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Authors

Wolff, Michael W
Hopmans, Jan W
Stockert, Christine M
et al.

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Effects of drip fertigation frequency and N-source on soil N₂O production in almonds



Michael W. Wolff^{a,*}, Jan W. Hopmans^b, Christine M. Stockert^a, Martin Burger^b,
Blake L. Sanden^c, David R. Smart^a

^a Department of Viticulture & Enology, University of California, Davis, CA 95616, United States

^b Department of Land, Air and Water Resources, University of California, Davis, CA 95616, United States

^c University of California Cooperative Extension, One Shields Avenue, Davis, CA 95616, United States

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ABSTRACT

Little work has been done on nitrous oxide (N₂O) emissions from nitrogen-fertigation systems, despite the greater degrees of control allowed over fertilizer N fate. More fertigation users are adopting high-frequency (HF) N application schedules to improve nitrogen use efficiency and reduce nitrate (NO₃⁻) leaching. The possibility is raised that this practice may also lower N₂O emissions, through effects on soil N concentrations and soil microbial populations. At the same time, NO₃⁻-based N-fertilizers are frequently selected for use in HF systems, where they should be more immediately available to plants than ammoniacal fertilizers. This choice of N-source is likely to affect N₂O emissions. We monitored surface emissions of N₂O in an almond orchard (*Prunus dulcis* [Mill.] DA Webb) in Belridge, California. Fertigation treatments were 4x year⁻¹ (Standard) urea ammonium nitrate (UAN), 20x year⁻¹ (HF) UAN and 20x year⁻¹ (HF) CaNO₃ + KNO₃. Estimated surface emissions were HF UAN > Standard UAN > HF NO₃, with differences only significant between the HF treatments, where HF UAN emitted 2.0 times the N₂O seen from HF NO₃. Net production was also monitored by depth in the soil using sampling tubes and Fickian diffusion calculations. UAN typically had highest N₂O production at 10–15 cm depth, while N₂O was generally reduced to N₂ below 20 cm in all treatments. Differences were seen in the distribution of NH₄⁺ and NO₃⁻ on the soil exchange complex and in soil solution, with data from 60 cm suggesting that leaching hazards could be greater from Standard UAN than from HF NO₃. Multiple linear regression of N₂O production with predictors had the best fit at 15 cm, where extractable NH₄⁺, WFPS and temperature together accounted for an adjusted R² of 0.68. Persistent soil microbial changes were seen in denitrification capacity, with HF UAN = HF NO₃ > Standard UAN, while 3% O₂ assays suggested high contributions of N₂O from nitrifier denitrification in the fertigation context. High-frequency fertigation with ammoniacal fertilizers did not mitigate N₂O emissions, but nitrate-based fertilizers did, suggesting that N sources for fertigation warrant careful study.

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1. Introduction

It is estimated that agriculture contributes 80% of anthropogenic nitrous oxide (N₂O) emissions (Davidson and Mosier, 2004), of which most are ultimately derived from nitrogen (N) applied as N-fertilizers and manure-N (Wrage et al., 2001; Davidson, 2009). These agricultural N₂O emissions account for about 5.5% of global annual anthropogenic GHG emissions (Smith et al., 2007). The N₂O emitted originates from microbial N pathways, the balance of

which is affected by the application of N fertilizers (Robertson and Grace, 2004) or by irrigation regime, aside from natural factors.

Application of N fertilizers through micro-irrigation systems (fertigation) presents certain advantages over dry fertilization, especially in allowing more precise administration of N in coordination with crop demand (Lamm et al., 2004). It has special relevance in arid and Mediterranean systems, where many high-value tree crops are irrigated. Insufficient work has been done to describe the N₂O emissions from N fertigation (Venterea et al., 2012) and to describe strategies of mitigation. Reports from fertigated tree crops (Schellenberg et al., 2012; Alsina et al., 2013; Maris et al., 2015) have so far been lower (uncorrected EFs of 0.23% – 0.68%) than global expectations of N₂O-N from mineral fertilizer

* Corresponding author.

E-mail addresses: michael.wolff@inia.cl, mwwolff@ucdavis.edu (M.W. Wolff).

applications, sometimes estimated as 0.9% of the total applied N (Bouwman et al., 2002), with a default Tier 1 value of 1% used by the IPCC (2006).

With various motives, renewed attention is being paid to “split applications” of nitrogen fertilizers, now usually called “high-frequency” in the fertigation context. “High-frequency” N-fertigation in tree crops currently refers to at least 7 applications over the course of a growing season (Kusakabe et al., 2006; Quinones et al., 2007; Alva et al., 2008), and can reach up to daily applications during certain stages of development (Ayars et al., 1999; Neilsen and Neilsen, 2002; Gao et al., 2012). Monitored systems which adjust water and nutrient delivery to tree crops on an hourly basis are best described as “open hydroponics” (Falivene et al., 2005; Schumann et al., 2009).

Increasing crop N-use efficiency may be the primary goal among growers who adopt high-frequency fertigation. Positive effects have been seen with daily application on low-OM, sandy soils with pomegranate (Phene et al., 2015), table grape (Howell et al., 2014) and tomato (Patel et al., 2015). In Australia, high-frequency N fertigation is becoming standard in citrus production, although yield differences in citrus have been elusive (Alva et al., 2008; Treeby et al., 2012). Further crop-specific research should allow finer tuning for greater synchronicity with demand, as is sought by almond growers in California (Lopus et al., 2010).

Risks of nitrate leaching below the crop root zone accompany N fertigation, a problem with both productive and ecosystemic facets. Mediterranean trees such as almond can have strong fall root flushes (Basile et al., 2007) with corresponding N uptake that aids against these losses. They can also have deep root systems, but they are not necessarily developed in such a way by farming practices, especially in drier climates (Franco and Abrisqueta, 1997). The utility of high-frequency fertigation in minimizing nitrate leaching is widely recognized (Ayars et al., 1999; Syvertsen and Sax, 1999; Alva et al., 2008) particularly in sandy soils, or in soils with low cation exchange capacity (Bhat and Sujatha, 2009). Physically, high-frequency reduces the peak soil solution N concentrations seen after fertigations and maintains root zones with more consistent levels of soluble nutrients (Silber et al., 2003). A larger fraction of applied N may be adsorbed on mineral or organic surfaces. And root physiological responses may increase N uptake efficiency.

For similar reasons, high-frequency N-fertigation may also meet a third priority: reducing N₂O emissions. At lower concentrations, N-processing soil microbes, whose rates are limited by a number of factors, are likely to transform a larger proportion of the applied N while the soil remains wet and conditions favorable. Among denitrifiers, with lower levels of available NO₃⁻, a lower fraction of this pool has been emitted as N₂O (Senbayram et al., 2012), possibly in part because of inhibition of N₂O reduction with greater NO₃⁻ (Weier et al., 1993). The only study we are aware of that has compared N₂O from high-frequency and standard N-fertigations was carried out under laboratory conditions (Ciarlo et al., 2008), where it was found that splitting an application of KNO₃ into 5 doses decreased total emissions. This result, too, would reflect the capacities of denitrifiers. Research at the field level is needed, since fertigation typically affects a large volume of soil, with relevant spatial variations in water-filled pore space, O₂ and substrate concentrations.

