRNA transcript numbers and either N$_2$O emission or total denitrification rates.

**DISCUSSION**

**Effects of Long-Term Compost Application Compared to Long-Term Mineral Application**

The long-term (8 yr) application of compost in the investigated Tuscan vineyard soil changed soil chemical properties, leading to an increased C and N content as previously reported (Tatti et al, 2012). In the current study, long-term compost application increased abundance of the \textit{nirS}, \textit{nirK}, and \textit{nosZ} gene-bearing communities 10, 2.2, and two-fold, respectively, compared to CS soils. There are currently no other studies that have reported effects of long-term compost application on abundance of denitrifying bacteria. With respect to other organic amendments, Hallin et al. (2009) reported greater \textit{nirS}, \textit{nirK}, and \textit{nosZ} gene abundance in long-term application of sewage sludge and cattle manure compared with ammonium sulfate. These results suggest that application of organic amendments over the long-term can change not only soil properties but also soil microbial abundance including denitrifiers.
Long-term compost application also changed soil denitrification activity. Total denitrification rate in mineral N amended soil, where nitrate supply was not limited, was significantly higher in soil from the OS management compared with CS management. Increased organic C availability commonly increases denitrification rate in soil incubation studies (Gillam et al., 2008; Miller et al., 2008; Miller et al., 2009), and therefore, the increased denitrification in the OS management soil likely reflects increased C availability. Similarly, Dambreville and colleagues (2006) reported an increase of potential denitrifying activity in soil treated for 7 yr with composted pig manure compared with soil treated with ammonium nitrate, due to a higher total C content and microbial biomass.

**Effects of Short-Term Mineral Nitrogen Amendment**

Addition of mineral N had no significant effect on the abundance of the *nirK* and *nosZ* gene-bearing communities, while in the CS soil, increased the *nirS* gene copy number up to 2.2-fold compared to the unamended soil. The experimental conditions used in this study created both anoxic and oxic conditions because of the high water content and the presence of large macro pores due to the high clay content of the soil, respectively. Therefore, it is not clear if mineral N availability (i.e., NO$_3^-$) could have given a competitive advantage to denitrifiers compared to other microbial communities. Some study demonstrated that denitrifier growth rate was better under anoxic conditions (Murray et al., 1992; Philippot et al., 1996) while other studies suggested that denitrifiers were more competitive under oxic conditions (Smith and Tiedje, 1979; Tiedje, 1988). Although addition of mineral fertilizer induced denitrification and N$_2$O emissions, NO$_3^-$ had no effect on *nosZ* gene mRNA transcript numbers. Given that soil has anoxic sites and denitrification gene induction occurs at low N-oxides concentration (Vollack and Zumft, 2001; Saleh-Lakha et al., 2009), it is possible that the *nosZ* gene expression was already induced in soil.

As expected, mineral N addition had no significant effect on soil respiration, which can be used as an indicator of soil C availability. Mineral N addition increased both N$_2$O emissions and total denitrification rates. In incubation studies, addition of NO$_3^-$ has resulted either in an increase in denitrification rate (Jordan et al., 1998; Strong and Fillery, 2002) or had no effect on denitrification rate (Myrold and Tiedje, 1985; De Klein and van Logtestijn, 1996). Gillam et al. (2008) concluded that NO$_3^-$ addition affects denitrification rate only when NO$_3^-$ is limiting the denitrification process, which can occur when the supply of NO$_3^-$ is low or the demand for NO$_3^-$ is high due to limited O$_2$ supply in combination with high availability of organic C. Nitrate addition increases N$_2$O emissions in most cases through either an increase in the denitrification rate or through a change in the N$_2$O molar ratio (Gillam et al., 2008). The N$_2$O molar ratio is controlled by the relative availability of C and NO$_3^-$ (Firestone et al., 1979; Miller et al., 2008) and is commonly high when there is an abundant supply of NO$_3^-$ in soil (Gillam et al., 2008; Miller et al., 2008). Interestingly, in this study the N$_2$O molar ratio was low (<0.1 in most cases) even when soil NO$_3^-$ concentration was high. Low N$_2$O molar ratios in the presence of high NO$_3^-$ concentration have been infrequently reported in the literature (Mahmood et al., 1998; Vallejo et al., 2006). The low N$_2$O molar ratio in this study may reflect the high (332 g kg$^{-1}$) clay content of the soils. Most pores within the soil aggregates are small and, consequently, are water-filled at high water content. This would substantially reduce the rate of N$_2$O diffusion in soil and may result in reduction of N$_2$O to N$_2$ before the N$_2$O can diffuse out of the aggregates (Smith, 1990).

