

AirJection Irrigation Mitigates Denitrification and Leaching

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Abstract. *Using a high efficiency venturi to inject air into water delivered through subsurface drip irrigation, commonly referred to as AirJection® Irrigation, has been shown to result in increased yields for a variety of crops. Studies have also indicated that the technology can positively affect photosynthetic activity, soil respiration rates, and stomatal conductance. In the current study, we compared the relative quantity of a series of genes known to be involved in the nitrogen cycle for soils subjected to AirJection® Irrigation for at least five years, with those that were not aerated. DNA was extracted using a PowerSoil™ kit and gene quantification was obtained via polymerase chain reaction. Distribution of the tested genes within the microbial populations was very distinct among the aerated and non-aerated soils. AirJection Irrigation had a clear selective impact on the distribution of the tested genes among the soil microbial population. While AirJection did not impact N fixation or ammonia oxidation, it did significantly change the denitrification genes population in manner that can positively affect nitrogen use efficiency. Furthermore, with judicious water management within the root zone, AirJection Irrigation can favor the dominance of bacteria that enhance plant nitrate uptake with a potential reduction in nitrate leaching.*

Keywords: Airjection Irrigation, Nitrate leaching, N fixation, Denitrification, Oxygenation

Introduction

Injection of air into the root zone environment has shown to enhanced crop productivity. However, the cost of an air-only injection system separate from the irrigation system, had previously remained cost-prohibitive. More than 75 years ago, Durell (1941) wrote, “a study of suitable oxygen carriers, which could be applied as fertilizer, and which would release oxygen slowly to the soil during the growing season, may be worthwhile.” With the acceptance of subsurface drip irrigation (SDI) by

commercial growers, implementation of an air injection system has become economically feasible. Nonetheless, the design of an air-injection system through a SDI tape requires thorough analysis and understanding of air movement within the soil profile and at the soil surface. When air alone is supplied to the SDI system it emits as a vertical “stream,” moving above the emitter outlet directly to the soil surface. As a consequence, the air affected soil volume is probably limited to a chimney column directly above the emitter outlet. Balancing the air/water relationships as well as changing soil temperature could affect growing conditions, yield, and time of harvest, particularly in locations with limited growing seasons. The concept of aerating the irrigation water increases the potential for the air to travel within the root zone, thereby positively affecting plant growth.

Through work in other areas, the Mazzei Corporation has developed high efficiency venturi injectors capable of aerating water with fine air bubbles. By combining the Mazzei injectors with SDI, it is possible to deliver “aerated water” close to the root zone. The technology has now been patented and is referred to as ***Air-jection® Irrigation***. In summary, the system allows for a fluid mixture to be delivered to the root zone of the plant, via the irrigation systems, in what can best be characterized as an air/water slurry. In previous work with growers on a commercial test plot basis, Air-jection has demonstrated bell pepper yield increases of 13 percent and 8 percent for premium and processed bell peppers, respectively. Findings from the initial CSU-Fresno study by Goorahoo et al (2001) justified follow-up fieldwork on larger commercial plots. On average, AirJection® Irrigation has resulted in a 13-18% yield increase in fresh market tomatoes, cantaloupes, honeydews, broccoli, strawberries and sweet corn (Goorahoo et al., 2008). Similar results have been obtained by a research group at Queensland University in Australia (Bhattarai, et al., 2004, 2005 & 2006), where the technology has been called “Oxygation”. Our work on organic farming systems indicated that AirJection® Irrigation also positively affected photosynthetic and soil respiration rates, stomatal conductance, leaf scale water use efficiency, plant tissue nitrate concentrations, and shoot and root biomass (Reddy, 2008).