Field studies must also explore whether frequency of mineral N applications modifies soil microbial populations over time scales greater than the interval between applications, increasing the rates of certain transformations involved in nitrification and denitrification. Nitrifier populations have been seen to change with repeated additions of ammonium (Wagenet et al., 1977) as has nitrification potential (Ding et al., 2010). And differences have been seen in denitrification enzyme activity (DEA) between soils from

drip zones that had been fertigated with UAN vs. KNO₃ for several years (Matiasek, 2012). More frequent applications of N may maintain higher levels of nitrous oxide reductase in denitrifiers, which shows an important lag in synthesis (Weier et al., 1993; Senbayram et al., 2012).

The N-species applied to a soil in fertilization or fertigation may affect N₂O emission levels. Overall, N applied as ammonium, or as urea which is hydrolyzed to ammonia within days after fertigation, is subject to possible emission as N₂O from more pathways than is N applied as nitrate, and may lead to greater N₂O emissions (Liu et al., 2007; Gagnon et al., 2011; Inselsbacher et al., 2011).

This possible disadvantage of urea- and ammonium-based fertilizers may be compounded in the field by the effects of N-species on the depth of N₂O production (Smith et al., 1997). Furthermore, differences in concentration of NH₄⁺ applied which accompany high-frequency fertigation, can also be expected to affect the distribution of adsorbed NH₄⁺ in the soil profile. To describe such effects, work must be done to monitor the vertical distribution of N₂O production and of available NH₄⁺ and NO₃⁻. A small number of studies have reported N₂O concentration profiles in the field following N-fertilization (Li et al., 2002; Müller et al., 2004; Hou et al., 2010; Riya et al., 2012), but few have used diffusion-based calculations to assess N₂O production at different depths of a soil profile in the laboratory (Clough et al., 2006) or in the field following fertilization (Yoh et al., 1997; Venterea and Stanenas, 2008; Nan et al., 2016); and we find no such reports on fertigation. The question whether high-frequency fertigation can reduce N₂O emissions was addressed in an almond orchard under surface drip. The studied field was in an arid zone but represents a system which straddles Mediterranean and arid zones within California's Central Valley. The most popular fertilizer for fertigation in the area, UAN, was compared in 4 vs. 20 annual applications. It was expected that high-frequency UAN would lower total N₂O emissions. It was hypothesized that such a result would be due in part to modified soil microbial capacities, assessable through laboratory tests of field soils including ammonium oxidation potential, denitrification rate and potential, and denitrification “product ratio” (N₂O/(N₂O + N₂)). The field soils were also tested under 3% O₂ conditions, as a representative level of oxicity for drip zones, to compare N₂O production from nitrifier denitrification with heterotrophic denitrification.

NO₃⁻-based fertilizers have often been proposed for high-frequency or “just-in-time” N application (Lamm et al., 2004), and are suggested as a means to improve NUE in tree crops (Quaggio et al., 2014). Therefore a NO₃⁻-based formulation was also tested against UAN within the high-frequency system. This formulation was not tested in the standard 4x year⁻¹ applications because the high NO₃⁻ concentrations entailed were considered to present an unacceptable leaching hazard. It was expected that the NO₃⁻ formulation would lead to lower N₂O emissions than from UAN; it was hypothesized that NO₃⁻ would be found deeper in the soil profile, and there would undergo more complete denitrification than the NO₃⁻ introduced directly and indirectly by UAN applications. The possibility that higher available NO₃⁻ in these treatments would increase a soil's denitrification capacity was tested in laboratory assays, as were consequent possible effects on the product ratio.

2. Materials and methods

2.1. Experimental design

The trial took place over the course of a growing season defined by the water and N application schedule, from March 2013 through October 2013, in an almond orchard (*Prunus dulcis*, cv. *Nonpareil*) in Belridge, Kern County, California (N 35°30'37" W 119°40'3").

Approximately 110 cm of irrigation were applied, while only 2.4 cm of rainfall occurred in the following winter months (CIMIS Station #146). Using surface drip fertigation, the following treatments were applied, all equivalent to $336.3 \text{ kg N ha}^{-1} \text{ year}^{-1}$ (Table 1).

High-Frequency Nitrate (HF NO₃) required separate injections of monoammonium phosphate (MAP) and Ca(NO₃)₂ in order to avoid precipitation of Ca₃(PO₄)₂. All fertigations lasted 24 h, starting and ending at 7:00 AM, and injection of the non-MAP fertilizers took place between 11:00 PM and 3:00 AM.

The soil at the site is classified as a Milham sandy loam (fine-loamy, mixed, superactive, thermic, Typic Haplargids) derived from granitic and sedimentary alluvium, deep and well-drained. In an existing experiment (P.H. Brown, UC Davis), five blocks are laid out as a randomized complete blocks (RCB) design, with eight strip-plot treatments of 15 trees. Treatments assessed had K applications of $224.17 \text{ kg ha}^{-1}$ and P applications of 74.46 kg ha^{-1} . Four blocks were studied for N₂O surface emissions, and three of these included soil gas and solute measurements.

2.2. Measured surface emissions of N₂O

Static gas chambers were used, of 20.3 cm diameter PVC piping 11 cm high placed over PVC collars installed 5 cm deep directly after fertigation, with aluminum insulation, rubber bottom seals, and manual internal fans. Two drippers were sampled in each plot per event, and different drippers were studied at each fertigation event; those studied were situated about 2 m from the nearest tree trunk, to represent average temperature, moisture and root presence. Chambers were positioned at 4 distances from drippers, along a transect into the center of the alley, at 0, 20, 50 and 90 cm. The maximum extent of the wetting zone at surface rarely exceeded 60 cm from drippers. Emissions from each collar were used to describe emissions from rings around the dripper, with borders midway between distances.

Three gas samples were taken over 10-min intervals. To calculate fluxes the ideal gas law was used as by Parkin and Venterea (2010) including ambient temperatures from a CIMIS weather station (#146) 2.5 km from the orchard, and an elevation of 120 m, corresponding to 0.985 atm of pressure. Nonlinearity was not apparent with these short flux times.

2.3. Calculated emissions of N₂O

Surface emissions of N₂O were expected to derive almost entirely from N fertigations and the first subsequent irrigations in the Standard treatment (Schellenberg et al., 2012), with strong seasonal (temperature) dependence that informed the sampling schedule (March: 5 daily measurements; April: 4; June: 3; July: 3; October: 3). Output rates of drippers were checked. Gas samples were taken around peak emissions (13:00–18:00) on the first day after fertigation, and thenceforth from 8:00–14:00 to represent daily averages.