**Effects of Short-Term Compost Amendment**

A single application of compost increased *nirK* and *nosZ* gene-bearing communities two-fold in soils from both CS and OS management systems. Compost has been shown to stimulate heterotrophs growth in the short term due to C availability (Viti et al., 2010). Moreover, previous studies have reported denitrifier abundance increasing with organic C availability (Miller et al., 2009; Bárta et al., 2010), although others authors showed no effect on denitrifiers (Henry et al., 2008; Miller et al., 2008; Henderson et al., 2010). We hypothesize that the contrasting response of denitrifiers community to C source addition observed in different studies may be due to the type of organic matter (i.e., more readily-available vs. more complex) and differences in experimental conditions used. Compost application increased the *nirS* gene copy number g$^{-1}$ dry soil around 20-fold in soil from the CS system, while the increase in OS system was around two-fold. The *nirS* gene-bearing community in soil from the CS system was more responsive to compost addition than the other denitrifying communities studied here. The contrasting long-term managements may have affected the *nirS* gene-bearing community structure, which then resulted in a differential response to the compost amendment treatment in this study. In contrast to *nirS*, the increase *nirK* gene abundance was evident only after 21 d regardless of the management system, thus indicating a different response to addition of complex organic C. These results, together with the different response to N addition, suggest a difference in ecological niches between *nirK* and *nirS* denitrifier communities, as previously shown in agricultural soils by other authors (Chen et al., 2010; Hallin et al., 2009).

Compost addition significantly increased *nosZ* gene transcripts compared to mineral N addition and unamended soils regardless of management system. *nosZ* gene mRNA transcription is induced by anoxic condition and nitric oxide (Vollack and Zumft, 2001). The higher microbial activity following compost addition, as indicated by increased soil respiration, may have depleted soil O$_2$ supply and, thereby, favored *nosZ* gene transcription. Very few studies have examined the response of denitrification gene mRNA transcription to complex carbon source addition. Henderson et al. (2010) reported a significant increase of *nosZ* gene mRNA transcript numbers in anoxic microcosms soils amended with plant residues. In this study, the increase in *nosZ* gene mRNA transcript numbers could be due to an increase
in number of transcripts per cell and/or an increase in the number of cells. The nosZ mRNA/DNA ratio was two-fold higher in CS soil compared to OS for all treatments: compost addition increased the number of nosZ gene bearing denitrifiers; thus, the lower mRNA/DNA ratios observed in compost amended soils compared to other amendments suggested that not all the nosZ gene bearing denitrifiers may have been producing transcripts or producing them at the same time.

Compost addition did not increase total denitrification rates and soil mineral N concentrations in both management systems but significantly increased soil microbial activity as indicated by respiration. This may reflect either limited release of N from the added compost or net immobilization following compost addition. The lack of a response of emissions to compost addition can be attributed to a limitation in nitrate supply, which was the main driving factor for this process. Our results were similar to those obtained by Alluvione et al. (2010), who found very low N₂O emission following compost addition compared with urea in field experiments due to less nitrate availability.

**Relationships between Denitrifier Abundance and nosZ mRNA Transcript Numbers and Soil Processes**

Significant correlations were observed between soil respiration and nirS, nirK, and nosZ gene abundance/mRNA transcript numbers. Soil respiration was increased in response to both a long-term compost addition in the OS management and to compost numbers. Soil respiration was increased in response to both a long-term compost addition and to compost addition as an amendment. The positive correlations are consistent with the key role of organic C in influencing microbial abundance. Miller and coworkers (2009) reported a positive correlation between respiration and nitric oxide reductase gene of *Pseudomonas mendelii* and related species (*env*Bₚ) copy number following liquid manure addition on soil, while other authors did not report such correlation in their analysis after soil addition of plant residues (Miller et al., 2008; Henderson et al., 2010).

No significant relationships were observed between denitrifier abundance or nosZ gene mRNA transcript numbers and N₂O emissions or denitrification rates in our study. Similarly, previous studies in anoxic soil microcosms also reported no significant correlation between denitrifier abundance or mRNA transcript numbers and denitrification (Miller et al., 2008; Henderson et al., 2010; Dandie et al., 2011). Although the presence of denitrification gene transcripts suggests their actual involvement in the soil processes, it is known that mRNA may not be directly linked to actual related-enzyme activity, considering all the regulatory steps from gene expression to a fully functional enzyme. Moreover, it could be hypothesized that other players not targeted in the present study (i.e., other bacterial denitrifier groups or denitrifying fungi) might have been involved in denitrification in our system.

In conclusion, our study demonstrated that long-term application of municipal compost increased abundance of soil bacterial denitrifying communities and denitrification gene mRNA transcript numbers. Long-term compost addition also influenced respiration and denitrification. When NH₄NO₃ was added, denitrification rates increased, mainly in the OS soil, where no limitation of both N and C was present. Compost amendment on both OS and CS increased the nirK, nirS, and nosZ gene abundance and mRNA transcript numbers, and soil respiration, having provided readily utilizable C. In both systems, denitrification and N₂O emission were not affected by compost treatments, which did not bring enough mineral N to soil to fuel these soil processes. Moreover, under the condition tested, we showed that despite the common assumption that N₂O molar ratio is high when nitrate supply is abundant, this may not be the case in clay-rich soil. Obtained result suggests that the use of inorganic fertilizer on long term compost treated soils could lead to a high N loss from the system under conditions favoring denitrification. The results highlighted that urban-waste organic management practices can have a profound effect on soil proprieties, microbial communities, and key soil processes and that these changes can have important implications in terms of soil health and environmental contamination.

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