Objective

In our ongoing research, we are evaluating the impact of AirJection® Irrigation on yield and soil salinity for tomatoes grown on salt affected heavy clay soils. The specific objectives are to: determine the impact of AirJection® Irrigation on yield and Brix level of fresh-market tomatoes grown on a salt affected heavy clay soils; and, evaluate the impact of Air-jection® Irrigation on the spatial and temporal variability of salinity levels as measured by the apparent electrical conductivity of the soil. Concurrently, we are also attempting to evaluate the long term impacts of Air-jection® Irrigation on the component so the nitrogen (N) cycle for soils used for vegetable crops. Hence, the objective of the current study was to quantify the relative proportion of a series of genes known to be involved in the N cycle for soils collected from non-aerated fields and from those subjected to Air-jection® Irrigation for at least five years.

Materials and Methods

The study was located at a commercial vegetable grower in Mendota, California USA, a Panoche clay soil. A replicated (four times) soil sampling protocol was implemented in 2015 in which soils were collected within the 0-6 and 6-12 inches depths in adjacent fields that were non aerated (water only) and those subjected to Aijection Irrigation (Table 1). Samples were collected at distances of approximately 1/4 (Head), 1/2 (Middle) and 3/4 (Tail) from the irrigation inlet along the distance of the drip tape run length.

Table 1. Summary of treatments used in evaluation of soil DNA series

Irrigation type	Distance	Depth	Treatment
Water	H	6in	WH6in
		12in	WH12in
	M	6in	WM6in
		12in	WM12in
	T	6in	WT6in
		12in	WT12in
AirJection	H	6in	AH6in
		12in	AH12in
	M	6in	AM6in
		12in	AM12in
	T	6in	AT6in
		12in	AT12in

Nitrogen cycling genes were selected to describe the entire N cycle from nitrogen fixation (*nifH*) to nitrification (ammonia oxidation), and denitrification (Nitrate, nitrite and nitrous oxide reduction) (Table 2). Bacterial quantification was carried out using protocols employing the primers described by Hilty et al. (2010). DNA was extracted using the PowerSoil™ extraction kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturers protocol.

Table 2. Tested genes

Role	Bacteria	Archaea
Nitrogen	<i>nifH</i> (nitrogenase reductase)	-
Ammonia oxidation	<i>amoA</i> (ammonia monooxygenase)	-
	-	Arch <i>amoA</i> (archaea)
	-	Cren <i>amoA</i> (Crenarchota)
Nitrate reduction	<i>narG</i> (Proteobacterial Membrane-	-
	<i>napA</i> (Proteobacterial Periplasmic	-
Nitrite reduction	<i>nirK</i> - Denitrifying nitrite reductase	-
	<i>nirS</i> - Denitrifying nitrite reductase	-
Nitrous oxide	<i>nosZ</i> - nitrous oxide reductase 1	-
Total	<i>16S rDNA region</i>	

A pooled sample of DNA extracted from all 48 soil samples were made for primer pair thermal gradient optimization tests. Dilutions (undiluted, 1/10, 1/20, 1/40, 1/100) of the pooled sample were used as template to assess the optimal annealing temperature of each of the primer pairs. The assay recipe and protocol for use with BioRad QX200™ ddPCR™ EvaGreen Supermix was in accordance to manufacturers instruction and was modified by inserting a thermal gradient between 52 °C and 64 °C for 1 min extension time in place of the annealing step. This insured that the correct dilution (which

turned out to be 1/10 for the 16S primers and no dilution for all others) as well as the correct annealing temperature were used for each primer pair to achieve optimum results.

Data were tested individually for each gene, employing a standard t-test to assess the differences of the means; equal or non-equal variance was considered as appropriate. Principal components analysis (PCoA) was employed to visualize the trends within the entire dataset.