Emissions for statistical comparisons were the sums of observed measurements per plot, considered as describing a

whole day. However, in order to be comparable to Standard, emissions from the HF applications required interpolations of unmeasured events, which were averages of each measured event's emissions with the previous or the next.

Previous experience of the patterns of emission demanded a different approach in order to estimate total growing season emissions per treatment. First day emissions were calculated assuming that N₂O emissions increased linearly from baseline at 7 A.M. until peak ambient temperature, which was close to the time of measurement, then a Q10 adjustment was made using hourly ambient temperatures for the remainder of the day and night (correlation to ambient temperature on this site established by Wolff et al., unpublished data). Both of these manipulations sought to avoid overestimation. Q10 values were derived from seasonal diurnal measurements. To estimate the (low) emissions over two additional days after sampling, an exponential curve of decline was fit to measured points averaged by treatment, as $\text{emissions} = y_0 + a^{(\text{day}^* - b)}$, which had the best fit to data. Baseline rates were calculated by treatment to describe time periods when the upper soil was dry, starting 5–7 days after each fertigation, where the curve of decline ended. These were extended until the first day of fertigation for emissions calculations.

2.4. Soil profile measurements

Soil gas, moisture, temperature and N-distribution profiles were assessed in 3 experimental blocks. 1/8" OD brass tubing was used to take gas samples from depths of 5, 10, 15, 20, 30, 45, 60 and 80 cm in the soil, at 20 cm from drippers. The tubing was reinforced with steel wire, for insertion, after which the wire was withdrawn and replaced with a second, close-fitting internal brass tube of 3/32" OD to minimize internal air volume. Tubes were topped with septa consisting of Nalgene tubing of 1/8" inner diameter injected with a dual plug of silicone and butyl rubber; butyl rubber blocks N₂O diffusion while silicone maintains a good seal through syringe use. A volume of air corresponding to the tube volume was extracted before sampling. 5-mL gas samples were taken from 5 and 10 cm depth, and 10-mL samples from 15 cm and greater depths. For samples at 5 and 10 cm, tubes with closed ends and lateral cuts near their tip were inserted at 45° to the soil surface, allowing the surface soil to be tamped down at the entrance point without compacting the soil to be sampled. On this sandy loam, samples could be taken 5 h after irrigation without apparent resistance due to soil water.

Gas samples were stored in glass vials (Exetainer[®], Labco Limited, Buckinghamshire UK) with silicone sealant applied over the butyl septum before evacuation to 45 mTorr. All samples were analyzed for N₂O using a ⁶³Ni electron capture detector (ECD), and larger vials were tested for CH₄ and CO₂ using a flame ionizing detector (FID), both on the same gas chromatograph (GC-2014, Shimadzu Inc., Kyoto, Japan).

Simultaneously, a custom-built thermocouple probe of diameter 3/16" took soil temperature at 5, 10, 15, 20, 30, 45 and 60 cm (Omega Instruments, Stamford, Connecticut). A neutron probe

Table 1
Fertigation Treatment Specifications.

Treatment	Frequency	Formula	Urea-N %	NH ₄ ⁺ -N %	NO ₃ ⁻ -N %
Standard UAN	4: 20% March, 30% April, 30% June, 20% October	UAN32 + MAP	44.9	32.6	22.5
High-Frequency UAN	20: 5% × 18 March–July, 5% × 2 October	UAN32 + MAP	44.9	32.6	22.5
High-Frequency NO ₃	20: 5% × 18 March–July, 5% × 2 October	KNO ₃ + Ca(NO ₃) ₂ + MAP	0	10.6	89.4

(Hydroprobe 503, Campbell Pacific Nuclear Model, Concord, California) provided measurements of volumetric water content at 20, 30, 45, 60 and 90 cm, using aluminum access tubes, and a soil moisture count curve fitted to measurements from this soil ($\theta_{vol} = 0.0629e^{1.0481x}$, $R^2 = 0.957$). A Theta probe (ML2x, Delta-T Devices, Cambridge, UK) was used to measure θ_{vol} in the upper 10 cm.

Texture and bulk density were tested at each site at 0–20, 20–30, 30–45, 45–60 and 60–90 cm.

To compare the vertical distribution of dissolved NH_4^+ and NO_3^- in the soil after fertigations, high-flow ceramic soil solution samplers (Soil Moisture Co., Santa Barbara, California) were installed at 15, 30 and 60 cm depth, 20 cm from the dripper laterally. Evacuation down to 600 mm Hg of vacuum was accomplished with an automotive brake pump, and samples were collected 6 h later, but on the third days after fertigation, samples adequate for analysis were only extracted from 38% of samplers, with fewer in summer, and very few in general from 15 cm depth.

Beginning in June, exchangeable NH_4^+ and NO_3^- were also assessed using soil samples from 0 to 5, 12.5–17.5 and 27.5–32.5 cm on each day. Approx. 20 g fresh weight soil was extracted in 80 mL of 2 M KCl, with one hour of shaking. These and the samples of soil solution were run for colorimetric analysis of NH_4^+ (Kempers and Kok, 1989) and of NO_3^- (Doane and Horwath, 2003).

2.5. N_2O production in soil

N_2O concentrations by depth were combined with moisture readings, bulk density and texture to estimate the soil diffusion coefficient for nitrous oxide using the Unified Diffusivity Model—Buckingham Burdine Campbell (UDM-BBC) (Moldrup et al., 2003). The Campbell parameter “b” was estimated using the clay fraction (Rolston and Moldrup, 2002). Net production or consumption of N_2O was estimated for each measured point in the soil except the deepest, using the one-dimensional mathematical model of Yoh et al. (1997) and field measurements described above. The model's temporal term $dc/dt \times V_a$, representing change in concentration of N_2O in a point over time (storage), was estimated by sampling gas concentrations and soil moisture twice in one dripper per treatment, after a lag of at least 1 h.

2.6. Microbial and pH effects

Persistent treatment effects were assessed on soil microbial N processing rates and potentials. The soils were collected in late August after a month of irrigations without fertilizer. One replicate per plot was used, and results analyzed as an RCB consistent with the field experiment.

pH was tested with 1 g:2 mL slurry in deionized distilled water, using samples from 0 to 20 cm depth at 20 cm from drippers.

Treatment effects on nitrification rate were tested with the same samples using an ammonium oxidation assay (Kandeler et al. (2011) citing Berg and Rosswall (1985)).

Treatment effects on denitrification were tested with the same samples using a modified denitrification enzyme activity (DEA) procedure (Tiedje, 1982) with N_2 as a flushing gas, with glucose,

and without chloramphenicol. 5% headspace acetylene, generated from calcium carbide, was added to a round of duplicate samples; in the first test on the soils, 10% headspace acetylene had appeared to impede the entire denitrification process. Headspace gas samples were nonlinear by 60 min, so data from 20 and 40 min were used. The microcosms were also tested at 24 h to describe Denitrification Potential (DP) (Yeomans et al., 1992); by 48 h, N_2O declined in some microcosms.

Soils from UAN treatments were tested for N_2O production over a 36-h incubation at 3% O_2 and 50% WFPS according to the method of Zhu et al. (2013) with some modifications. Samples of 15 g dry weight underwent a 24-h pre-wetting at 25% WHC, then were put into 180 mL Erlenmeyer flasks with butyl rubber stoppers and twice evacuated to 1000 mTorr and flushed for a minute with N_2 . Flasks were then injected with O_2 for a 3% O_2 headspace, then fertigated to 50% WHC with NH_4^+ or NO_3^- , with and without acetylene, and pure water (details in Table 4). 5 mL headspace samples assessed N_2O production rates through measurements at 5 and 15 h; by 25 h they had declined in NH_4^+ microcosms, although N_2O production continued.

2.7. Statistical analyses

All statistical comparisons between treatments were carried out using PROC GLM for ANOVA (SAS, Cary, NC), with three blocks used for soil N_2O , NO_3^- and NH_4^+ tests, and four blocks for surface emissions, soil microbial tests and pH. Separate analyses were run between the two relevant treatments for surface emissions and 3% O_2 incubations. All three treatments were included for pH and other soil microbial tests, and the pairwise differences assessed with Tukey's range test.

3. Results and discussion

3.1. Surface emissions

Estimated growing season N_2O emissions were greatest from HF UAN fertigation, followed by Standard UAN, and lowest from the HF NO_3^- treatment, but comparisons between the two UAN and the two HF treatments only showed a significant difference between the HF systems (Table 2).

The failure to find differences in the Standard UAN—HF UAN comparison may have been due to high variability within and between blocks for the Standard UAN plots; in the UAN comparison the significance of blocking was $p < 0.69$ vs. $p < 0.12$ in the HF comparison. CVs over the measured period were 52% in Standard, 24% in HF UAN and 27% in HF NO_3^- .

Emissions from the irrigations following Standard UAN fertigations totaled 26% of the treatment's growing-season emissions. In previous work, little effect had been seen in subsequent (3rd and 4th) irrigations, even in loam soils (Wolff et al., unpublished data; Schellenberg et al., 2012). In the HF treatments such residual effects were indistinguishable from effects of the next fertigation. There were important seasonal effects; higher temperature caused earlier and higher peaks of N_2O emission, as well as higher event totals (Fig. 1).

Table 2

Growing season emissions, including interpolated data for high-frequency treatments and statistics.

Treatment	Emissions Estimate (g N_2O -N ha ⁻¹)	Emission Factor	Pairwise Significance	Statistical Mean ± S.E. of Mean ^a
Standard UAN	780.56	0.23%	NS vs. HF UAN	713 ± 187
High-Frequency UAN	1036.13	0.31%	–	818 ± 97
High-Frequency NO_3^-	511.17	0.15%	S vs. HF UAN	376 ± 50

^a Statistical mean differed from growing season emissions estimate. See Methods.

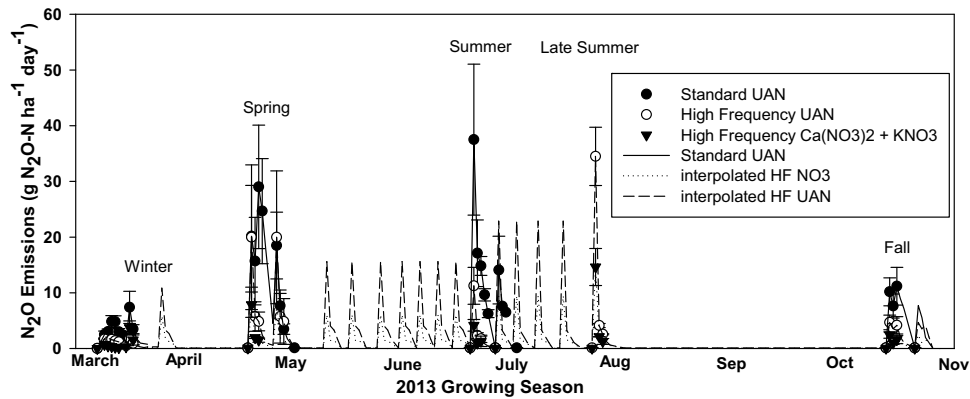


Fig. 1. Observed and interpolated emissions from three treatments over 2013 growing season. Error bars indicate standard errors of the mean.

The average (winter) precipitation at the site is 18 cm. The winter of 2013–2014 which followed our work was the driest on record in California; the experimental site received only 2 cm of precipitation and so was not monitored for emissions. However, previous work in the orchard (Schellenberg et al., 2012), monitoring UAN-derived N_2O emissions from static sprinklers at similar application rates, included a winter with 12 cm precipitation. Of the $730 \text{ g } N_2O\text{-N } \text{ha}^{-1}$ yearly average emission, those data allowed an estimate that $18.5 \text{ g } N_2O\text{-N } \text{ha}^{-1}$ were emitted in winter. The latter quantity, if it had been seen in all the treatments studied here, would increase estimated N_2O emissions by 3.7–8.5%.

At the same time, Mediterranean perennial crop systems usually see more winter rainfall, and are capable of supporting cover crops in the winter. In such cases, post-season emissions can be expected to constitute a larger portion of the total (Kallenbach et al., 2010; Kennedy et al., 2013) and cover crop management, can be an important control, as studied in vineyards (Steenwerth and Belina, 2008; Garland et al., 2014).

3.2. Spatial distribution of fertigated N

To analyze the transport and fate of applied fertilizer, NH_4^+ and NO_3^- in soil solution were sampled by suction lysimeters (Fig. 2) and in soil by KCl extraction (Fig. 3). Urea from Standard UAN was hydrolyzed to NH_4^+ and further oxidized to mobile NO_3^- in the soil profile, which by the third day led to 14x higher total NO_3^- concentrations at 60 cm depth under Standard UAN than under HF NO3 (Fig. 3). Low dissolved NO_3^- in the HF NO3 treatment (Fig. 2) may be ascribed to higher diffusion to outer parts of the wetting zone (Abalos et al., 2014), greater root uptake of nitrate through mass flow soon after fertigation, and the observed high retention of nitrate in the upper part of the soil (Fig. 3). NH_4^+ in solution near surface agreed with differences in application strength (Standard UAN = $5 \times$ HF UAN) (Fig. 2), but such differences were not apparent in KCl-extractable NH_4^+ , which described much greater quantities.