Results

Abundance of the tested genes was normalized to the abundance of the bacterial or archaeal indicator ribosomal genes (16S rDNA). Thus results represent the possible intensity of the respective function among the bacterial or archaeal populations but not necessarily the absolute counts of each gene per unit soil mass or volume. A principal component analysis was carried out (Figure 1) in which the proportional gene quantities were considered as independent variables with the treatment as dependent variables. This analysis allowed for an evaluation of the relationship between the independent variables and also their relationship with the treatments. There was a clear separation between the AirJection and the control treatments. This was obviously associated with a decrease in the proportional counts for three denitrification genes describing the entire denitrification pathway. Generally, AirJection minimizes the count of genes known to effect denitrification along the entire denitrification sequence (i.e. inhibits reduction of nitrate, nitrite and nitrous oxide = likely less NO_x gaseous losses = likely increased nitrate-N availability to plants)

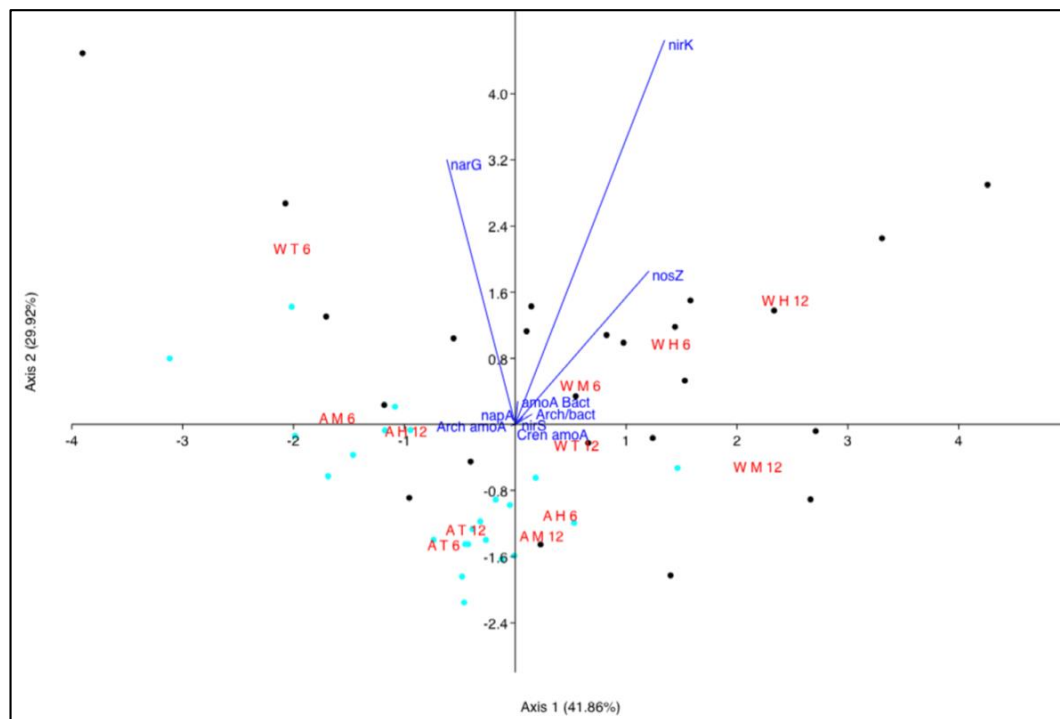


Figure 1. Principal component analysis. Blue dots = AirJection (A) treatment samples (A); Black dots = water (W) only treated samples (control); H, M and T = location along the delivery irrigation line; 6 and 12 = sample depth in inches; Red labels located at the centroid of the samples describing the respective treatment. The graph describes about 72% of the total variability. Lines indicate the direction and the discriminant capacity of the tested genes (all genes were normalized in units of gene per bacterial count).

N fixation: The *t-test* analyses indicated that the means for the AirJection and control data were statistically significantly different ($pH_0=0.07$), this was mainly due to a couple of extreme data points in treatment W-H 12in (Figure 2). Thus it may be stated that there is not sufficient evidence to indicate a decline in nitrogen fixation due to AirJection, however, the trend justifies future testing.