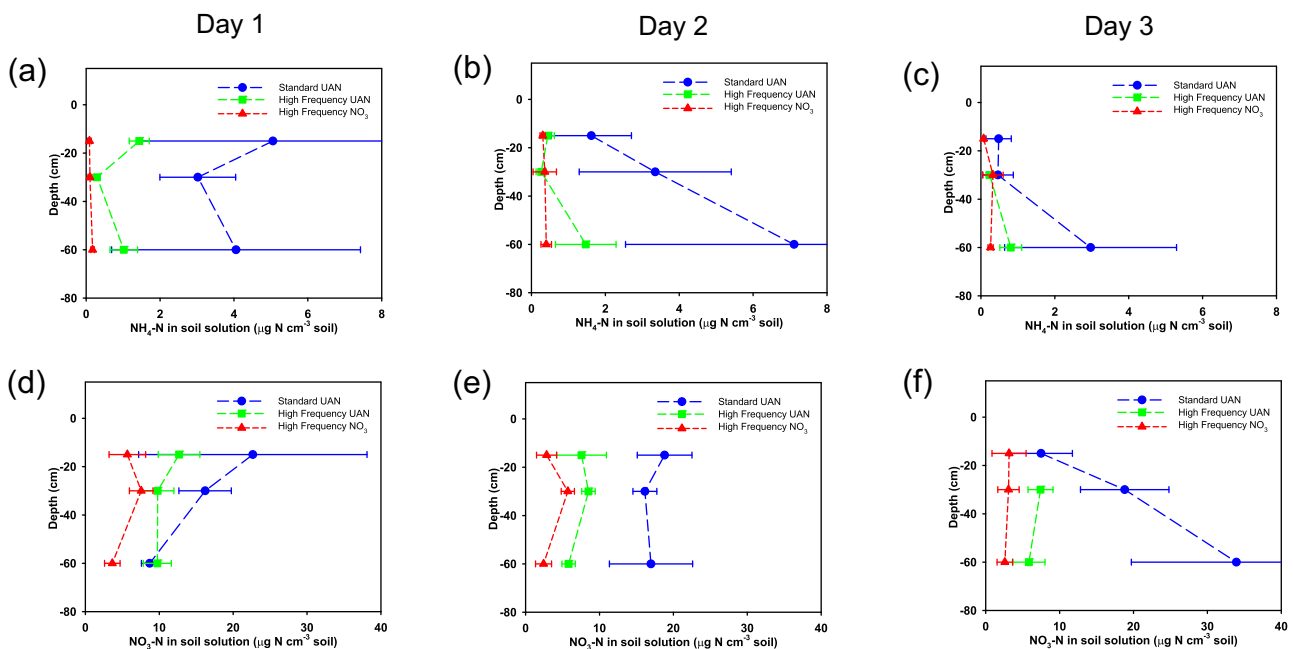


Fig. 2. Averaged soil solution NH_4^+ and NO_3^- over the first three days following summer and early fall fertigations, adjusted for soil moisture to allow comparison of quantities present. Error bars indicate standard errors of the mean.

(a) NH_4^+ solution Day 1; (b) NH_4^+ solution Day 2; (c) NH_4^+ solution Day 3; (d) NO_3^- solution Day 1; (e) NO_3^- solution Day 2; (f) NO_3^- solution Day 3.

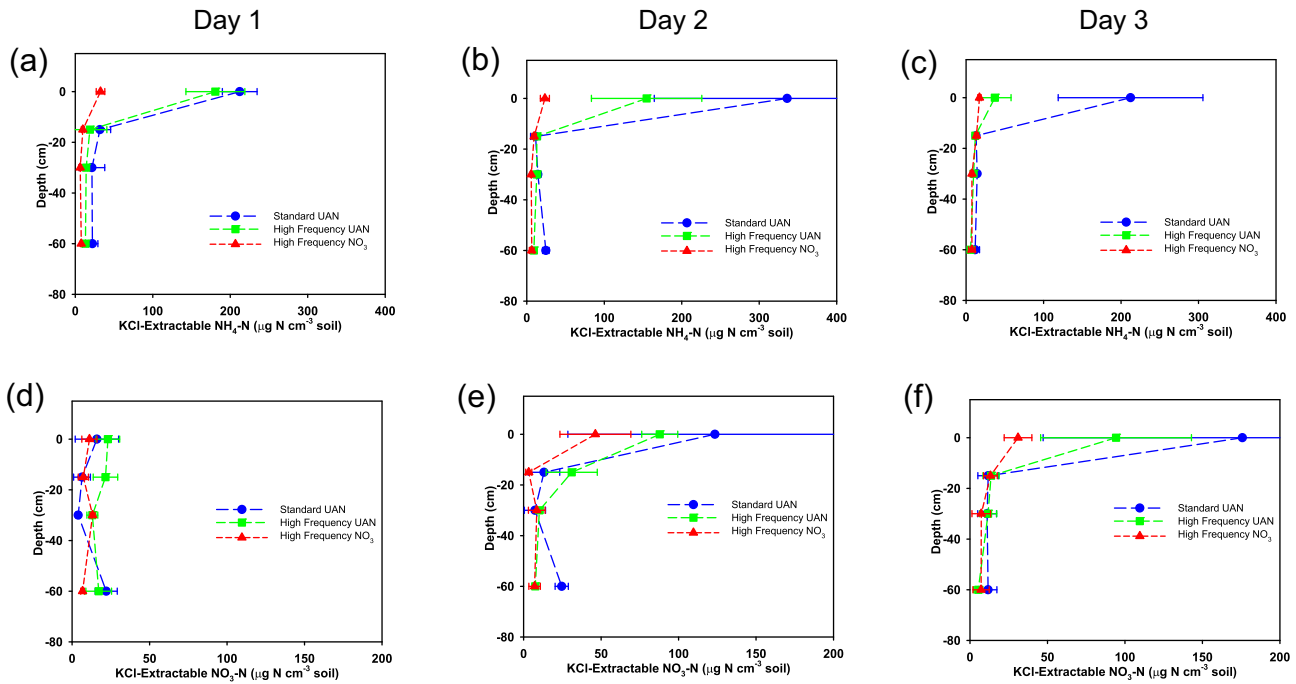


Fig. 3. Averaged soil KCl-extractable NH_4^+ and NO_3^- over the first three days following summer and early fall fertigations, per volume of soil to describe quantities present. Error bars indicate standard errors of the mean.

(a) NH_4^+ extract Day 1; (b) NH_4^+ extract Day 2; (c) NH_4^+ extract Day 3; (d) NO_3^- extract Day 1; (e) NO_3^- extract Day 2; (f) NO_3^- extract Day 3.

3.3. Production and fate of N_2O in the soil profile

In general, a better understanding of the spatial origin and fate of N_2O under different conditions should lead to improved fertilization and fertigation practices. In this investigation, net N_2O production by depth was calculated from soil gas concentrations, soil diffusion parameters, temperatures and water contents (Fig. 4) (Yoh et al., 1997) along a one-dimensional vertical profile at 20 cm from the dripper. A 1-D model cannot give a mass balance for N applied with water from a point source; unaccounted lateral diffusion will probably amount to a net loss and lead to underestimates of production. However this problem was judged to be ameliorated by conditions observed before the experiment. Maximum concentrations of N_2O were observed in the soil at 10–15 cm, shallow depths where lateral diffusion away from the dripper could only account for a small loss of N_2O compared with losses to the atmosphere. It was also observed that water tended to flow laterally, past the surface wetting front, through a sandy horizon above a clayey horizon which begins at around 50 cm depth, also diminishing net lateral gas diffusion.