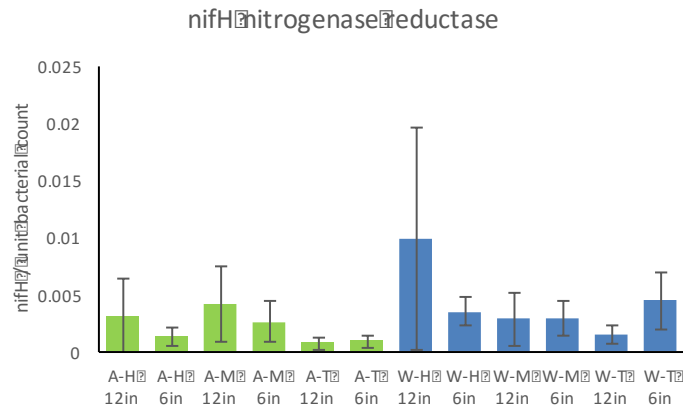
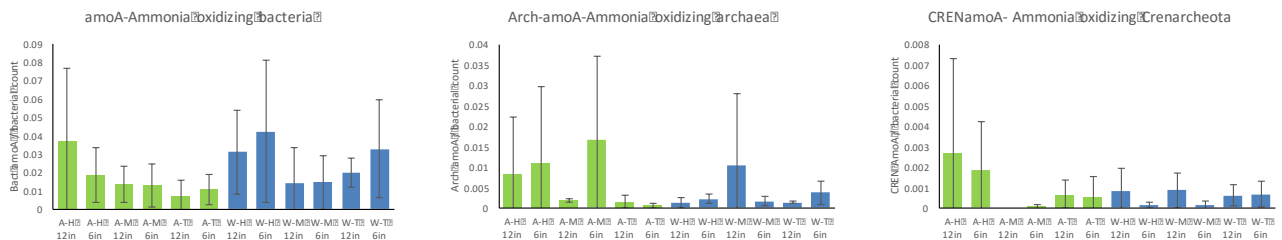


Figure 2. Distribution of *nifH* proportional gene counts across treatments. Error bars describe the statistical 95% Confidence Interval

a. Bacterial and Archaeal *amoA* genes normalized to total Bacterial counts



b.

Archaeal *amoA* genes normalized to total Archaeal counts

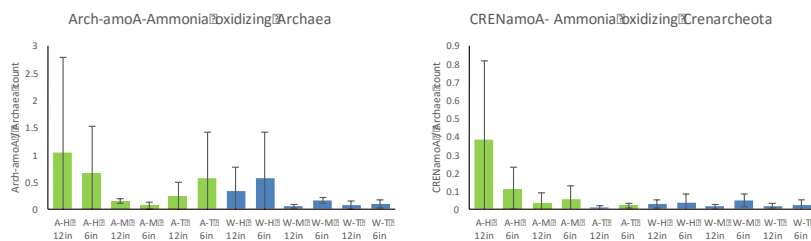


Figure 3. Ammonia monooxygenase distribution; top row describes its intensity (*amoA*) within or against (*Arch-amoA* and *CRENamOA*) bacterial population; the second row describes its intensity within (*Arch-amoA*) or against (*CRENamOA*) Archaeal population. Error bars describe the statistical 95% Confidence Interval.

Nitrification: Ammonia oxidation potential was tested for both Bacteria and Archaea (Figure 3). Bacterial nitrification activity was similar between AirJection and control ($pH_0=0.18$). Archaeal denitrification per unit bacteria was also not distinct across treatments ($pH_0=0.27$ for *Arch-amoA*;

$pH_0=0.36$ for *CRENamOA*). It should be noted that this comparison integrates both changes in archaeal counts and changes in archaeal associated nitrification and therefore reflects the contribution of archaeal nitrification to the entire microbial population (Figure 3a). A verification of the changes in nitrification among the archaeal population only (Figure 3b) while might show a trend for more activity in the AirJection tests, such trend is not statistically significant ($pH_0=0.22$ for *Arch-amoA*; $pH_0=0.1$ for *CRENamOA*). Our results indicate that aeration of the irrigation water was not sufficient to minimize the density of nitrification genes in the population. Nevertheless, these tests did not verify the actual gene expression which would more accurately describe the role of bacteria and archaea in nitrification under the treatment.