The model is highly sensitive to variability in N_2O concentration data, and at the plot level the calculations showed fluctuations between production and consumption by depth which were not plausible or consistent. Therefore the concentration data and soil parameters for each treatment/day were averaged. Where possible, curves were fit to the concentration data of the form $\text{N}_2\text{O conc} = a \times e^{(-b \times \text{depth})}$, which provided the dc/dz terms. Furthermore, concentrations of N_2O measured at 5 cm were generally much lower than at 10 cm and led to frequent estimates of net consumption near surface. It was deemed likely that these samples had been contaminated by atmospheric air. Production of N_2O at 10 cm was therefore calculated with reference to ambient N_2O concentrations at 0 cm instead of the 5 cm concentration data. Measurements spaced several hours apart determined that an

average 4.4% of total production was accounted for by change in concentration over time (“storage”) during the measured days.

Ultimately no treatment statistics could be reported with the profile production data, but the model revealed general patterns and treatment effects on the depths of N_2O production. The highest emissions, which were seen in summer and fall, were associated with the most consistent patterns of N_2O distribution in the soil profile. Results from Days 2, 3 and 4 after fertigation, not shown, had similar distributions to those measured on Day 1, although with a slightly higher fraction of N_2O concentrated in the deeper soil, 40–60 cm. The relative stability in depths of production was seemingly contradictory to the changes in N distribution seen in soil solution (Fig. 2); but it was notable that distribution of extractable N (Fig. 3), showed less change at the same points (15 cm and below).

N_2O concentration patterns under most days and treatments were bimodal, with a shallow peak at 10–15 cm and a deeper peak around 45–60 cm, in the zone of higher clay content. The deeper peaks were sometimes strong 3–4 days after fertigation, especially in the Standard UAN treatment, illustrating the deeper distribution of N under higher rates of UAN application.

Calculations in UAN treatments after winter typically showed points of highest production at 10–15 cm depth, usually underlain directly by the points of greatest consumption, at 15–20 cm (Fig. 4). The calculations for 20 and 30 cm might underestimate production, because of more significant lateral diffusion of N_2O around 30 cm, where WFPS generally declined (Fig. 4). Production was seen at the lower peaks around 45 cm, but calculations suggested that N_2O produced in these lower peaks was generally consumed before reaching surface (Fig. 4), consistent with the findings of Nefel et al. (2000). This helps to explain why emitted N_2O was less per unit applied in Standard UAN than in HF UAN.

Calculations in HF NO_3 profiles generally showed much lower net N_2O production/consumption than the UAN treatments. This is credited to the more even distribution throughout the soil of

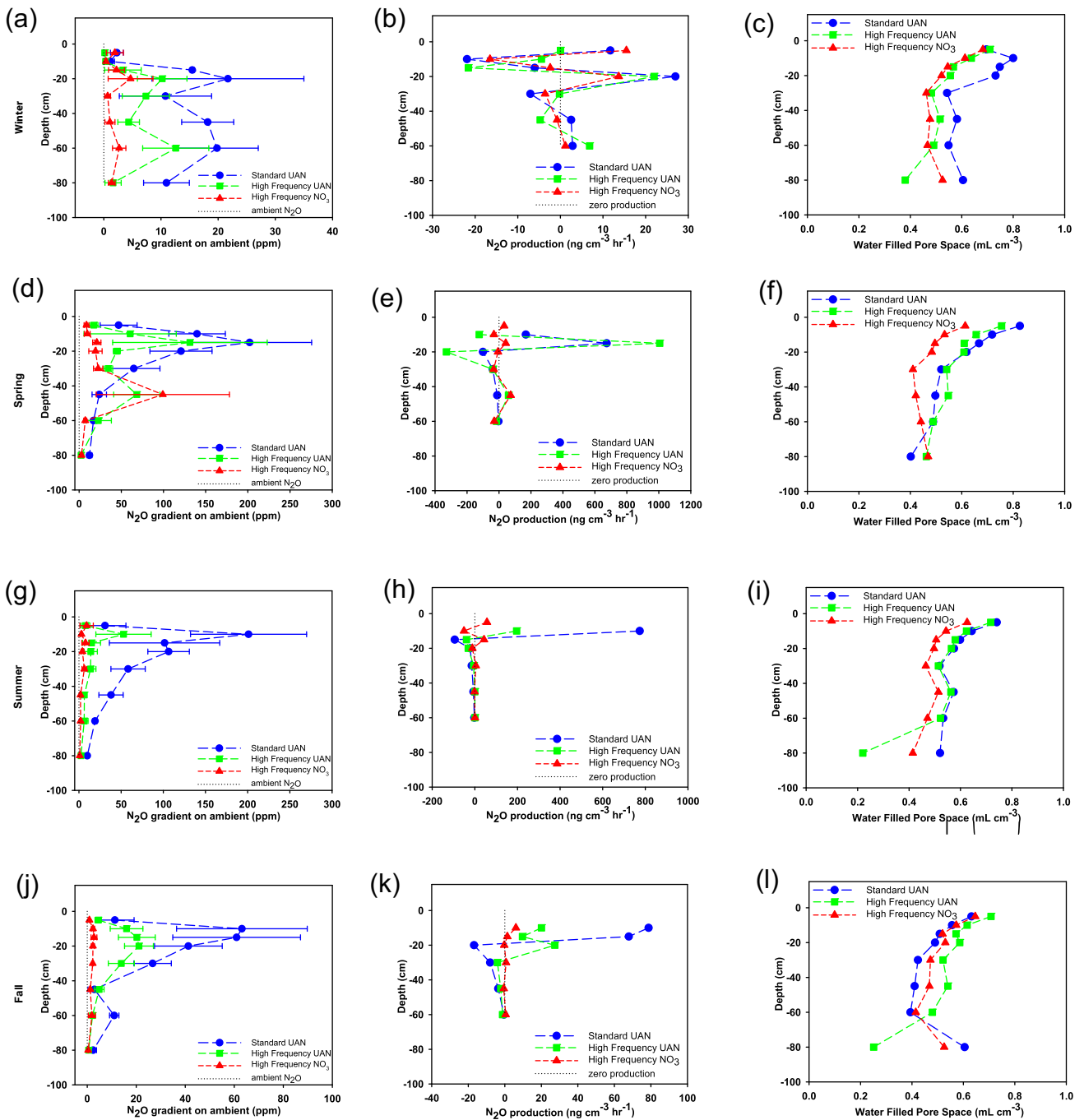


Fig. 4. Profiles of N_2O concentration, calculated N_2O production/consumption and WFPS on peak days of major fertigrations. (a) Winter Day 2 ppm; (b) Winter Day 2 production; (c) Winter Day 2 WFPS; (d) Spring Day 1 ppm; (e) Spring Day 1 production; (f) Spring Day 1 WFPS; (g) Summer Day 1 ppm; (h) Summer Day 1 production; (i) Summer Day 1 WFPS; (j) Fall Day 1 ppm; (k) Fall Day 1 production; (l) Fall Day 1 WFPS.

applied NO_3^- , vertically and laterally, which led to low concentrations. Production profiles (Fig. 4) further suggest that a high proportion of the N_2O produced in this treatment was consumed before it could be emitted from the surface.