Denitrification: The genes density for this N gaseous loss, whose expression is favored under anaerobic conditions, were the most clearly affected by AirJection treatment (Figure 4).

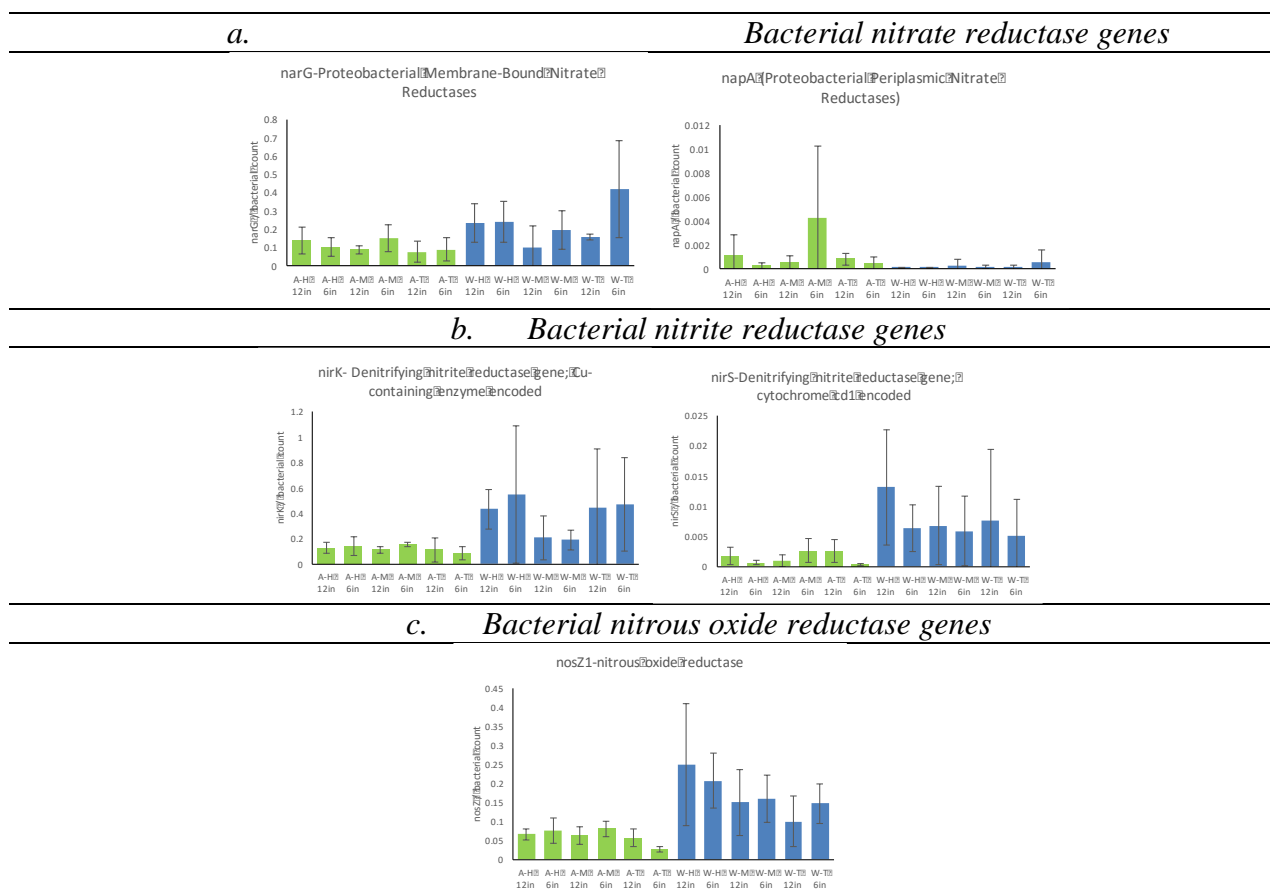


Figure 4. Distribution of genes involved in the denitrification pathway; nitrate reductases (*narG* and *napA*), nitrite reductases (*nirK* and *nirS*), and nitrous oxide reductase (*nosZ*). Error bars describe the statistical 95% Confidence Interval.

Nitrate reductase participate in the reduction of nitrate (NO_3) to nitrite (NO_2). Of the two tested genes one, *narG*, was depressed in the AirJection treatment a trend statistically significantly different from control ($pH_0=0.002$). The second relevant gene, *napA*, was enhanced by the treatment but not this was statistically not significant ($pH_0=0.08$) (Figure 4a). It is considered that *narG* is active at high nitrate concentration while *napA* may more actively reduce nitrate when the nitrate concentration is

reduced (Stewart et al., 2002). Thus in our control experiment the larger narG suggest active denitrification linked to likely large nitrate availability and more anaerobic conditions. The depressed napA activity is likely linked to the large nitrate concentration and high narG activity. A reduction of narG activity under aerobiosis (i.e. AirJection) might occasionally enhance napA activity to the detriment of narG. Nevertheless the consistent decrease of narG gene copies with AirJection suggest continuous selective pressure that limits nitrate reduction potential within the microbial population.

Nitrate reductase participate in the reduction of nitrite (NO_3) to nitrous oxide (N_2O). For both genes tested here there was a significant decrease in copy number in the bacterial population ($p_{H_0}=0.001$ for nirK and $p_{H_0}<0.001$ for nirS). The results are self-evident; aerobic conditions associated with AirJection depleted these genes (Figure 4b) from the microbial population clearly decreasing the capacity of these microbes to reduce nitrite. This is likely a cascade effect whereby lower nitrate reduction potential (see narG above) produces less nitrate that may be available for further oxygen loss.

Concluding Remarks

- The relative quantity for a series of genes known to be involved in nitrogen cycle was estimated for soils collected from non-aerated fields and those subjected to AirJection Irrigation for at least five years.
- The ratio between total archaea and total bacteria were estimated. Archaea have been shown to be active in matter cycling in soils and to be more resilient than bacteria. On the other hand, bacteria dominance indicates a likely shift to more luxurious growth conditions.
- Nitrogen fixation potential was evaluated using the most commonly known relevant gene, *nifH*. Ammonification genes (ammonia monooxygenases), related to the rates of mineralization of organic matter, were tested for both Bacteria (*amoA*) and Archaea (Arch-*amoA*, CREN-*amoA*).
- Denitrification potential, common in anaerobic conditions and a process directly linked to gaseous losses of nitrogen (as NO_x 's), was verified through the quantification of a number of genes related to various metabolic pathways known to be of relevance. Thus nitrate reductase genes (narG, napA), nitrite reductase genes (nirS, nirK), and nitrous oxide reductase (nosZ) were evaluated.
- The distribution of the tested genes within the microbial populations was very distinct among the two treatments, aerated and non-aerated. This indicates that AirJection had a clear selective impact on the distribution of the tested genes among the population. It may thus be hypothesized that total diversity also changed.
- Generally, AirJection Irrigation led to a proportional increase of Bacteria versus Archaea. While the AirJection Irrigation did not have a significant impact on nitrogen fixation or ammonia oxidation, the practice of adding aerated water via the buried drip line did have a significant impact on denitrification genes suggesting lower NO_x production potential and thus likely increased availability of nitrate in the root zone. This might be hypothesized to enhance nitrogen use efficiency potential with AirJection, and with the judicious water management within the root zone, plant nitrate uptake can be enhanced with a potential reduction in nitrate leaching.

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