Overall, surface emissions of N_2O decreased more quickly over the days following fertigrations than did soil gas concentrations and calculated in-soil production rates, suggesting greater importance of production near surface (<10 cm) during the first and second days. Under the driest conditions, seen on Day 3 after fertigation in late summer, increased N_2O concentrations at 60 and 80 cm were concurrent with the lowest post-fertigation surface emissions. Calculations of N_2O production for that date showed consumption

at 45 cm in both HF treatments (there was no Standard fertigation), supporting the conclusion that N_2O produced deeper was being consumed at points immediately above, as well as possibly diffusing downwards.

Although the averaging of soil gas profiles by treatment limited the options for statistical analysis, the factors driving N_2O production in the soil could still be assessed. Multiple linear regressions of surface emissions and of production at 15, 30 and 60 cm were carried out using calculated N_2O production per treatment per day at those depths, and the corresponding averaged NH_4^+ in solution, NH_4^+ in soil extracts, NO_3^- in solution, NO_3^- in soil extracts, WFPS and temperature. Treatments were pooled

because the dataset was limited within each treatment and the differences seen when HF NO₃ was separated were minor.

Regressions had little predictive capability at 30 and 60 cm depth. Nevertheless, it was notable that WFPS had negative coefficients at both depths, indicative of more complete denitrification with greater soil moisture.

At 15 cm, the Adjusted R² was only 0.14 but several alternative analyses gave better predictions. When excluding negative production values, an Adj. R² of 0.58 was seen, which rose to 0.68 when reduced to extractable NH₄⁺, WFPS and temperature. If production at 10 cm was averaged with that at 15 cm, most negative values were eliminated, and using all data and variables the Adj. R² was 0.21, or 0.26 with extractable NH₄⁺, NO₃⁻ in solution, WFPS and temperature.

These results caused some questioning of the calculations of N₂O production and consumption, which were volatile even in averaged forms, so regressions were carried out with soil gas concentrations as well. At 15 cm, all variables regressed to Adj. R² of 0.26; reduced to NH₄⁺, WFPS and Temperature, the Adj. R² was 0.32. Concentration averaged between 10 and 15 cm had an Adj. R² of 0.41, while reduced to NH₄⁺, WFPS and Temperature, the Adj. R² was 0.49.

Regression of surface emissions followed the same pattern, being compared to NH₄⁺ and NO₃⁻ in soil extracts at 2.5 cm depth, WFPS and temperature, where NO₃⁻ was found insignificant. The adjusted R² of this regression is not reported because it is less complete than the analyses above.

The superior predictive capability of extractable NH₄⁺ at 15 cm and near surface was unexpected, since it is usually assumed that only the NH₄⁺ (and NH₃) in solution is available for microbial consumption (Prosser, 2011). However, little relevant investigation has been done in soils and the question can be raised whether microbial foraging on clays can desorb ammonium (Gisvold et al., 2000).

3.4. Microbial assays for persistent treatment effects

The persistence of input effects on the functioning of the soil microbial community is an important agro-ecological concern. Here several assays of nitrification and denitrification capacity tested for persistent treatment effects which could influence N₂O emissions. Soils were collected in late August after a month of irrigations without fertilizer. Treatment differences were of interest, not the comparison of assay results to field rates.

The most ready metric of a soil's denitrification response to NO₃⁻ amendments is its denitrification enzyme activity (DEA), designed to assess soil process rates before they are affected by the synthesis of additional enzymes. Since fertigation applications make a large amount of NO₃⁻ available in a short time, the pre-event DEA of a soil may play a significant role in denitrification-

derived N₂O emissions. Results showed very similar N₂O production by the two HF treatments in a DEA assay, which were significantly higher than Standard UAN (Table 3). Over 24 h, characterized as Denitrification Potential (DP), this initial difference was persistent, although it lost statistical significance.

Given that drip fertigation saturated zones are not entirely dissimilar from the conditions of these assays, it was expected that DEA and DP modified with acetylene might also suggest differences in the product ratio (N₂O/(N₂O + N₂)) of denitrification in the field treatments. Results were inconclusive, with widely dispersed values.

Rates of ammonium oxidation to nitrite, as an index of nitrification potential, supported the importance of frequency and rate of NH₄⁺ application, HF UAN > Standard UAN > HF NO₃, but differences were only significant between the HF UAN and HF NO₃ treatments (Table 3). Strict chemoautotrophs typically dominate nitrification in cropped soils (Taylor et al., 2010), and their numbers are more likely to be affected by availability of NH₄⁺ than are the heterotrophs largely responsible for N₂O emission through denitrification. Higher amounts of available nitrite are known to stimulate nitrifier denitrification and associated N₂O losses (Venterea, 2007; Ni et al., 2014), so a persistent effect of NH₄⁺ application on ammonium oxidation to nitrite could increase N₂O emissions under HF fertigation.

Ammonium oxidation and DEA assays are predicated upon standard conditions, the former being oxic, open, shaken slurry, and the latter completely anoxic. Actual oxygen availability in drip zones may cover a wide range between those points, but is expected to be limited. Little data is available, but Gil et al. (2011) found 4.97% O₂ in the sampled soil air of a clay loam in an avocado orchard under drip. It can be assumed that many surfaces within larger aggregates would have lower O₂ (Sexstone et al., 1985), being well suited for nitrifier denitrification, which takes place at <5% O₂, while denitrification requires <0.05% O₂ (Goreau et al., 1980; Zhu et al., 2013).

It was therefore deemed useful to test the persistent effects of HF fertigation (HF UAN vs. Standard UAN) on potential soil production of N₂O at 3% O₂ (Zhu et al., 2013). The only treatment differences were in microcosms with NO₃⁻ amendments (Table 4), where N₂O was presumably derived mainly from denitrification inside aggregates, supporting DEA results (Table 3). The lack of HF treatment effects with NH₄⁺ may be due to high rates of adsorption on soil surfaces expected with this N source (and seen in the field), leading to gradual liberation. Nevertheless, emissions of N₂O with NH₄⁺ amendments were higher than those with NO₃⁻, confirming the large potential contribution of nitrifier denitrification from drip zones. The alternative explanation, being a general, rapid turnover from nitrifier-produced NO₂⁻ and NO₃⁻ to denitrifier-produced N₂O, has not consistently been supported by isotopic studies in laboratory (Kool et al., 2011; Zhu et al., 2013).

Assessments of N₂O/(N₂O + N₂) product ratio using acetylene in DEA, DP, and 3% O₂ incubation assays did not give robust support to the hypothesis that greater microbial capacity for nitrification and/or denitrification should correlate to a higher portion of complete reduction of N to N₂ (Tables 3 and 4). It must be noted that N₂O is more likely to be reduced to N₂ when NO₃⁻ is limited (Senbayram et al., 2012), which it was not in the DEA test and DP tests. Further, the reduction of N₂O to N₂ dominates under anoxic conditions (Davidson et al., 2000), which were not prevalent in the 3% O₂ test. The factors affecting the "completeness" of nitrifier denitrification to N₂ have been little studied (Poth, 1986; Müller et al., 2014) and may be distinct from those affecting denitrifier denitrification. Lastly, tests of residual NO₃⁻ suggested that acetylene may have slightly inhibited NO₃⁻ reduction.

The comparison of N₂O from HF UAN with a HFNO₃⁻-based treatment including Ca(NO₃)₂ raises the question of whether

Table 3
Soil microbial assays in samples taken at 0–20 cm depth with Tukey pairwise differences ($p < 0.05$).

Assay	Standard UAN	HF UAN	HF NO3			
Soil pH	7.23	ab	7.09	a	7.52	b
2:1 slurry						
Ammonium Oxidation ng NO ₂ -N g ⁻¹ soil min ⁻¹ 5 hours	23.03	ab	29.97	a	18.93	b
Denitrification Enzyme Activity ng N ₂ O-N g ⁻¹ soil min ⁻¹ 20 min	0.1	a	0.22	b	0.188	b
DEA Mole Fraction N ₂ O-N/(N ₂ O-N + N ₂ -N)	0.87	a	0.98	a	0.96	a
Denitrification Potential ng N ₂ O-N g ⁻¹ soil 24 h ⁻¹	18733.33	a	49516.85	a	41291.36	a
Denit. Pot. Mole Fraction N ₂ O-N/(N ₂ O-N + N ₂ -N)	0.53	a	0.35	a	0.45	a

Table 4

Relative importance of nitrification- and denitrification-derived N₂O emissions ($\mu\text{g N}_2\text{O-N g}^{-1}\text{ soil } 15 \text{ h}^{-1}$) from UAN-fertigated soils under 3% O₂ and 50% WHC, preincubated for 24 h with differences by ANOVA ($p < 0.05$).

Low Oxygen Soil Assays	Emissions ($\mu\text{g N}_2\text{O-N g}^{-1}\text{ soil } 15 \text{ h}^{-1}$)			
	Standard UAN		HF UAN	
Ammonium amendment $100 \text{ mg N kg}^{-1}\text{ soil}^{\text{A}}$	233.48	a	200.14	a
Ammonium amendment with blocked ammonium oxidation $100 \text{ mg N kg}^{-1}\text{ soil}^{\text{B}}$	30.78	a	82.45	a
Nitrate amendment $100 \text{ mg N kg}^{-1}\text{ soil}^{\text{C}}$	45.64	a	141.31	b
Nitrate amendment Mole Fraction $\text{N}_2\text{O-N}/(\text{N}_2\text{O-N} + \text{N}_2\text{-N})^{\text{D}}$	1.01	a	0.87	a
Averaged controls <i>No amendments, same irrigations</i>	39.505	a	46.405	a

N^A as ammonium sulfate; B^B as amm sulf + .01% acetylene; C^C as potassium nitrate, D^D using 5% acetylene.

differences may be ascribable to the opposite pH effects of the fertilizers. HF Ca(NO₃)₂ + KNO₃ did produce a significantly higher pH than HF UAN within 6 months of the treatment's inception (Table 3). This could partly explain lower N₂O emissions from the HF NO₃ treatment (Simek and Cooper, 2002; Raut et al., 2012; Sun et al., 2012), but the effect is likely not a strong one because all were in neutral range (Morkved et al., 2007).

Our observation of 2.0× greater N₂O emissions from HF UAN than from HF NO₃ agrees well with Abalos et al. (2014), who saw 2.4× greater N₂O emissions from urea than from calcium nitrate in a drip-fertigated melon field in Spain. The greater predictive capacity of extractable NH₄⁺ over NO₃⁻ provided evidence of a high contribution of nitrifier denitrification to N₂O emitted in the field. This was supported by laboratory tests of our field soils at 3% O₂, and concurred with findings by Vallejo et al. (2014), as well as by Sanchez-Martin et al. (2008), who calculated that with drip-fertigated ammonium sulfate, 45% of N₂O came from nitrification.

Considering both field and laboratory data, frequency effects in the application of UAN were only seen in nitrate denitrification rates and in N present at 60 cm depth. Nitrifier capacities do not seem to have been affected, due perhaps to the adsorption of fertilizer NH₄⁺ and its gradual release over time. Still, rates of nitrifier denitrification in the field may have seen concentration effects, as a corollary of frequency differences.

Direct investigation of N₂O production and fate in the soil profile has not advanced far, and is only beginning in the fertigation context. It is increasingly clear that much, if not most, soil N₂O might be reduced before emission from surface. It is commonly assumed that the upper 20 cm play the central role in surface emissions (Smart et al., 2011); in this study, production of emitted N₂O was centered at the 10–15 cm depth. Calculations of net N₂O production at different depths in the soil showed more overall N₂O consumption than production at 20 cm and below, helping to explain lower emissions under NO₃⁻ treatments, where points of production were deeper. N₂O production near surface (upper 5 cm) has been difficult to measure, but is a missing link of great importance.

The measurement and simulation of soil O₂ availability, varying as it does at different points in soil aggregates, remains an obstacle that impedes comparison of field and laboratory soils. Basic questions also persist over fertilizer N availability in its different stages of transformation. And field trials have not verified whether fertilizer management can affect the completeness of N reduction in classical or nitrifier denitrification.

4. Conclusion

There are important differences between the conditions normally underlying N₂O emissions from row crops with solid

fertilizers, and the semi-saturated soils of fertigated drip zones. While few researchers expect lower N₂O emissions from nitrate fertilizers in the former context, N₂O production was cut in half by preference of nitrates in a high-frequency fertigation system. Furthermore, from various metrics of N available in the soil, N₂O production was found to be most dependent upon extractable NH₄⁺. This suggests that nitrifier denitrification can be a major source of N₂O with ammoniacal fertilizers applied through fertigation.

It is also generally assumed that nitrate fertilizers pose a high leaching hazard. Here, however, limited evidence taken at 60 cm depth suggested that such fears may be misplaced in systems where water supply is well-controlled.

Fertilizer management choices affect the soil microbial community, and the assays presented here show that those effects can be persistent through a growing season in fertigated drip zones, affecting both nitrifiers and denitrifiers. Nitrifier populations responded to the quantity if not the frequency of NH₄⁺ administered, and denitrification rates increased with increased frequency of N application.

Both frequency and source of fertigated N can affect the distribution of N in the soil profile, its uptake by crops, its leaching potential, and surface emissions of N₂O. Our results do not give a basis to expect N₂O mitigation with high frequency application of ammoniacal fertilizers. However, preference of nitrate fertilizers did lower N₂O emissions, reaffirming the importance of N source in the fertigation context.

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